

The Lichens

Edited by Vernon Ahmadjian and Mason E. Hale



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THE LICHENS

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THE LICHENS

Edited by

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PREFACE

Lichens are nature's most remarkable alliances. Combinations of certain fungi and algae have been so successful that there are now some 20,000 species of lichens distributed in most of the environmental habitats of the world. Ironically, the success of lichens has caused a major problem for those who study them—the one of identity. Logically, they should be classified with the fungi. Practically, however, this causes difficulties because of the wide differences between the two groups and because of the large number of lichens—they are the single largest group of Ascomycetes. For these reasons mycologists have been reluctant to deal with the lichens, and lichenologists have been content to maintain the separation. In recent years there has been movement toward incorporating lichens in a fungal classification. For example, the sixth edition of the "Dictionary of the Fungi" includes lichens for the first time. We are encouraged by such action and hope that this volume will stimulate further efforts to integrate lichenized and nonlichenized fungi.

The suggestion for this work came from the editors of the multivolume treatise "The Fungi" published by Academic Press. The lichenized fungi were not included in these volumes. The editors wanted a treatment that would complement the treatise and we hope that this volume fulfills this goal.

Investigations on lichens have differed from those on fungi. Topics such as genetics, cell biology, and ontogeny dominate mycology but remain virtually untouched among lichenized forms where the emphasis has been on more classic organismic level research and chemotaxonomy. We believe that mycologists have much to learn from their neglected relatives just as lichenologists have from developments in the nonlichenized fungi.

While there are several college-level texts on lichen available, none can match the depth of a multiauthor treatment to which each author brings a voice of authority and imparts some of the excitement in his own specialty. We are aware, of course, that multiauthored texts lack continuity and differ from chapter to chapter in emphasis and approach.

It has been difficult to find authors to cover all of the topics which seem

essential. However areas which we consider to be most important are included: structure and development, physiology of the intact thallus, environmental response and effects, secondary metabolic products, and symbiont interactions. The appendices consist of a taxonomic scheme, methods for isolating and culturing lichen symbionts and thalli, and methods for isolating and identifying lichen substances. Hopefully gaps will be filled in future editions of this treatise. A few areas were deliberately omitted; phytosociology, for example, is extensively covered in Barkman's book.

Ultimately, the real value and usefulness of this treatise derives from the efforts of the individual authors. They have been patient with editorial badgering, deadlines, and other problems that arise when so many authors from different countries come together in a common cause. We can only hope that this pioneering effort brings to the scientific community a new appreciation of the scope and diversity of lichenology.

*VERNON AHMADJIAN
MASON E. HALE*

Part I

STRUCTURE AND DEVELOPMENT

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Chapter 1

ANATOMY, MORPHOLOGY, AND DEVELOPMENT

H. M. JAHNS

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I. Introduction

The thallus of the lichenized ascomycetes exhibits such a complexity of form and color that the inexperienced observer may be forgiven for failing to realize what diverse organisms belong to this group of plants. At the beginning of the nineteenth century botanists were making their first attempts to classify the lichens, and in order to systematize the immense variety of growth habit they first divided the class on the basis of several major growth forms. For example, lichens covering rocks, trees, or soil with a thin, more or less well-developed crust were separated from leaflike species which adhere more or less firmly to the substrate and from upright, branching,

bushlike forms. It will become obvious that this principle of classification is more or less arbitrary accentuating only the most striking stages on a scale of continuous development from a primitive to a highly differentiated organism. There are numerous intermediates between the three basic growth forms.

Certainly the growth form of lichen thalli cannot be considered as a principal characteristic on which taxonomy can be based. The foliose lichens, for example, do not form a taxonomic group of related species but only a morphological unity. The lichens of one family, even of one genus, may belong to the crustose, foliose, and fruticose growth form.

A theory proposed by Reinke (1894–1896) suggests that the lichens of different taxonomic groups have developed independently from crustose species to foliose forms and finally to fruticose plants. Fruticose lichens are regarded as the most highly developed peaks of several parallel lines of evolution. This theory, although attractive, is not acceptable. As will be seen, the structure of foliose thalli is not less complicated than that of fruticose thalli. On the contrary, some highly differentiated organs such as cypellae are confined to foliose genera. For this reason, the foliose and fruticose lichens must be regarded as forms equal in their level of development in a line of evolution leading from a crustose organization to a more highly differentiated habit. Crustose lichens can, with some certainty, be considered as primitive or secondarily derived.

In spite of the above-mentioned objections it is still convenient to divide the lichens into growth forms. The habit being often the most obvious characteristic for distinguishing lichen species, the growth form is the most useful starting point in the construction of artificial keys for their determination.

The habit of a lichen is due not only to the overall growth form, but is often the result of special anatomical characteristics. For example, the shape of the thallus surface depends on the anatomy of the cortex. External appearance and internal structure are interdependent.

The tissues of the thallus consist of certain cell types, which are derived from the simple cells of the fungal hyphae. For a better understanding of the habit and structure of the lichen thallus it is, therefore, convenient to describe the original fungal cell and to follow its development into the specialized cells of which the various tissues and thalli are composed.

II. Anatomy of the Thallus

A. Cells

The spores of lichenized and nonlichenized fungi germinate and produce hyphae which are divided into cells by means of cross walls called septa. These cells are characterized by their basic cylindrical form and thin walls

(Fig. 4). Cells of different hyphae may become secondarily connected. This happens at points where adjacent hyphae touch one another; where their cell walls fuse interconnecting pores are developed. These points of contact between two cells are referred to as anastomoses (Fig. 17). Cells which are connected by pores are chiefly confined to lichens with highly differentiated thalli. In *Peltigera*, pores can be found in the cross walls between the cells as well as in the anastomoses of the adjacent longitudinal walls. The hyphae of *Parmelia* are often swollen at the cross septa thereby appearing bone-shaped. The cross septa in this genus are thickened and perforated by pores (Fig. 16).

The fungal cell retains its cylindrical form in loosely organized tissues. In modified lichen tissues the shape of the cells changes because of their special growth and differentiation. Adjoining cells may also influence the shape. For example, cells may be flattened or become angular by the mutual pressure of adjoining developing cells.

In cell differentiation, one of the most important features is the distance between the septa of the hyphae. If the septa are close together, the cells are nearly square in longitudinal section; if the septa are far apart, the cells will appear more rectangular. Many of these cells continue to grow and they begin to swell. The square cells tend to become spherical (Fig. 2) while the rectangular cells take on a more ellipsoid form (Fig. 3). If only one end of the cell swells, it becomes clavate. These asymmetrical cells are often found in the fruiting body at the tips of the paraphyses. In *Aspicilia* and *Coenogonium*, for example, several club-shaped cells are formed successively (Fig. 19).

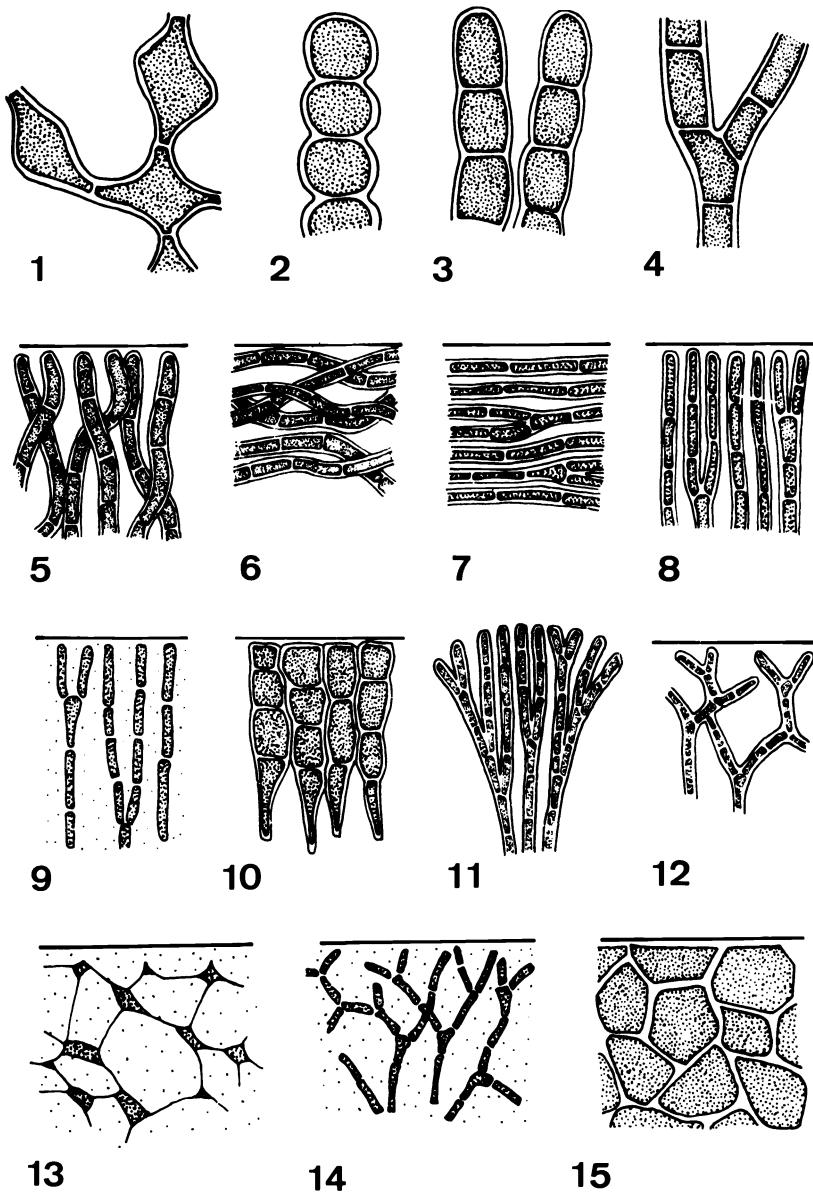
In most lichen tissues the cells show a less regular form than in the ultimate cells of the paraphyses. Usually the lumina are irregularly enlarged and multiangular with thin protuberances (Fig. 1). In cross section these cells are three- to many-cornered and only a part of the cell retains a rounded form.

The shape of the lumen can be influenced by changes in the structure of the wall. Substances may be deposited on the walls or they may become gelatinized and swollen. In this way the cell lumen may be reduced to a thin, often attenuated cavity. In some tissues the cell walls become indistinct and form a homogeneous substance around the lumina (Figs. 9, 13, and 14).

B. Tissues

1. DEVELOPMENT OF TISSUES

The structure and development of the tissues depends on the form of the cells and on the particular type of contact between them. This is achieved either by the mutual adherence of the cell walls, by the formation of anastomoses, or by the gelatinization of the cell walls. Other important factors are the direction of growth and the orientation of the hyphae to the surface of the thallus and to each other (Figs. 5-8).



FIGS. 1-15. Hyphae and tissues. Fig. 1, hyphae with multi-angular cells; Fig. 2, hyphae with globose cells; Fig. 3, hyphae with ellipsoid cells; Fig. 4, branched hyphae with cylindrical cells; Fig. 5, interwoven hyphae in anticalinal arrangement; Fig. 6, interwoven hyphae in pericalinal arrangement; Fig. 7, parallel oriented hyphae in pericalinal arrangement; Fig. 8, parallel oriented hyphae in anticalinal arrangement; Fig. 9, prosoplectenchymatous tissue

In the tissues the hyphae are either parallel, resulting in a fastigiate arrangement (Figs. 7 and 8), or they are irregularly bent to produce a tissue of interwoven threads (Figs. 5 and 6). Only rarely do the hyphae branch at right angles; usually they branch at an acute angle and form a fan-shaped tissue (Fig. 11). In lichens the most characteristic tissue arrangement is a netlike structure composed of branched, anastomosing hyphae (Figs. 12–14). The cells of this tissue usually have angular or irregular lumina (Fig. 13). This netlike tissue is rarely found in unlichenized ascomycetes and is therefore absent in the description of the *textura* types given by Korf (1958). The other tissue arrangements described here correspond to the types given by Korf.

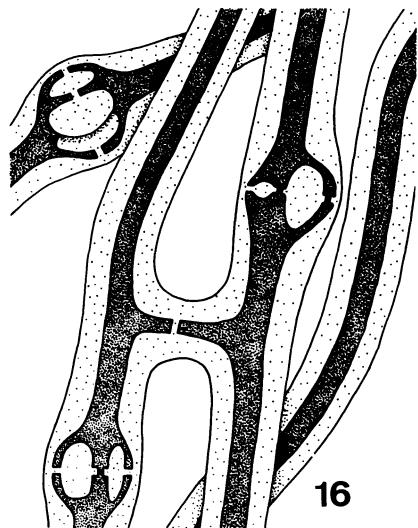
2. TYPES OF TISSUES

The development of a true parenchymatous tissue in lichens is rare. Such a tissue is formed by cells dividing in three planes. This kind of cell division, which is characteristic for higher plants, is found in the stroma of some ascocolocular fungi and in the muriform ascospores of some lichens, for example, in *Polyblastia* and *Staurothele*.

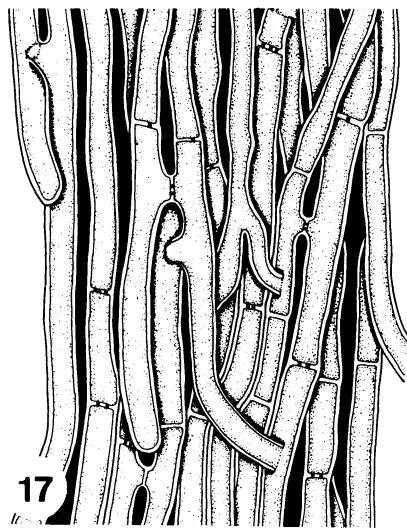
With the exception of these special cases, all lichen tissues are plectenchymatous in origin. The cells divide in only one plane forming cellular hyphal threads. In plectenchyma, the hyphae are loosely interwoven, interconnected by anastomoses, or firmly glued together. The secondary contact between different hyphae can be so close and united that the individual hyphae may be indistinguishable. Some plectenchyma are similar to tissues of higher plants and accordingly are given names that express this resemblance. If the cellular structure of a plectenchyma, consisting of closely packed cells, resembles the parenchyma of higher plants, the tissue is called pseudoparenchymatous or paraplectenchymatous. If the walls of the cells are strongly gelatinized, so that the tissue is similar to prosenchyma (collenchyma) of the higher plants, it is called prosoplectenchymatous.

These two types of compacting tissues can develop from different basic cell forms. For example, a pseudoparenchyma may arise from short, rounded thin-walled cells of different hyphae, which are pressed together, finally forming an unbroken tissue of angular isodiametric cells (Fig. 15). It may be impossible to recognize that this tissue really consists of individual

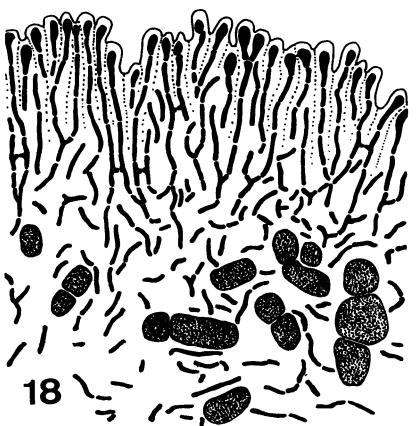
developed from anticlinal hyphae with strongly gelatinized walls; Fig. 10, pseudoparenchymatous tissue formed from thin-walled anticlinal hyphae; Fig. 11, fan-shaped arrangement of hyphae; Fig. 12, branched hyphae in a netlike arrangement; Fig. 13, cell lumina of netlike hyphae lying in the homogeneous substance of the gelatinized walls; Fig. 14, prosoplectenchymatous tissue formed by hyphae in a netlike arrangement with gelatinized walls; Fig. 15, pseudoparenchymatous tissue. (Figs. 1–15 from Henssen and Jahns, 1973.)



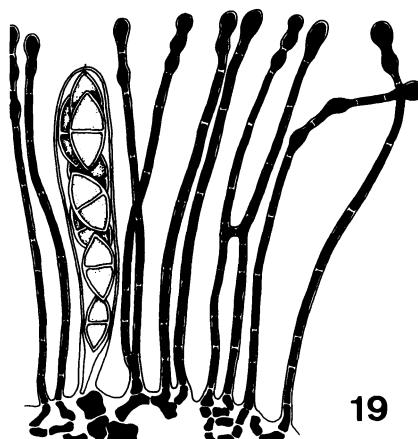
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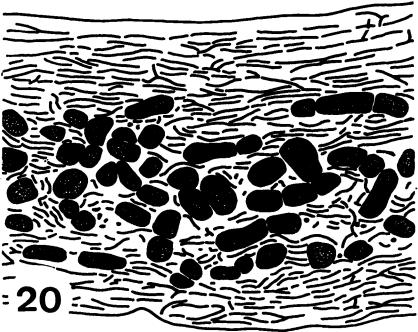
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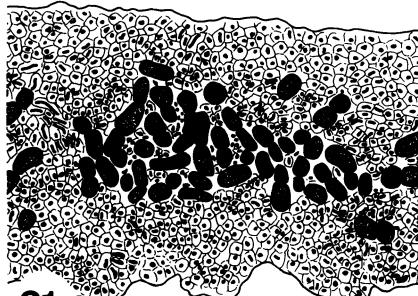
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hyphae. In other pseudoparenchymatous lichen tissues the individual hyphae are still discernible (Fig. 10). This kind of tissue develops from elongated swollen cells of loosely interwoven hyphae. In pseudoparenchyma the cell walls may become gelatinized.

Most prosoplectenchymatous tissues develop from plectenchyma with a netlike structure of multiangular or irregularly shaped cells. The walls of the cells gelatinize and become a homogeneous mass in which it is no longer possible to distinguish individual hyphae. Frequently, the shape of the cell lumen changes during the growth of the tissue and accordingly the appearance of the tissue can vary considerably in detail. Not only short-celled hyphae, but also long-celled hyphae in parallel orientation, can form prosoplectenchyma. The cell walls, already connected by anastomoses, may become gelatinized and firmly cemented together.

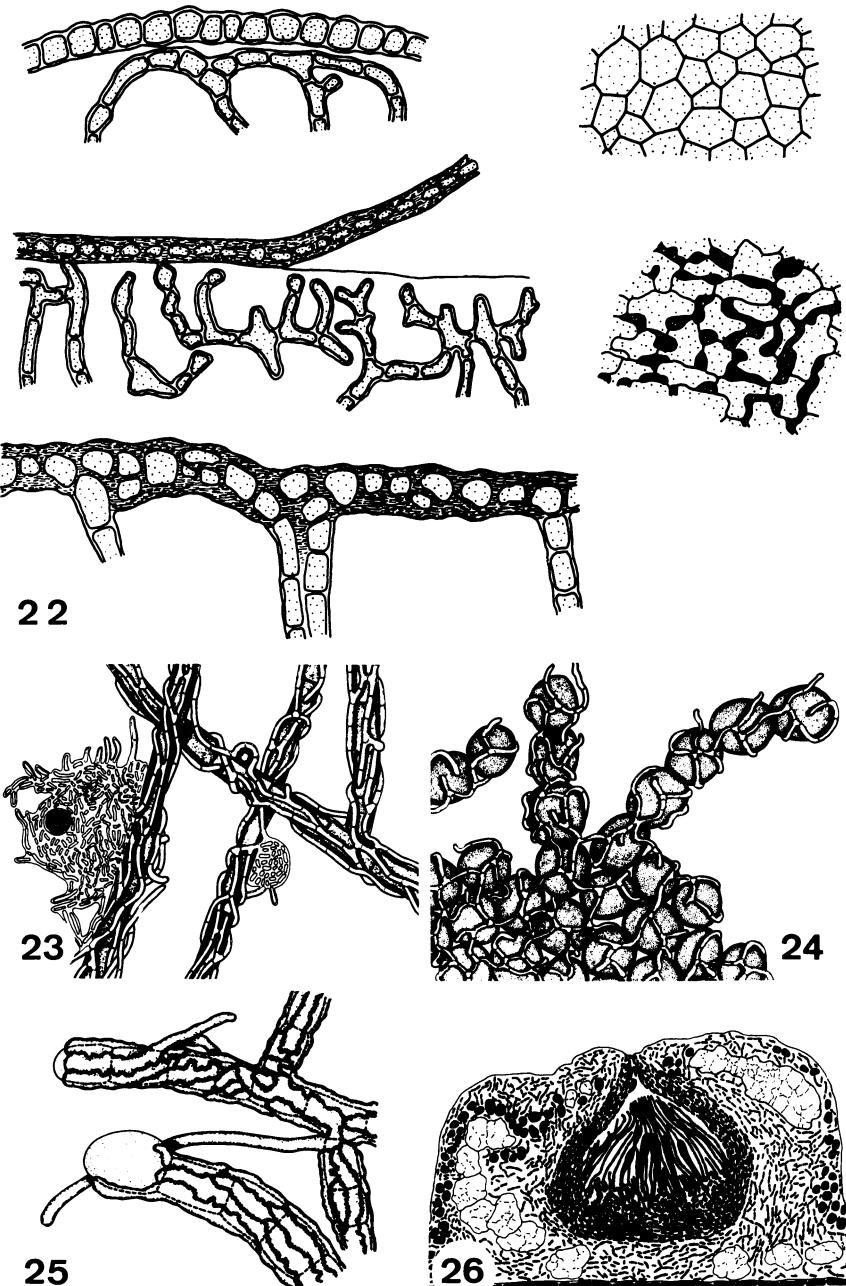
The hyphae of loosely interwoven plectenchymatous tissues are either irregularly bent or parallel. In tissues with a fastigiate arrangement, the hyphae lie parallel or perpendicular to the surface of the thallus (Figs. 7, 8, 18, 20, and 21). The second type is referred to as a palisade tissue while the perikline structure has no special name.

In some gelatinous lichens, for example in *Leptogium*, the hyphae are compacted at the surface of the thallus into a pseudoparenchymatous layer which is only one cell thick. Seen from above, this tissue consists either of isodiametric cells pressed together in an unbroken layer or of loosely organized irregular cells (Fig. 22).

C. Structure of the Thallus

The habit of some primitive lichens, especially of those species where the process of lichenization is not far advanced and the relation between mycobiont and phycobiont is not yet definitely stabilized, resembles the thallus of free-living fungi or algae. In some species, the primitive lichen thalli consist of a loose fungal mycelium enclosing scattered groups of algae, which spreads over the substrate, while other thalli resemble a gelatinous algal colony penetrated and interwoven by fungal hyphae. An example of a thin mycelium with loosely associated algae is the genus *Lepraria*, a lichen that grows on soil, rocks, or tree bark. Alternatively, the thallus of

FIGS. 16–21. Fig. 16, hyphae from the medulla of *Parmelia cetrariooides* with bone-shaped cross-septa perforated by pores; Fig. 17, hyphae from a rhizine of *Peltigera praetextata* showing anastomoses and pores; Fig. 18, palisade tissue in the cortex of *Roccella phycopsis*; Fig. 19, ascus and paraphyses of *Coenogonium* with club-shaped cells; Figs. 20–21, longitudinal and vertical sections through the thallus of *Darbshirella gracillima* showing the algal layer and arrangement of cortical hyphae. (Figs. 16–21 from Henssen and Jahns, 1973.)



Figs. 22–26. Fig. 22, types of cortex in the Collemataceae. Above: cortex of isodiametric cells in *Leptogium sinuatum* seen in cross section (left) and from above (right). Middle: primitive cortex of *Leptogium apalachense*; the cell lumen lies inside a gelatinous substance and forms an irregular pattern when seen from above (right). Below: cortex of *Physma byrsinum*

some species of *Collema* consists largely of the blue-green alga *Nostoc* and resembles a colony of this alga (Fig. 66). The hyphae of the mycobiont of *Collema* grow inside the gelatinous matrix of the phycobiont.

The lichen thalli described above, characterized by a simple and undifferentiated thallus with irregularly distributed algae, are termed homoiomerous. Only a few lichen genera have this type of thallus. Though the thallus of most lichens is separated into several distinct layers, there exists one other group with an unstratified thallus, which nevertheless is not usually referred to as homoiomerous; these are lichens with extremely short and hairlike thalli consisting of strands of filamentous alga closely wrapped in fungal hyphae (Figs. 23–25).

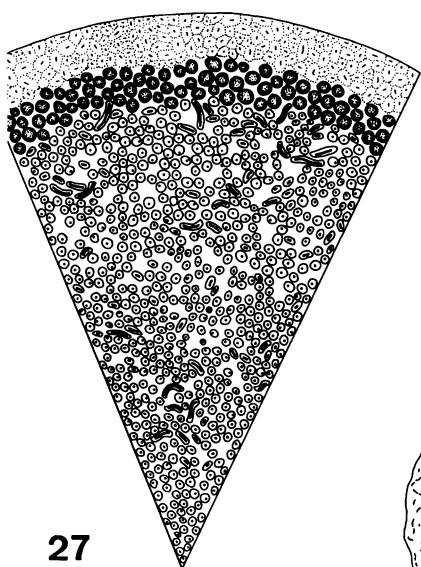
Most lichens are more complex in structure. The algae are restricted to a particular layer in the thallus and besides the algal zone there is at least one other defined layer, the medulla, which contains no algae. Other layers, a cortex for example, may also be developed. These thalli with a stratified organization are called heteromerous (Fig. 93).

1. ALGAL LAYER

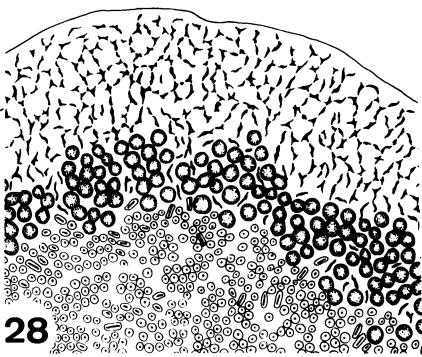
Within the algal layer the contact between the partners of the symbiosis is established. The relations between algae and hyphae vary considerably. Mycobiont and phycobiont are either without direct contact or the hyphae of the fungus more or less completely clasp and surround the algae. In some lichens, cells of the mycobiont are pressed against the algal cells and are called appressoria. In other genera haustoria penetrate the algal cell membrane. Haustoria that penetrate the living cells may kill the algae. In some lichens algae and attacking haustoria divide simultaneously. The two daughter cells of the alga are clasped by two branches of the divided haustorium.

In the algal layer the algae multiply by mitotic cell division and by aplanospores. In *Trebouxia*, for example, the protoplast of an algal cell divides into several protoplasts, each of which subsequently secretes a cell wall. These aplanospores are freed by the rupture of the wall of the mother cell. The stages of this process can easily be observed in sections of the thallus. Sexual reproduction by zoospore formation has not been observed within the lichen

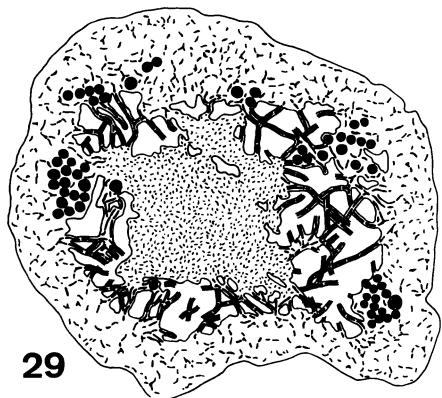
which is several cells deep in some places. Fig. 23, hairlike thalli of *Coenogonium* sp. with *Trentepohlia* as phycobiont; in two places the hyphae gather to form a fruiting body; Fig. 24, hairlike or granular thallus of *Coenogonium moniliforme* with *Physolinum* as phycobiont; Fig. 25, hairlike thallus of *Cystocoleus niger* with *Trentepohlia* as phycobiont; the alga is completely covered by hyphae; Fig. 26, young peritheciun of *Porina nucula* within a thallus granule; the thallus contains large crystals of calcium oxalate. (Figs. 22–26 from Henssen and Jahns, 1973.)



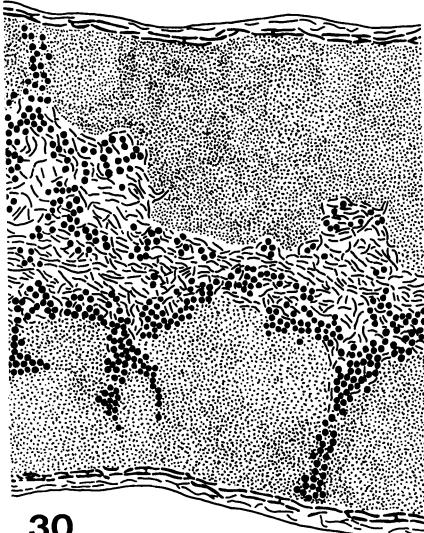
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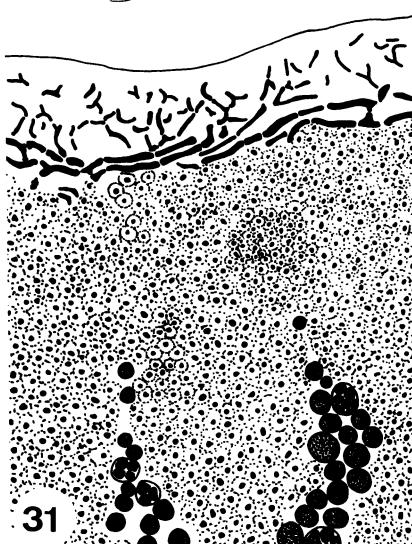
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Figs. 27-31. Fig. 27, part of a cross section of the radial thallus of *Sphaerophorus globosus* showing a strongly gelatinized cortex; Fig. 28, cross section of the thallus of *Sphaerophorus melanocarpus* showing cortical hyphae with gelatinized walls in a netlike arrangement; Fig. 29,

thallus, although the algae produce these motile stages in pure cultures of the phycobiont.

The thickness of the algal layer varies in different lichen genera and the position of the algal zone in the thallus is not invariable. The algae are situated in that part of the thallus where the hyphae are sufficiently loosely interwoven to leave enough space for the algae and where they have an optimum light intensity. The algae are therefore seldom located at the surface or deep within the thallus. The fact that the algae are not strictly confined to a specific layer of the thallus becomes apparent if, by chance, the position of a lichen thallus is changed in nature. If a lobe of a foliose thallus is reversed, the algal layer, which usually lies near the upper surface of the thallus, migrates to the new upper surface and establishes itself inside a tissue which was originally part of the medulla in the lower part of the thallus before reversal (Jahns, 1970).

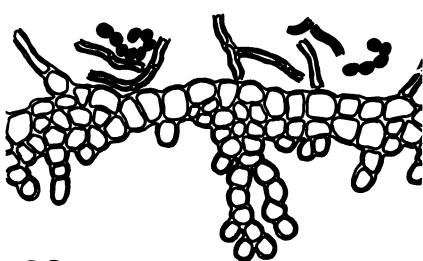
2. MEDULLA

The medulla consists of loosely interwoven hyphae in a periclinal arrangement. The hyphae are in general only weakly gelatinized and often have a fibrous or a cottony appearance. The medulla has a greater water-holding capacity than any of the other tissues and is a region of food storage. The individual hyphae are not easily moistened and this, together with their loose interweaving, facilitates gas exchange within the thallus. Many lichen substances are deposited extracellularly in the medulla and other layers of the thallus. Besides the deposits of typical lichen substances the thallus may be interspersed with large crystal clusters of calcium oxalate. This occurs, for example, in species of *Cladonia*, *Porina*, and *Usnea* (Fig. 26).

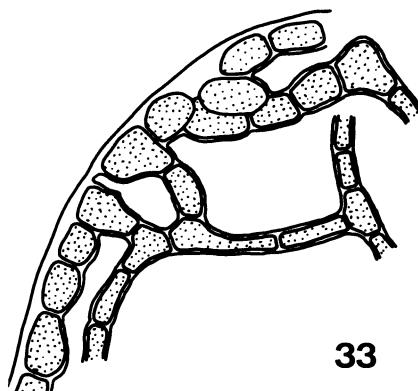
In some fruticose lichens, such as *Usnea* which has a radial arrangement of the tissues, a central axial strand can be distinguished internal to the medulla (Fig. 46). The structure of the central axis is dense and consists of paraplectenchymatous or prosoplectenchymatous tissue giving considerable tensile or skeletal strength to the thallus. In other genera, i.e., *Alectoria*, *Cladonia*, and *Ramalina*, the central axis is absent. Its place can be taken by a central hollow or by gelatinous or spongy tissues. In *Letharia* a central cord is formed by fusion of several smaller strands (Fig. 29).

In fruticose thalli which are held upright by the tube-shaped cortex, the cortical hyphae are either arranged netlike or periklin or they form a palisade tissue (Figs. 18, 27, and 28).

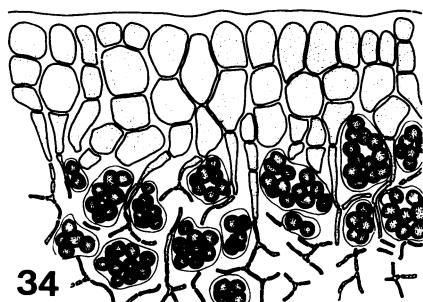
cross section of the thallus of *Letharia vulpina* with a central cord formed by several smaller strands; Fig. 30, cross section of the isolateral thallus of *Ramalina siliquosa*. The algal cells are either situated in the medulla or in the cortex; Fig. 31, the double cortex of *Ramalina siliquosa*. (Figs. 27–31 from Henssen and Jahns, 1973.)



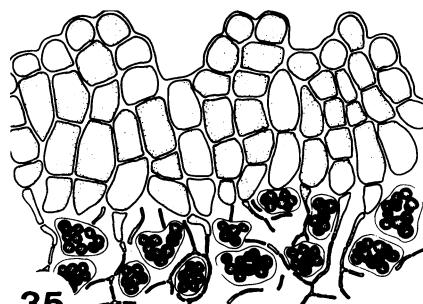
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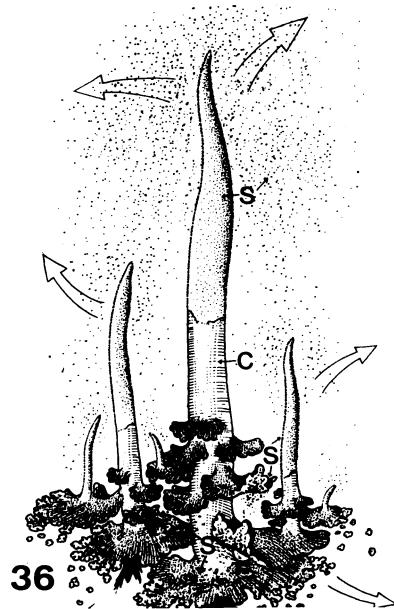
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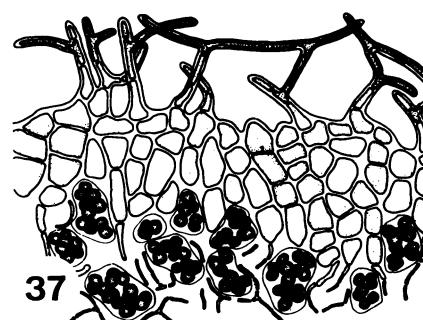
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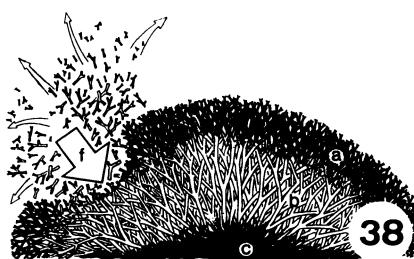
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Figs. 32–38. Fig. 32, hairs on the lower side of the thallus of *Leptogium americanum* resembling a string of pearls; Fig. 33, development of the cortex in *Collema occultatum*; Fig. 34, thallus of *Peltigera horizontalis* with a smooth cortex; Fig. 35, thallus of *Peltigera scabrosa*

3. CORTEX

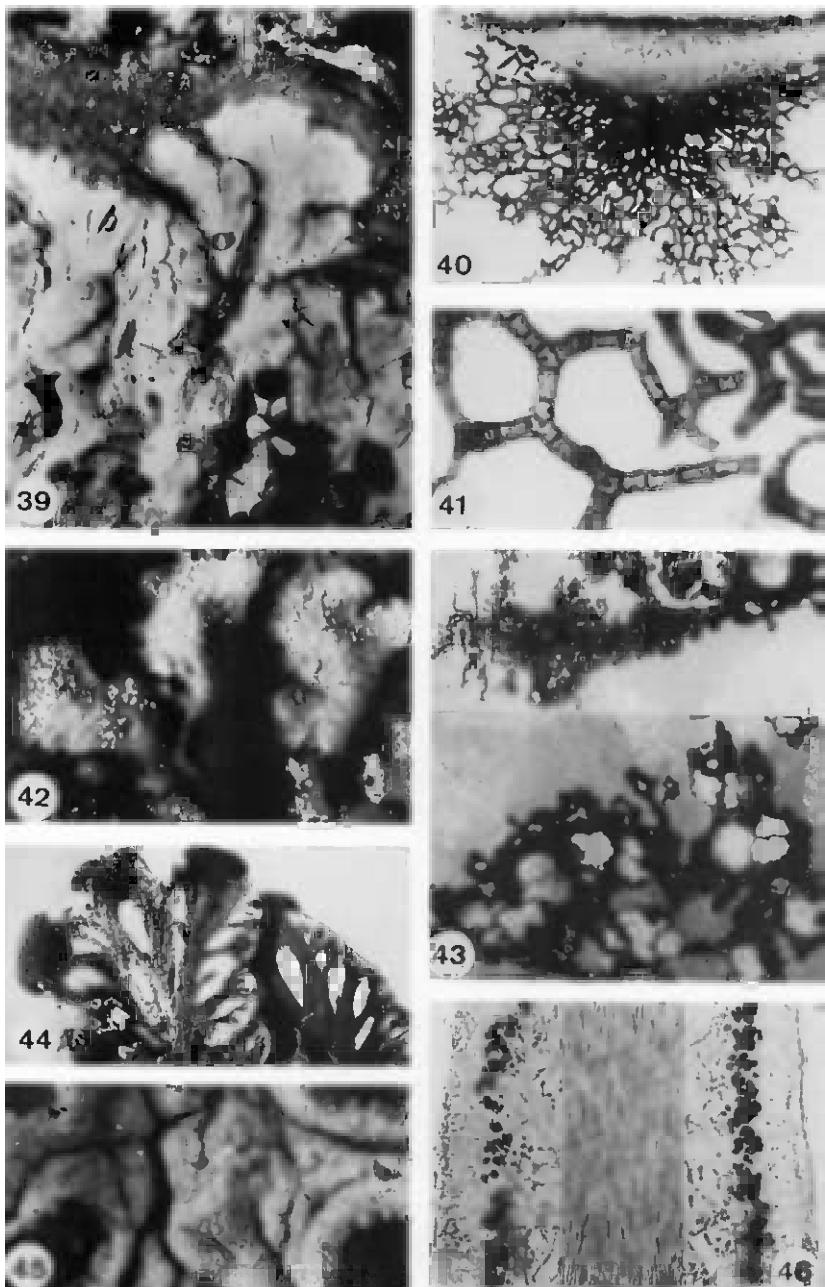
Lichens which consist only of medulla and algal layer have a granular or powdery appearance. Most lichens are protected by a cortical layer which is sometimes pigmented and always covers the upper side of the thallus and sometimes also the lower surface. The cortical layers are comparable to the epidermis of a green leaf. The thickness of the cortex varies in different lichen genera and the layer does not always form a continuous stratum. For example, in *Ramalina* and *Solorina*, the cortex can be broken by clefts or can be thinner in places thereby allowing the algae to penetrate into the covering layer (Figs. 30 and 115).

All tissue types can build a lichen cortex. Occasionally, two different tissues form a cortex which then appears as a two-layered structure. For example, in *Ramalina siliquosa* the outer part of the cortex is formed by a few parallel to reticulately orientated hyphae, connected by anastomoses. The rest of the thick cortex is formed of hyphae with gelatinized walls with a fastigiate arrangement. The hyphae of the two tissues lie at right angles to one another (Fig. 31).

The homoiomerous thalli of some gelatinous lichens show a few of the phylogenetic steps by which a cortex has been formed. Most species of *Collema* have a simple uncorticated thallus, but in some species the first stages in the development of a primitive cortex can be observed. Hyphae growing from the inner part of the thallus towards the surface bend at right angles and continue their growth parallel to the surface but still inside the gelatinous substance of thallus (Fig. 33). The species of *Leptogium* show all steps of development from a cortex formed by loosely organized irregular cells to a layer of isodiametric cells pressed together forming an unbroken stratum. In *Leptogium* the cortex is always formed outside the gelatinous substance of the thallus. In this genus the cortex is usually one cell thick (Fig. 22) but in some related genera it is several cells deep.

VEGETATIVE STRUCTURES OF THE CORTEX. The anatomy of the lower cortex of the thallus can differ from that of the upper cortex even in the same species. The shape of the outermost cells of the cortex has an important influence on the habit of the lichen. The surface is often covered with a thin homogenous cuticle, but in a number of lichens the outermost cells

with a granular cortex; Fig. 36, podetia of *Cladonia* showing dispersal of soredia; the upper part of the podetia is ecoricate and completely sorediate (S, soredia; C, cortex); Fig. 37, thallus of *Peltigera canina* covered with hyaline hairs; Fig. 38, bushlike group of podetia of *Cladonia* showing distribution by fragmentation of the thallus (a, zone of growth; b, older parts of the podetia which do not grow; c, decaying podetia; f, fragmentation). (Figs. 32–35 and 37 from Henssen and Jahns, 1973; Figs. 36 and 38 from Hennipman, 1969.)



Figs. 39–46. Fig. 39, thallus of *Parmeliella plumbea* with dark, hairy prothallus ($2 \times$); Figs. 40–41, netlike tomentum on the underside of *Anzia ornata* ($65 \times$ and $330 \times$); Fig. 42, thallus of *Physconia grisea* covered with white pruina ($9 \times$); Fig. 43, black prothallus of

become necrotic and give the thallus a scurfy appearance. These tiny granules are called pruina. They may also be an accumulation of carbonates and oxalates. For example, the margins of foliose thalli of *Physconia* and the disks of the apothecia in particular are covered with a whitish dust (Figs. 42 and 45).

Some cortical cells may continue their growth and develop into thin, hyaline hairs (Fig. 72), each consisting of one or more cells. The hairs are long and pointed or branched and connected by anastomoses (Fig. 37). In some species the cells of the hairs are globose and resemble a string of pearls (Fig. 32). Hairs may form a felted, hirsute, or cottony mat called a tomentum (Figs. 83, 85, and 123). Many foliose species, especially those without a cortex or with only a poorly developed one, are characterized by a tomentum on the lower surface of the thallus. *Peltigera* and *Lobaria* are well-known examples of this type. The tomentum can become a thick, spongy layer of netlike branched hairs as in *Anzia* (Figs. 40 and 41). The blackened hypothallus of *Parmeliella* and *Pannaria* also resembles a tomentum (Fig. 39). The thalli of some crustose lichens bear bristly hairs.

The form of the marginal cells of the cortex, and as a result the habit of the thallus, may vary in closely related species. In *Peltigera horizontalis* all marginal cells of the cortex end in an unbroken layer, covered by a gelatinous cuticle (Fig. 34). The thallus appears smooth and glossy (Fig. 121). The cortex of *Peltigera scabrosa* is characterized by little granules (Fig. 122), each consisting of several cells equivalent to abbreviated bundles of hyphae which have grown above the general surface of the thallus (Fig. 35). In *Peltigera canina* the cells of the cortex end in interwoven, unorientated hyaline hairs (Fig. 37) giving the thallus its felted appearance (Fig. 123).

The upper surface and the lower surface of foliose lichens can be covered by netlike veins (Fig. 44). For example, the underside of *Peltigera* always has veins of a spongy and felted appearance. In this genus the veins are formed by the medullary hyphae multiplying at certain places. In *Hydrothyria*, an aquatic lichen belonging to the same family as *Peltigera*, the veins consist of single medullary hyphae with strongly enlarged cell lumina.

The layers of the thallus—upper cortex, algal layer, medulla, and lower cortex—are more or less present in all heteromerous lichens. Those lichens with a radially organized thallus are no exception. Here the only difference is

Rhizocarpon geographicum growing on white quartz stone; at some places squamules of the thallus have developed (15 \times); Fig 44, lower surface of the thallus of *Peltigera venosa* showing black veins (3 \times); Fig. 45, thallus of *Diploschistes* covered with whitish pruina and bearing apothecia (18 \times); Fig. 46, longitudinal section through the thallus of *Usnea ceratina* showing the central cord (140 \times). (Figs. 39–42 and 44–46 from Henssen and Jahns, 1973).

that the medulla lies in the center of the thallus and is enclosed by a cylindrical algal layer and cortex. The medulla may have a hollow center or an axial strand.

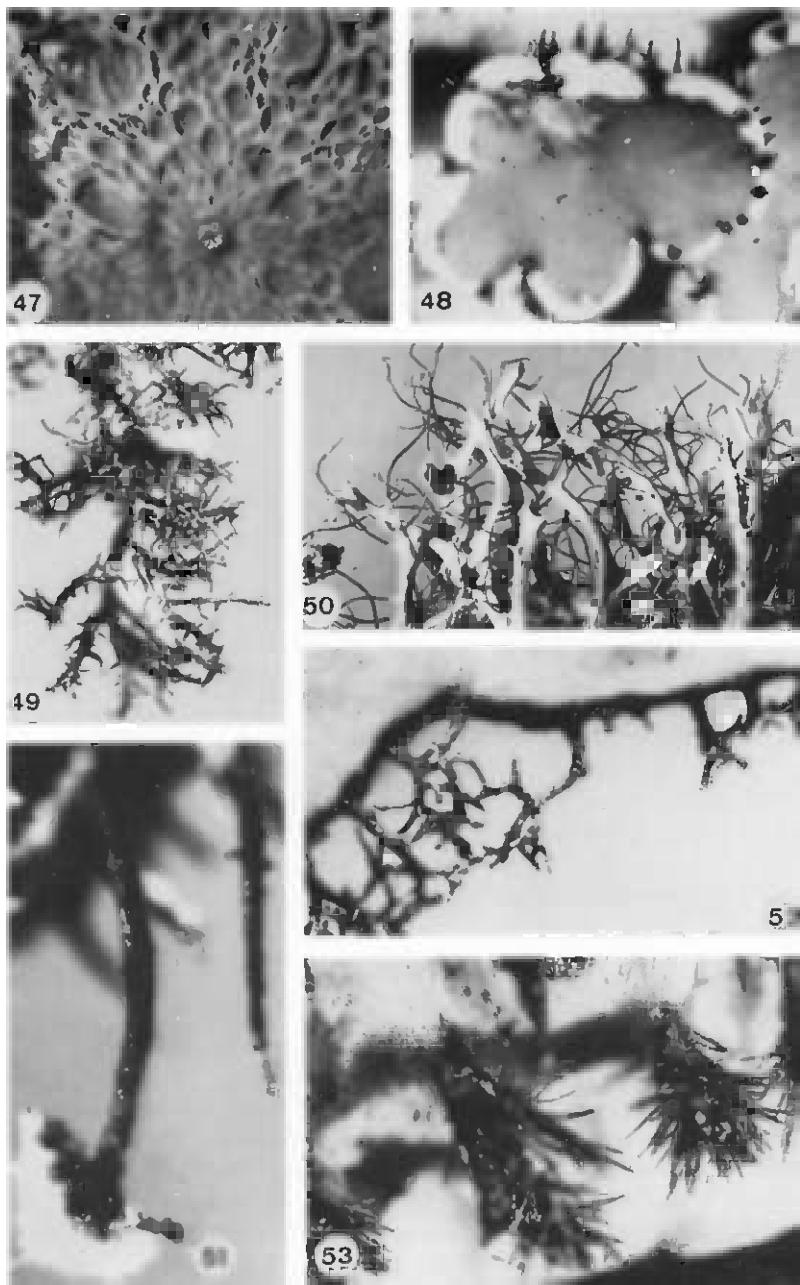
Foliose lichen thalli resemble the leaves of higher plants. The cortex of the lichen corresponds to the epidermis and the algal layer to the palisade layer. The cyphellae and pseudocyphellae of lichens have the same function as the stomata of the leaf, while their anatomical structure resembles the lenticels of higher plants. There are significant differences in thallus thickness between lichen specimens growing in the shade and others exposed to the sun (Scott, 1971). For example, thalli of *Xanthoria parietina* growing on shaded tree trunks are thinner than thalli growing on exposed rocks, a feature corresponding to the sun and shade leaves of angiosperms. Not only the thickness of a lichen thallus as a whole, but also the relative thickness of the different layers, varies under different circumstances. The cortex and medulla of lichens growing in the shade are thinner while the algal layer is thicker than in specimens exposed to the sun.

D. Attachment of the Thallus to the Substrate

The thallus of homoiomerous lichens is fastened to the substrate by the basal hyphae. The same simple way of attachment also is found in some heteromerous lichens. The rhizoidal hyphae, which anchor the thallus by clasping little particles of the substrate, are like the tomental hyphae. Lichens growing on soil incorporate grains of sand between the hyphae of the lower part of the thallus.

Rhizines have the same function as rhizoidal hyphae. They are composed of bundles of more or less parallel aligned hyphae and develop in three different ways. In some lichens, i.e., in the *Parmeliaceae*, the hyphae of the rhizines are cemented together as soon as they start to develop. They form a direct elongation of the cortex tissue. The walls of the hyphae are glued together by gelatinization. When the tip of the rhizines reaches the substrate the growing hyphae spread and form a disklike holdfast attaching the lichen to the substrate (Fig. 51). In this disk the hyphae and particles of the substrate are glued together. In other groups of lichens, for example, in the *Peltigeraceae* and *Stictaceae*, young rhizines consist of loosely associated hyphae, which later become closely connected by anastomoses. The mature rhizines of this type may spread at their tip and become brushlike (Fig. 53). In *Leptogium* the rhizines are formed by a tuft of individual hyphae not connected with one another.

To what extent rhizines can transport dissolved mineral or organic metabolites from the substrate to the thallus has not yet been established. Probably there is some correlation between the type of rhizine and its ability to transport water which varies considerably. For example, the compact



FIGS. 47–53. Fig. 47, underside of *Umbilicaria pustulata* with central holdfast ($1.5\times$); Fig. 48, bulbate cilia at the margin of apothecia of *Parmelia abstrusa* ($25\times$); Fig. 49, squarrose rhizines of *Parmelia ecuadorensis* ($25\times$); Fig. 50, thallus of *Heterodermia leucomela* with long dark cilia ($8\times$); Fig. 51, rhizine of *Parmelia sulcata* showing point of attachment to the substrate ($50\times$); Fig. 52, dichotomous rhizines of *Parmelia revoluta* ($25\times$); Fig. 53, rhizines of *Peltigera aphthosa* ($12\times$). (Figs. 47, 49–51, and 53 from Henssen and Jahns, 1973.)

rhizines of *Parmelia* are not quickly wetted by water while the treelike rhizines of *Peltigera* function like a wick.

Rhizines always grow from the underside of the thallus. Vegetative structures emerging from the margin of the thallus and closely resembling the rhizines are called cilia. The habit of rhizines and cilia varies in different genera. The simplest type is an unbranched strand of hyphae (Figs. 50 and 51). Branched rhizines and cilia are of a squarrose or dichotomous type (Figs. 49 and 52). Short cilia can have a bulbate inflated base (Fig. 48).

Unbranched cilia, for example in *Usnea*, are sometimes called fibrillae (Fig. 76) and short pin-shaped protuberances are named papillae. As it is unknown whether there are any fundamental differences between these vegetative structures, it is really impossible to give a meaningful definition of the different names.

Some lichens, especially those growing on rocks or tree bark, are attached by a disklike holdfast (Figs. 47 and 74).

III. Morphology of the Thallus

A. Color of the Thallus

Most lichens are gray or brown when dry. In wet thalli the color of the algae can be seen more distinctly through the cortex and these lichens become more or less green. Many species are brightly colored by incrustations of special lichen pigments in the cortex. For example, the orange or yellow substance called parietin is found in the *Teloschistaceae*. Green and yellow tints are common in lichens while red, blue, and violet colors are rare. Many of these pigments are not confined to a single genus but often occur widely throughout the lichens. They are absent in most gelatinous lichens and rare in pyrenocarpic species.

B. Growth Forms of the Thallus

The segregation of lichens into the large groups of crustose, foliose, and fruticose lichens has already been mentioned. However, the hairlike or filamentous lichens with their short, thin branches and the gelatinous lichens form two extra groups which are not satisfactorily incorporated into one of the three main types. The gelatinous lichens are cartilaginous when dry, but immediately swell and become gelatinized in wet conditions. To a certain extent all lichens, which are hard and brittle when dry, may swell when wet and become soft and flexible. When the traditional classification into growth forms is applied to all the different species of lichen, intermediate forms are usually arbitrarily placed in that group to which the majority of closely related species belong.

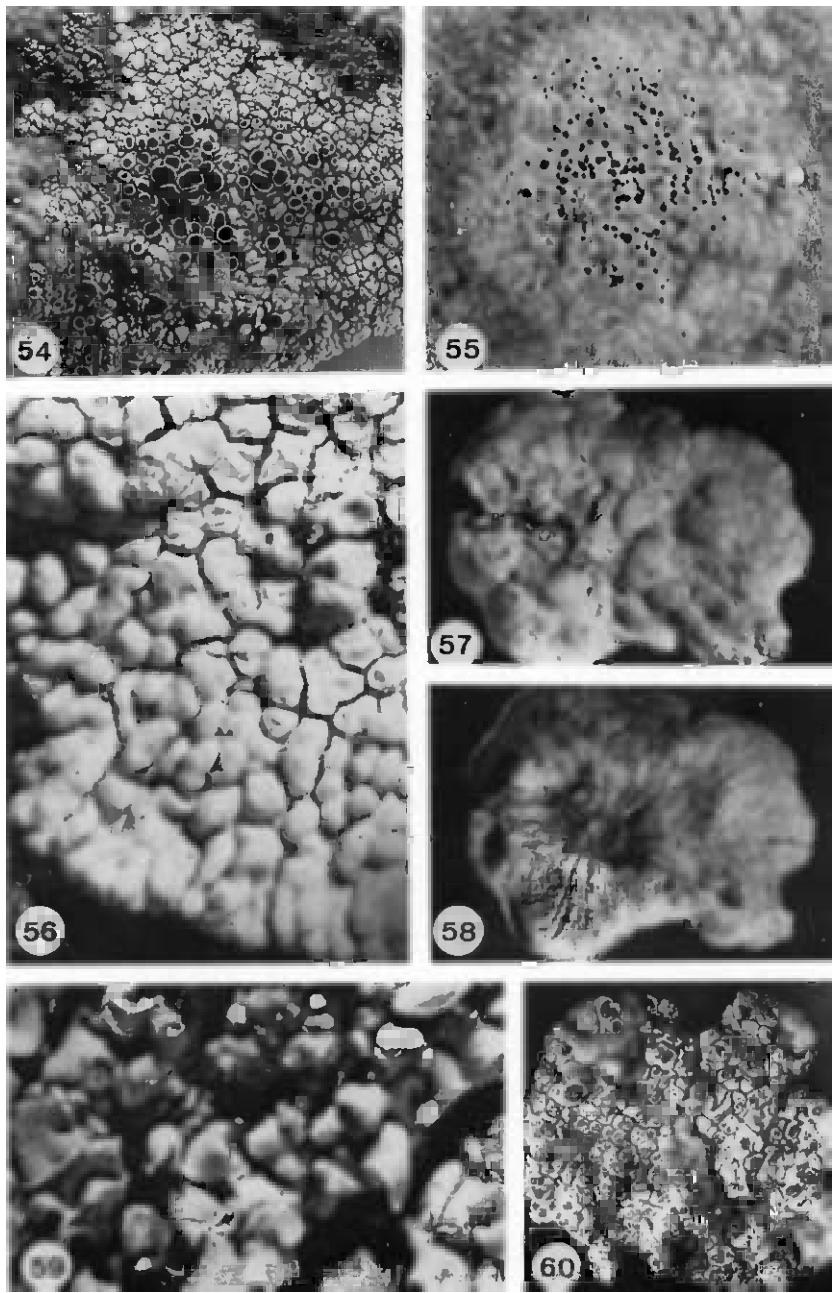
1. CRUSTOSE LICHENS

Crustose lichens never possess a lower cortex. They are attached to soil, rock, or tree bark by the hyphae of the medulla and the contact is so intimate that they are practically inseparable from the substrate. A patch of crustaceous lichen may belong to one species and yet be composed of many individuals which have fused together. Simple crustose lichens are homoiomeric. They lack a cortex and are therefore granular in structure. The mycelium spreads over the substrate in a thin filamentous mat enclosing the algae.

The thallus of most crustaceous lichens consists of little scales called areoles (Fig. 54). The lower hyphae of the areoles usually grow faster than the main part of the thallus and form a thin spreading layer around it. This mat of hyphae is usually dark in color and is called a prothallus (Fig. 43). The name hypothallus, also previously used for this structure, is more correctly applied to the thin filaments which link the areoles of the inner part of the thallus. A cracked surface in lichens may develop in one of two ways. In many species the thallus is initiated evenly and becomes cracked, often incompletely, at a later stage. In other groups of lichens, small defined areas of the thallus develop on an advancing prothallus appearing as separate entities and gradually becoming more closely compacted towards older parts of the thallus (Fig. 43).

Lichens with very small areoles are frequently homoiomeric. Bigger areoles begin to show the first signs of a differentiation into layers. The algae are accumulated in the upper part of the thallus, and at the surface a kind of cortex is formed by necrotic, gelatinized cells. This layer of dead cells is continuously sloughed off, but is always reformed by the growth of the thallus. An example of this type is *Acarospora*. Other crustose lichens, especially the intermediate forms between the crustose and the foliose type, have a true heteromeric thallus. The cortex may cover only the upper surface of the thallus or it also may include the margin of the individual areoles.

An extreme example of the crustose type are lichen thalli which grow completely inside their substrate, whether it be wood or stone. Species growing inside rock are called endolithic, and those penetrating wood are termed endophloeodic. Sometimes the thallus of these lichens can be seen as a discoloring of the substrate (Fig. 55), but frequently only the ascocarps in pits or on the surface of rock or bark indicate the presence of a lichen. The hyphae of endolithic lichens appear to excrete lichen substances which are able to dissolve the stone and thus make it possible for hyphae and algae to penetrate several millimeters into the rock. Species growing inside limestone develop special oil cells that are intercalated along the hyphae. The irregularly swollen cells appear clustered with oil drops.



Figs. 54–60. Fig. 54, areolate thallus of *Lecanora frustulosa* ($2\times$); Fig. 55, endolithic thallus of *Lecidea*; the black apothecia emerge from the stone ($0.6\times$); Fig. 56, thallus of *Acarospora oxytona* with effigurated margin ($3\times$); Figs. 57–58, placoid thallus of *Xanthopeltis rupicola* seen from above (with apothecia) and from below (with umbilicus) ($3\times$ and $3\times$); Fig. 59,

2. INTERMEDIATE FORMS BETWEEN CRUSTOSE AND FOLIOSE LICHENS

In some crustose lichens elongated, small lobes replace the areoles. These lobes can be fastened to the substrate by the entire lower surface or the margin of the thallus can be free and ascending. Different combinations of these characteristics lead to a variety of described growth types.

If the margin of the thallus consists of small, elongated lobes while the inner part is composed of small areoles, the lichen is said to have an effigurated margin (Fig. 56). If the whole thallus is formed by elongated lobes, the lichen belongs to the placoid type. Thalli of the effigurate and placoid type are closely appressed to the substrate by their whole lower surface. The squamulose thallus of some species of *Heppia*, *Lecanora*, *Lecidea*, and *Placynthium* consists of little scales. At one side their margin separates from the substrate and bends upwards (Fig. 59). Several scales can be arranged in a rosette. The squamules may overlap like the tiles of a roof and are then imbricate.

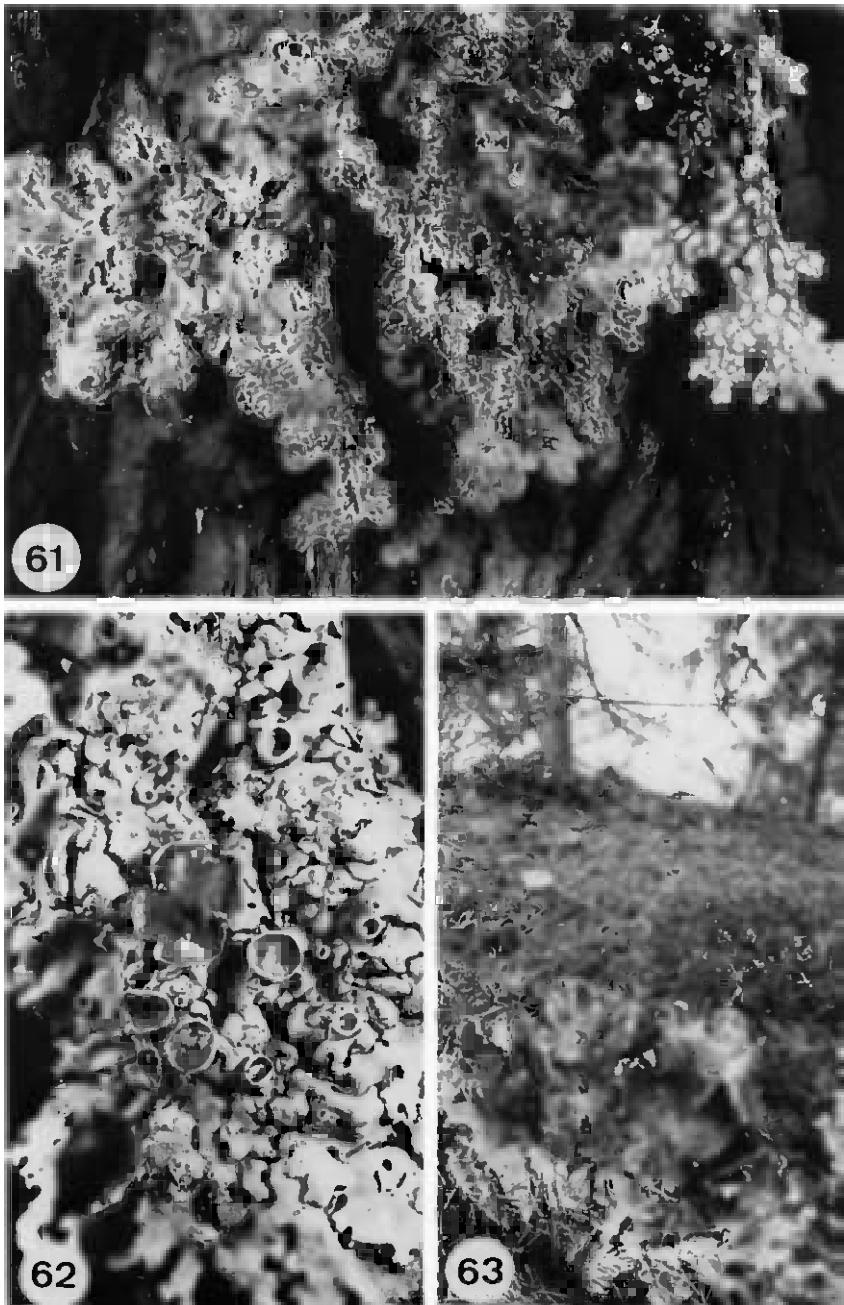
Further development of the squamulose thallus is seen in peltate lichens. In this type only the central part of the scales is fastened to the substrate, the whole margin becoming free. Lichens of this type have more or less the same habit as the umbilicate foliose genera (Figs. 57, 58, and 60). The scales and lobes of some squamulose and peltate thalli are bent upwards and begin to resemble fruticose growth forms. For example, some species of *Peltula* with upright lobes and with a corresponding radial anatomy could be classified as having a fruticose growth habit (Fig. 65).

3. FOLIOSE LICHENS

The thallus of foliose lichens is formed by flattened lobes, which are heteromerous and dorsoventral in structure. Two principal types, the laciniate and the umbilicate growth form, can be distinguished. Laciniate thalli adhere more or less firmly to the support on which they grow. Either the whole lower surface is in contact with the substrate or the margin of the lobes becomes free and bends upwards. The thalli are usually attached by rhizines or rhizoidal hyphae. The umbilicate lichens are platelike and attached by a central discoid holdfast called the umbilicus (Fig. 47).

a. **LACINIAE FOLIOSE LICHENS.** The laciniate lichens form an extremely polymorphous group. Their habit and the mode of attachment of the thallus vary and their anatomy is the most complex of all lichens. Some are

squamulose thallus of *Lecidea scalaris*, the margin of the squamules becoming sorediate (8 \times); Fig. 60, umbilicate thallus of *Glypholecia scabra* (1 \times). (Figs. 54 and 57–60 from Henssen and Jahns, 1973.)



Figs. 61–63. *Lobaria pulmonaria*; note on the right side an inverted part of the thallus ($\frac{1}{8} \times$); Fig. 62, foliose, laciniate thallus of *Parmelia quercina* ($2.5 \times$). Fig. 63, foliose laciniate thallus of *Peltigera canina* ($\frac{4}{3} \times$). (Figs. 61 and 62 from Henssen and Jahns, 1973.)

very large plants. Lobes of *Lobaria pulmonaria* (Fig. 61) may reach a length of 30 cm and multilobed thalli of *Parmelia* (Fig. 62) reach $\frac{1}{2}$ m in diameter (Fig. 64). The thalli of *Lobaria* are covered on both sides by a cortex, while in *Peltigera* (Fig. 63) the cortex is restricted to the upper side, the underside being tomentose with veins. In *Parmelia* the whole lower surface of the thallus or only part of it is attached to the substrate by rhizines. Rhizines, cilia, veins on the thallus surface, and other vegetative structures are common in foliose lichens. The cortex layers are derived from different types of tissue.

b. UMBILICATE FOLIOSE LICHENS. Umbilicate lichens have a disklike thallus that is attached to the substrate with a central holdfast. The holdfast causes a small depression in the surface of the thallus. All species of *Umbilicaria* (Fig. 67) have this type of thallus, as the name of the genus indicates. An umbilicate thallus occurs in other lichens which are not closely related to *Umbilicaria*, as, for example, in the pyrenocarpous genus *Dermatocarpon* (Fig. 68) and in the gymnocarpous genera *Glypholecia*, *Omphalodium*, and *Xanthopeltis* (Figs. 57, 58, and 60). The thallus of *Umbilicaria* is heteromerous and fully corticated. Many species also are characterized by a veined or rugose thalline surface.

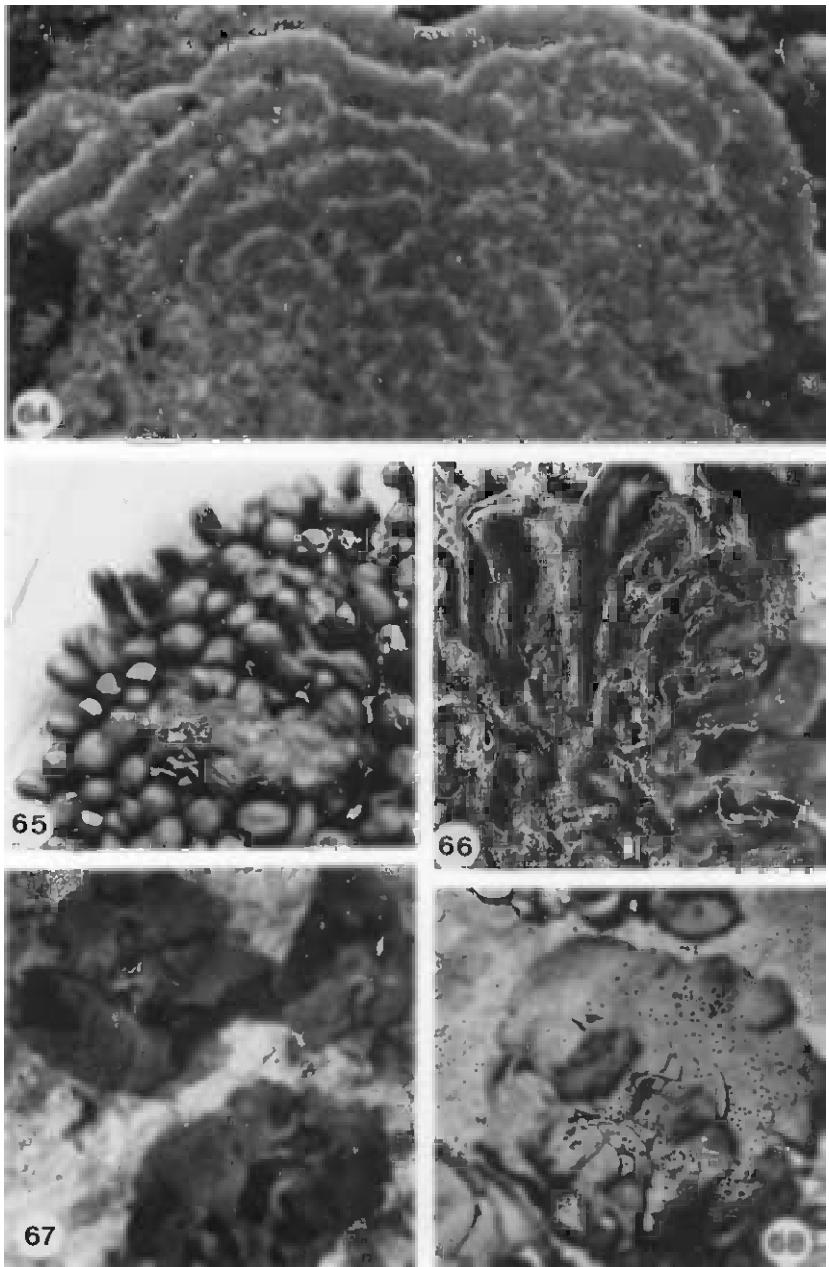
4. INTERMEDIATE FORMS BETWEEN FOLIOSE AND FRUTICOSE LICHENS

The thalli of foliose lichens of the laciniate type, for example, those of *Cetraria* (Fig. 73), are sometimes nearly erect so that they are often considered to be fruticose. The lobes are fastened by the lower surface and in older thalli the base begins to rot. This further proves the arbitrariness of classification based on thallus types.

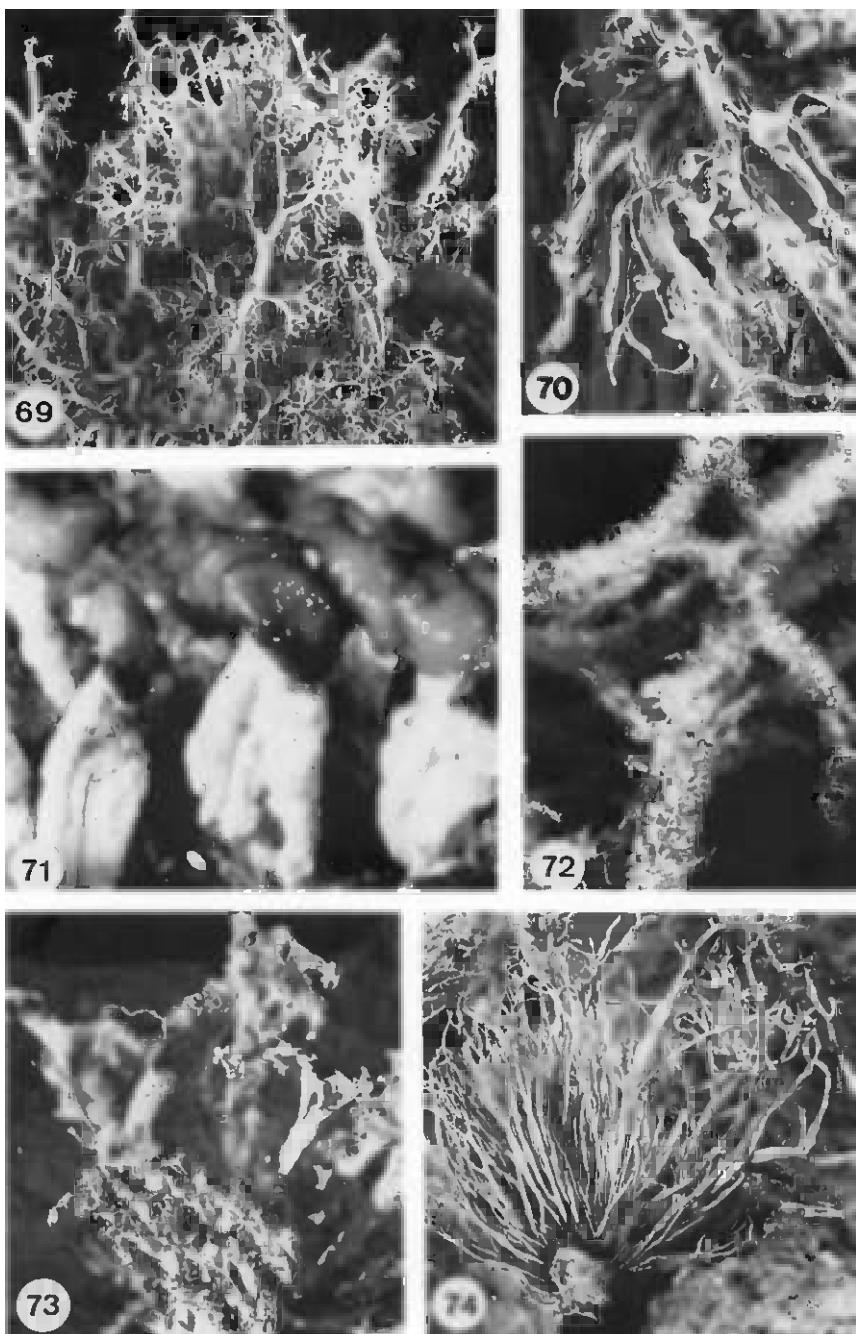
5. FRUTICOSE LICHENS

The lobes of fruticose lichens are strap-shaped or threadlike with a radial or dorsiventral thallus. *Ramalina* (Fig. 70) and *Roccella* (Fig. 79) are good examples of strap-shaped, radial thalli, while *Usnea* (Fig. 75) consists of thin strands up to 5 m long. *Evernia* and *Pseudevernia* have strap-shaped, dorsiventral thalli.

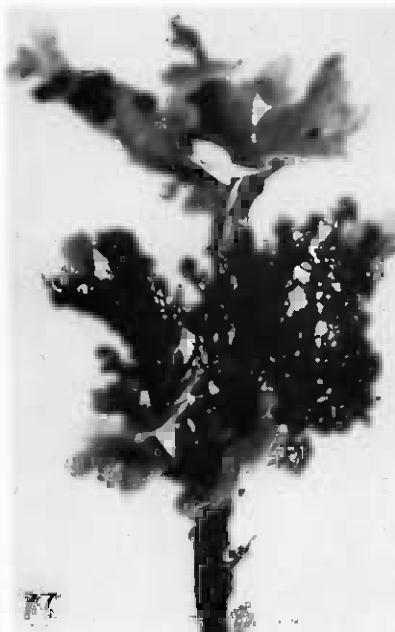
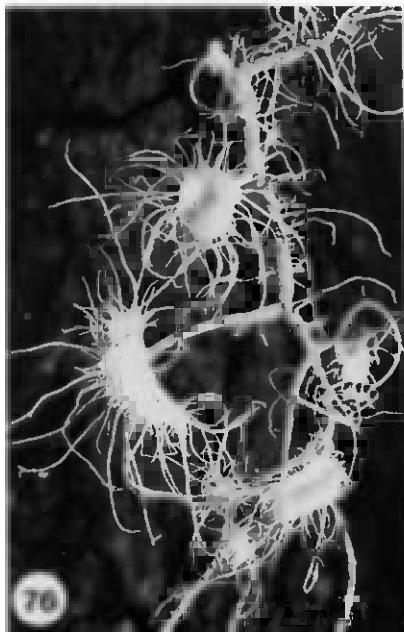
Many strap-shaped and radiate thalli are attached to the substrate by a holdfast (Fig. 74). Some long, pendulous, threadlike strands of certain species of *Usnea* hang from the branches of trees, without any organized attachment to the bark. Of these, *Usnea longissima* may reach a length of several meters. Other fruticose lichens that grow on soil form little cushions which consist of separated upright lobes. Frequently, they are not attached



Figs. 64–68. Fig. 64, zoned thalli of *Parmelia centrifuga*: new thalli are continuously formed in the center of the outer growing zones ($\frac{1}{10} \times$); Fig. 65, fruticose thallus of *Peltula* ($4 \times$); Fig. 66, gelatinous thallus of *Collema subfurvum* ($1 \times$). Fig. 67, umbilicate thallus of *Umbilicaria rigida* ($1 \times$); Fig. 68, umbilicate thallus of *Dermatocarpon miniatum*; the ostioles of the perithecia can be seen as blackpoints ($3 \times$). (Figs. 64, 67 and 68 from Henssen and Jahns, 1973; Fig. 65 from A. Henssen.)



Figs. 69–74. Fig. 69, fruticose thallus of *Cladonia impexa* ($1 \times$); Fig. 70, fruticose thallus of *Ramalina fraxinea* ($1 \times$); Fig. 71, fruiting bodies of *Baeomyces placophyllus* ($9 \times$); Fig. 72, hairy thallus of *Teloschistes flavicans* ($10 \times$); Fig. 73, thallus of *Cetraria cucullata* bearing apothecia ($1 \times$); Fig. 74, thallus showing holdfast of *Ramalina curnowii* ($1 \times$); (Figs. 71 and 74 from Henssen and Jahns, 1973.)



Figs. 75–77. Fig. 75, thallus of *Usnea longissima* ($\frac{1}{10} \times$); Fig. 76, thallus of *Usnea ceratina*; note fibrillae on the apothecial margins (2.5 \times); Fig. 77, *Sticta filix*; the dark fruticose thallus with *Nostoc* as phycobiont bears the foliose thallus which has a green phycobiont (2 \times). (Figs. 75–77 from Henssen and Jahns, 1973.)

to the soil. Some species degenerate at the base and become completely free. They may be dislodged by the wind and blown over the ground. Good examples of this type are species of *Cladonia*, sect. *Cladina*, and *Cornicularia*.

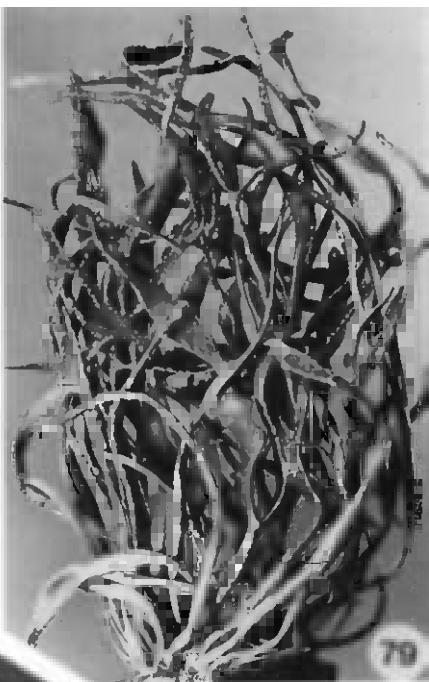
The stiffness of fruticose lobes is achieved by two different types of basic construction. In some lichens the hyphae of the cortex serve as supporting tissue. They form a cylindrical tube at the lateral edge of the thallus, while the center of the lichen is hollow or filled with a cottony medulla. This type of construction serves to keep the plant upright and to withstand lateral pressure. The supporting tissue is a prosoplectenchyma or pseudoparenchyma with the hyphae being closely cemented. In the second type of fruticose lichen the supporting tissue is situated in the center of the medulla. A central cord or axial strand is constructed from thick-walled, perpendicular, agglutinated hyphae. *Usnea* has a single threadlike elastic cord, while other lichens develop several individual strands which later fuse. This central axial strand gives the requisite tensile and skeletal strength to pendulous lichens.

6. LICHEN THALLI WITH A TWOFOOLD CHARACTER

In some lichens the thallus consists of a horizontal part lying on the substrate and of a vertical, fruticose part, bearing the fruiting bodies. The horizontal thallus can be crustose, as in some species of *Baeomyces* (Fig. 71) or foliose, as in *Cladonia* (Fig. 78). The horizontal thallus of a lichen may be evanescent, being only found in a very young specimen, disappearing as it matures. The adult lichen consists only of a vertical fruticose thallus (Fig. 69).

The twofold thallus has been independently developed in several lichen families and can be of different origin. In *Cladonia* the thallus verticalis is formed from the generative tissue, a tissue which surrounds the sexual organs and usually gives rise to the ascocarp. Thus, the thallus verticalis of this genus is ontogenetically a part of the fruiting body. This kind of fruticose stalk is called a podetium. The podetia may be simple or richly branched with pointed apices or apical cups.

The development of the thallus verticalis in *Stereocaulon* (Fig. 80) and *Pilophorus* (Fig. 81) is different. A part of a squamule of the thallus horizontalis or a complete granule of the thallus grows vertically upwards and develops into a simple or branched more or less erect thallus verticalis. The primordium of the fruiting body is formed only at the top of this stalk. The generative tissue builds only the ascocarp while the thallus verticalis is differentiated from vegetative thallus tissue. This kind of stipe is called a pseudopodetium.



7. HAIRLIKE THALLI

The habit of hairlike lichens, with their threadlike thalli, resembles that of fruticose lichens. These lichens, however, are much smaller and usually not more than a few millimeters high. In contrast to most lichens, the habit is principally determined by the phycobiont. Filamentous algae, belonging to the *Chlorophyceae* or the *Cyanophyceae*, are more or less closely ensheathed by hyphae of the mycobionts (Figs. 23–25).

8. GELATINOUS LICHENS

The consistency and growth form of gelatinous lichens are for the most part determined by the blue-green phycobiont. The characteristic swelling of the wet thallus is due to the gelatinous sheath of the phycobiont. Frequently, the structure of the thallus is homoiomeric but the anatomy is different from the homoiomeric crustaceous lichens. In crustose lichens the algae are scattered in a mycelium of loosely interwoven hyphae, while the hyphae of gelatinous lichens usually grow inside the gelatinous algal substance that fills the thallus. The hyphae often do not touch the algae. The lateral margin of the thallus is also sometimes formed by the gelatinous sheath of the algal cells.

In some genera the fungus provides a cortex at the surface of the thallus. A gradation of differentiation can be found among genera of the *Collemataceae*. Most species of *Collema* are noncorticate, but in some species vertically oriented hyphae reach the surface of the thallus, bend at right angles, and spread parallel to the surface (Fig. 33). *Leptogium* has a cortex which is one cell layer thick. It consists of irregularly formed cells which are arranged in either a broken pattern or in a regular layer of isodiametric cells when viewed from above (Fig. 22). The cortex of other lichens belonging to the *Collemataceae* is several cell layers thick.

All types of growth forms are found in the gelatinous lichens. Most species are very small and only the foliose thalli reach a diameter greater than 10 cm (Fig. 66). The color of the thallus is olive-green, blackish, or gray. The bright colors of lichen substances or pigments are not present in this group. Some species are red or violet when wet, but this color is due to the gelatinous sheath of certain blue-green phycobionts such as *Gloeocapsa*.

FIGS. 78–81. Fig. 78, cup-shaped podetia of *Cladonia chlorophaea* growing from a foliose primary thallus; the older podetia bear apothecia (3 \times); Fig. 79, thallus of *Roccella fuciformis* with soralia (1 \times); Fig. 80, pseudopodetia of *Stereocaulon alpinum* with phyllocladia and apothecia (7 \times); Fig. 81, *Pilophorus strumaticus*; upright pseudopodetium bears black apothecia; note black cephalodium on the thallus (25 \times); (Figs. 78–80 from Henssen and Jahns, 1973.)

C. Development of the Thallus

A review of lichen development has been presented by Steiner (1965), and only selected aspects of this subject will be described in this chapter.

New lichen thalli can only develop in a favorable environment. If the biotope is unsuitable for the lichen, the development stops at an early stage and only a more or less homoiomerous layer of sorediate appearance is formed. These half-developed thalli can frequently be observed in humid and shadowy places. But even under the most favorable conditions a lichen thallus can only evolve if both partners of the symbiosis are present at the same time. Therefore, those methods of reproduction which disperse both partners in close union are the most valuable to the lichen. It is to the advantage of the lichens that in many species the vegetative diaspores, such as isidia, soredia, and thallus fragments have taken the place of sexual spores. If the mycobiont is dispersed by means of ascospores, basidiospores, conidia, or gemmae the germinating mycelium has to find suitable free-living algae for a new lichenization.

The hyphae emerging from the germinating spores can be of different shapes. In *Xanthoria parietina* they consist either of short, rounded, or of long, thin cells (Werner, 1931). Those hyphae which make contact with the algae become hook-shaped and clasp the algal cells. The fungus is the active partner in the development of the primary stages of the thallus, although the hyphae do not grow towards the algae.

Probably only very few germinating spores succeed in forming a new lichen (Scott, 1971). This difficulty leads to some adaptations, by which the chance of success is increased. The most successful method is the forming of hymenial algae that occur, for example, in *Endocarpon*. These algae lie in the fruiting body between the asci and are ejected together with the spores. Both partners are together at the time of germination. The spores of some lichens after being ejected are dormant until contact with algae is made. In other genera the spores germinate directly but the mycelia seem to live as saprophytes for a short time. The spores of *Verrucaria margacea* germinate inside the perithecium and only the young mycelium is ejected (Tobler, 1925). Perhaps this mycelium, being larger than germinating spores, can make contact with algae more easily.

If lichenization is achieved, the young mycelium and the acquired algae at first form an undifferentiated lump. In *Xanthoria parietina* it takes nearly a month before any signs of different layers can be observed. The same undifferentiated mass of algae and hyphae can be observed if the development starts from a vegetative diaspore. The first hyphae growing from a vegetative propagule function as hapteres, attaching the diaspore to the

substrate. The subsequent differentiation of thallus layers is as slow a process as in thalli developed from germinating ascospores. In *Xanthoria parietina* it takes two months before cortex, algal layer, and medulla are differentiated. After eight months the rosette-shaped thallus has reached 2–6 mm in diameter. Apothecia are formed after 1 or 2 years.

The mycobiont is responsible for the growth of the thallus. At the margin of the thallus the growth of the fungal hyphae may be so vigorous that an undifferentiated zone free of algae is formed around the lichen. This zone is called a prothallus and is hairy or netlike in *Parmeliella*, *Placynthium*, and other genera and cartilagenous in *Pertusaria*. The hyphae growing ahead of the thallus naturally need algae to build a complete lichen, but as the phycobionts of the lichens do not form motile stages inside the thallus they are not able to move to the new parts of the lichen by themselves. There seem to be two fundamentally different means by which growth and development are promoted. Either algae from the old part of the thallus are pushed passively towards the prothallus by the fungal hyphae or free-living algae are incorporated into the prothallus. The first of the two methods can be observed in *Cladonia*. The primordium of the podetium growing vertically from the primary thallus carries with it algal cells from the algal layer. In the thallus of *Pertusaria* the algae are pushed into the new parts of the thallus by special bundles of hyphae showing a fastigiate arrangement (*Schiebehypfen*) (Nienburg, 1926). The same process of differentiation can be found during the development of internal cephalodia in *Lobaria* (Fig. 127).

The incorporation of free-living algae during the growth of the lichen is also well known. In principle it resembles a new lichenization from spores and algae. The trapping of algae by the mycobiont has been observed as the first stage of development of cephalodia and of certain isidia. It has been claimed that the algal layer of the podetia of *Cladonia* also develops from free-living algae coming into contact with the stalk (Weise, 1937). This seems questionable as *Trebouxia*, the phycobiont of *Cladonia*, is probably not sufficiently abundant in the free-living state. Perhaps the conditions of the experiments leading to the observation differed too much from the natural environment.

In *Placynthium nigrum* the trapping of new algae and the transport of algae from old parts of the thallus to younger areas has been combined in an interesting manner (Geitler, 1933–1938). The squamules of the thallus, which contains a symbiotic blue-green Rivulariaceae, are surrounded by a hairlike prothallus. Hormogonia of the alga are freed from the margin of the scales by water and come to rest on the prothallus. Here they are enmeshed by the hyphae. Where they are incorporated into the tissue new squamules

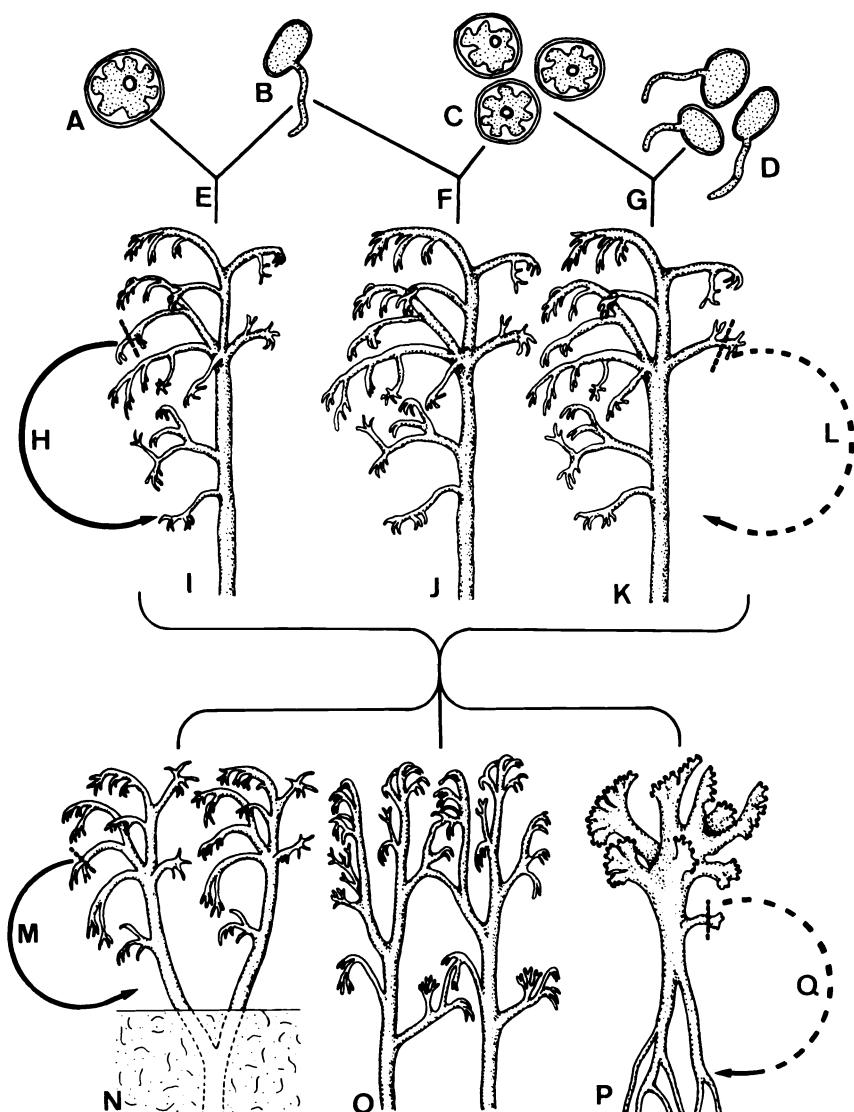
are formed on the prothallus. These continue to grow and finally reach the older squamules, forming a continuous system of scales.

Although lichens never stop growing, the typical morphological habit of the species is preserved as the growth is limited in some directions. For example, the vertical growth of foliose lichens is so slow that it only replaces the uppermost cells of the cortex which are continuously sloughed off. Fruticose lichens, on the other hand, show an unlimited vertical growth. They develop either from foliose stadia growing upwards and secondarily becoming fruticose and erect or the primordia of the thallus directly becoming fruticose. A good example of the first method of development is *Lichina* (Henssen, 1969). Most frutescent species of this genus are foliose when young. The other type of development is represented by *Stereocaulon* subgenus *Holostelidium* (Lamb, 1951). The pseudopodetia of these lichens develop from granular primordia that directly grow into vertical, branched stalks. In *Cladonia* subgenus *Cladina* the annual growth can be extremely regular. Every year a new nodium with lateral branches is formed at the top of the treelike thallus.

The mechanisms regulating and controlling the growth are mostly unknown. Humidity, light, and gravity seem to influence the vertical orientation of frutescent lichens. The growth of hyphal tissues sometimes seems to be stimulated by the presence of algae (Tobler, 1928).

In lichens growth is usually restricted to the tip of the thallus. The zone of growth does not exceed a few millimeters a year and intercalary growth is negligible. In fruticose lichens the proportion of newly developed to dead parts of the thallus becomes increasingly unfavorable as only the upper parts of the lichen continue to grow, while the lower, unproductive parts accumulate. In crustose lichens the annual increase of thallus surface measured as the percentage of the actual size of the thallus is smaller in old thalli than it is in young ones (Steiner, 1965). The inner parts of the thallus have no means of transporting photosynthetic products to the growing outer squamules, so that only the photosynthetic products of the marginal parts of the thallus can be used for the growth process.

In many foliose lichens the old parts in the middle of the thallus die and disappear while the young marginal parts of the lichen continue to grow outward. The result is a ring-shaped thallus as found in *Parmelia centrifuga* (Fig. 64). The rings may reach a diameter of 1 meter. As the lichen grows about 2 mm each year such a thallus began its development some 500 years ago, but as the living, ring-shaped part of the thallus measures only about 6–10 cm in diameter the oldest living part of the lichen is 30–50 years old. Crustose lichens may become much older. Thalli of *Rhizocarpon* are said to reach an age of 1000 years or more.



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FIG. 82. Diagrammatic representation of possible origins of lichen thalli, i.e., (I) from one spore and one algal cell (J) from one spore and several algal cells (K) from several spores and several algal cells. The question of what constitutes individuality of these thalli is discussed in the text. (From Henssen and Jahns, 1973.)

D. Individuality of the Thallus

The growth forms of lichens and some special characteristics of their ontogeny make it difficult to define the term "individual" in this group of plants. The schematic drawing in Fig. 82 gives a detailed review of some of the problems using the fruticose lichens as an example. In the most simple case, one germinating spore (B) contacts (E) one alga (A). From these two bionts the whole thallus descends (1). The thallus does not fuse with other thalli. This plant certainly is an individual, which either forms spores or reproduces by vegetative diaspores (H). In most cases the germinating ascospore (B) will achieve lichenization (F) by including more than one alga (C) into the mycelium. The developing lichen (J), therefore, contains algae of different genetic origin; but as the mycobiont dominates these lichen thalli the individuality of the plant is not questioned. In many fruticose lichens and frequently in crustose lichens the thallus not only is formed by several algae (C) but mycelium from more than one germinating fungal spore (D) is incorporated (G). The tissue of this lichen (K), therefore, is not genetically uniform. Theoretically, it is possible that a vegetative diaspore of this plant does not contain all of the genetically different hyphae which have descended from the different spores. These diaspores would not contain all the genetic information which determined the habit of the mother plant and vegetative reproduction may lead to a certain variability (L).

The thalli of frutescent lichens grow at the top and die away at the base. As a result branched thalli may split and become separate plants (N). These are genetically identical if the mother plant was uniform. The individuality of the two daughter plants, which can reproduce by vegetative diaspores (M), is evident. The process of division by growth is known from other plants, for example, from the moss *Sphagnum* where it occurs regularly.

Lichen thalli tend to fuse, especially in the dense cushions of certain fruticose lichens such as *Cladonia*. In most lichen species only the branches of different plants grow together at points of contact (O). This type of connection should be clearly distinguished from the complete fusion achieved by other lichens (P). The second type of contact is typical for *Cladia* (Jahns, 1972). In this genus nearly all fructifying branches arise from the complete fusion of small branches belonging to different plants. In these lichens it is quite probable that different parts of the big branches contain genetically different hyphae. Therefore, in *Cladia* the reproduction by vegetative diaspores in connection with the fusion of branches leads to a recombination of morphological characteristics (Q). The lichens of the genus *Cladia* cannot be called individuals in the strict sense of the definition.

IV. Vegetative Structures and Their Development

Lichens have unique vegetative structures that are found frequently in foliose and fruticose lichens and sometimes in crustose forms. Some of these structures serve as vegetative diaspores for the dispersal of the lichen and may have special physiological functions. All vegetative structures are of vital importance in taxonomic studies of lichen speciation.

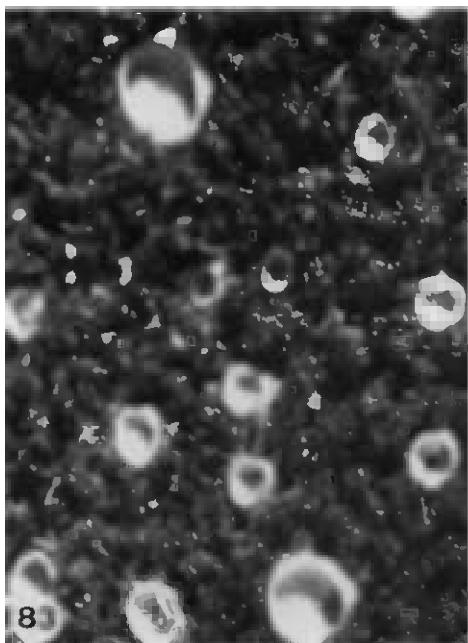
A. Aeration Pores

The cortex of the larger heteromerous foliose lichens with its closely agglutinated hyphae seems to be a serious obstacle to the exchange of gases. Recently, investigations with the scanning electron microscope have shown that some lichens have small pores between the cortical hyphae (Peveling, 1970). Some genera of lichens are characterized by special openings in the cortex which are believed to assist in the aeration of the thallus. They are macroscopically visible. It has never been experimentally demonstrated, however, that these openings really have a function in promoting the exchange of gases.

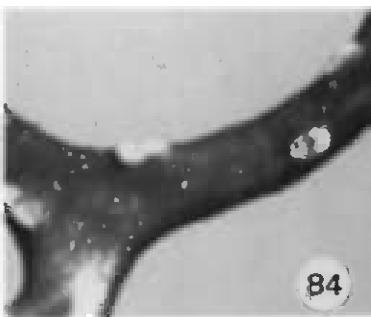
1. CYPHELLAE

Cyphellae are rounded pores that are characteristic of the foliose genus *Sticta* (Fig. 83). They develop as little rounded pits in the lower surface of the thallus. The lower cortex extends as an encircling protruding rim, enclosing a small craterlike depression. At the bottom of the crater the texture of the tissue is looser, the hyphae becoming separated to form a layer of globose cells (Fig. 90). This structure is similar to that of the lenticels of higher plants. The cyphellae may enlarge and become much bigger and irregular in outline. Old cyphellae lose their function as the rounded cells adhere and are embedded in the gelatinized remnants of the dying outer cells (Fig. 91).

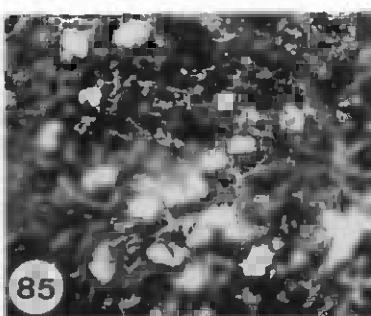
Only the aerating organs of *Sticta* are called cyphellae in the strict sense, although the pores of other genera are not fundamentally different in structure. The openings, called tubercles (Fig. 85), in the lower side of *Nephroma* are also raised above the thallus but exhibit no central depression as those in *Sticta*. They too are filled with short separating cells (Fig. 93). Protuberances on the upper side of *Parmelia aspidota* are synonymous with cyphellae or pseudocypellae. They have an elevated central opening where the cortex is reduced and loose hyphae of the medulla penetrate through the algal layer to reach the surface (Fig. 86). The difference between cyphellae



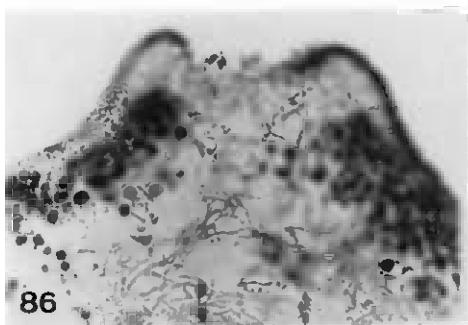
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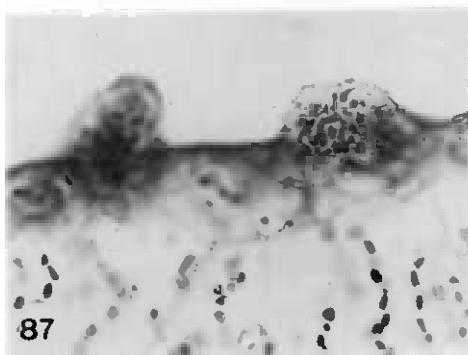
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and pseudocyphellae is not great and the distinction is arbitrary. The variety of differentiation is far greater than the two names indicate.

2. PSEUDOCYPHELLAE

Pseudocyphellae are formed on the upper and lower surface of several foliose and fruticose lichens. In *Pseudocypsellaria* they form irregular warts. The openings are filled by a network of short cells (Fig. 89). In very old and abnormally large pseudocyphellae algal cells may be deposited between the loose hyphae. These pseudocyphellae resemble soralia but the similarity is misleading as no soredia are produced.

The pseudocyphellae of the fruticose lichen *Cornicularia divergens* (Fig. 84) are either shallow depressions in the cortex or they form pores that penetrate to the medulla (Fig. 94). The pseudocyphellae on the upper side of *Cetraria cetrariooides* show a similar development (Fig. 95).

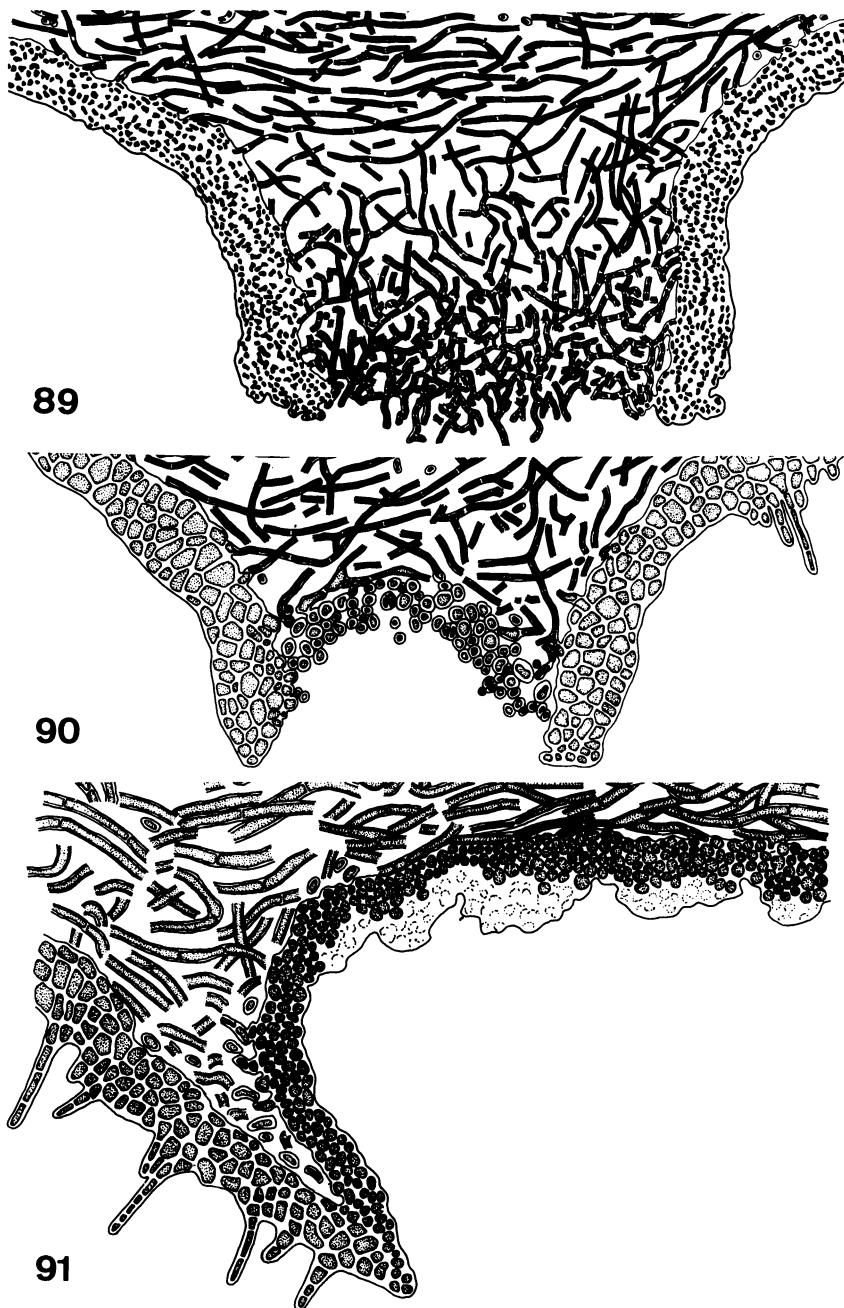
In some lichens, i.e., *Parmelia exasperatula* and *Placopsis cibellans*, the remnants of isidia serve as aerating organs. When the isidia break off, they leave on the thallus little warts which have a central opening filled by loosely interwoven hyphae.

The size of pseudocyphellae is quite constant in most species and has considerable taxonomic significance (Hale, 1967). This fact recently has helped to separate populations of *Cetraria chicitae* and *Parmelia olivetorum*, two vegetatively extremely similar foliose lichens in eastern North America. At first thought to be indistinguishable when sterile except by chemical tests (olivetric and alectronic acids, respectively), these two species were found to differ significantly in pore size, the average maximum size in *P. olivetorum* being 0.48 mm and in *C. chicitae* 1.20 mm.

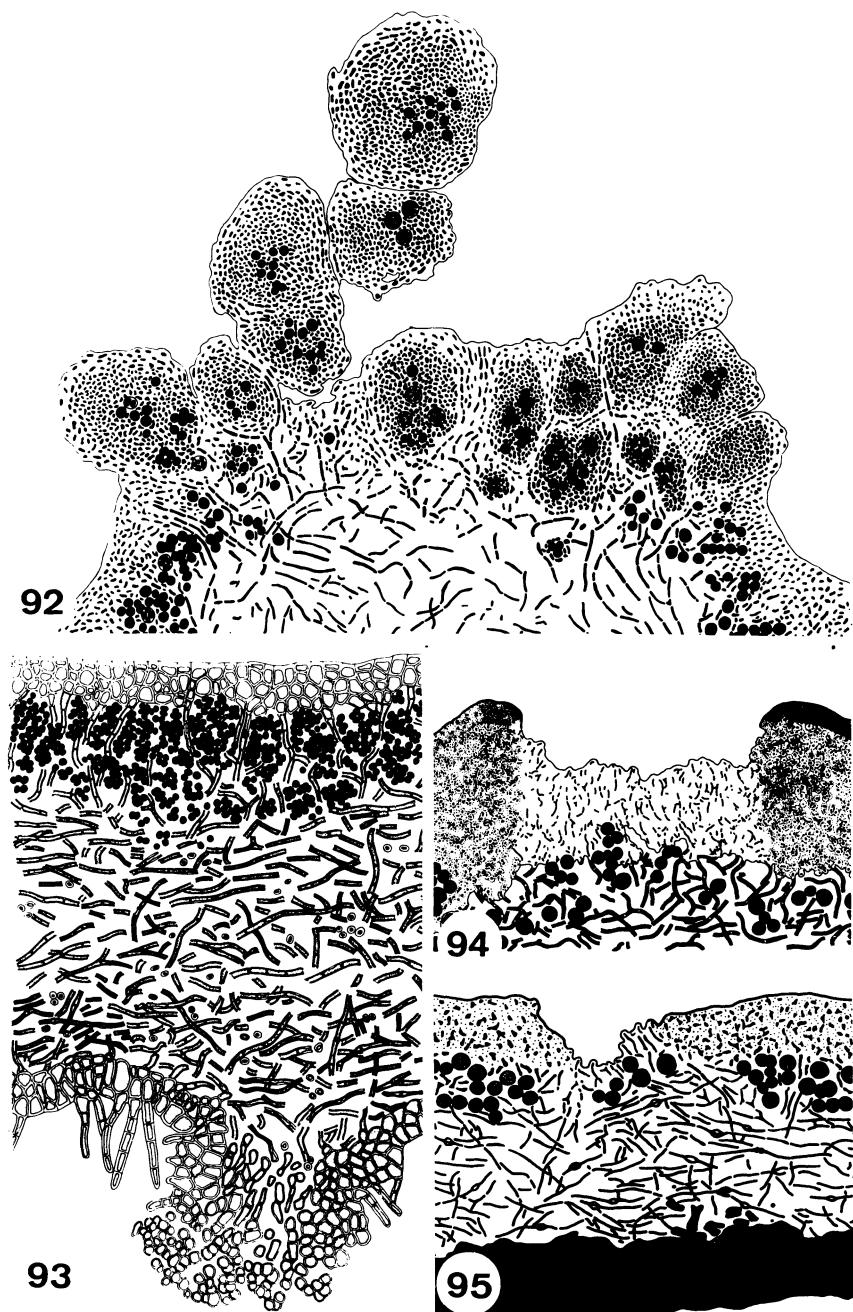
B. Vegetative Diaspores

Many lichens develop vegetative organs of dispersal, called isidia, soralia, and hormocystangia. It is open to question whether isidia always act as vegetative propagules, but it is interesting that lichens producing isidia or soredia do not freely produce ascocarps. In some lichens isidia probably only increase the surface area. In the soralia, vegetative diaspores—the soredia—are produced. Lichenized hormocysts are the diaspores formed in hormocystangia of some lichens with blue-green phycobionts. Soredia

Figs. 83–88. Fig. 83, cyphellae on the tomentous underside of *Sticta* (20 \times); Fig. 84, pseudocyphellae of *Cornicularia divergens* (16 \times); Fig. 85, tubercles on the underside of the tomentous thallus of *Nephroma* (8 \times); Fig. 86, aeration pore of *Parmelia aspidota* (200 \times); Fig. 87, young isidia of *Collema flaccidum* in section (350 \times); Fig. 88, hormocystangium of *Lempholemma versiculiferum* (60 \times). (Figs. 83, 84, and 86–88 from Henssen and Jahns, 1973.)



FIGS. 89–91. Fig. 89, pseudocyphellae of *Pseudocyphellaria*; Figs. 90–91, young and old cyphellae of *Sticta sylvatica*. (Figs. 89–91 from Henssen and Jahns, 1973.)



FIGS. 92-95. Fig. 92, soralium of *Lobaria pulmonaria*; Fig. 93, respiration pore of *Nephroma resupinatum*; Fig. 94, pseudocystellae of *Cornicularia divergens*; Fig. 95, pseudocystellae of *Cetraria cetrariooides* (Figs. 92-94 from Henssen and Jahns, 1973.)

and hormocysts are separated from the lichen by the active growth of the thallus, while isidia can only serve as organs of dispersal if they are passively broken off. Reproduction by isidia is similar to the dispersal of a lichen by thallus fragments that are readily produced when a dry, crisp lichen is crushed (Fig. 38).

Soredia, hormocysts, and isidia are an adaptation to the symbiotic role, as these diaspores disperse both partners of the symbiosis together. Hormocystangia are only known from *Lempholemma* while there are many sorediate and isidiate lichens. Dispersed diaspores grow into new lichen thalli. At first, algae and hyphae multiply to form an undifferentiated mass. Later, the algae arrange themselves in an algal layer and the hyphae start the differentiation of a cortex. Diaspores falling on unsuitable habitats may provide a source of algal partners to be taken over by other lichen fungi characteristic of the habitat.

1. ISIDIA

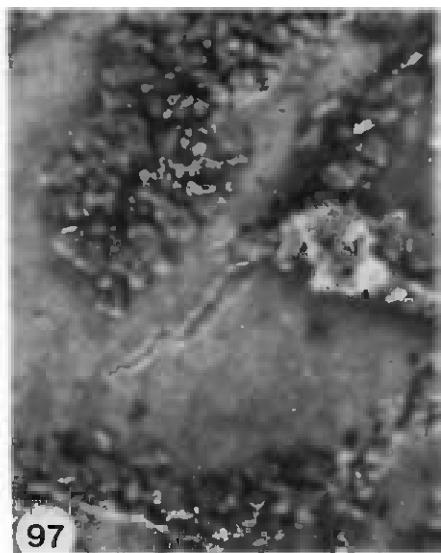
Isidia are small protuberances of the thallus incorporating algal and medullary tissue covered by a cortex. They are globose, cylindrical, coralloid, or scale-shaped (Figs. 96–101) and range from 0.01 to 0.03 mm in diameter and from 0.5 to 3.0 mm in height. It is difficult to separate isidia from other protuberances of the thallus. Small warts on the surface of the thallus are called papillae and larger, dorsiventral bodies, resembling small lobes of the thallus, are named lobules. Neither is fundamentally different from an isidium. Fibrillae of *Usnea* also can be confused with isidia, especially the young stages which do not differ in size from isidia.

Isidia are formed by crustose, foliose, and fruticose lichens and by some gelatinous lichens. The isidia of many crustose lichens are easily broken off and without question serve for dispersal. In many foliose and gelatinous lichens this does not seem to be the case as one very seldom finds places where the isidia have broken off. In some species, for example, in *Parmelia tiliacea*, the isidia may be liberated on death of the lobe. It is possible that in other species the isidia serve to increase the surface area and presumably the assimilative capacity of the thallus, but this theory has not been tested and there is no proof that isidiate species have any advantage over non-isidiate species. Up to 25–30% of the species in foliose and fruticose genera have isidia, but they are much rarer in crustose groups.

Figs. 96–101. Fig. 96, Bushlike isidia on the thallus of *Umbilicaria pustulata* (20 \times); Fig. 97, Isidia of *Peltigera praetextala* formed at places where the thallus is torn (15 \times); Fig. 98, Cylindrical isidia of *Pseudevernia furfuracea* (35 \times); Fig. 99, Isidia of *Parmelia saxatilis* (50 \times); Fig. 100, wartlike isidia of *Parmeliella atlantica* (10 \times); Fig. 101, scale-shaped isidia of *Collema flaccidum* (30 \times); (Figs. 96–99 and 101 from Henssen and Jahns, 1973.)



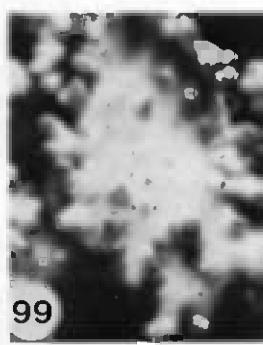
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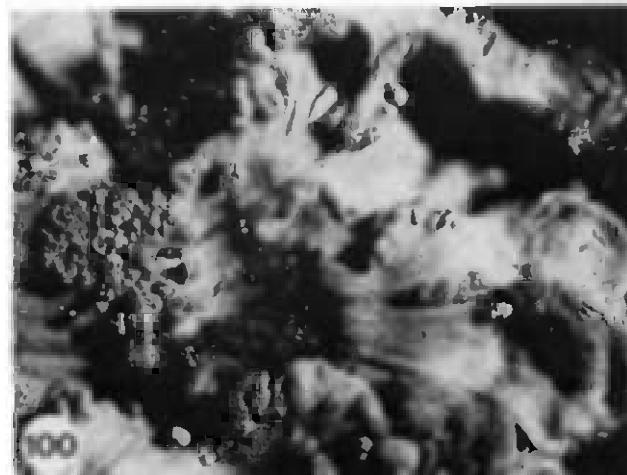
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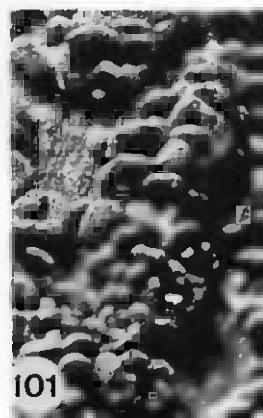
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DEVELOPMENT OF ISIDIA. It is still unknown what factors cause the formation of isidia. In *Peltigera praetextata* it has been shown experimentally that the development of isidia or lobules is stimulated by the tearing or wounding of the thallus (Fig. 97). The development of isidia seems to be genetically determined. Their presence and shape is an important taxonomic characteristic.

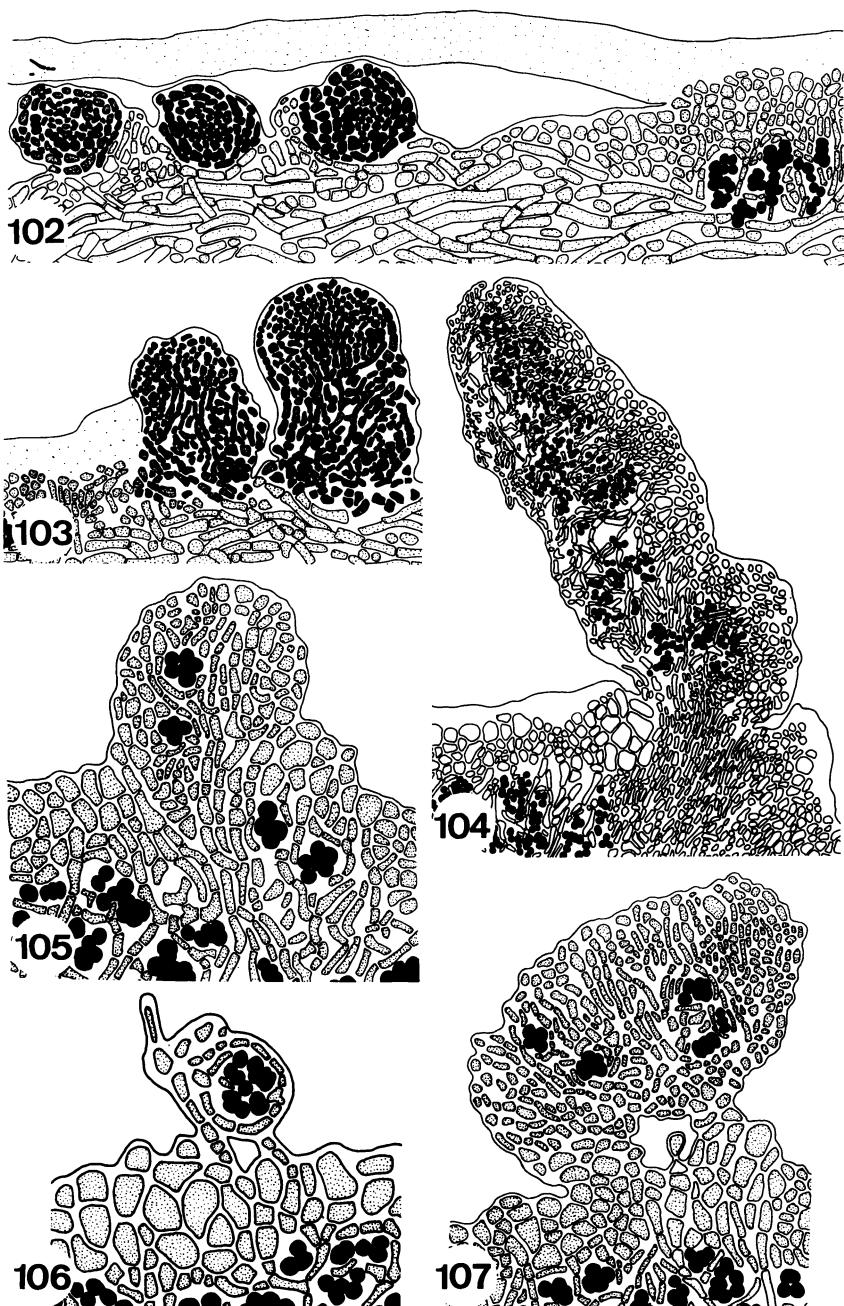
In heteromerous foliose lichens the formation of isidia is initiated by the medullary hyphae. These penetrate between the cells of the cortex, at the same time pushing the algal cells upwards. This primordium, composed of both algal and medullary tissue, rises above the thallus and is secondarily covered by a newly formed cortex. An example of the development of isidia from the medulla is shown in *Peltigera praetextata*. The isidia are formed under the lacerated cortex, which is bent upwards in some parts (Fig. 102). The isidium continues to grow (Fig. 103). The mature isidium has a dorsiventral structure and a secondarily formed cortex (Fig. 104). If the term isidium is to be restricted to protuberances from the thallus showing a radial structure, then the dorsiventral bodies on the thallus of *Peltigera praetextata* must be called lobules or folioles.

In *Umbilicaria pustulata* the cortex of the isidia (Fig. 96) is not formed secondarily but the cortex of the thallus grows upwards and surrounds the medullary tissue from their initiation.

The isidia of *Peltigera lepidophora* sometimes develop from hyphae of the medulla (Fig. 105) (Linkola, 1913) in the same way as in *Peltigera praetextata*, but sometimes a quite different method of construction can be observed. The hairs on the upper surface of the thallus enmesh free-living algae which have fallen onto the thallus (Fig. 106). These mature isidia are completely indistinguishable from those formed in the medulla and it is impossible to tell which way they have developed (Fig. 107). This second method of development is the same as in the formation of cephalodia in *Peltigera aphthosa* (see p. 51), the only difference being that the isidia contain the same algae as the thallus.

The formation of isidia in gelatinous lichens is initiated by the algae at the edge of the thallus. These divide to form a small protuberance arising from the ecorticate thallus (Fig. 87), and this structure becomes secondarily enmeshed by the hyphae. If the thallus has a cortex, it surrounds the young isidium from the beginning, elevating it together with hyphae and algae of the medulla.

In the fruticose, filamentous lichens of the genera *Usnea* and *Alectoria*, isidia can develop from soralia. A characteristic example is *Alectoria nidulifera*, which forms typical, short isidia in older soralia (Fig. 118). In *Usnea* these isidia frequently continue to grow and become fibrillae. In *Parmelia subaurifera* isidia can also become sorediate.



Figs. 102–107. Figs. 102–104, development of isidia in *Peltigera praetextata*; Fig. 105, development of an isidium of *Peltigera lepidophora* by growth of medullary hyphae; Fig. 106, development of an isidium in *Peltigera lepidophora* by trapping of algae; Fig. 107, older isidium of *Peltigera lepidophora*. (Figs. 102–107 from Henssen and Jahns, 1973.)

2. SORALIA

Soralia differ from isidia in being noncorticated. They are powdery breaks in the cortex of the thallus and contain minute soredia. Soredia consist of several algae closely enveloped by hyphae; they serve as diaspores. Individual soredia are 25–100 μm in diameter. The soredia adhere, forming powdery or granular, nonwettable masses. The structure of a soredium is different in unrelated lichen genera. Soredia of *Parmelia* are formed by algae which are only loosely enmeshed by hyphae, while in *Peltigera* the algae are closely enveloped by hyphae. The porelike soralia not only form the vegetative diaspores but may also be important for the exchange of gases.

Soredia need not be confined to delimited soralia but can originate over the whole surface of a lichen, which assumes a powdery appearance (Fig. 36). True delimited soralia may be divided into several types on the basis of their shape (Bitter, 1901; Du Rietz, 1924; Frey, 1963):

a. *Maculiform soralia* are rounded or oblong patches or depressions on the upper surface of the thallus. They may originate as very small, dot-shaped structures (*punctiform soralia*) and may extend until they cover nearly the whole surface (Fig. 108).

b. *Rimiform soralia* (fissure-shaped soralia) are oblong and sometimes branched. They develop from fissures of the cortex. This type is rare and is characteristic for *Parmelia sulcata* (Fig. 112).

c. *Convex-globular soralia* form globose heaps of soredia on the laminar part of the thallus (Fig. 111). If these globose soralia originate at the tips of swollen, dorsiventral lobes or at the ends of fruticose branches they are called *capitiform* (capitate) *soralia*.

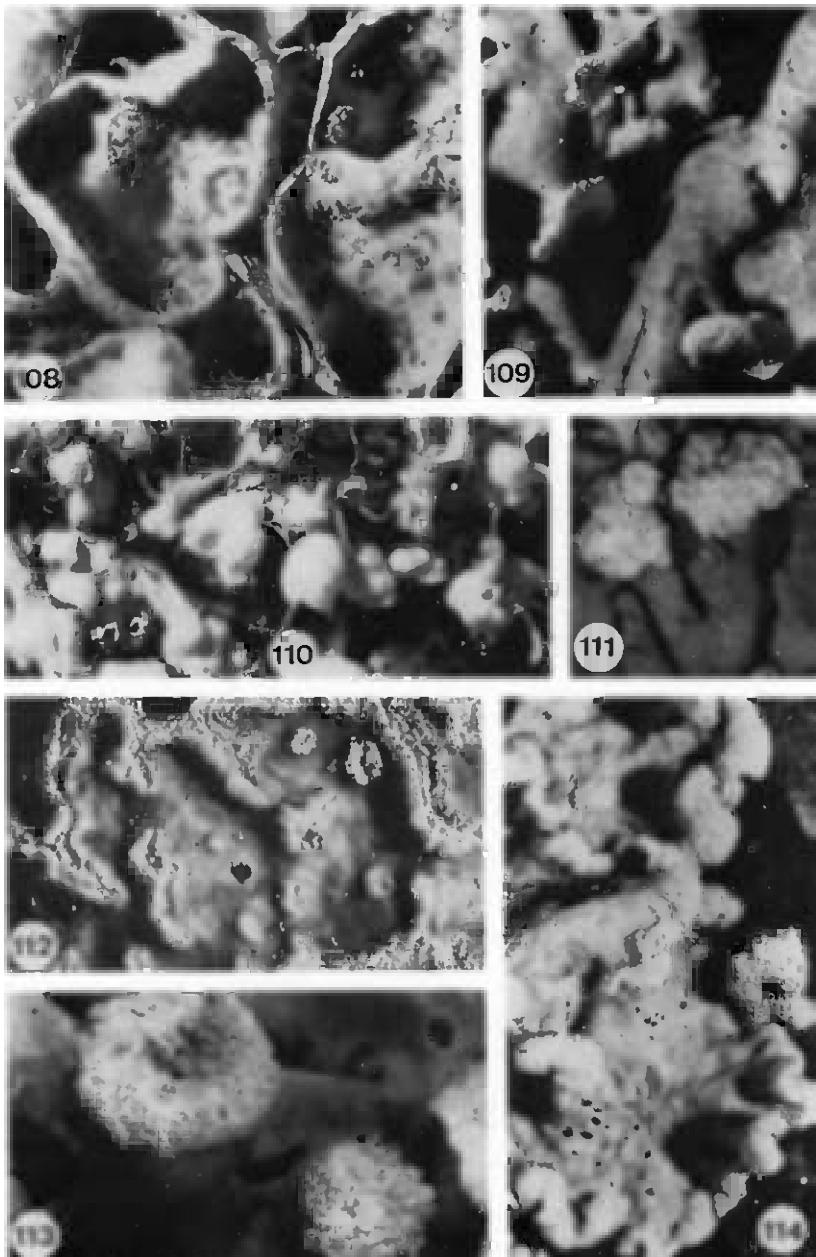
d. *Maniciform* (cuff-shaped) *soralia* are not much different from globular soralia. They develop on circular, lobelike protuberances of the thallus and have a central perforation (Fig. 113). This type occurs in *Hypogymnia* and *Menegazzia*.

e. *Labriform* (lip-shaped) *soralia* develop at the ends of the thallus lobes, splitting the ends into two lips. The upper lip usually curls upwards partly exposing the soralia (Fig. 109). If the upper lip is inflated and becomes vaulted the structure is called a *forniciform* (helmet-shaped) *soralium* (Fig. 110).

f. *Marginal soralia* develop along the margin of dorsiventral lobes, forming a coherent border (Fig. 114).

g. *Parietal soralia* are deep seated in the thallus and cause swellings and vesicles on the surface. Release of soredia occurs with rupture of the vesicles. This type, which is rare, occurs in *Ramalina obtusata*.

DEVELOPMENT OF SORALIA. The formation of a soralium may be initiated by the medullary hyphae. In heteromerous, foliose, or fruticose lichens



Figs. 108–114. Fig. 108, Thallus of *Peltigera spuria* covered with maculiform soralia ($5 \times$); Fig. 109, labriform soralia of *Hypogymnia physodes* ($8 \times$); Fig. 110, forniciiform soralia of *Physcia adscendens* ($8 \times$); Fig. 111, globose soralia of *Physcia caesia* ($5 \times$); Fig. 112, rimiform soralia of *Parmelia sulcata* ($16 \times$); Fig. 113, maniciform soralia of *Menegazzia terebrata* ($8 \times$); Fig. 114, marginal soralia of *Cetraria pinastri* ($11 \times$). (Figs. 108, 109, and 112–114 from Henssen and Jahns, 1973.)

these hyphae divide at certain places, forming short, thin-walled cells. The algae lying in the vicinity are also stimulated and begin to divide. The resulting algal cells are closely enclosed by the dividing hyphae. Young soredia are continuously formed in the medulla thereby pushing the older soredia upwards until they break through the cortex and form a soralium (Fig. 92). The soredia are dispersed by water, wind, or insects.

Other vegetative structures of lichens can change into soralia; for instance, the isidia of some species of *Parmelia* may become sorediate and the pseudocyphellae of *Pseudocyphellaria* can develop into soralia. This development is not always completed as, under these conditions, the relation between the symbionts sometimes appears disturbed.

In general, the development of soralia is increased in moist, shady habitats. Ecological observations have shown that a low light intensity especially has a stimulating influence. Shaded rocks are nearly always covered with sorediate lichens but aquatic lichens never produce soralia.

The formation of soralia sometimes may depend on the age of the lichen. Young thalli of *Peltigera spuria* (Fig. 108) are covered with soralia, but as fruiting bodies are formed the soralia are reduced so that old fruiting thalli are devoid of soralia. It is possible to find communities of this species that include sorediate, sterile thalli, thalli bearing soralia and apothecia, and nonsorediate thalli with abundant apothecia. Before the discovery of this astonishing observation all three morphotypes had been described as separate species.

In general, the formation and shape of soralia seem to be genetically determined. Their presence or absence, color, shape, and development are often important in taxonomic studies. There are a number of nonsorediate lichens which are indistinguishable from sorediate species except for their lack of soralia. Usually, in these pairs of species the sorediate lichen does not have apothecia while the other, nonsorediate species produces fruiting bodies.

3. HORMOCYSTANGIA

Hormocystangia are swollen areas of the thallus situated at the ends or margins of the lobes in some species of *Lempholemma*. They are filled with lichenized or unlichenized hormocysts which are liberated with the decay of hormocystangia (Fig. 88). The hormocysts are short algal filaments derived from the symbiotic *Nostoc* and enclosed by a thick gelatinous sheath. The fungal hyphae do not enmesh the algae as they do in soredia but penetrate into the gelatinous sheath. Not all hormocysts are lichenized and this type of vegetative reproduction seems better adapted for the dispersal of the phycobiont.

C. *Cephalodia*

Some lichens not only consist of one mycobiont and one phycobiont, but of one mycobiont associated with two different algae. The main algal partner always belongs to the *Chlorophyceae*, the second to the *Cyanophyceae*. The importance of the latter for the mycobiont lies in the fact that the additional blue-green alga is able to fix atmospheric nitrogen and has been shown to pass this to the mycobiont in the main part of the thallus (see Chapter 9).

In some lichens these blue-green algae are found within the thallus which already contains green algae. In *Solorina crocea* they form a second algal layer (*Nostoc*) beneath the *Chlorophyceae* (*Coccomyxa*) of the first layer (Fig. 115). In *Compsocladium*, a genus of the *Stereocaulaceae*, single filaments of *Cyanophyceae* lie in the medulla of the fruticose thallus, while the *Chlorella*-like green algae form an algal layer beneath the cortex.

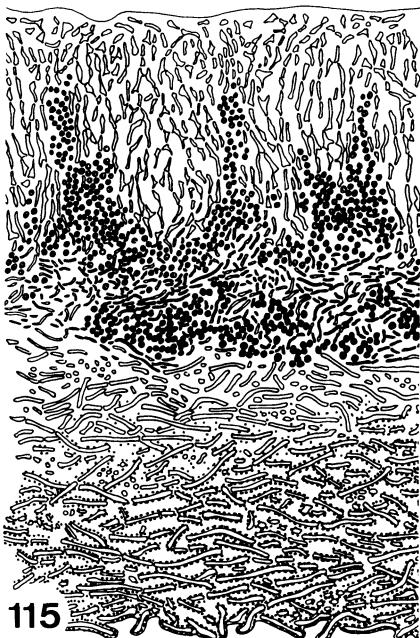
In most lichens with two algal symbionts the blue-green algae are not distributed within the thallus but lie in special, delimited, swollen parts of the thallus. These structures, sited either in the medulla or on the upper or lower surfaces of the thallus according to the species, are called cephalodia.

Some lichens always associate with the same genus of blue-green algae as the second phycobiont, while other lichens can utilize different blue-green algae. Even adjoining cephalodia on the same thallus may contain different algae, for example, *Gloeocapsa* and *Stigonema* (Fig. 128) (Forssell, 1883).

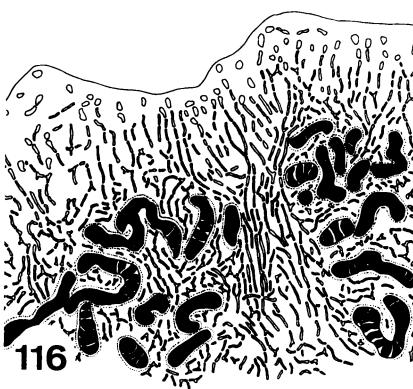
The ability to form cephalodia has been acquired independently in several lichen families. Their formation appears to be genetically determined and is therefore of important taxonomic value. For example, all genera belonging to the family *Stereocaulaceae* have cephalodia. Other genera which regularly form cephalodia are *Placopsis* and *Psoroma* and some species of the *Caliciaceae*, *Lecideaceae*, and *Peltigeraceae*.

It is possible to distinguish between external and internal cephalodia. Internal cephalodia are found frequently in *Lobaria* and *Sticta*. They consist of clumps of algae which lie in the medulla of the thallus. They become so large that they can be seen from the outside as swollen warts on the thallus. They always contain *Nostoc*.

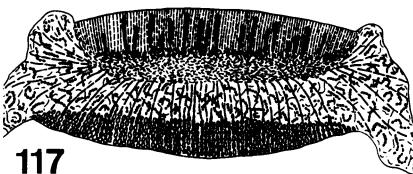
The shape of external cephalodia varies considerably. In *Peltigera aphthosa* they are disk-shaped and slightly darker in color than the thallus (Fig. 124). In *Placopsis* the disks of the cephalodia are pink-red and much larger than the apothecia (Fig. 125). The cephalodia of *Pilophorus* and *Stereocaulon* are round (Fig. 81) or branched and bushlike. In *Stereocaulon* the cephalodia are divided into several types on their anatomy. They can be ecarticate (Fig. 129) or covered by a cortex (Fig. 130) (sacculate type) (Lamb, 1968). The cortex consists of netlike, branched hyphae which sometimes end in thickened cells (Fig. 116).



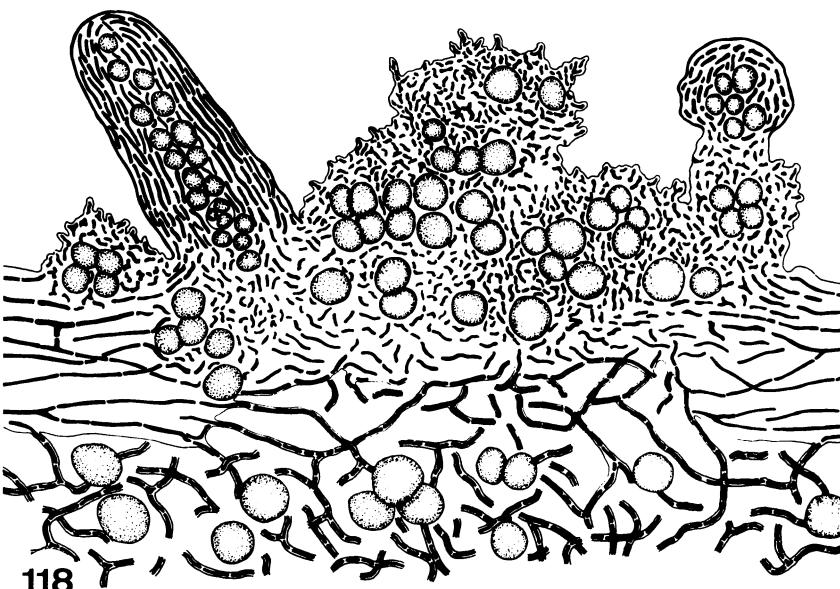
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Figs. 115-118. Fig. 115, section through the thallus of *Solorina crocea*. The cells of the *Coccomyxa* phycobiont reach into the cortex at several places. The *Nostoc* phycobiont (black) forms a layer beneath the green algae; Fig. 116, saccate cephalodium of *Stereocaulon conio-phylum* containing *Scytonema*. The terminal cells of the hyphae forming the wall are swollen;

The external cephalodia do not always sit on the thallus. In crustose lichens with an areolated thallus they usually form little scales of their own between the areoles. These cephalodia are frequently distinguished from the rest of the thallus by their different color and rounded shape. Their anatomy can be either nearly identical with the rest of the thallus or quite different. *Solorina spongiosa*, for example, consists of a circular lobe, containing green algae and bearing a large sunken apothecium on a coralloid warted cushion containing blue-green algae.

DEVELOPMENT OF CEPHALODIA

The cephalodia of *Placopsis* arise as separate scales between the areoles of the young thallus. The thallus grows much more quickly than the cephalodium and thus the tissue of the thallus is pushed under the cephalodium lifting the latter upwards (Fig. 125). The cephalodia of the old thallus are often found towards the middle of its lobes.

In *Peltigera* the *Nostoc* symbionts of the cephalodia come from outside the thallus and are trapped by hairs on the surface of the thallus (Darbishire, 1927). The algae fall on the thallus and stimulate the growth of the hairs which envelope them (Fig. 131). More hyphae then participate resulting in the formation of the cephalodium (Fig. 132). This older cephalodium seems to stand on stilts (Fig. 133). In its lateral layer a cortex is differentiated. The cortex of the thallus disintegrates beneath the cephalodium and a direct contact between medullary hyphae and cephalodium thus is achieved.

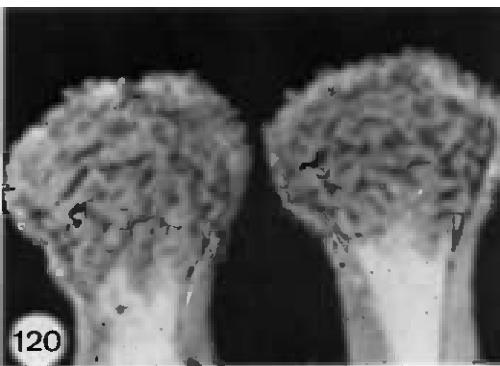
Internal cephalodia are formed in a similar manner. Here, too, the *Nostoc* algae are trapped by the hyphae of the cortex (Jordan, 1970; Jordan and Rickson, 1971; Moreau, 1928). They are enmeshed by a thick layer of fungal cells and pressed into the thallus, where the cephalodium is eventually formed (Fig. 126). The enmeshing hyphae form a bundle which pushes the algae inside. Usually, the algae penetrate via the lower surface of the thallus but sometimes they seem to enter via the upper surface of the lichen. If this happens the cephalodium lies above the algal layer, which is pushed deeper into the medulla (Fig. 127).

It is a striking fact that the lichen is able to select the right alga it needs to build a cephalodium from the multitude of algae found in the vicinity of the thallus. In nearly all lichens new cephalodia must be formed with free-living algae. Only one example is known where the cephalodia themselves undergo vegetative reproduction which results in their dispersal (P. W. James, unpub-

Fig. 117, apothecium of *Collema coilocarpum* with basal supporting tissue formed by the anchor hyphae; Fig. 118, development of isidia in a soralium of *Alectoria nidulifera*. (Figs. 115–118 from Henssen and Jahns, 1973.)



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Figs. 119–125. Underside of an apothecium of *Peltigera leucophlebia* with small patches of cortical tissue ($5 \times$); Fig. 120, undersides of apothecia of *Peltigera aphthosa* completely covered by cortical tissue ($5 \times$); Fig. 121, smooth cortex of *Peltigera horizontalis* ($10 \times$); Fig. 122,

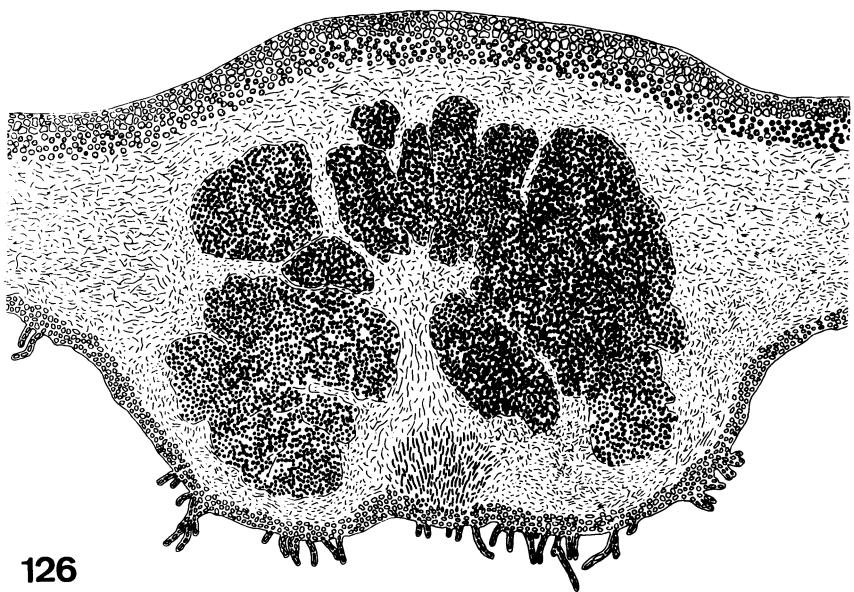
lished results). The margins of the scalelike cephalodia of a species of *Psoroma* from Tasmania and New Zealand becomes sorediate. These soredia grow into new cephalodia, either on the same lichen or on another thallus of the same species.

V. Influence of Fungus and Alga on the Habit of the Lichen

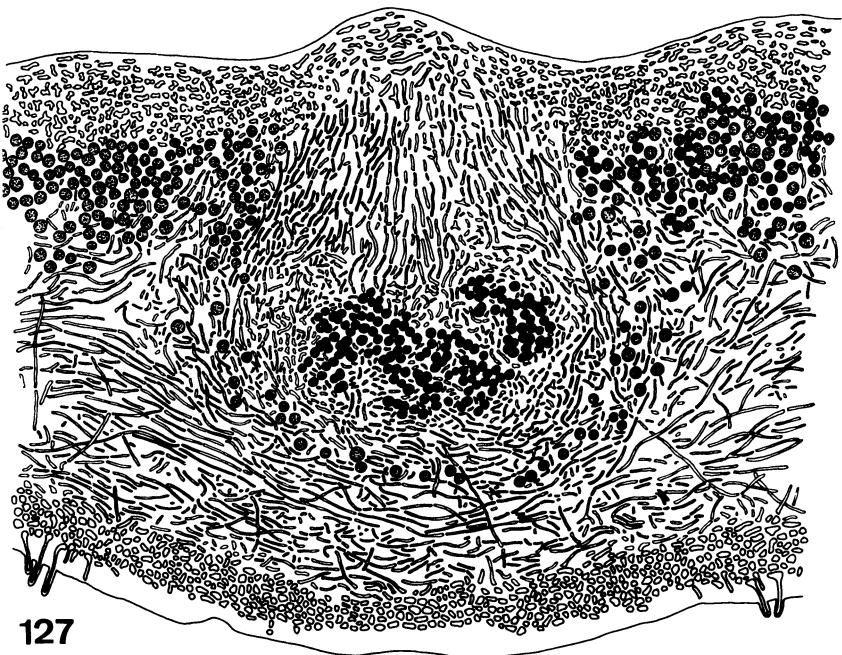
The habit of a lichen is usually stated to be determined only by the mycobiont, but in the case of one special kind of cephalodium, which has been misinterpreted for a long time, the alga also may have considerable influence. While most species of *Lobaria* are characterized by the development of internal cephalodia, the thalli of *Lobaria amplissima* bear shrublike, external cephalodia. Similar shrublike structures also are described as independent, living lichens and are named *Dendriscocaulon*. As a result, the protuberances on *Lobaria amplissima* were thought either to be cephalodia or were regarded as parasitic lichens. New light has been shed on this problem by a recent discovery (James, unpublished results). In New Zealand, a lichen belonging to the genus *Dendriscocaulon* has been found bearing on its fruticose, treelike thallus, small but characteristic lobes of *Sticta filix* (Fig. 77). As a rule cephalodiate lichens develop a thallus containing green algae, which secondarily captures blue-green algae that form the cephalodia, but here the situation is reversed. James has suggested that the first stage is a *Dendriscocaulon* with *Nostoc* as phycobiont, which subsequently, under certain environmental conditions, traps green algae and forms the normal thallus of *Sticta filix* which bear the characteristic ascocarps. The free-living species of *Dendriscocaulon* may be interpreted as incomplete stages of lichens from the genera *Sticta*, *Lobaria*, and possibly *Pseudocyphellaria*. More importantly, the example of *Dendriscocaulon* suggests that by association with different algae the same fungus can be induced to form thalli which are morphologically and anatomically completely different from one another.

Nevertheless, in most lichens the mycobiont is the dominant partner, and as a rule different fungi in association with the same alga always result in differently organized thalli. For example, some genera belonging to the Lichinaceae or to imperfect groups all contain the blue-green *Scytonema* as phycobiont. In *Lichenothrix* and *Thermitis* the appearance of the algae is unchanged by the fungal hyphae (Henssen, 1963, 1964). In *Thermitis* the

granular cortex of *Peltigera scabrosa* ($15 \times$); Fig. 123, hairy cortex of *Peltigera canina* ($15 \times$); Fig. 124, cephalodia of *Peltigera aphthosa* ($20 \times$); Fig. 125, thallus of *Placopsis gelida* bearing cephalodia and soralia. A small cephalodium has been formed near the main thallus and is half surrounded by a small scale of the thallus ($8 \times$). (Figs. 120–123 and 125 from Henssen and Jahns, 1973.)



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Figs. 126–127. Fig. 126, section through an internal cephalodium of *Lobaria laetevirens* containing large groups of *Nostoc*. The green algae form a small layer beneath the upper cortex; Fig. 127, section through a thallus of *Lobaria pulmonaria* with young internal cephalodium.

fungal hyphae grow inside the gelatinous sheath of the alga, while in *Lichenothrix* they grow on the outside of algal filaments. Only the haustoria of this genus penetrate the gelatinous sheath and reach the algal cells. The phycobiont of *Zahlbrucknerella*, another genus of the Lichinaceae, is closely enveloped by the hyphae, and it is hardly possible to see the characteristic pseudobranches of the alga. Only the tips of the pseudobranches are free and can be seen, while in *Lichenodium* even this is no longer possible. In *Polychidium* the fungus is so dominant that the alga can no longer be seen from the outside at all. In *Coccocarpia* the filamentous phycobiont is completely incorporated into a foliose thallus.

En masse the mycobionts are the dominant partners of the symbiosis and are responsible for the complicated thalline structures, but it must not be forgotten that the influence of the alga is vital in order to achieve these results. Nonlichenized ascomycetes may form a stroma which sometimes resembles a lichen thallus, but the highly differentiated thalli of foliose and fruticose lichens are much more complicated. Their vegetative structures reach a high level of differentiation paralleled by the organs of higher plants.

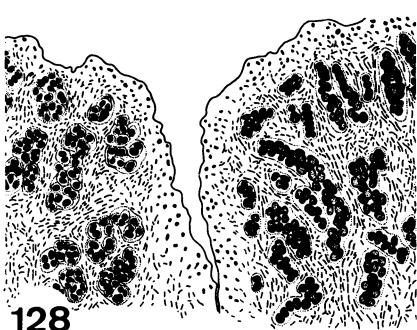
VI. Influence of Fruiting Bodies on the Development of Thallus Tissues

Many of the types of tissues and cells found in the cortex and medulla of lichens are present also in the fruiting body, especially in the exciple. These structures are treated in Chapter 2 but there are some examples of the way these tissue types may become altered in the anatomy of the thallus adjoining the ascocarp.

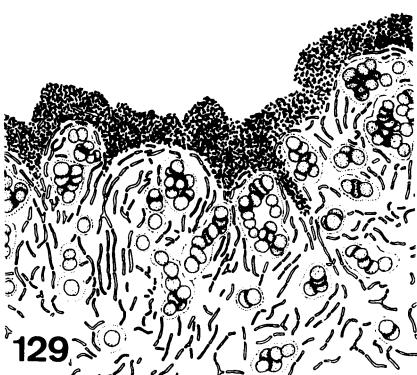
The thallus of *Peltigera aphthosa*, for example, is normally ecorcticate on its lower surface but beneath the marginal apothecia there is a well-developed, wrinkled cortex (Fig. 120). The formation of this layer seems to be induced by the algae of the thallus. A part of the algal layer is pushed to the lower surface of the thallus by the growth of the fruiting body. Only where these algae are found is a cortex developed. This fact can more easily be seen in *Peltigera leucophlebia*. In this species only scattered groups of algae reach the lower part of the thallus and therefore the cortex too consists of scattered patches (Fig. 119).

The influence of the ascocarp on the anatomy of the thallus is most conspicuous in gelatinized lichens. The thallus of these species seems to be too soft to bear the fruiting bodies and therefore either secondary supporting

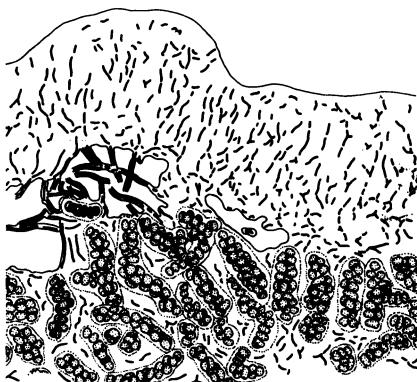
The *Nostoc* cells seem to have penetrated to the upper cortex and lie above the green algae, which is unusual. (Figs. 126 and 127 from Henssen and Jahns, 1973.)



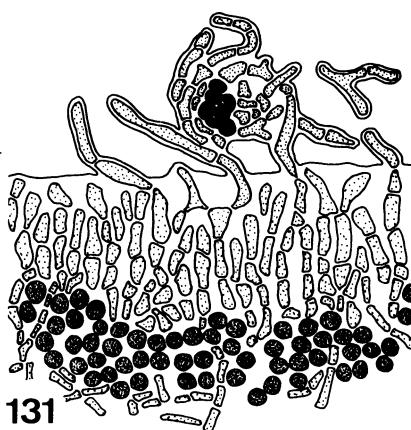
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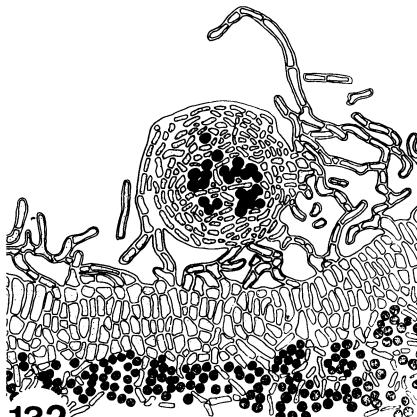
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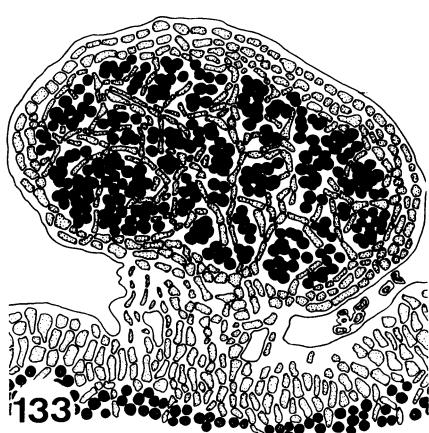
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tissues are formed or the cortex of the thallus is strengthened. The fruiting body may be attached in the thallus by special hyphae, called anchor hyphae, which grow from the tissue of the ascocarp (Fig. 117).

Acknowledgments

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FIGS. 128–133. Fig. 128, two cephalodia of *Lecidea pelobotrya*, the left one containing *Gloeocapsa* and the right one *Stigonema*; Fig. 129, cephalodium of *Stereocaulon* sp. with *Nostoc* as phycobiont. The cephalodium is ecorcticated; Fig. 130, sacculate cephalodium of *Stereocaulon ramulosum* with a netlike cortex tissue containing *Stigonema* as phycobiont; Fig. 131–133, development of cephalodia in *Peltigera aphthosa* by trapping algae with the hairs of the upper cortex. (Figs. 128–133 from Henssen and Jahns, 1973.)

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Chapter 2

SEXUAL REPRODUCTION

MARIE-AGNÈS LETROUIT-GALINOU

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I. Ascolichens

The sexual reproduction of ascolichens is basically similar to that in the other Ascomycetes and will be discussed with reference to those.

A. *The Basic Cycle of Sexual Reproduction in Ascolichens and Ascomycetes (Fig. 1)*

According to Chadefaud (1953, 1960), the basic reproductive cycle in Ascomycetes is not digenetic, as often claimed, but is in principle trigeneric, as in the algal family Floridaceae or, among the Basidiomycetes, the Uredinales with alternation of (1) a gametophyte producing male and female sexual elements; (2) sporophyte I (= prosporophyte), siphonaceous and mictohaploid, that is, with cells containing several nuclei, some male and the others female but still immature and not coupled; and (3) sporophyte II (= ascosporophyte), dikaryotic and giving rise to ascii. Sporophytes I

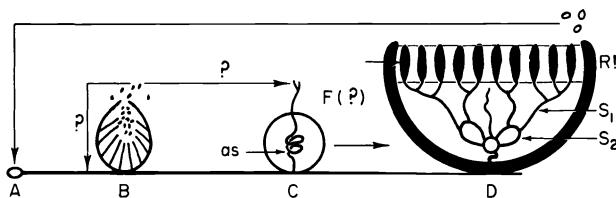


FIG. 1. Basic scheme of sexual reproduction in ascolichens. A, Haploid spore (ascospore or conidiospore) producing a thallus; B, pycnidium in which spores arise, some considered to be direct spores (left arrow), others spermatia (right arrow); C, ascogonial apparatus (as) included in the gametophytic primordium, trichogamy may possibly occur; D, sporophytic generating ascocarps (a) apparatus which develops on the ascogonial apparatus and is included in an ascocarp derived from the primordium. Chromosomal reduction (R!) takes place in the ascospores (S_1 and S_2 , prosporophyte and ascosporophyte).

and II together constitute the sporophytic apparatus and develop parasitically on the gametophyte in the interior of fruiting bodies that are called ascocarps.

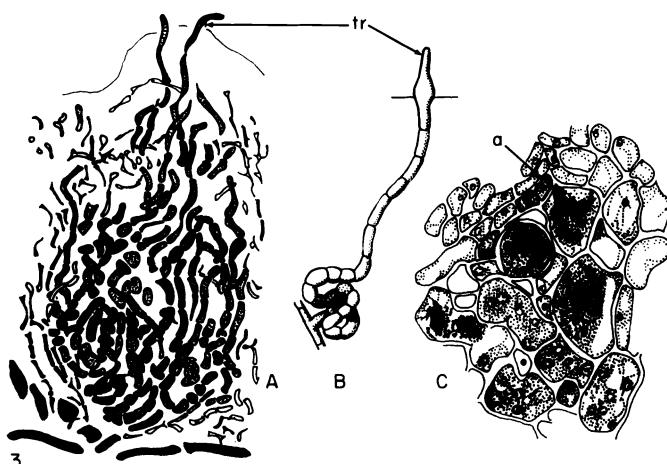
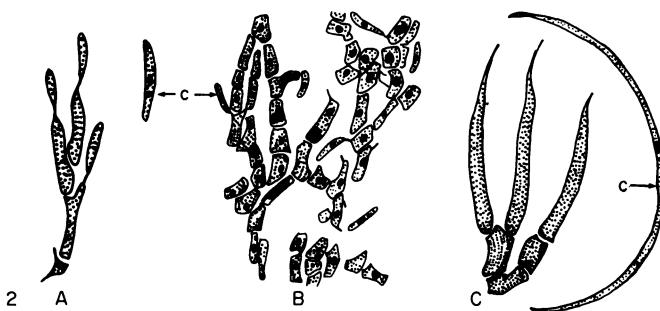
B. Reproductive Apparatus of the Gametophyte

In the nonlichenized Ascomycetes, the gametophyte consists of mycelia and appendages, sclerotia and stromas, and it produces three types of reproductive filaments: (1) asexual reproductive filaments, or conidiogenous filaments, directly producing nonmotile spores called conidial spores or conidia; (2) male filaments which are either spermatogenous filaments producing nonmotile male gametes (called spermatia) or parascogonial filaments associated with ascogonial filaments and which ensure fertilization directly; and (3) female or ascogonial filaments, certain cells of which (ascogonial cells) have a role as female elements. According to Chadefaud (1960), the existence of spermatia to which parascogonial filaments have not been substituted might be a primitive character indicative of an early stage of evolution.

In ascolichens, the gametophyte consists of the thallus which produces or can produce only two types of reproductive structures: microsporogenous filaments and ascogonial filaments. Microsporogenous filaments produce microspores, the nature of which (whether conidial or spermatial) is under discussion. They are generally confined in fructifications that resemble pycnidia. Ascogonial filaments which are lodged in the primordia of the ascocarps are never accompanied by fertilizing parascogonial filaments. They are formed in the primordia or these secondarily develop around them, depending on the species.

1. THE MICROSPOROGENOUS FILAMENTS (Fig. 2)

Microspores of various shapes and forms are produced by more or less differentiated sporogenous cells. These spores are always exogenous, that is, arising from buds. They are formed on a sporogenous cell, quite often at the tip of a style, never at the bottom of a neck or a ring collar as is seen in certain nonlichenized Ascomycetes.



FIGS. 2 AND 3. Reproductive apparatus of the gametophyte. Fig. 2, microsporogenous filaments. A, endobasidial filament of *Buellia canescens*; B, part of the endobasidial microsporogenous plexus in *Lobaria laetevirens*; C, exobasidial filament of *Roccella montagnei* (c, microspore). Fig. 3, ascogonial filaments. A, complex ascogonial apparatus formed of a large number of ascogonial filaments of the *Collema* type in *Lecidea elaeochroma*; B, ascogonial filament of *Collema* sp. (after Stahl, 1877); C, vesiculate multinucleate cells (a) of the ascogonial apparatus of *Peltigera rufescens* (a, ascogonial cells; c, microspore; tr, trichogyne).

The sporogenous cells, in general, belong to differentiated sporogenous filaments. Always multicellular, they are simple or branched and free or anastomosed, depending on the species. In this case, they can form a loose network or a kind of lacunose paraplectenchyma (Fig. 2B).

Two types of sporogenous filaments may be distinguished (Glück, 1899). In the *exobasidial* type (Fig. 2C), the sporogenous cells, in this case called "sterigmata," arise singly on lateral or subterminal sporophores. They are homologous to lateral branches and, as a rule, are elongated and morphologically distinct from sterile cells. In the *endobasidial* type (Fig. 2A, B) the sporogenous cells are generally less distinct from sterile cells and by contrast lie in an intercalary or terminal position. The resulting chainlike arrangement is similar to that in some nonlichenized Ascomycetes such as *Neurospora sitophila*.

The sporogenous filaments are joined into pycnidia except in some poorly known groups (Ozenda and Clauzade, 1970). Pycnidia are spherical or flask-shaped, opening at the apex with an ostiole. Rarely projecting or stalked (*Cladonia*), they are often included in the thallus. They have a wall, sometimes dark. The sporogenous filaments can fill the entire cavity in a lacunose mass when anastomosed. They very often form a fertile layer, sometimes plicate (*Cladonia*, *Ramalina*, etc.), covering the inner surface of the wall.

According to some authors (Möller, 1888), these microspores are asexual spores capable of germinating, but according to others they behave as spermatia, functional or not, for one can observe them adhering to trichogynes of ascogenous filaments (Stahl, 1877; Baur, 1898; Ahmadjian, 1966; Jahns, 1970). If they are indeed spermatia, the pycnidia from which they emerge must be called spermogonia.

2. THE ASCOGONIAL FILAMENTS (Fig. 3)

The female reproductive organ in the Ascomycetes as a group is made up of differentiated filaments that are always multicellular, ascogonial filaments apparently homologous to the male sporogenous filaments. They are formed mainly of three parts, going from the base to the apex (but not all the parts will always be present): a foot composed of sterile cells; the ascogone, the essential part composed of the fertile cell, sometimes several (= ascogonial cells); and the trichogyne, slender and erect, sterile, and when there is trichogamy attached either with spermatia or a fertilizing cell of parascogonial filaments. The ascogonial filaments do not give rise to free gametes. It is the ascogonial cells which play the role of fertile elements. Each of these can be considered to be a nongametogenous gametocyst (Chadefaud, 1960).

Ascogonial filaments were first observed in lichens by Fuisting (1868) for *Lecidea fumosa* and later by Stahl (1877). Stahl interpreted them as female

elements and carefully described their structure and evolution in various species of *Collema*. The filaments were eventually observed in all species examined, with the exception of *Phlyctis agelaea*.

The ascogonial filaments in lichens are always composed of a large number of cells, contrary to many of the Ascomycetes. This applies especially to the fertile part that presents a clearly filamentous aspect not unlike that of thalline hyphae. For the most part the foot is generally little differentiated.

Moreau and Moreau (1928) distinguished two kinds of ascogonial filaments. The *Collema* type (Fig. 3B), by far the most common, has uninucleate, cylindrical, and isodiametric ascogonial cells; the *Peltigera* type (Fig. 3C), found in *Peltigera* and *Solorina*, has ellipsoidal and multinucleate ascogonial cells. Trichogynes in both types are differentiated slowly and often branched.

The ascogonial filaments may be simple and originate singly. More often they are branched and their parts together make up the ascogonial apparatus. At times one finds an *arbuscular* type where each branch remains free. At other times we have a more or less glomerular type where the branches are intertwined in a more or less dense cluster or glomerule, sometimes giving a paraplectenchymatous aspect (Fig. 3A), from which trichogynes arise. In such a case only the central elements in the glomerule are fertile. Another type of ascogonial apparatus has been found in *Pertusaria pertusa* (Letrouit-Galinou, 1966), *Baeomyces* and *Icmadophila ericetorum* (Nienburg, 1908), and various *Cladoniae* (Krabbe, 1891). This is the spreading type, composed of a branched system of sterile supporting hyphae on which fertile hyphae develop from place to place, and contain ascogonial cells.

Nothing is known about the basis of differentiation of the reproductive apparatus. There are no reports in lichens of either heterothallism, so common in nonlichenized Ascomycetes, or of a dioecious condition.

C. Problem of Fertilization of the Ascogones

There has been heated discussion on the question of whether fertilization of the ascogone is involved in the developmental stages. According to several authors microspores of lichens are spermatia which do in some cases become attached to the trichogyne and fertilize the ascogone. Consequently, spermatial trichogamy would be a basic phenomenon. In support of this thesis one should note that (1) the asexual nature of these microspores is not well established, (2) spermatia have often been found adhering to the apex of trichogynes (Stahl, 1877; Baur, 1898; Ahmadjian, 1966) and they can result in structural modifications of the trichogyne and ascogonial cells, and (3) according to Ahmadjian (1966), the great variability in monospore cultures of *Cladonia cristatella* can be explained only by genetic factors incompatible with apogamy.

The opposite theory, stressed by Moreau and Moreau (1928), holds apogamy to be the rule. This is clearly the case when microspores are lacking; when present it is stated that they are always either asexual spores or non-functional spermatia. One of the more serious arguments for partisans of this theory is that the initial hyphae of the ascosporophyte in a number of lichens (especially many of those cited as examples of trichogamy) are uninucleate, not dikaryotic. This is easily explained by apogamy, and with greater difficulty by trichogamy. In this last instance one must allow for a hypothesis referred to as double fertilization (Gwynne-Vaughan and Williamson, 1931), according to which the fertilized ascogone and the sporophytic apparatus derived from it would have diploid nuclei (conjugated or not) and in no case conjugated haploid nuclei. This hypothesis, however, was rejected some time ago (see Martens, 1946; Chадеfaud, 1960).

Until better informed, we will take the following point of view:

1. Apogamy is probable if primary ascosporophytic hyphae with uninucleate cells are formed in the developmental stages. It is then always accompanied either by a fusion between cells of the ascosporophyte (= perittogamy) or by other phenomena leading to the formation of dikaryotic cells.
2. One could admit fertilization of the ascogone, resulting in the formation of a dikaryon, if the hyphae of the ascosporophyte are dikaryotic from the outset. According to the species, this fertilization would be classified as spermatial trichogamy (basic case); somatogamy, the conjugated nuclei being issued from normal gametophytic cells [for example, *Lecidea elaeochroma* (Letrouit-Galinou, 1966)]; or autogamy, in other words the transformation of nuclei of the ascogone, some into male nuclei, others into female nuclei (that seen perhaps in *Peltigera*).

D. The Sporophytic Apparatus

1. ASCOMYCETES IN GENERAL

The sporophytic apparatus is normally formed by the combination of two phases of the reproductive cycle: that of sporophyte I (the prosporophyte) and that of sporophyte II (the ascosporophyte).

a. SPOROPHYTE I. This phase of the trigenetic cycle, often reduced and appearing absent, is intercalated between the ascogonial cell, fertilized or apogamous, and the dikaryotic ascogenous filaments described by Claussen (1912). Unsuspected for a long time (Martens, 1946), it was first defined and interpreted by Chадеfaud (1953, 1960).

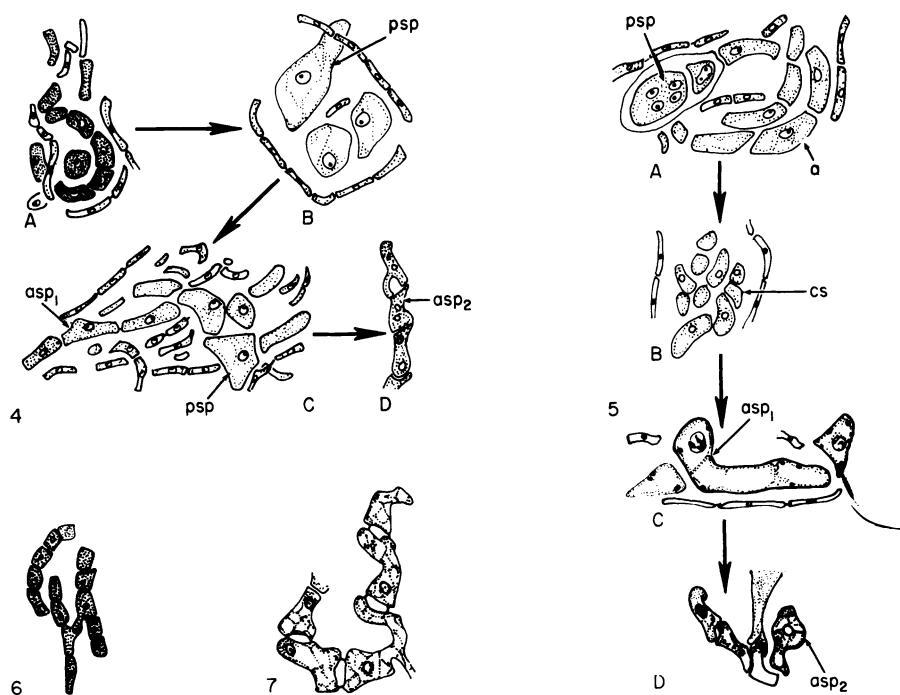
Basically the fertilized ascogonial cell, which takes the place of the zygote, gives rise to some vesicles or tubes (cf. *Pyronema confluens* and *Peltigera*

spp.) that are, as a rule, siphonaceous, multinucleate, and mictohaploid, that is, provided with separate haploid nuclei, some male and some female but still immature and not conjugated. These vesicles and tubes constitute the prosporophyte which develops as a parasite on the gametophyte. Later, in certainly the most primitive case (*Dothidea puccinoides*; Luttrell, 1951b), they are transformed into sporocysts, in the interior of which small spores with dikaryons are differentiated. The two included conjugated nuclei are one male and one female, semimature, so that they are coupled without fusing. These spores (= Chadefaud's carpospores) are not liberated; they give rise to the ascosporophyte at this point.

The prosporophyte is, in fact, never this typical since (1) it is often little developed, sometimes reduced to the ascogonial cells alone; (2) the spores are only very rarely individualized: sometimes their formation is only present in outline; sporulation is often replaced by a simple division into spore cells (*Pertusaria pertusa*). There is also often complete apospory and then the prosporophytic vesicle gives rise directly to sporophyte II. In some of the Discomycetes, especially *Pyronema confluens*, and perhaps also in *Peltigera rufescens*, sporulation is replaced by the emission of dikaryotic carposporous tubes on which the ascosporophyte then arises.

b. SPOROPHYTE II. Sporophyte II or the ascosporophyte develops from dikaryotic spores, the spore cells, or the carpospore tubes produced by sporophyte I, or directly from sporophyte I. As a parasite on the gametophyte it is formed by "ascogenous filaments" with dikaryons, each dikaryon being composed of two coupled haploid nuclei, one male and one female, which divide by conjugated mitoses. These filaments generally consist of hyphae with clamp connections comparable to those of sporophytes in the Basidiomycetes and, like them, composed of successive dangeardia. The type of dangeardium involved here is the well-known croziers. They are made up of a group of cells which arise by division of one cell with a dikaryon. They are most often formed with two dikaryotic cells, but there are other rarer types (Chadefaud, 1943, 1960). Certain of them, situated at tips of hyphae, are ascogenous dangeardia: their dikaryotic cells give rise to an ascus in which ascospores are formed, and these will produce new gametophytes when liberated.

c. THE PERITOGAMOUS SPOROPHYTE (Fig. 6). In certain apogamous species, especially with many lichens, the ascosporophyte is not initially dikaryotic. It consists of primary ascosporophytic filaments with uninucleate, haploid cells, connected to dikaryotic secondary ascosporophytic filaments. Each secondary filament begins either with one cell of a primary filament becoming binucleate by a mitosis, not followed by septation, or by two primary filaments, fusing without karyogamy. Such a fusion has been



Figs. 4-7. Sporophytic apparatus of ascolichens. Fig. 4, evolution of the sporophytic apparatus in *Buellia canescens*. A, ascogonial apparatus; B, vesiculate, uninucleate prosporophytic cells; C, primary part formed of uninucleate cells of the peritrogamous ascosporophyte; D, secondary dikaryotic part of the ascosporophyte. Fig. 5, evolution of the sporophytic apparatus in *Pertusaria pertusa*. A, mictohaploid prosporophytic apparatus; B, mass of uninucleate cells coming after the prosporophyte (carposporal cells?); C, uninucleate cells of the primary part of the ascosporophyte; D, dikaryotic, ascogenous secondary part of the ascosporophyte. Fig. 6, peritrogamy in *Collema pulposum* (after Moreau and Moreau, 1928). Fig. 7, *Lecanora subfuscata*: matured part of the ascosporophyte with secondarily uninucleate cells. (a, ascogonial filament; asp₁, primary part of the ascosporophyte; asp₂, secondary part of the ascosporophyte; cs, carposporal cells; psp, prosporophyte.)

described by Killian (1938) in the pyrenomyctete *Lasiobotrys lonicerae* under the term peritrogamy. According to drawings by Moreau and Moreau (1928), it also occurs in the lichen *Collema pulposum* and, according to Erbisch (1969), in *Pertusaria pertusa*.

2. THE SPOROPHYTIC APPARATUS IN LICHENS

The development of this apparatus is known in only about 15 discolichens. Sporophyte I appears to be more or less reduced to the ascogonial cells alone

except in *Peltigera* where it gives rise apparently to carposporic tubes. Formation of the sporophytic apparatus at the expense of the carpospore cells has been observed in *Pertusaria pertusa*, and this phenomenon may also occur in *Lobaria laetevirens* (Letrouit-Galinou, 1966, 1971). The ascosporophyte in all other cases arises directly from prosporophytic vesicles.

Sporophyte II begins as a primary part of uninucleate cells in almost all species that have been studied. To this a dikaryotic secondary part is next added by perittogamy or otherwise. This secondary part with a few exceptions (perhaps certain Baeomycetaceae and Peltigeraceae) is formed of hyphae with clamp connections or with lateral hooks (Moreau and Moreau, 1925) and is then "dangeardian."

This sequence can be illustrated with the five examples that follow. The ascosporophyte in the first three examples comprises a primary part with uninucleate cells.

a. *Collema* sp. (Baur, 1898; Moreau and Moreau, 1928). The ascogonial apparatus is an "archicarp" coiled in a helix and terminated by a long multicellular trichogyne, the tip of which projects above the thallus. The ascogonial cells in this archicarp enlarge and become vesiculate and multinucleate and so are transformed in prosporophytic vesicles. The vesicles give rise to branched hyphae which form the primary part of the ascosporophyte and are made of uninucleate cells. One would be led to believe that there is apogamy in accordance with the views of Moreau and Moreau. Next, hyphae of the secondary part are developed; these are dikaryotic and provided with lateral clamps and give rise to asci at their tips. The first dikaryotic cells can originate from the fusion of two adjacent uninucleate cells, and this would then be considered perittogamy (Fig. 6).

b. *Pertusaria pertusa* (Baur, 1901; Letrouit-Galinou, 1966; Erbisch, 1969) (Fig. 5). The ascogonial apparatus is complex and branched with ascogonial filaments of the *Collema* type (Fig. 5A). Certain cells evolve into multinucleate prosporophytic vesicles which later become masses of uninucleate carposporal cells (Fig. 5B). From these cells come primary ascosporophytic hyphae with uninucleate cells (Fig. 5C). Next, through perittogamy, dikaryotic cells are formed (Fig. 5D) which give rise to secondary ascosporophytic filaments with lateral clamps, producing asci. According to Erbisch the development varies from one species to the next in *Pertusaria*.

c. *Buellia canescens* (Letrouit-Galinou, 1966) (Fig. 4). The ascogonial apparatus is formed of isolated filaments of the *Collema* type (Fig. 4A). Certain of these elements consists of ascogonial cells which enlarge and are transformed into apparently prosporophytic vesicles; these nevertheless

remain uninucleate (Fig. 4B), contrary to the situation in the preceding case and reminiscent of apogamy. The resulting ascosporophyte is formed of a primary part with uninucleate cells (Fig. 4C) and a secondary ascogenous dikaryotic part with lateral clamps (Fig. 4D).

d. *Lecidea elaeochroma* (Letrouit-Galinou, 1966). The ascogonial apparatus with a glomerular aspect consists of ascogonial filaments of the *Collema* type in which the ascogonial cells become binucleate, perhaps as a consequence of somatogamy. They enlarge and become transformed into a vesicular prosporophytic apparatus, probably siphonaceous and multinucleate. From this apparatus come the ascosporophytic hyphae which are at first dikaryotic and provided with lateral clamps. The asci form slowly on the hyphae.

e. *Peltigera* (Moreau and Moreau, 1928; Letrouit-Galinou and Lallement, 1971). The ascogonial cells are initially multinucleate. They give rise immediately, or after transformation into prosporophytic vesicles, to long siphonaceous tubes which are apparently carposporous tubes. The hyphae of the ascosporophyte, dikaryotic ascogenous hyphae with lateral clamps, arise from these tubes either directly or after division into dikaryotic cells.

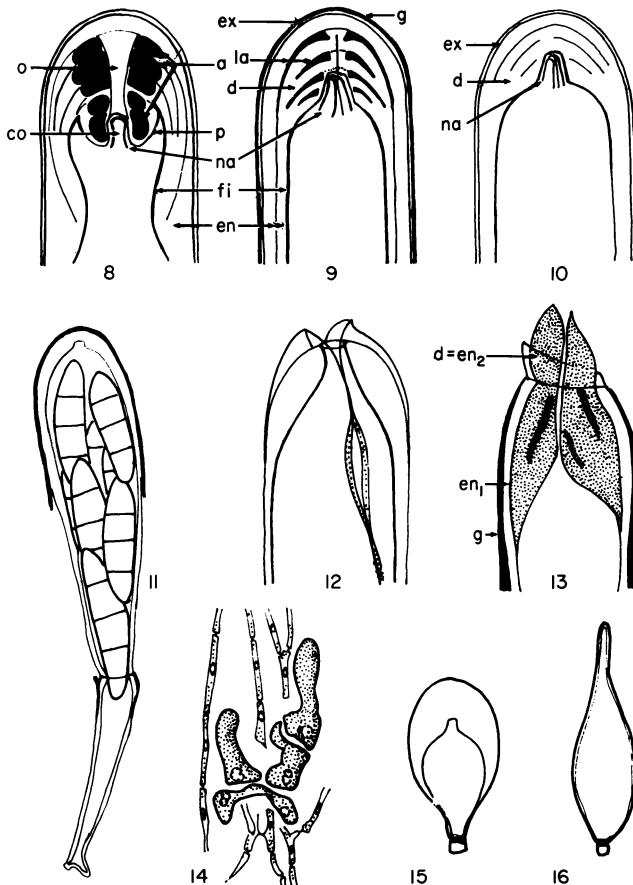
The oldest ascosporophytic cells in *Buellia canescens*, *Lecidea elaeochroma*, and *Lecanora subfuscata* seem to become secondarily uninucleate (Fig. 7) but the reason for this phenomenon is unknown.

E. The Ascus

Asci in lichens are always derived from dikaryotic proascal cells so that karyogamy takes place at the beginning of their development and the same if the ascogonial apparatus is apogamic. The ascus originates most often from a fertile cell of a dangardium with formation of a lateral clamp or a hook; in that case, one can see at the base of the ascus traces of the two septa which separated it from the basal cell of this dangardium and from its clamp or hook. A single ascus can be formed initially from an ascogenous dangardium, but many are often formed because the clamp of the dangardium produces a secondary ascogenous dangardium of which the clamp in turn produces a third, etc.

The three elements of the ascogenous dangardium in *Icmadophila ericetorum* (Chadefaud, 1960)—the basal cell, fertile cell, and hook—are oriented in a nearly straight line; the ascus rises laterally on the fertile cell in this case. At other times, as in *Pertusaria pertusa* according to Erbisch, there is no ascogenous dangardium. The ascus comes directly from a dikaryotic cell without producing such a dangardium at first.

Asci are generally claviform, a character that could be interpreted as primitive according to Chadefaud (1960), but they may also have more characteristic shapes in certain families: subglobose or pear-shaped in the Arthoniaceae and Cryptotheciaceae (Fig. 15), flask-shaped in the Thelocarpaceae (Fig. 16), or cylindrical in the Caliciaceae.



FIGS. 8–16. Asci of lichens. Fig. 8, basic scheme of the apical apparatus of the ascus (adapted from Parguey-Leduc and Chadefaud, 1963); Fig. 9, the archaeasceous type; Fig. 10, the nassaceous type; Fig. 11, jack-in-the-box-type dehiscence in *Roccella montagnei*; Fig. 12, bivalve dehiscence in *Pertusaria pertusa*; Fig. 13, rostrate dehiscence of *Parmelia caperata*; Fig. 14, young asci at the extremity of ascosporophytic hyphae; Fig. 15, ascus of *Arthonia*; Fig. 16, flask-shaped ascus of *Thelocarpon*. (a, amyloid ring; co, ocular chamber; d, apical dome; en, endoascus; en₁, external layer of the endoascus; en₂, internal layer of the endoascus; ex, exoascus; fi, internal film; g, amyloid gelatin; la, annular amyloid lamellae; na, apical nasse; o, oculus of the dome obscured here by the apical cushion and its manubrium; p, pendant of the dome.)

1. THE ASCAL WALL, APICAL APPARATUS, AND METHOD OF DEHISCENCE

According to observations with the light microscope, which are almost the only ones available, juvenile asci have a thin wall which is covered in discolichens with an I+ blue gelatin (= amyloid gelatin) at a very early stage. Later on, this wall thickens so that the asci in lichens look more like those of bitunicate nonlichenized Ascomycetes than those of unitunicate nonlichenized Ascomycetes.

The ascal wall in lichens, fundamentally conforming to the general type (Chadefaud, 1942, 1960, 1969), consists of two layers, both complex: (1) an external firm layer, the *exoascus*, often covered with the gelatin mentioned above, and (2) an internal layer, the *endoascus*, sometimes thick and basically double. An internal membrane can be added to these layers, in connection with the plasmalemma of the ascus.

The structure or staining qualities of these layers are often modified at the apex of the ascus. Various formations that are differentiated make up the *apical apparatus* of the asci as studied by Chadefaud (1942, 1960, 1969, Fig. 8). This apparatus can include the following three parts:

1. An apical calotte derived from the exoascus.

2. An apical dome, an essential part of the apical apparatus. Laminate, it arises from the endoascus, particularly from the inner layer. Its sides extend far down toward the base of the ascus. The summit is thickened, and its axis is often hollow with one ocular chamber open at the base or with the oculus open at both ends. If there is one oculus, it is generally plugged apically by an apical cushion elongated into the oculus by a manubrium. The dome can produce around the lower orifice of the ocular chamber or oculus a tubular pendant projecting toward the base in the ascal cavity. Various parts of a more or less complex apical ring are differentiated in the dome and in its pendant. These are always amyloid in lichens.

3. An apical nasse (trap), characteristic of the nassaceous type. This is composed of straight or coiled longitudinal rods lodged in the ocular chamber, lying against the walls of this chamber and joined at the summit. These rods seem to be in connection with the epiplasm.

All lichens are inoperculate as far as the type of dehiscence is concerned (Ziegenspeck, 1926; Richardson and Morgan-Jones, 1964; Letrouit-Galinou, 1966; Richardson, 1970), with the following main variations:

1. Bitunicate jack-in-the-box dehiscence (Fig. 11) (pyrenolichens, some discolichens): the exoascus ruptures at the tip or in a circle at a distance from the tip; the endoascus is ejected and elongates carrying along the ascal contents.

2. Bivalvate dehiscence (Fig. 12) (e.g., *Pertusaria* and *Baeomyces*; *Calopla-*

caceae, Gyalectaceae): the exoascus splits longitudinally into two valves. The endoascus does not come out.

3. Rostrate dehiscence (Fig. 13) (most discolichens): the exoascus and the external layer of the endoascus split only at the tip; the apical dome bursts out, sometimes with eversion.

4. Poricidal dehiscence: dehiscence by a more or less well-defined apical pore (*Graphis elegans*; Richardson, 1970).

5. Rupture, disarticulation, or gelification of the ascus wall (Caliciaceae). Some asci seem to be indehiscent (e.g., *Phlyctis*).

Particulars of the asci, their walls, apical apparatus, and manner of dehiscence may be defined under several broad ascus types (Chadefaud, 1942, 1969; Luttrell, 1951a). Most lichens fall under two types, and these may in fact be connected: the nassaceous bitunicate and the archaeasaceous types:

1. The bitunicate nassaceous type (Fig. 10) is found in nearly all pyrenolichens and in certain groups of discolichens (e.g., *Arthonia*, *Opegrapha*, *Chiodescon*, etc.). It is the type known in the dothideaceous pyrenomycetes and some nonlichenized Discomycetes, in particular *Patellaria atrata* which is classified in the Lecanorales. The exoascus is thin and the endoascus thick. The apical dome is nonamyloid and hollowed with an ocular chamber containing a more or less distinct apical nasse with dehiscence of the jack-in-the-box type.

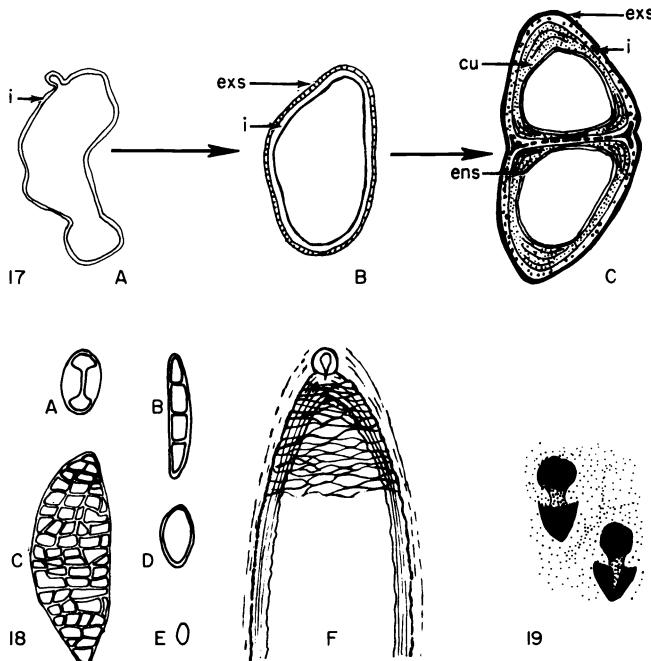
2. The archaeasaceous type (Fig. 9) is characteristic of lichens in the order Lecanorales, apart from some of the Patellariaceae. The exoascus is covered with a very characteristic amyloid gelatin; the endoascus, also amyloid, is made up of two layers. The apical dome arises from the innermost layer which may even be reduced to this dome, a thick, laminate, and usually amyloid structure. It is often hollow with an ocular chamber. This chamber can include an apical nasse which leads to the nassaceous type. It can be surrounded by an amyloid ring differentiated in the dome which also leads into the nassaceous type. Dehiscence is rostrate or bivalve. Several variants are distinguishable by the fine structure of the dome (Chadefaud *et al.*, 1963; Letrouit-Galinou, 1973).

2. CYTOLOGY OF THE ASCUS: SPOROGENESIS AND ASCOSPORES

We know very little of the cytoplasmic features of the ascus and its inclusions, nature of reserves (sometimes lipids, other times glycogens), or evolution. Some authors (Maire, 1905; Moreau and Moreau, 1919; Stevens, 1941; Letrouit-Galinou, 1966) have observed various configurations in nuclear division, confirming in the species investigated that the first mitosis is indeed reductional.

Some aspects of spore delimitation in *Roccella montagnei* resemble stages

described by Harper (Fig. 19), but one should realize that these stages are not recognized today, and it appears rather certain that they would not be found in all species. In *Physcia aipolia* (Fig. 17) (Rudolph and Giesy, 1966) with brown bisepitate spores, the mechanism of ascospore formation is in effect one described by Carroll (1969) for *Ascodesmis* and *Ascobolus*. A very thin, flexible double membrane, related to the endoplasmic reticulum, completely surrounds the sporoplasma with the eight spore nuclei at first. The sac subsequently formed by invagination subdivides into eight sections, each containing a nucleus and forming the primordial envelope of each spore. The primary membrane of the spore (Fig. 17A) is first laid down between the two layers of this envelope, next becoming the epispose. Later the secondary membrane with radial structure is laid down between this and the external layer and becomes the exospore (Fig. 17B). The intercellular septum appears as an annular fold of the primary membrane which extends



Figs. 17–19. Ascospores. Fig. 17, differentiation of the spore wall in *Physcia aipolia* (based on photographs of Rudolph and Giesy, 1966). A, deposition of the primary membrane (i) (= epispose); B, formation of the secondary membrane (exs) (= exospore); C, differentiated spore (cu, epilocular cupules; ens, endospore; exs, exospore; i, epispose). Fig. 18, examples of ascospores. A, *Caloplaca cerina*; B, *Roccella montagnei*; C, *Phlyctis agelaea*; D, *Lecanora subfuscata*; E, *Acarospora*; F, *Pertusaria pertusa* (half-spore). Fig. 19, beginning of spore differentiation in *Roccella montagnei*.

centripetally. This septum contains a very thin interlocular lamina (Fig. 17C). Moreover, a thin endospore forms around the cytoplasm of each cell, and a lamellar deposit around this endospore thickens the episporule considerably. The endospore of each cell seems to be what Chadefaud (1960) calls a locula and the layers contributing to a thickening of the episporule might then be the epilocular cupules.

There are generally eight spores per ascus, but there may be more (several hundred in the Acarosporales) or less, either because some of the nuclei abort or because the spore membrane includes two or more nuclei per spore.

Spores (Fig. 18) are sometimes unicellular (and these uni-, bi-, or multi-nucleate), uniseptate, or multiseptate and muriform or not. Size varies in length from a few microns (Acarosporales) to several hundred microns (some *Pertusaria* and *Varicellaria* species).

F. Morphology and Anatomy of the Ascocarp

The ascosporophyte and ascii of lichens develop in the interior of the specific gametophyte formations, the ascocarps, comparable to those of nonlichenized Ascomycetes, with the exception of those in the *Cryptotheciaceae* which seem directly immersed in the thallus. We will examine in turn the morphology, anatomy, and ontogeny of lichen ascocarps.

1. MORPHOLOGY OF THE ASCOCARP

Two broad types of ascocarps, the perithecioid and apothecium, are traditionally recognized in lichens, just as in the nonlichenized Ascomycetes.

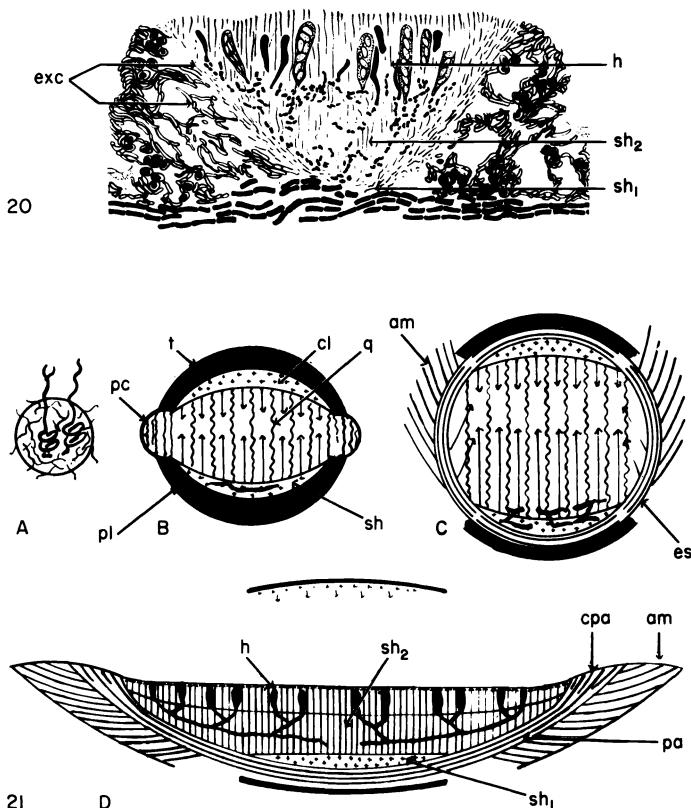
The ascii of perithecia are immersed in a perithecial cavity which is bounded by an excipulum distinct from the thallus. This cavity opens at the top through a narrow pore, which is sometimes located at the end of a more or less elongated neck.

With apothecia the ascii are by contrast directly exposed to the exterior. Apothecia are as a rule round but they can also be lirelline, that is, elongated and sometimes branched. They may or may not have an excipulum.

2. ANATOMY OF THE ASCOCARP

The ascocarps of lichens, as in other ascomycetes, are made up of three parts: the hymenium, the subhymenium, and the excipulum (Fig. 20):

a. The hymenium consists of the ascii, among which are very often intermingled sterile interascal filaments, uni- or multicellular, simple or branched, and free or anastomosed. There are three kinds of filaments depending on their origin: (1) true paraphyses, free filaments produced from the sub-



FIGS. 20 AND 21. Basic structure of ascocarps. Fig. 20, basic structure of a mature ascocarp. (in this case *Lecanora subfuscata*). Fig. 21, basic developmental scheme of ascocarps of ascolichens. A, the primordium; B, primary corpus; C, formation of a secondary parathecial envelope; D, mature ascocarp provided with a typical parathecial apparatus. (am, amphitheium; cl, epiphytinal cupula which gives rise to pseudoparaphyses; cpa, parathecial crown; es, secondary parathecial envelope; exc, often double; h, hymenium; pa, paraphysis; pc, circumcentral plexus; pl, pericentral base; q, paraphysoid apparatus; sh, subhymenial meniscus; sh₁, primary part of the subhymenium; sh₂, secondary part of the subhymenium; t, pericentral segment.)

hymenium and extending upward in the same direction as the asci; (2) pseudoparaphyses, free or anastomosed filaments produced from the epiphytinal cupola and growing out in directions opposite to the asci. They are sometimes attached to the subhymenium and break off apically, a condition that is capable of mimicking true paraphyses; and (3) paraphysoids, attached from the start at both ends and formed by stretching and intercalary elongation of carpocentral elements to be described below.

A pulverulent mazaedium containing spores is formed in the Caliciales by transformation of the hymenium after breakdown of the ascal walls and fragmentation of the paraphyses.

b. The subhymenium contains the sporophytic apparatus. In addition it consists of sterile filaments, the primary ones being part of the carpocenter (see below), the secondary ones consisting of the bases of the interascal filaments.

c. The excipie, sometimes lacking (Arthoniaceae, Phlyctidiaceae), consists solely of sterile hyphae. It more or less completely surrounds the hymenium and subhymenium. It is called lecideine when no algae are present and is clearly distinguished from the thallus by texture and color. Lecanorine is in the alternative case where algae are incorporated in the excipie. As a matter of fact, the nature of the excipie varies considerably from case to case depending on its ontogeny.

G. Basic Sterile Structures of the Ascocarp

Development of ascocarps is a complex phenomenon which involves many structures of which some are transitory, ones being primary, the others secondary (Letrouit-Galinou, 1968). There are many types and variations which will be discussed below.

1. PRIMARY STRUCTURES

These consist of the primordium and the primary corpus derived from it.

a. The primordium (Fig. 21A) (= *generative Gewebe* of Henssen, 1968) is a small gametophytic mass of homogeneous texture, containing the ascogonial apparatus. The constituent hyphae arise at the foot of the ascogone in the Collemataceae (Stahl, 1877; Henssen, 1965). It is derived directly from the thalline hyphae in all other cases, often preserving their structure (e.g., *Nephroma*, *Peltigera*) or are ramifications of them.

As a rule the primordium is formed before ascogonial filaments, more rarely later (e.g., *Collema*, *Buellia*, *Physcia*). It is paraplectenchymatous in some Arthopyreniae (Janex-Favre, 1970), as the structure called an ascostroma in some of the nonlichenized ascomycetes. It is arbuscular in some cases.

b. The primary corpus (Fig. 21B) is derived directly from the primordium, from which are differentiated a central mass or carpocenter (containing the ascogonial apparatus, then the sporophytic apparatus produced by it) and a pericentral envelope. This includes, generally speaking, a tectal part or tegment, a basal part or floor. It may be lacking when the primordium is totally transformed into a carpocenter (e.g., *Lecanora*).

From the carpocenter are formed (1) the subhymenial apparatus which

contains the sporophytic apparatus and is sometimes paraphysogenous and gives rise to true paraphyses; (2) an epiphytial apparatus often in the form of a bell; it can give rise to descending filaments which remain short or develop into definite pseudoparaphyses; and (3) a paraphysoid apparatus composed of paraphysoidal filaments which are often anastomosed and joined with the other two apparatus described above and which may or may not persist in the form of paraphysoidal interascal filaments.

The primary corpus can enlarge by adding on elements of the same nature at its periphery, with this aggregate making up the *peribase*. It is formed at the expense of the circumcentral plexus, lateral and annular, probably in nature similar to that of the primordium.

The primary corpus in *Lobaria laetevirens* is probably differentiated at the expense of the thallus.

2. SECONDARY STRUCTURES

In contrast to the primary structures these are not derived directly from the primordium. They are added secondarily to the primary elements. Some of these are preparathelial *sensu lato* (Bellemère, 1967); others comprise the parathelial apparatus *sensu stricto*.

a. Secondary preparathelial structures (Fig. 21C), still poorly known, develop after the differentiation of the carpocenter and before that of the parathelial apparatus when this is present. They could be derived from the carpocenter but possibly also from the thallus. These structures are complete if they entirely surround the carpocenter and incomplete otherwise. They are usually formed of erect, parallel, or contiguous filaments which gives them a parathecoid appearance. They may be provided with amphithecid or amphithecial filaments on the exterior face and with secondary paraphyses on the inner face. The tip may be provided with a parathelial ring composed of divaricate filaments.

In this way the following are (or could be) secondary formations: the proparathecium of *Buellia canescens*; the lateral wall and inner lining of the lirellae in the Graphidales; the periphyses-invested wall of the Thelotrema-taceae; the secondary envelope (and perhaps the carpocentral envelope as well) of certain pyrenolichens; the perihymenial envelope of *Pertusaria*; that in *Lichina*; and, finally, the parathecoid envelope of *Peltigera* and *Lobaria*. These formations are of special interest because their structure is intermediate between that of the pericentral envelope, to which it is added, and that of the parathelial envelope which follows.

b. The parathelial apparatus (Figs. 21D and 32D) was first carefully described by Corner (1929–1930) and Dughi (1952, 1954). It comprises (1) a parathecium, often cup-shaped, flaring, and composed of filaments which,

oriented directly obliquely toward the top and exterior, are elongated and branched; (2) an amphithecum, lecideine or lecanorine, formed of hyphae that arise at the outer face of the parathecium and are oriented toward the exterior; this makes up part of the excipulum; (3) a system of secondary paraphyses which, by contrast, arise at the inner face of the parathecium and encircle the primary part of the hymenium; these remain extrahymenial in some of the ascohymenial nonlichenized Pyrenomycetes; (4) a more or less distinct parathelial ring at the summit of the parathecium composed of short filaments intercalated between those of the amphithecum and the secondary paraphyses.

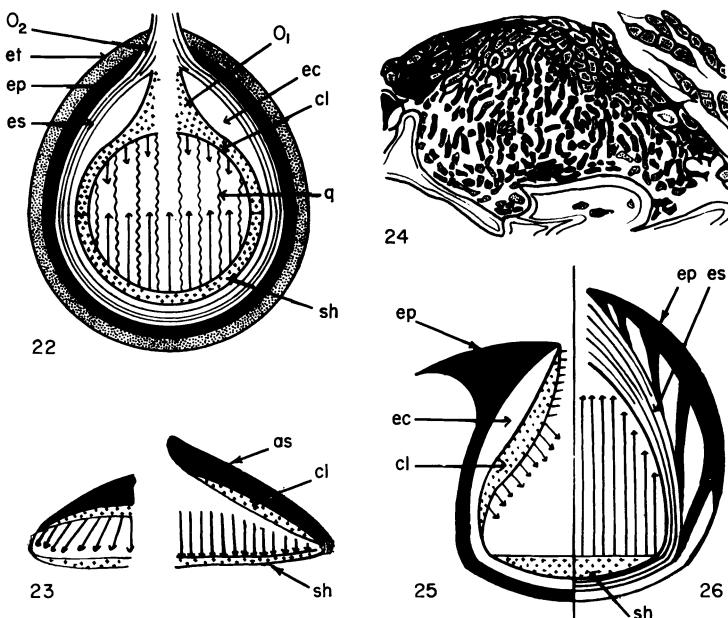
The parathelial ring forms first. It elongates and branches, its hyphae yielding the parathecium, the amphithecum, and the secondary paraphyses. The hyphae reform at the same time, in proportion to structures derived.

H. Basic Types of Perithecia and Apothecia

The basic types are differentiated by the arrangement and degree of development of the ontogenetic structures which were described above. Unfortunately, we still know the sequence of ascocarp development for only a small number of species and actually, from this limited information, can gain no more than a partial insight into the problem.

1. PERITHECIAL TYPES IN THE PYRENOLICHENS (FIGS. 22-26)

Doppelbaur (1959) and Janex-Favre (1970) have studied perithecial development in various species in the Arthopyreniaceae, Pyrenulaceae, and Verrucariaceae. Janex-Favre has concluded the following in these lichens (Fig. 22): (a) the primordium is normally plexiform, rarely paraplectenchymatous; (b) the evolution of the carpocenter conforms to a type which has been called advanced; when the epihymenial apparatus and the subhymenium coexist, they constitute a perilocular lining homologous to that in some nonlichenized ascolocular pyrenomycetes (Wehmeyer, 1955; Moreau and Moreau, 1956; Chadefaud, 1960; Parguey-Leduc, 1966); (c) four envelopes of distinct origin and structure may contribute to the formation of the excipulum, the *thalline* envelope derived from the thallus, the *primary* perithecial envelope derived from the primordium at the same time as the carpocenter, the *secondary* envelope, developed after differentiation of the carpocenter and comparable to a parathecoid envelope since it is formed of contiguous hyphae parallel to the meridian of the peritheciun and is sometimes provided with an apical ring, and a *carpocentral* envelope derived from the carpocenter as the primary envelope but very slowly and at the outside of the perilocular lining; and (d) an ostiolar apparatus at the top of the peritheciun in various species may include an inner formation coming



Figs. 22-26. Types of perithecia in ascolichens (after Janex-Favre, 1970). Fig. 22, scheme; Fig. 23, ascostromatic type with pseudoparaphyses: two developmental stages; Fig. 24, *Arthopyrenia submicans*: ascostromatic type perithecium in stage A of development (the subhymenial meniscus is lacking in this species); Fig. 25, ascocolular type with noninterascal pseudoparaphyses of the Verrucariaceae; Fig. 26, ascohyphomeric type with true paraphyses and secondary envelope. (as, ascostroma; cl, epihyphomeric cupola; ec, carpocentral envelope; ep, primary envelope; es, secondary envelope; et, thalline envelope; O₁, inner structure of the ostiolar apparatus; O₂, intermediate structure of the ostiolar apparatus; q, paraphysoid apparatus; sh, subhymenial meniscus.)

from the carpocenter, an intermediate formation derived from the inner layers of the wall, and an outer formation related to the outer layers of it.

Janex-Favre (1970), then, distinguishes three perithecial types among the species she examined:

1. An ascocolular type with a paraplectenchymatous primordium and interascal pseudoparaphyses, comparable to that in the dothideal ascocolular pyrenomycetes (*Arthopyrenia fallax*, *A. submicans*) (Figs. 23 and 24). This is the primordium called a stromatic sphere or pyrenosphere by Chadeaud (1960). There is no paraphysoid apparatus. The carpocenter may be reduced to the epihyphomeric apparatus, producing long hymenial pseudoparaphyses. The perithecial wall derived from the primordium is paraplectenchymatous and lacks an ostiolar apparatus.

2. An ascocolular type with a plexiform primordium and short extra-hymenial pseudoparaphyses (several species in the Verrucariaceae *sensu*

stricto) (Fig. 25). The perilocular lining is complete; the epihydrial apparatus gives rise to short pseudoparaphyses which resemble periphyses. The paraphysoidal apparatus is generally lacking, and the subhydrial apparatus is only very rarely paraphysogenous. The perithecial wall includes an inner, generally carpocentral envelope and an outer either pericentral or thalline envelope. An ostiolar apparatus is present but usually reduced to its inner formation.

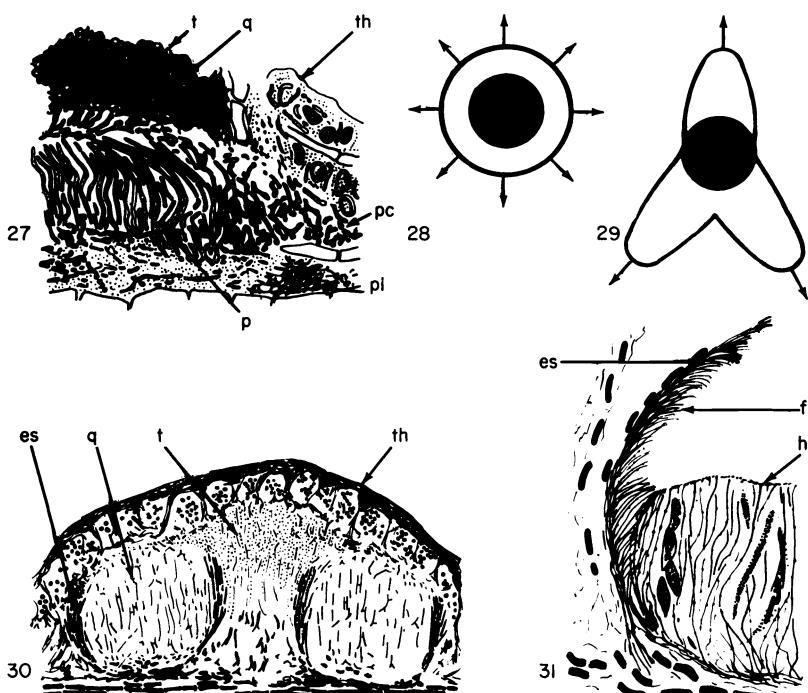
3. An ascohydrial type with true paraphyses and a secondary envelope present (*Dermatocarpon miniatum*, *Pyrenula nitida*, *Porina* sp., *Arthopyrenia sublittoralis*, *A. conoidea*) (Fig. 26). There is no epihydrial apparatus and the carpocenter is reduced to a subhydrial apparatus that is always paraphysogenous and to which a transitory paraphysoidal apparatus may or may not be added. The perithecial wall always includes a secondary envelope. Derived from this envelope, the ostiolar apparatus is reduced to its intermediate formation. A number of variants have been discovered.

2. BASIC APOTHECIAL TYPES IN DISCOLICHENS

These include the graphidian type, the prelecanorian types and the lecanorian type.

a. THE GRAPHIDIAN TYPE. (Figs. 27–29). The graphidian type is characteristic of the Graphidales, especially *Arthonia* (Zogg, 1944), *Opegrapha* (Letrouit-Galinou 1966), *Graphis* (Janex-Favre, 1964), *Roccella* (Letrouit-Galinou, 1966), as well as *Phlyctis* (Letrouit-Galinou, 1966). The mature round or lirelliform ascocarp is derived directly from the primary corpus which enlarges by adding on a peribase. A parathelial apparatus is lacking. The primordium is plexiform and the carpocenter composed of a frequently paraphysogenous subhydrial apparatus, persistent or not persistent. Consequently, the interascal filaments could be true paraphyses, paraphysoids, or a combination of the two. The pericentral envelope is sometimes well developed and carbonized (Graphidaceae), incomplete or absent at other times (Arthoniaceae). The primary corpus and afterwards the mature ascocarp grows at the expense of the circumcentral plexus, which is continuous and circular in the case of round apothecia (*Phlyctis*, *Roccella*), discontinuous and localized at some points in the periphery in lirelline species. In the latter case growth is localized at points where the plexus is present. Thus, the apothecium will elongate later if there are just one or two growth points but branch if there are more than two. Between these points a lateral wall is formed, homologous perhaps to a secondary preparathelial formation.

b. THE PRELECANORIAN TYPES (Figs. 30 and 31). We group here, perhaps artificially, the types known for *Lichina* (Henssen, 1963; Janex-Favre, 1967),



FIGS. 27–31. Graphidian and prelecanorian types. Fig. 27, extremity of a lirella of *Graphis scripta* (graphidian type) (after Janex-Favre, 1964); Fig. 28, growth of a circular graphidian ascocarp; Fig. 29, growth of a lirella; Fig. 30, young secondary ascocarps of *Pertusaria pertusa* (prelecanorian type); Fig. 31, detail of the secondary envelope of a mature ascocarp of *Thelotrema lepadinum* (prelecanorian type). (es, secondary envelope; f, periphysoid filaments of the secondary envelope; h, hymenium; p, paraphyses; pc, circumcentral plexus; pl, pericentral base; q, paraphysoid apparatus; t, pericentral tegment; th, thallus.)

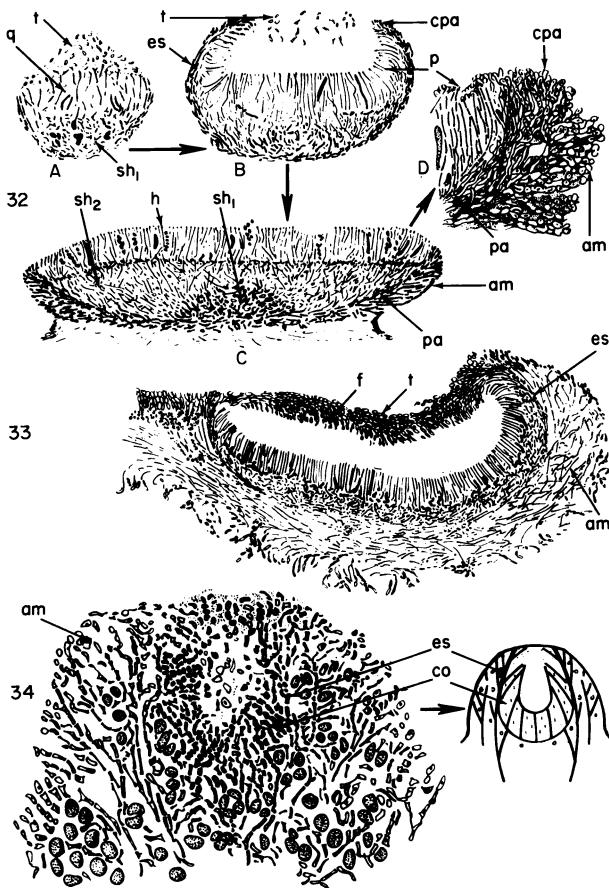
Pertusaria, and *Thelotrema* (Letrouit-Galinou, 1966). These types are difficult to interpret. All three cited have in common a secondary preparathecial envelope but lack a parathelial apparatus *sensu stricto*. Furthermore the primary corpus is reduced because the circumcentral plexus is lacking and the apothecia are more or less perithecioid.

The primary corpus in *Lichina* is reduced to the carpocenter, consisting of the subhymenial base and a persistent paraphysoidal apparatus. The carpocenter is later surrounded by a secondary envelope.

Secondary carpocenters are formed in *Pertusaria pertusa* (Fig. 30) in the abortive primary corpus. These are transformed into mature perithecioid, coalescent ascocarps, each composed of a nonparaphysogenous subhymenial base with a persistent paraphysoid apparatus and of a perihymenial envelope provided on the outer face with amphithecioid filaments, giving a thalline aspect.

In *Thelotrema lepadinum* (Fig. 31) the mature ascocarp is made up of a paraphysogenous subhymenial base to which is added a lateral wall provided with extrahymenial filaments (= periphyses), reminiscent of the nonlichenized discomycetes in the order Ostropales (Bellemère, 1967).

c. THE LECANORIAN TYPE (Figs. 32–34). This is the commonest type, characterized by the formation of a parathelial apparatus in the strict



Figs. 32–34. Lecanorian type. Fig. 32, development of ascocarps in *Buellia canescens*. A, differentiation of the primary corona; B, placement of the secondary envelope and the parathelial corona; C, mature ascocarp; D, detail of the margin of the parathelial apparatus. Fig. 33, immature ascocarp of *Peltigera rufescens*, further provided with an epicentral velum (t). Fig. 34, *Parmelia caperata*: very young ascocarp at the time of differentiation of the paraplectenchymatous husk (right, explanatory diagram). (am, amphitheciun; cpa, parathelial corona; co, paraplectenchymatous husk; es, secondary parathecoid envelope; f, descending filaments; h, hymenium; p, paraphyses; pa, parathecium; q, paraphysoid apparatus; sh₁, carpocentral subhymenium; sh₂, secondary subhymenium; t, pericentral tegment.)

sense and true paraphyses as interascal filaments. Different variants are exemplified by the following three species:

1. *Buellia canescens* (Fig. 32): The primary corpus (A) is derived from a plexiform primordium. It consists of a pericentral envelope, a paraphysogenous subhymenial apparatus, and a transitory paraphysoid apparatus. Next, a proparathecium (B), probably homologous to a preparathecial formation, is added to the primary corpus. It has an apical parathecial ring from which is derived the parathecial apparatus *sensu stricto*. The much reduced primary corpus in *Lecanora subfuscata* and *Lecidea elaeochroma* produces the parathecial apparatus directly.

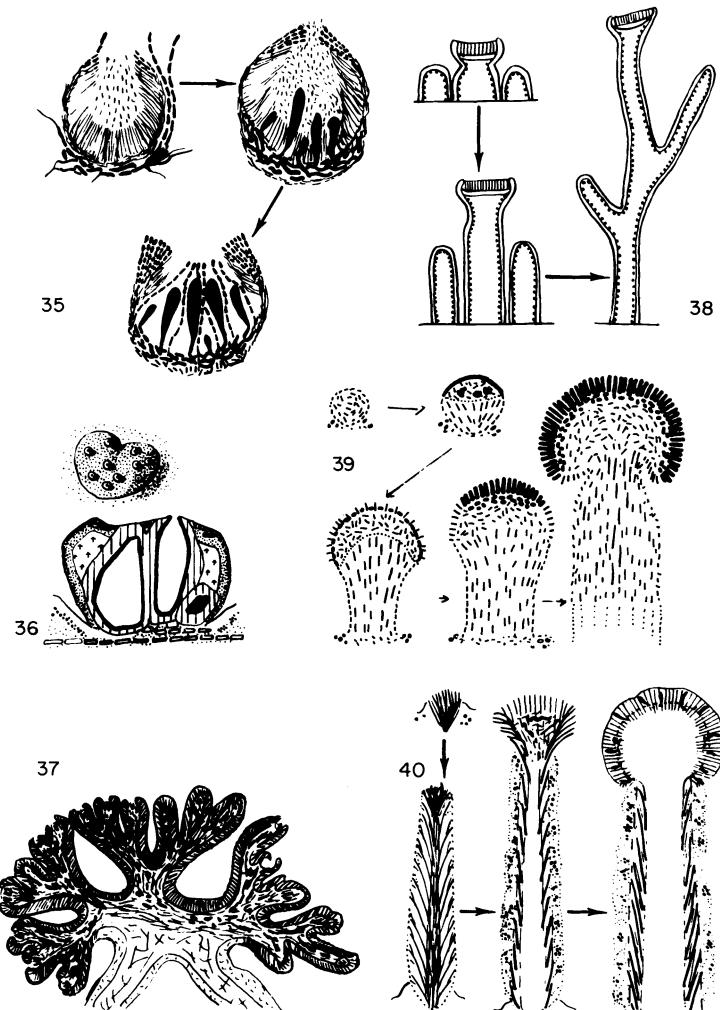
2. *Peltigera rufescens* (Letrouit-Galinou and Lallement, 1971) (Fig. 33): The primordium consists of a palisade of branched erect thalline filaments. It gives rise to a primary corpus reduced to the carpocenter, including a paraphysogenous subhymenial base and an epicentral palisade, itself divided into a paraphysoid apparatus and an epihymenial apparatus. This has descending filaments. Next, a secondary preparathecial envelope of remarkable structure is formed consisting of erect filaments much as a real parathecial apparatus, and with lateral branches. Filaments oriented toward the interior, however, are not transformed into paraphyses; instead they add to different parts of the primary corpus, much as circumcentral hyphae would do. A parathecial apparatus *sensu stricto* is derived next from this envelope. The primary corpus and the parathecioid envelope are considerably reduced in *Nephroma resupinatum* (Letrouit-Galinou and Lallement, 1970). That in *Lobaria laetevirens* (Letrouit-Galinou, 1971) is formed directly at the expense of the thallus.

3. *Parmelia* (Fig. 34; Baur, 1904; Moreau and Moreau, 1925; Letrouit-Galinou, 1970) as well as *Usnea* (Nienburg, 1908). The development in these genera obviously differs from the above types. In effect, an envelope is organized around the primordium, at least partly at the expense of the thalline hyphae. This envelope is at the same time comparable to the parathecioid envelope of *Lobaria laetevirens* and to a true parathecial apparatus but whose internal ramifications are not transformed into paraphyses. The ramifications form a paraplectenchymatous husk (proper rim of the ascocarp) and a paraphysogenous subhymenial plexus. The outer ramifications are thalloid and are incorporated at the thalline rim, as well as the parathecioid envelope itself.

I. Special Types of Ascocarps in Pyrenolichens and Discolichens

1. PYCNOASCOCARPS (Fig. 35)

The ascogonial filaments in *Physma* (Stahl, 1877) and various Ephebeaceae (Henssen, 1963) form beneath the pycnidia. Asci later develop in these,



Figs. 35-39. Fig. 35, development of pycnoascocarps (after Henssen, 1963); Fig. 36, stromatoid structure in *Laurera sanguinaria*: general aspect and transverse section; Fig. 37, *Umbilicaria proboscidea*: transverse section of an old apothecium (after Henssen, 1970); Fig. 38, schematic representation of the ontogenetic and phylogenetic development of pseudopodidia in different *Stereocaulon* (according to Lamb, 1951); Fig. 39, development of podetia in *Baeomyces rufus*; Fig. 40, development of podetia in *Cladonia floerkeana*.

between the conidiophores that are sometimes still functional, and eventually true paraphyses are born.

2. PSEUDOSTROMATA (Fig. 36)

Perithecia in some of the pyrenolichens in the Trypetheliaceae are aggregated in specific structures often designated as stromata. This stroma is, in

fact, an accessory structure, slowly differentiated around the ascocarps that were already well developed and at the expense of the thallus or perithecial envelope (Johnson, 1940; Letrouit-Galinou, 1957). This structure is, therefore, not comparable to the stroma of mycologists which forms early in development before the appearance of ascogonial filaments.

3. GYROPHORINE APOTHECIA (Fig. 37)

The formation of asci in the hymenium of the Umbilicariaceae is most often confined to fertile zones that often project in an annulate or stellate manner (= gyri). This arrangement results from the repeated formation of sterile zones (Scholander, 1934; Frey, 1936; Henssen, 1970) and dichotomously dividing fertile zones. These gyri, according to Henssen, project because their growth is arrested in the central part of the sterile portion while they continue to grow actively at their periphery in the fertile zones.

4. PODETIA (Figs. 38–40)

The ascocarps in some discolichens formerly united in the family Cladoniaceae develop on special structures that are erect, often branched, and thalline in appearance. These are classified as podetia, of which three types may be distinguished:

a. In *Stereocaulon* and related genera they are actually pseudopodetia (Fig. 38; Wolff, 1905; Lamb, 1951; Jahns, 1970), purely thalline structures formed by the vertical growth of some of the thalline granules. The apothecia that develop later seem to be lecanorian at least in *Stereocaulon*, with a plexiform primordium and a well-developed primary corpus.

b. The podetium of *Baeomyces* (Fig. 39) differs fundamentally from that in *Stereocaulon* in the sense that it becomes the ascocarp (Nienburg, 1908; Letrouit-Galinou, 1966; Jahns, 1970). The primary corpus is derived from a superficial plexiform primordium. It consists of a carpocenter, which contains the ascogonial apparatus and extends marginally, and a pericentral envelope consisting of a thin transitory cover and a thick foot. The foot elongates and becomes the podetium. The carpocenter is later covered by epicentral filaments, some of which become paraphyses while others at the periphery remain extrahymenial. The podetium of *Baeomyces*, derived from the lower part of a primary corpus, is the same as the structure called a pseudodiscopodium by Bellemère (1967).

c. The podetium of *Cladonia*, as in *Baeomyces*, is part of the ascocarp but its structure and mode of development are different (Krabbe, 1891; Letrouit-Galinou, 1966; Jahns, 1970). It terminates in a point or a cup and is often branched, being derived from a bundle-shaped primordium composed of divaricate hyphae that elongate and branch and within which the ascogonial

filaments arise, as in the primary corpus of a typical apothecium. These filaments are often differentiated quite early, in the initial bundle itself, but most frequently appear only after the podetia develop at branch tips or along the margin of the cup. Hyphae are drawn aside around them, forming a parathecoid or parathelial envelope. These produce paraphyses, the bases of which form a paraphysogenous subhymenial plexus.

J. Summary

Lichenized Ascomycetes are characterized as follows in comparison with nonlichenized Ascomycetes.

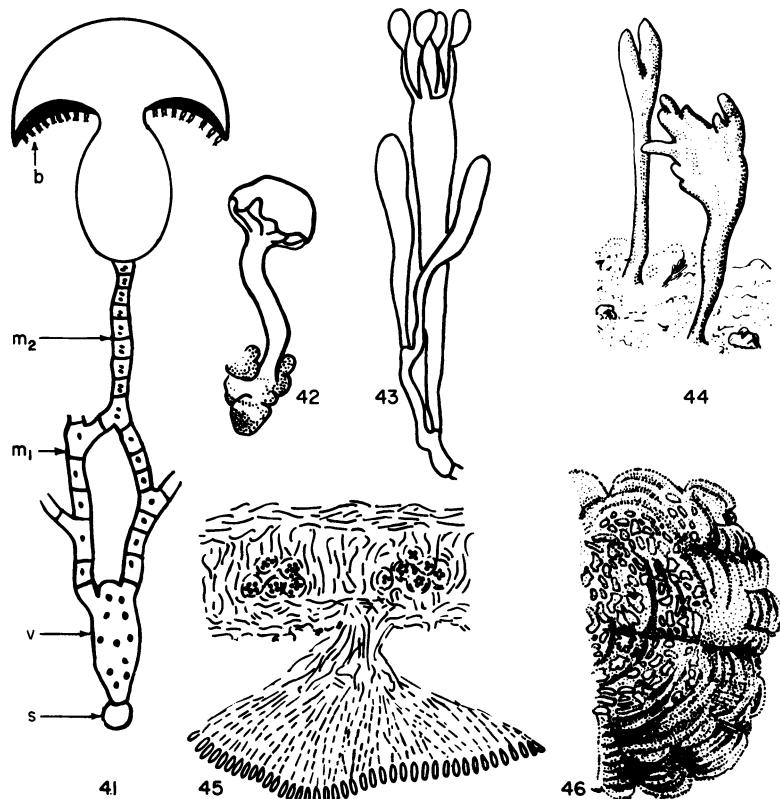
1. THE SPOROPHYTIC APPARATUS

- a. The ascogonial apparatus is often complex and always composed of long multicellular filaments, in general with uninucleate cells (the *Collema* type) but rarely multinucleate (the *Peltigera* type).
- b. This apparatus produces a reduced but still recognizable prosporophyte.
- c. Next, in many species, the ascosporophyte formed from the prosporophyte consists of a primary part with uninucleate cells and later, after peritogamy or otherwise, a secondary dikaryotic ascogenous part with lateral clamps.
- d. As a rule the asci are bitunicate–nassaceous or archaeasaceous.

2. THE ASCOCARP

- a. The lichenized fungi do not differ basically from nonlichenized Ascomycetes as far as ontogeny and organization of the ascocarps is concerned. In particular, the interascal filaments may be represented by paraphysoids (as in *Opegrapha*), pseudoparaphyses (as in *Arthopyrenia fallax* and *A. submicans*), or true paraphyses (the majority of species). However, it is only in some pyrenolichens (*Arthopyrenia fallax* and *A. submicans* with pseudoparaphyses) that the ascocarp is derived from an ascostroma, though reduced to a pyrenosphere, whereas this happens in many of the ascolocular pyrenomycetes (*Pleospora*, etc.). No true stroma is present in other lichens, and one could believe in this case either the thallus itself replaces it or the primordium is a particular type of ascostroma.
- b. In discolichens, (1) it is possible (cf. Chadefaud *et al.*, 1968) that the ascocarp in its primitive form is comparable to that of the less evolved discomycetes (cf. Bellemère, 1967), ascostromatic, lenticular, and with paraphysoids, but the ascostroma replaced by a plexiform primordium; (2) the

graphidian type would be, therefore, the closest relative to this primitive hypothetical form; (3) for the more highly evolved types we can say that the one in *Thelotrema* is reminiscent of the Ostropales; the podetal types in *Baeomyces* and *Cladonia* correspond to those of *Cudonia* (Duff, 1922) and *Mitrula* (Corner, 1929–1930), respectively; the lecanorian type (= parathelial; Chadefaud, 1965) is by far the most common type in lichens but very poorly represented among the nonlichenized Discomycetes; and finally, the eudiscopodian type, so very common in these latter, is lacking in lichens.



Figs. 41–46. Sexual reproduction in basidiolichens. Fig. 41, basic reproductive cycle in Basidiomycetes (after Chadefaud, 1960); Fig. 42, *Omphalina ericetorum* (Agaricales): general aspect of the carpophore and the thalline lobes (after Poelt and Oberwinkler, 1964); Fig. 43, basidia and basidiospores of *Omphalina ericetorum*; Fig. 44, columnar carpophore of *Clavulinopsis septentrionalis* (Aphyllophorales) (after Poelt, 1959); Fig. 45, *Cora pavonia*: transverse section of the carpophore on the level with a fertile papilla (after Grassi, 1950); Fig. 46, lower surface of the carpophore with fertile papillae in *Cora pavonia* (after Mattiolo, 1881). (b, basidium; m₁, primary mycelium; m₂, secondary mycelium; s, spore; v, multinucleate vesicle.)

II. Basidiolichens

There are very few basidiolichens—according to recent accounts, only about 20 species and 10 genera. They all are Basidiomycetes with carpophores and neobasidiates with typical basidia (Fig. 43).

The reproductive cycle has not been studied in any of the species. One would judge, however, that it should resemble that in nonlichenized species (Fig. 41), where in principle a haploid basidiospore gives rise to a haploid primary mycelium. A secondary dikaryotic mycelium is then produced by intercellular fusions (= perittogamies), often with lateral clamps. The mycelial hyphae become aggregated in the form of carpophores. The mycelial elements of the fertile layer produce the basidia, and these give rise to basidiospores after nuclear fusion and meiosis.

The primary and secondary mycelia, according to Chadefaud (1944, 1960), are homologous with primary and secondary sporophytes of peritogamous Ascomycetes; there would be no gametophytic phase.

The carpophores of basidiolichens are only rarely of the Agaricales type where the hymenium develops simultaneously and is located on lamellae (*Omphalina*, Fig. 42). More frequently we find the Aphyllophorales type with a continuously developing hymenium. In addition, they do not have a vallicular hymenium (not localized in the pits of particular podia). They are in columns (*Clavaria* and *Clavulinopsis*; Fig. 44), in sheets (*Stereum*), or multiple ridges or segments (*Cora*). In *Cora* the brackets are formed of intertwined mycelia, in the center of which a gonidial layer is lodged. The lower surface (Fig. 46) bears fertile papillae (Fig. 45). According to Tomaselli and Caretta (1969) the branched hyphae in an umbell comprising these papillae have their origin in the gonidial layer.

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Chapter 3

SYSTEMATIC EVALUATION OF MORPHOLOGICAL CHARACTERS

JOSEF POELT

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I. Introduction

The following treatment will present first of all those systematic criteria which are being employed today as important for the definition of taxa in different hierarchical steps. The divisions that are ultimately developed and proposed might be considered as way stations; they can only reflect our present knowledge, misinformation, and problems.

The number of papers on which this contribution is or should be supported is extremely large. Just to cite all of them would exceed by a wide margin any reasonable number of pages, and the reader will forgive me if some are chosen in a perhaps very subjective and one-sided way. More exact defini-

tions and descriptions of the various morphological and anatomical traits may be found in Chapter 1 by Jahns and Chapter 2 by Letrouit-Galinou. An outline of generic and familial classification is presented in Appendix A.

II. Systematic Criteria

The earliest scientific knowledge of flowering plants recognized flowers and fruits as carriers of the essential features. Thus, phanerogamic taxonomy could develop as a progressively deeper comprehension of principles which were viewed correctly from the very beginning. This was not so with many cryptogamic groups, least of all with lichens. The recognition of determinative characters was either impossible or inadequate for a long period because of the lack of technical aids. In the course of time, this has led to a continual change in the range of characters regarded as important (in line with improvements in optical equipment and similar aids). While the gross thallus form—now called growth form or organizational state—was considered to be a basic criterion at first, the gross structure of ascocarps, spore types, and then the properties of pycnidia were taken as primary characters. More recently we have seen developmental history of the ascocarps and lately the structure of the ascus as having ultimate value as well as chemical characters derived from comparative studies of lichen substances.

The following discussions will attempt to arrange the characters chiefly in order of importance with regard to their interdependence. They are based largely on information drawn from various texts (des Abbayes, 1951; Fünfstück, 1926; Hale, 1967; Moreau, 1927; Nienburg, 1926; Ozenda, 1963; Smith, 1921; Tobler, 1925, 1934).

A. Asci

The ascus type has stood in the foreground of interest in the last 20 years. It had been studied in an isolated fashion since the middle of the last century (see de Bary, 1884, p. 90). Luttrell (1951, with references to earlier literature) was the first to demonstrate comprehensively its importance in the broad system. He divided the pyrenomycetes into two series: the bitunicate series with a typical bitunicate ascus which splits into two layers at maturity and the unitunicate series where the wall, while still two-layered, does not split [designated later by Dughi (1956) as the nonfissitunicate ascus on semantic grounds (see Lamb, 1964, p. 18)]. This division was next applied without difficulty to the discomycetes where the inoperculate Helotiales and operculate Pezizales were assigned to the unitunicates, while a series of forms with an earlier unsettled association as Dothiorales or Hysteriales had to be referred to the bitunicate fungi (see below for the Lecanorales). Richardson (1967) has summarized data on lichen fungi based on these principles.

In France, the problem has been studied from other aspects, quite independent of the results obtained primarily by the Anglo-American school. The French workers regard certain structures in the ascus as basic; these are for the most part very difficult to observe. The nassaceous ascus type, for example, corresponds to the bitunicate ascus, which has a nasse corona in the ocular chamber of the tholus (this term proposed by Lettau, 1932). The anellasceous type in unitunicates has a different ring structure in the tholus, an apical wall thickening (Chadefaud, 1960). Both types have a phylogenetic origin in an archaeasceous prototype, according to Chadefaud *et al.* (1963), with the ring and nasse united so that a whole series of intermediate stages may be distinguished, in part running the gamut in ontogeny, in part strongly restricted to taxonomic groups. According to this viewpoint, the most primordial ascus type would be most conspicuous in the lichenized ascomycetes, the derived ones also in their nonlichenized relatives. In any event, the results of the French workers alter the relatively simple concepts of unitunicate and bitunicate asci, the cardinal point lying in the lichen fungi. Meanwhile, new difficulties occur in our understanding of them. The majority of discocarp lichen fungi brought together in the Lecanorales have long been regarded as unitunicate. Contrary to this concept we find, for instance, investigations by Butler (1939) that motivated Müller and von Arx (1962) to show that *Buellia*, a lichen genus that appeared to have a sure place in the Lecanorales, was in fact ascolocular. The question is whether the Lecanorales are heterogeneous or uniformly bitunicate or whether the application of this definition to all ascomycetes in principle has been carried too far. There appears to be consistency for multilayering or at least two-layering of all asci; there are discrepancies in the question of whether violent expulsion of the endoascus from the exoascus, often valued as a criterion, corresponds to the natural function in each case.

Even greater difficulties arise with the pyrenocarpous lichens. Originally, only comparatively few groups were classified in the ascoloculares, but subsequently largely equated with the bitunicates, bitunicate asci are being demonstrated for more and more pyrenocarpous lichen fungi (Morgan-Jones and Swinscow, 1965; Swinscow, 1966; Chadefaud, 1960, p. 642; Vézda, 1968, p. 367), so that in the end (Vézda, 1968, p. 368) we are left with only the Porinaceae *sensu stricto*, and a few genera that are probably closer to the discocarp lichens. Janex-Favre (1971, p. 567) recently found that the ascus in *Porina* is derived bitunicately. Thus, the pyrenocarpous lichen fungi have been transferred virtually *in toto* to the bitunicates. The problem of classifying them, however, is still not resolved. One is left with the feeling that a large part of the lichen fungi cannot be meaningfully subdivided along the classical definitions of unitunicate and bitunicate. The degree of complication in the relationship is illustrated by *Nephroma* and *Peltigera*, highly evolved foliose lichens, which have customarily been classified in the same

family, Peltigeraceae. Ziegenspeck (1926, p. 355) and Galinou (1955) found that only *Nephroma* has typically nassaceous bitunicate asci, whereas the anellaceous asci of *Peltigera* should establish their position in the unitunicates but they are actually bitunicate (Letrouit-Galinou and Lallement, 1971). The two genera have so much in common, however, that the possibility of an analogous origin from completely different stocks would have to be considered out of the question.

There is no doubt that characters of ascus structure have considerable significance. They have basic significance in distinguishing orders, families, genera, and perhaps in many cases even species. The study of asci demands exhaustive examination, not in the least with the aid of the electron microscope [see Bellemère (1969) for the nonlichenized genus *Bulgaria*]. One must also take into consideration ontogeny, function, permanence and, not the least, chemistry which involves differences in the iodine reaction (see Vézda, 1968). Only then can we fully assess the taxonomic significance of asci in lichen systematics. At the present time, the discrepancies in evaluation of ascus structure lead us to conclude that ascus types cannot be evoked alone as decisive taxonomic criteria.

B. Spores

Spores assumed great systematic value with the introduction of the microscope for morphological analysis. Their value has changed very little although evaluation will change with increasing experience. Ascospores are normally the products of meiosis (reduction division) in the ascus and, at least initially, are unicellular and colorless. They remain in this stage with many groups of different systematic affinity. The one-celled, colorless type is therefore found widely and is of little value in the absence of other specific features. One may generalize by saying that the more complicated the spore type the greater is its value for systematic purposes, and a more sound evaluation is dependent on the degree of maturity of the spore.

There is also a distinct systematic relationship of the simpler types with major taxonomic groups. Thus, spores of the classic bitunicates (Arthoniales, Dothideales) always contain at least two cells, often different in form or size. The cells are often also strongly dissimilar in size or mode of formation. If more than two cells are present, one will often find cells in the series that are clearly different in form or size. As a rule, two- and multiseptate plurilocular spores in the unitunicate species are symmetrical when viewed transversely.

Complex spore types can be family-specific, as the polar-diblastic type in the Teloschistaceae, or as the spores with lens-shaped lumina as in the Graphidaceae *sensu stricto* and the Thelotremaeaceae. However, even in such cases, derived types will naturally occur within the families. This is the

same for the thick-walled polyenergide spores in the Pertusariaceae in which characteristically there is a trend for a reduction in spore number as well.

The essential features of spores and their systematic significance are discussed briefly below.

Virtually all groups of lichenized fungi have eight spores per ascus. Deviations from this to higher numbers occur in various isolated families and are generally useful at the species level. The number may vary somewhere between 12, 16, 24, and 32. Frequent deviation from the base number is specific for certain families such as the Lichinaceae, Heppiaceae, and Candelariaceae. The Acarosporaceae (including Thelocarpaceae) are characterized by numerous spores as a family trait and this is correlated with other characters. There may be abnormal occurrences of less than eight spores. The tendency for fewer spores is, however, constitutionally limited, for the number may vary freely, as in *Pertusaria pertusa*, where one will find four spores per ascus in an apothecium but occasionally three, five, or six. Reduction in spore number is usually correlated with increasing size of individual spores.

Spores are predominantly ellipsoidal in shape and usually quite uniform if the length-width index is low. Generally speaking, the longer a spore in relation to its width, the greater is the variability in length.

Spore size depends on the number, form, and size of asci. In connection with the tendency for a lower than average number and other characters, size can be specific for genera and even for families. As a rule, spore size has value only at the species level or is characteristic for a species group. Size varies over very wide limits in many genera, even small ones (e.g., *Tapellaria*, *Asterothyrium*; Santesson, 1952, pp. 319 and 494).

Spores are colorless in the great majority of cases. There is an analogous tendency for a slow ontogenetic pigmentation going from colorless to yellowish or grayish-green to black in a series of unrelated genera with transversely septate or muriform spores (*Polyblastia*, *Staurothele*, *Endocarpion*, *Graphis* s. lat., *Lopadium*, and *Rhizocarpon*). This trait is usually species-specific, and definite stages of maturity are necessary for its definition. When used to characterize genera, as in the Graphidaceae, Thelotremales, and Opegraphaceae, the genera should be regarded for the most part as artificial entities. Few-celled types of spores darken very quickly, a similar color change at most being recognized. Such a type is normally genus-specific and sometimes family-specific (*Physciaceae*, *Encephalographa*, and Caliciales).

Ornamentation of spores is exceedingly rare among lichenized fungi. It is of specific value above all in the Caliciales, where in various genera it is a very good species character (see Tibell, 1971), and sometimes in *Physconia* and *Melaspilea*.

Little research has been done so far on the spore wall. Its fine structure ought to elucidate important characters. Recent studies have been made on the multilayered wall of the large polyenergide spores of the Pertusariaceae (Erbsch, 1969). In particular, still to be analyzed are, among others, the thin deformable walls of *Aspicilia* spores as well as the types in the Teloschistaceae and Physciaceae (for recent work, see Sheard, 1967; Lamb and Henssen, 1968).

Multinucleation of the protoplasts appears to be a character of higher value that is still little examined.

The value of cell number and septation cannot be categorically stated. We can find very clearly defined types where special wall differentiation brings out additional characters (Teloschistaceae, Ramalinaceae, etc.) but at the same time others in which the number of septa varies within certain limits from spore to spore. This falls into a pattern such that the greater the number of average septa the greater is the variation (*Bacidia*, *Sporopodium*, *Tapellaria*, *Rhizocarpon*, Graphidaceae, etc.). Too little is known of the mode of division, a potentially good character as used in many of the nonlichenized fungi (for example, *Pleospora*).

Halo formation in spores can be regarded in many groups as a character of at least generic value, but one should consider that a halo can be demonstrated only in certain stages of maturity.

One often finds abnormal formation of spores in many groups of lichens. They are sometimes easy to recognize, but at other times are difficult and can give rise to systematic errors.

Little attention has previously been given to the ecological aspects of spore evolution. There is a tendency in many quite different foliicolous genera for the development of very large spores. Spore halos should also be regarded as ecological adaptations which have already become organization characters for many groups. What functional and systematic role the expulsion of spores with ascus (Schmidt, 1970) or even paraphysis fragments in the Caliciales (Tibell and von Hofsten, 1968, for *Texosporium*) plays is uncertain.

C. Paraphyses

The term paraphyses (see Richardson, 1967, p. 353, for a discussion of terminology) has been used in many different plant groups, mostly for threadlike organs which lie between sexual organs or sporangia and serve to protect them or to promote their function. Accordingly, the term paraphyses has long been used in all Ascomycetes for the sterile hyphae which fill the space between asci in the ascocarp. With the discovery of the differing nature of ascocarps came the realization that paraphyses of ascolocular fungi originated in a completely different way from those in the ascohymenial

fungi. These were thus designated as paraphysoids and the whole as a paraphysoidal net owing to the netlike structure. Both types are sufficiently clear in theory but often very difficult to differentiate in practice. They occasionally occur together (e.g., in *Graphis*; see Janex-Favre, 1964). This netting, considered occasionally as a criterion for paraphysoids, is widely distributed in many genera of the Lecanorales and as such is hardly useful for separating paraphyses and paraphysoids.

It is recommended for descriptive purposes that all interascal hyphae be designated as paraphyses and the expression "true paraphyses" and "paraphysoids" or "paraphysoidal net" be used for an exact definition. This is done in the following discussion.

Most paraphyses persist for a long period of time. In some groups (e.g., Verrucariales) there is a rapid gelatinization long before the asci mature. Although the chemistry of this process is still unclear, it can be regarded as systematically relevant with some caution.

Paraphyses are almost always divided into cells, although the septa often remain indistinct and cannot be recognized adequately with the microscope. Structure of the septa appears to be important in many groups but has received little attention in the past. Distinct nonseptate paraphyses have been described by Vězda (1965) for *Absconditella*. Branching and netting as characters for genera and species groups are to be used only with care. It is not uncommon to find anastomoses of paraphyses in genera in which no mention of such is made in the diagnosis (for example, in *Lecidea*, *Lecanora*, and *Caloplaca*). They often appear to be quite different, however, in different groups and probably originate in various ways. Stiffness or flexibility are related to the thickness of the paraphyses or their walls (stout, stiff paraphyses in various families with blue-green algae). Length of cells of paraphyses varies widely in most cases, and the length of the paraphyses themselves can apparently be used to some extent for genera and species groups. Still this character must be confirmed to be statistically better than it has been in the past. Paraphysis cells are mostly cylindrical. A tendency for rounding off of at least the upper cells is characteristic of *Aspicilia*, and more so for *Harpodium*. This gives rise to a more or less moniliform row of cells (Magnusson, 1939, from Hue, 1911). The end cells of paraphyses, also called paraphysis knobs (commonly the next to last cell is also involved in their formation), are usually more or less strongly thickened in the Lecanorales but typical only for paraphyses which reach the hymenial surface. Only these have any diagnostically useful value.

The epithecium, in the mycological sense of structures originating from paraphyses and overlying the asci, is rare in lichens but when present may be usable as a character (*Sporopodium*, *Ochrolechia*). In many lichens, the upper zone of the hymenium (10–40 μm high, averaging 20 μm) is distinct

as an epiphyllum from the lower zones, which are separable mostly by lack of pigmentation, by granular excretum over the paraphyses as epipsamma, or by pigmented wall layers of paraphyses. Either possibility may occur and would be useful in diagnosis, but, on the other hand, they may occur in addition or successively. Because the chemistry of these appearances is largely undetermined, they can be used provisionally only with careful statistical confirmation. The epipsamma often appears to be identical with the cortical pigment.

Adspersion of paraphyses occurs as a useful valid character in several groups although the chemical nature is still unknown (*Lecidella*). The same holds true for the inclusion of oil drops in the hymenium (*Buellia*). Coloration of the whole hymenium (*Lecidea*, *Rhizocarpon*, *Lecanora atra*) is useful at the species or group level, at least in the tendency toward this trait, but the chemistry involved is quite unknown.

D. Types of Fruiting Bodies

The form of the fruiting body has played an important role in lichen systematics from the beginning (Acharius, 1810), when at first only the external appearance or histological differentiation recognizable in a crude section was considered. Knowledge of the diversity of types came about with improvements in the microscope, resulting in an extensive literature. This is not the appropriate place to discuss the literature on the systematic usefulness of individual characters or the question of developmental history which suffers from great differences in terminology used by different authors. Below are some characters examined only for their systematic value.

The division into apothecia and perithecia, used here in the usual descriptive sense, has long been used to distinguish major groups. Nevertheless, many examples of transitional types are known, but these are limited in systematically well-characterized ways to definite families [Pertusariaceae, Acarosporaceae (*Acarospora*, *Thelocarpon*, partly with "perithecia"), Gyalectaceae, Ostropales]. It is not certain what the original type of fruiting body originally was. It would probably be the apothecium in most instances.

In perithecia, we have characters related to the structure of the perithecial wall, but these have been investigated very little, especially in the carbonized forms. The degree of carbonization varies considerably phenotypically and is useful as a character only statistically. The occurrence of periphyses is obviously systematically important but it must be carefully examined. Involucrella, known in the Verrucariaceae, have proven to have very useful characters by Doppelbaur (1959). The question remaining to be answered here is to what extent their significance goes beyond the cate-

gory of species and whether they should be used to separate out genera as was done by Servít (1954).

The type of fruiting body in the Caliciales obviously goes back to a basic type which has undergone various systematically relevant modifications. Nádvorník (1942) and Schmidt (1971) in particular have demonstrated that the fine structure is of significance.

Difficult questions arise in any discussion of lirelliform apothecia. They have originated obviously at very different places in the system of ascostromatal and ascolocular groups and are quite dissimilar systematically. Frey (1949) and Henssen (1970) have shown that the complex furrows in the much discussed genus *Umbilicaria*—a trait used by Scholander (1934) and Llano (1950) to separate out several genera—can be used for the definition of species through a consideration of their ontogeny and that all possible transitions occur within natural species groups. This would, in their opinion, nullify any application above the species level.

Lirellae form the basis of a family character for the Graphidaceae and Opegraphaceae. One might first examine actual transitions from *Opegrapha* to *Lecanactis*, a discocarp genus, if *Lecanactis* were better studied. There is no doubt that transitions between apothecia and lirellae occur in the genus *Sarcogyne* in the Acarosporaceae. One can generalize by saying that the character "lirellalike fruiting bodies" may be familial, generic, or specific in particular cases or is even merely an ontogenetic end stage.

Apothecia have long been distinguished by the nature of the relationship to the thallus and to the algae (Dughi, 1952; Sheard, 1967). In many instances, the generic level value is granted for the basic types. Cryptolecanorine apothecia are, of course, constant and characteristic within certain limitations for large groups (*Aspicilia*), but in *Buellia* (Lamb and Henssen, 1968) they are connected by all possible systematic and ontogenetic transitions with lecideine ones. Marginal cryptolecanorine apothecia can be marginate in a lecideine fashion. The extent of penetration of algae into the margin can vary over extraordinary lengths (Poelt and Wunder, 1967, for *Caloplaca*).

Carbonization of structures is obviously due to still unknown chemical and cytological processes that are quite diverse. It can, as a rule, be regarded as a good character at the generic level with consideration to a certain partly ontogenetic variability. Carbonization may be confined only to the hypothecium (*Dirinaria*, *Roccella*, etc.). The great range of possibilities for carbonization is used for defining species in *Lecidea*. The ontogeny in species of *Pyxine* is noteworthy: the juvenile apothecia are lecanorine, with the intervening carbonization leading to destruction of the algae and ending with a superlecideine apothecium. A fuller knowledge of the chemical aspects would result in a more important systematic criterion.

The type of apothecial margin is often used in the *Lecanora subfuscata* group (Magnusson, 1932; Poelt, 1952). It is constant here and characteristic for species groups. This character has so far received too little attention, as have the various deposits of substances of differing chemical quality, nature, and quantity. They are sometimes identical with thallus compounds but are not rarely confined to apothecia. There is no reason why they could not be used as species characters with consideration of quantitative differences.

E. Stromata

The systematic value of the stroma, in which a number of perithecia, and more rarely apothecia, are combined in a syncarpous body, has been considerably overrated by earlier workers, perhaps because European lichenologists were not familiar with it. Zahlbrückner (1926) recognized families on the basis of this character. Vainio (e.g., 1890), followed by Watson (1929), had already made reference to the questionable value of such a division. The concept of the stroma was obviously so vague and little studied that Redinger (1938) could still conclude that the genus *Enterographa*, classified in the Chiodectionaceae which are characterized by presence of a stroma, does not have a true stroma. Santesson (1952, p. 129, with other literature cited) presents a discussion of the whole problem. Some other properties are closely tied in with the formation of stromata; these were used by Zahlbrückner to define families and are likewise useless: the position of single perithecia embedded in the stroma and the eventual formation of a common opening of obliquely or horizontally positioned perithecia. Letrouit-Galinou (1958) was able to show with the genus *Laurera* that all possible transitional stages can exist between only weakly implied stromata, from barely modified thallus plexus with the substratum to highly complex partially carbonized, sharply contrasting structures.

The all-inclusive character "stroma" can be employed for the definition of genera, subgeneric taxa, and sometimes only species, as, for example, in *Pertusaria*, where stromata are found in only some of the species and then usually just weakly delimited. It is remarkable that reproductive stromata are predominantly of tropical origin. The tendency to develop stromata may serve as a family-specific character in the Trypetheliaceae and Laureraceae.

Stromatic fruiting bodies, the ascomata, of typical bitunicate lichen fungi can contain one or several loculi as those of nonlichenized relatives. Families were based on the presence of "simple" or more or less complete "chambered" perithecia. The resulting groups were, on the one hand, even associated with the Pleosporales and, on the other hand, with the Myrangiales. As Riedl (1961) has demonstrated, all possible transitions may be found in size and "septation" of ascomata. These characters are useful at best for the definition of species or genera.

F. Pycnidia

The structure of pycnidia, their position in the thallus, and the size and shape of pycnospores, has been used since Nylander as a very important character frequently used to delimit genera. Unfortunately, very little has been published on these organs since the basic investigation by Glück (1899) and the improved system proposed by Steiner (1901). There is not much doubt about the basic significance of the types of structures, although Glück himself mentioned the existence of transitional stages between them. The form of the pycnospores appears to be constant by group with respect to variation in length of long filamentous types. Shape and length can be significant for family, for genus, or only for species in genera which have a great range of variation, as in *Opegrapha* and *Aspicilia*, in which Magnusson (1939, p. 16) was able to show a whole spectrum of species-specific length range. Pycnidia merit more exhaustive study with the newer technical equipment now available.

Terminology for the organs of pycnidia has changed in the past decade in accordance with a worker's conception on the significance of pycnospores. It seems plausible to me that they function normally as spermatia because of their small size, the very small amount of protoplasm, and their occurrence on trichogynes. In other cases their function has perhaps changed toward asexual reproduction. In this chapter the following neutral terminology is used: pycnide and fulcrum (instead of basidia and conidiophore) and pycnospore (instead of spermatium and conidium).

G. Growth Form

Growth form was used as a basic criterion in the earlier systems as the most easily recognizable macroscopic character, beginning with Micheli (1729) to Linnaeus (1753) and Acharius (1798). It remained as a character even with the advent of the microscope (Massalongo, 1855; Koerber, 1855, 1865; see also von Krempelhuber, 1869; Tuckerman, 1882, 1888), although more or less modified, and was finally employed as late as the early 1900's as a primary classification character (Harmand, 1905). It had long been realized, however, perhaps with the "Lichenes blastenospori," that quite different growth forms could occur together in natural groups and conversely similar growth forms can develop in quite different groups. Growth form was used by Zahlbrückner (1907, 1926) to differentiate families within a homogeneous developmental series, but this principle was not always followed.

The impossibility of a schematic division is illustrated in the Teloschistaceae as shown by Malme (1926), where one finds a complete coherent developmental series from primitive crustose to foliose and fruticose types. On the other hand, one should not overlook the fact that most foliose and

fruticose lichen genera may not be recently connected to definite crustose groups and show no closer relationship between themselves. These discontinuities lead necessarily to lending greater weight to the growth form as a character, of course only in particular cases, especially for distinguishing families. One may consult Barkman (1958, p. 182), Frey (1929), Hilitzer (1925), Klement (1955, p. 18), or Mattick (1951) for greater details on growth and life forms of lichens.

It can be determined with some exceptions that growth form, as an organizational stage, is far-reaching in natural genera and completely uniform, in principle, with species, insofar as it is dependent on characteristic anatomical structures. In addition, refinements such as form of lobes, type of lobe axis, and type of areolation, are also genetically determined in principle, the degree of development, to be sure, being dependent on the environment.

H. Auxiliary Thallus Organs

I will discuss here only the systematic significance of these organs; they are normally thoroughly covered in textbooks. They are often useful as family characters, as the pores in the Stictaceae, the tufted rhizines in the Peltigeraceae, or the umbilicus of Umbilicariaceae and are frequently specific to a particular genus, as rhizines in *Parmelia*, holdfasts in *Hypogymnia*, or cephalodia in *Placopsis*. Cephalodia in the Lecanorales—Peltigerineae may even be evaluated as a highly valuable characteristic if one considers the alternative occurrence of species with blue-green algae without cephalodia and species with green algae with cephalodia found in all genera. Rhizine structure is significant mostly for species groups [simple versus squarrose as in *Parmelia* (Hale and Kurokawa, 1964, p. 123)] as is the case with cilia in groups of species in *Teloschistes*, *Physcia*, *Anaptychia*, and *Heterodermia*. The corresponding situation in *Physconia* is smooth as opposed to perpendicular fibrous rhizines (Nádvorník, 1947, p. 117).

Auxiliary organs as ecologically significant formations must be the result of convergent evolution. This holds for rhizines, for pseudocyphellae which occur in individual species and species groups of many genera, for rhizine strands in placodial lichens (Poelt and Baumgärtner, 1964), and for the remarkable spongy layer which has a very similar aspect in the genera *Anzia* and *Pannoparmelia* but which arises in quite different histological ways. In a few cases, surface hairs may be characteristic of a whole genus (*Erioderma*); they are significant as a rule for species groups (various sections of *Parmelia*) or even for individual species of groups of otherwise hairless taxa (*Physcia*, *Physconia*). There are, moreover, characteristic distinctions in the structure of hairs and their density. The systematic value of hairs has occasionally been open to question. After a detailed study of this problem, I have not been able

to find a case in which presence of hairs is not constant for a taxon. In many cases the so-called vitreous hairs (in the Physciaceae) are so easily broken off that it is no easy matter to prove their existence.

The accessory organs of the thallus deserve more consideration systematically from various aspects.

I. Thallus Anatomy and Histology

Anatomical characters of thallus structure play a large role in the delimitation of foliose and fruticose genera. The constancy of axil formation of different types (*Usnea*, *Alectoria*, *Letharia*, and *Anzia*), veins (*Peltigera*, *Hydrothyria*), supporting tissues (*Ramalina*), and ground structure of cortical formations (hyphae anticlinal versus periclinal, in *Physconia* versus *Anaptychia*, *Physcia* versus *Heterodermia*, etc.) have frequently been confirmed. They are valid systematic criteria and will not be discussed further here.

It is another case with anatomical characters which are difficult to recognize that demand an estimation of quantitative differences. Cell diameter, thickness of layers, etc., in particular have been investigated in great detail by Hue (e.g., 1911) and systematically evaluated. The value of such results based on a single section is questionable, as seen with Magnusson (1944, p. 14). Again it was Magnusson (e.g., 1929), who employed criteria such as height of thallus cortex and thickness of cortical cells (height of the hymenium) for differentiating species (here in *Acarospora*) and used them to characterize groups. Almborn (1965, p. 454), among others, has raised doubts concerning this method. It is rather difficult to discuss the actual utility of such characters when the supporting data were derived for the most part from single specimens and the dependence of environmental factors was not critically proved (Ertl, 1951). There are hardly any investigations on the range of variability for these characters. One should also realize that many of the anatomical details may be traced back to special hyphal structures, the stages of which provide better, more directly usable results.

Experience based on identification work, on the other hand, has taught us that these characters, used with care, can be significant. Frey (1929, p 229) was able to show convincingly for *Umbilicaria* (syn. *Gyrophora*) that, while variable, some anatomical details could be uniform in species widely distributed over great areas.

One gets the impression that anatomical quantitative characters should rank well as characters of taxa of different levels, that they must still be verified by access to a broader range of material, and that it is not feasible to define rigid numerical data on the basis of the description of single specimens, later proved to be much too narrow after a second specimen is examined.

J. Algae

The algae which participate in lichen symbiosis (see Ahmadjian, 1967; Letrouit-Galinou, 1968) first attained significance in systematic classifications in connection with Schwendener's theory, although the groups with blue-green algae have long before been recognized separately (for example, Flotow, in Koerber, 1848; Nylander, 1854). It should be understood that with the acceptance of the symbiosis theory great importance would come to be attached to the type of alga. Fries (in particular 1871–1874) made the most consistent attempt to use algae in lichen classification. Still, it was realized that this principle was unnatural, especially since lichenologists had begun to comprehend lichen systematics based on lichen fungi. The principle has actually remained significant in practice to this day, not the least because the adaptation of lichen fungi to distinct algae is obviously the result of a long phylogenetic development and therefore worthy of attention.

Each case, to be sure, should be examined individually. Taxonomic entities of the more highly differentiated lichens will usually combine consistently with a definite alga, as *Parmelia* with *Trebouxia*. At the other extreme, there are different species in the same genus containing different algae (Stictaceae, *Petractis*). Cephalodia form an exception, for they occur in a series of rather distantly related genera (*Peltigera*, *Solorina*, *Stereocaulon*, *Pilophorus*, *Placopsis*, *Lecidea*, *Thysanophoron*) as morphologically more or less distinctly delimited formations; both types of algae occur intermixed in *Compsocladium* (Lamb, 1956, p. 160). The blue-green algae are often not specific; one and the same cephalodium might contain different species of alga at the same time (e.g., Lamb, 1951, p. 542). The structure of cephalodia in *Stereocaulon* exhibits a whole series of characters specific to species or species groups (Lamb, 1951, p. 531).

Replacement of an algal species with another must occur repeatedly in the phylogeny of a family such as the Stictaceae. Otherwise it is difficult to understand the occurrence of morphologically and chemically identical species which can only be distinguished by type of alga (see Yoshimura, 1971, for *Lobaria*).

We can generalize as follows: A given species of lichenized fungus will always occur with a definite algal species which may be differentiated into geographical races. The often reported occurrence together of different lichen algae in a thallus appears to be very rare, except for species with cephalodia, so far as one demands a symbiotic relation of the fungus to both.

Moser-Rohrhofer (1966b) discovered in *Maronella* a remarkable combination of a name-giving fungus with two different algae and a further torulose fungus species. Lichens in especially moist habitats will often have colonies of unrelated algae growing in thallus cracks and pores, but this type of occurrence is not germane to our problem.

Hymenial algae are restricted to the genera *Staurothele* s. lat., *Endocarpon*, and *Thelenidia*. They have nothing in common with the occasional literature references to algal packets in the hymenium of different species which seem to be the result of some kind of disturbance (Schade, 1963, p. 323).

K. Relation between Fungus and Algal Cells

The morphological and physiological relations between fungus and alga in symbiosis are of a varied nature. The occurrence of distinct haustoria—the term used in a broader sense, not in the narrow definition of Moser-Rohrrofer (1966a)—such as in the family of Lichenaceae (including Pyrenopsidaceae) has long been known (Plessl, 1963, and literature cited there), although different contact forms are found in the Cyanophili. The type of behavior can vary in exceptional cases in the same thallus. Geitler (1963) was able to show for *Lecidea globifera* that haustoria are obligately present in the older parts of the thallus but absent in younger parts. Plessl found, too, that there was a distinct systematically relevant connection between level of organization and type of haustorium with regard to ontogenetic and annual variation. To be sure, Ben-Shaul *et al.* (1969) and Galun *et al.* (1970) have shown that this picture should be modified. The form of contact can differ under different ecological conditions for the species they studied. Distinct haustoria were demonstrated in taxonomically remote species in xeric areas, whereas there is only close contact of hypae and algal cells under more moderate environmental conditions.

Further study of the questions raised ought to provide valuable support for systematics. For the present it would seem to offer little help for taxonomy in the realm of species for technical reasons.

L. Vegetative Diaspores

The systematic value of vegetative diaspores has gone through various changes. They were used by Acharius as criteria for defining genera (*Variolaria*, *Isidium*); later, sorediate phenotypes were designated simply as forms, especially under the influence of G. F. W. Meyer (1825). They were determined to be a sorediate condition, not a sorediate species. The constancy of these structures, however, induced Nylander to describe a large number of species in *Parmelia* on the basis of soredia and isidia. In this way their value was affirmed *de facto*. Investigations by Bitter (1901) on *Hypogymnia* resulted in general recognition of types of soralia as good species characters. The pendulum, however, swung to the other extreme at the same time. Bitter delimited superposed systematic entities with his soralia types so that subsequently it was the fashion to combine, at least formally, the sorediate, isidiate and nonsorediate, and nonisidiate species of the genus into special

groups. In this way any reference to possible relationships between groups would have been lost. Du Rietz (1924) ultimately developed some rules for types of soralia and isidia, ascribed basic taxonomic value to these formations, at different levels of rank, to be sure, and finally put pairs of species together that were morphologically and chemically identical but differed in presence or absence of vegetative diaspores. The frequency of apothecia bore a reciprocal relation to this order. This concept of species pairs was taken up and expanded by Poelt (1963, 1970). Hale (1965), studying *Parmelia* subgenus *Amphigymnia*, and Yoshimura (1971, p. 239) studying *Lobaria*, both found a series of other examples.

Since the different methods of vegetative dispersal have very unequal taxonomic value, I will briefly discuss special features below. This does not include thallus growth with subsequent division of thallus parts by dying away of the center or dispersion of branched fruticose lichens by dying off at the base.

a. UNDIFFERENTIATED FRAGMENTS. These often have a role in dispersal, especially in fruticose lichens (see Du Rietz, 1931). They are not tied in to definite structures, however, and for the most part have no taxonomic value. They could be used, nevertheless, to characterize populations if combined with generative sterility (as in unattached *Parmeliae* which rarely fruit). This method of division is common in *Cladonia* (Ullrich, 1955) and *Cetraria* [Compare the sterile race of *Cetraria islandica* from Iceland described by Kristinsson (1969)]. It is altogether possible that the polyphyllia found in many foliose lichens are of significance here. They are normally associated with lack of or sparse development of apothecia and in many cases appear to be rigidly controlled genetically (for example, in *Parmelia panniformis* and *P. laciniatula*), while in other cases the character is constitutionally limited and its manifestation obviously influenced by environmental factors (*Physconia*, *Physcia*, and *Anaptychia*). The taxonomic value must be determined individually for each case.

b. DIFFERENTIATED FRAGMENTS. These are found in a number of foliose lichens. They are fine fragile protuberances which cannot be called isidia or soredia [as, for example, in *Tornabenia atlantica* (Tavares, 1957) or *Anaptychia roemeri*]. Such structures ought to occur more often but are overlooked. In the same category are the results of lacerations which Frey (1929, p. 232) has discussed in *Umbilicaria*. The taxonomic value of these types should be studied more closely.

c. SCHIZIDIA. This term, first proposed by Poelt (1965a, p. 580), identifies vegetative diaspores that cannot be referred to isidia or soredia. First of all are the undifferentiated appearing areoles and lobes that split

in the lower part of the medulla parallel to the upper surface, the lower layer remaining in place. The corresponding upper part which comes loose by curling plays a role in dispersal. This has been described for *Fulgensia* where it is specific in all corresponding populations and is combined with rarity of apothecia. Only the scale of schizidial formation seems to be environmentally determined. The dispersal organs of *Parmelia taylorensis*, *Baeomyces* p. p., *Trapelia* p. p., and other lichens also belong in this category. I am convinced that this trait is genetically controlled.

d. PHYLLIDIA (SINGULAR PHYLLIDIUM FROM THE GREEK WORD FOR BLADE). These comprise those organs for vegetative dispersal which Du Rietz (1924) calls "Isidia squamiformia" and Culberson and Culberson (1956, p. 679) and Hale (1967, p. 22) call lobulae or lobules. They are distinctly foliose, flattened, and dorsiventral growths, of the upper cortex or thallus margin, usually with a constricted base, and fall away as diaspores. They are found in numerous species of *Parmelia* (but are rare in Europe), *Collema (flaccidum)*, *Lasallia*, *Physconia*, *Stictaceae*, and lastly in *Peltigera* where the often discussed "wound isidia" (Thomson, 1948, 1950; Lindahl, 1960) are better regarded as wound phyllidia. The capacity for producing phyllidia appears in all to be genetically fixed, even in *Peltigera*, as shown by Lindahl (1960).

e. ISIDIA. These are understood as thallus outgrowths with a more or less radial structure, which are usually somewhat scattered over the upper surface of the thallus but rarely in restricted isidangia. We can refer to Du Rietz (1924) for the different types; he was not familiar with the isidia scutelliformia (Santesson, 1952, pp. 30 and 559). Isidia should not be confused with tubercles and wrinkles of wind forms and senile thalli. They always appear to be genetically controlled and are generally good species characters. One of the exceptions is sorediale isidia which originate from soredia, especially in dry shaded habitats. These are not carried away by water or other forces but grow out on the parent plant and become corticate; they are, properly speaking, soredia. The ability of soredial granules to grow out like isidia on the parent thallus is, to be sure, genetically controlled; it is quite common in the Physciaceae and rare in the Parmeliaceae with many sorediate species. Formation of isidia in *Collema*, as used by Degelius (1954) chiefly for the delimitation of varieties and forms, seems by comparison to be little stabilized morphologically or systematically.

f. SOREDIA AND SORALIA. Both of these terms are used here in the usual, not original sense employed by Du Rietz (1924). Soredia are the granular diaspores; soralia are the more or less clearly defined places of formation (see Du Rietz for typology). Soredia and soralia are good charac-

ters for populations at the species level as a rule. This applies first of all for the occurrence on the whole, secondly for type of soralia, and thirdly for shape and size of soredial granules. It does not apply to a series of structures which are often misunderstood, including, for example, the pruina on fruiting bodies (as in *Pertusaria* spp.) or the leprarioid structures of very different lichens which primarily have nothing to do with vegetative dispersal but represent a specialized life form in habitats that receive no precipitation (and as such naturally have taxonomic significance). Neither should one consider useful the globular soralia of very irregular dispersion on the thallus (*Cladonia* sect. *Cladina*, *Cetraria*, *Parmelia*, and *Pseudevernia*). These occur most often on old or moribund specimens of foliose and fruticose lichens. These soralia do contain soredia but they seem to occur only under special unusual conditions and do not belong with the combination of characters of the species in question. We know nothing of the reason for their occurrence.

Species with genotypically restricted soralia are usually without apothecia. This is especially true for the majority of foliose and fruticose lichens. A noteworthy situation obtains in a whole series of crustose Lecanorales where adjacent thalli with apothecia and soralia and fertile thalli without soralia will be found together. These soralia appear to result neither from environmental factors nor from aging; they are very irregularly distributed and often irregularly large. These species usually occur on overhanging acid silicate rocks and more rarely on bark. Examples are *Lecanora subcarnea* var. *sorediata*, *L. cenisia* var. *soredians*, *L. rupicola* var. *sorediata*, *L. allophana* var. *sorediata*, *Lecidella elaeochroma* var. *sorediata*, and *Lecidea cyathoides* var. *sorediata*.

The rank of variety has usually been assigned to these always sympatric types which exist more rarely as the nonsorediate parallel forms. This division seems even sharper in other forms of the same genera: *Lecanora atra*—*L. pertusarioides*, *L. subradiosa*—*L. lojkaeana*, *L. intricata*—*L. soralifera*, *L. chlorophaeodes*—*L. chloroleprosa*, *Lecidea albocaerulescens*—*L. glaucophaea*, and *Placodiella olivacea*—*P. olbiensis*. In these instances the sorediate form is usually without apothecia and occurs more frequently alone. It occurs, nevertheless, in similar habitats. The rank of species would be appropriate since we are dealing predominantly with partial or complete apomicts, while the category of form would be recommended in the first mentioned group.

g. LICHENIZED HORMOCYSTS. This type of diaspore, described in 1945 by Degelius, is produced in so-called hormocystangia. According to Henssen (1968, 1969) it is restricted to a number of species in the genus *Lempholemma*. The occurrence of hormocysts is taxonomically significant and related to reduced apothecial formation.

III. Modifiability and Its Taxonomic Significance

Lichens lack the capacity to develop special resting stages for survival during unfavorable periods. Thus, as long-lived organisms, they may be subjected to the effects of an extreme and hostile environment over a period of many years. This leads to considerable environmentally induced modification, and the range of phenotypic variation, especially among many of the crustose lichens, is often much greater than the phenotypically expressed genotypic difference between species and even species groups (Poelt, 1965b; Lamb and Henssen, 1968). Phenotypes of a single species growing in different habitats can appear so unlike that it calls for a detailed analysis and long experience to recognize them as relatives of one and the same population. On the other hand, convergent phenotypic change arising at identical habitats can be so similar (Sandstede, 1931, p. 3) that very careful analysis and experience may be needed to confirm their true identity. Further discussions on the effects of substrate per se on morphological changes are presented by Brodo (Chapter 12).

A. Abiotic Factors

If one looks at the mean habitat of a lichen, it will be seen that the shade forms are less prominently pigmented and, for foliose and fruticose forms, are poorly branched and often have narrower lobes. In many groups the expression of species characters is to some extent heightened by exposure to light. The brown pigmentation of the upper surface of many species in *Hypogymnia*, associated with strong light intensity, becomes weaker as the light decreases. Shade forms are often difficult to recognize. Pendulous lichens are longer and less branched in shade but become short and shrubby when exposed to full sun and wind. Other examples of these well-known phenomena are enumerated by von Krempelhuber (1861, p. 72).

The grinding and polishing effect of wind exerts a strong influence on the phenotype, the thalli being abraded by sand or ice crystals. In this way, severely damaged lichen specimens are often incorrectly identified by inexperienced workers. Their normal characters can be totally altered, and not a few of them have been described as distinct species. Aquatic lichens, as in *Verrucaria*, show the effects of sand grains which abrade broad sections of the thalli exposed to rapidly flowing water.

Pruinosity of the thallus and/or apothecia is a much discussed character (Weber, 1962). In past decades, a large number of taxa have been described on the basis of the very variable occurrence of pruina. In actuality, there have been very few investigations into the true nature of pruina. Deposits of dust are sometimes mistaken for pruina in lichens that grow in xeric habitats. A

white, often thick epinecral layer is produced on crustose lichens in these regions. This occurs as a continuous incrustation or cracks apart into sections. Although environmentally induced in many taxa, its expression is undoubtedly genetically controlled in other groups, as in *Physconia*, which has large species-specific flocks of pruina (Poelt, 1966). Here the actual production of pruina, as well as its manner of formation, are genetically determined. Pruina in other cases consists of more or less distinctly crystallized deposits. These are the result of specific metabolic processes in the lichen and for this reason have taxonomic value. Here the capacity to form pruina is genetically determined while the amount produced may be subject to environmental factors.

It is unknown to what extent the occurrence of calcium oxalate crystals in lichens also has a specific genetic base. Schade (1970) discusses a series of examples which show that at least the quantity of crystals formed is dependent on environmental factors. The size of crystals in the discocarps of various taxa in the *Lecanora subfuscata* group is species-specific (Magnusson, 1932).

B. Biotic Factors

Schade (1933, with older references, 1956, 1963) should be credited with pointing out the great significance of feeding damage on the appearance and development of lichens. Damage caused by snails, mites, and insects is extremely widespread, and it is quite difficult in many, especially eutrophic areas to find any undamaged thalli for certain species. Fresh damage is fairly easy to recognize as having exogenous origin. Deformations arise on aging of the feeding areas (a bluing of the medulla in *Rhizocarpon geographicum* coll.) and especially through regeneration stages and repeated damage and regeneration. These stages may alter the phenotype significantly, providing the basis for the extraordinarily large number of new taxa created in the literature. Examples may be found in all the larger crustose genera. Taxonomists studying some species in *Caloplaca* would be hard put to define the "typical" (undamaged) appearance. Specimens preserved in herbaria have almost always been collected in the damaged state, and one may be forced to include damage in the description as the normal state of the population. Grumann (1954) proposed a nomenclature of damaged forms as "terata" and a more recent treatment of the problem can be found in Hertel (1967).

Aging phenomena can strongly alter the appearance of lichens in groups where individuals remain intact without vegetative dispersion, as well as in species of *Umbilicaria*. Schade (1963) and Lamb and Henssen (1968, p. 4) have referred to this problem most recently. A special form of habitual change occurs when lichen diaspores develop too closely together for still

unexplained reasons. Adnate dwarf thalli are produced, often with dense initials of pycnidia or apothecia which do not develop further.

Gall formation is not rare among lichens (see the comprehensive study by Grummann, 1960). This has also given rise to taxonomic errors, although these are far fewer than in the case of damage caused by insect feeding. Little has been written on erroneous determinations based on lichen parasites growing on lichens. The genus *Abrothallus* DeNot., for example, was based on the combination of a fungus with a *Parmelia* species (Santesson, 1960, p. 514), just as in *Aspidelia* Stirt. (Culberson, 1966). The parasitic lichen *Lecanora gisleri* attacks three different species in a distinct group in *Lecanora* and some combinations of host and parasite have been described as species (Poelt and Ullrich, 1964).

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Chapter 4

LICHEN PROPAGULES

F. BRIAN PYATT

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I. Introduction

The wide distribution of lichens suggests that these organisms possess efficient means of dispersal. Indeed, quite a wide variety of propagules have been recognized, some characteristic of fungi in general and others unique to lichens. They include both sexual and vegetative types. Ascospores, for example, are produced by the fungal partner of the association and prior to discharge are contained within special chambers: apothecia or perithecia. In addition, there are two asexual spore types of fungal origin, these being small and large conidia, produced within chambers termed pycnidia (or sphaerogonia). The structure and cytological details of these organs are discussed in detail by Letrouit-Galinou in Chapter 2 of this book.

There is a great variation of types of vegetative propagules (or vegetative diaspores) produced by lichens. Such structures are composites containing both fungal and algal contributions. Certain of these propagules, namely, isidia and soredia, are of special interest because they are not produced by nonlichenized fungi. Other types of vegetative propagules include thallus fragments, whole thalli, squamules, and portions or whole lobes of the thallus. These structures are heavier than spores and are hence probably generally carried shorter distances.

Thus, potentially, the lichen thallus often has a variety of propagation "techniques" open to it, and this has led to a certain amount of controversy over the significance and likely success of the various types of propagules. But Gregory (1952) believes that the possession, by a fungus, of any organ should be taken as evidence that it does have some function.

II. Vegetative Propagules

A. Isidia and Soredia

The morphology of these unique lichen diaspores is described in Chapter I by Jahns. Their importance, or at least potential importance, in lichen dispersal has been stressed in most texts on lichenology. They have rather high frequency, at least in lichens in temperate regions (Hale, 1967).

Most work has been concerned with soredia. Animals have been recorded as agents of soredia dispersal by Darbshire (1897), Barkman (1958), Hale (1961), and Bailey (1970). Dispersal of soredia by wind has been discussed by des Abbayes (1951), Barkman (1958), Gregory (1961), Smith (1962), Haynes (1964), and Bailey (1966a). Brodie and Gregory (1953) experimentally showed soredia being removed by wind from *Cladonia* cups. Brodie (1951) and Bailey (1966a) demonstrated splash dispersal of soredia, and dispersal by water trickles has been recorded by Bailey (1968). Soredia are described as being water repellent; they are probably airborne and are likely to be carried farther than isidia but not as far as spores.

There are no known records of active soredia dispersal. Indeed, in all cases, an external source of energy must be applied to the thallus to facilitate soredia dispersal. From this it does appear that no rhythm of soredia dispersal can exist other than that in some ways rather unpredictable one imposed by the environment itself. The only control that the thallus can exert is with regard to the time of production of these propagules. And so it appears that wind dispersal of soredia could occur at a time when other conditions are unsuitable for further development. However, it can be argued that ascospores can be discharged during a favorable period and carried to a region where conditions are unsuitable for germination; but ascospores are able to survive adverse conditions in a state of dormancy while soredia frequently become infected during periods of enforced dormancy.

Regarding distances of soredia distribution, Bailey (1966a) illustrated that soredia of *Lecanora conizaeoides* are carried 61.0 cm by 2.2-mm-diameter "rain" drops falling 366 cm. He noted an increased wind speed was necessary for maximum soredia dispersal as the water content of the thallus increased.

Isidia, being much larger than soredia, probably have a more limited range, and perhaps play a more important role by increasing the surface area

TABLE I
ISIDIA REGENERATION ON THALLI OF *Parmelia saxatilis* EXPOSED TO SULFUR DIOXIDE^a

Concentration (vpm)	0	5	10
Percentage disks regenerating	15	11	5
Average number of isidia on each regenerating disk	2.8	1.7	1.2

^aFrom Pyatt (1969a).

of the thallus. Barkman (1958) points out that the isidia, which are not water repellent, are likely to be carried down the tree trunk by the stem flow of water. While most isidia are produced diffusely over the upper cortex of the thallus, Kershaw and Millbank (1970) describe the development of squamulose isidia from algal cells underneath the hypothecium of apothecial initials in *Peltigera aphthosa*.

Production and regeneration of isidia can be influenced adversely by air pollution, thereby reducing the reproductive capacity of lichens in polluted areas. Pyatt (1969a), for example, used 1-cm disks of *Parmelia saxatilis* from which isidia had been removed. The disks were left in a damp petri dish for 6 weeks, and he found that disks removed from material collected near a source of pollution showed less regeneration of isidia than material obtained farther from the pollution source. Disks from material collected from a nonpolluted area were maintained in Perspex boxes subjected to either pure air, 5 vpm million sulfur dioxide, or 10 vpm million sulfur dioxide. After 10 days the material was removed and placed in moist petri dishes for 4 weeks. From Table I, it can be seen that sulfur dioxide limits the ability of lichen disks to regenerate isidia.

B. Other Vegetative Propagules

Various other propagules have been described in lichens and are discussed in detail in Chapters 1 and 3. Most of these, as Barkman (1958) indicates, are the heavier type of vegetative diaspore and, while probably removed by wind, are subsequently carried only short distances. An early example is found in von Schrenk (1898), who recorded dispersal of thallus fragments of *Usnea barbata* and pointed out that pendulous forms of *Usnea* rarely produce apothecia.

III. Sexual Propagules

A. Introduction

Spores occur in a great variety of plant groups. Ascospores are found in both lichenized and nonlichenized fungi, and this type is the most frequently

encountered sexual spore in the lichens. The ascocarps of lichens are relatively long-lived structures and produce asci, generally at a certain time, each year. Production will continue until, according to Verseghy (1965), "the ascogen hyphae become exhausted."

The division of the ascomycetes into various categories based on hymenial structure (ascohymenial versus ascolocular) and on ascal dehiscence and wall structure (unitunicate versus bitunicate) is elaborated in Chapter 2 by Letrouit-Galinou and Chapter 3 by Poelt.

Dennis (1960), with reference to nonlichenized fungi, indicates that the bitunicate type of ascus generally has a short stalk and contains colored spores which are multiseptate or muriform. However, Richardson and Morgan-Jones (1964) illustrate that in lichens neither of these criteria can be applied. Indeed, they agree with Groenhardt (1962) that one can only be sure the ascus is bitunicate if both endoascus and exoascus can be shown separately and this is rarely possible in lichens.

In the bitunicate ascus the outer wall or exoascus is thin and inextensible while the inner wall or endoascus is thicker and extensible. Richardson and Morgan-Jones (1964) describe how, toward maturity, the exoascus ruptures and the inner wall extends to form a cylindrical sac. Consequently, the ascospores are carried above the surrounding immature asci. Finally, the ascospores are discharged through an aperture at the apex of the ascus.

1. ASCOCARPS*

Ascohymenial lichens possess apothecia or perithecia. Apothecia may be stalked or sessile, disk- or cup-shaped. The uppermost region is the fertile hymenium which contains asci in various stages of development and sterile paraphyses. Eventually, with the exception of the mazaedium of members of the Caliciales, the ascospores are actively discharged.

Ascolocular lichens have rather scattered bitunicate asci and no real hymenium. Organized fruiting bodies, which resemble perithecia, are termed pseudothecia.

2. NATURE OF THE SPORE

There are usually eight ascospores present in each ascus; however, one does occasionally find ascospores in lower numbers and in multiples of eight. Erbisch (1964) in a study of two species of *Pertusaria* found two spores present in each ascus. He points out that the fusion nucleus divides to form four nuclei. Spore walls are subsequently formed and each potential spore

*See also Chapter 2.

captures one to three of the nuclei. The nuclei further divide until at maturity each spore has approximately 100 nuclei.

However, the author has found more variation in the number of ascospores contained within each ascus. In the case of *Pertusaria pertusa* two ascospores are generally produced within each ascus; asci containing one to three ascospores have been observed. Furthermore, in the case of asci containing two ascospores, the ascospores occasionally differ greatly in size; probably this results from the unequal cleavage of the cytoplasm of the ascus.

Hale (1967) points out that the smallest spores are probably those of *Acarospora* which barely exceed 1 μm ; while the largest are 510 μm (Santesson, 1952) in the tropical lichen *Bacidia marginalis*. Spores lack sculpturing and may be colorless, brown, or greenish.

The following classification of spores according to septation is taken from Hale (1967), and some of these types are illustrated in Fig. 1.

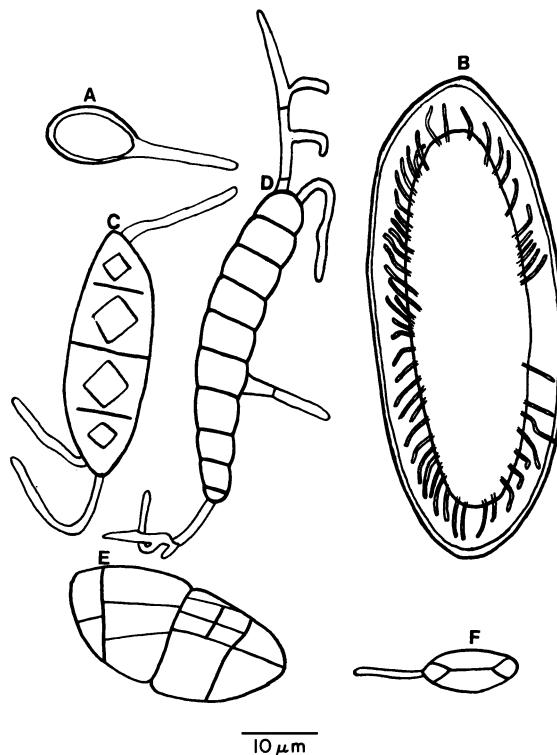


FIG. 1. Ascospore types.

- a. Simple spores: unicellular and unseptate, often small and thin walled (*Lecidea*, *Lecanora*, *Parmelia*, *Usnea*), more rarely very large (to 300 μm and thick walled (*Pertusaria*))
- b. Transversely septate spores: elongate and multicellular with 1–30 or 40 transverse cross walls (*Catillaria*, *Graphis*, *Pyrenula*)
- c. Muriform spores: multicellular with both transverse and longitudinal walls, often large (*Phaeographina*, *Lopadium*, *Umbilicaria*, *Diploschistes*)
- d. Polarilocular spores: two-celled spores with a thick median wall and a thin isthmus, or conversely a single-celled spore with a median constriction (*Teloschistaceae*).

Pyatt (1969a) has found much variation in spore sizes in a given species; e.g., those of *Caloplaca heppiana* measure 10.5–13.0 \times 6.3–8.4 μm , while those of *Rhizocarpon geographicum* measure 25–40 \times 11–18 μm . Septation is also a variable character. Figure 2 shows ascospores of *Rhizocarpon geographicum* drawn 1 hour after the start of sporulation (Pyatt, 1969a). It appears that the simplest type is that with three transverse septae and that further development occurs to give the more complex types. However, as the ascospores are discharged in various stages of septation, this does reflect some problems in the use of ascospores for species identification.

The multilayered sporangial wall of the fungus *Allomyces* has been described by Skucas (1967), and Reeves (1967) has described the fine structure

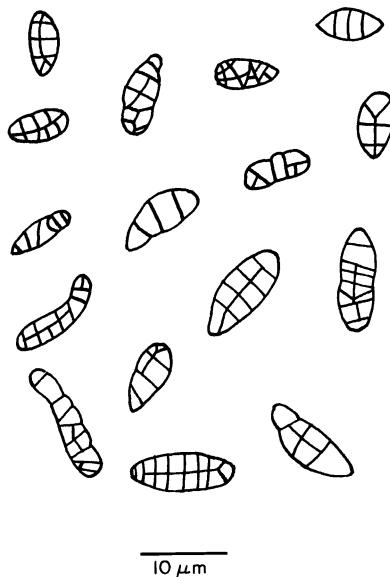


FIG. 2. Septation patterns in *Rhizocarpon geographicum*.

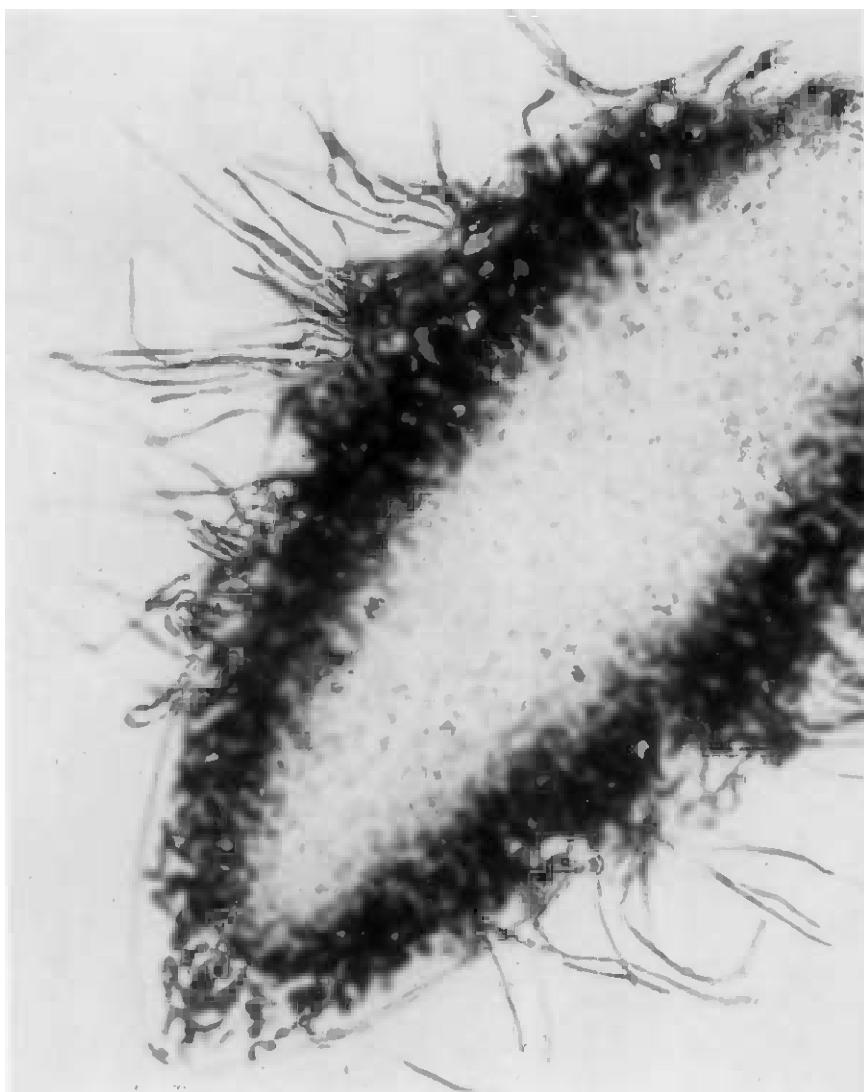


FIG. 3. Ascospore of *Pertusaria pertusa*.

of ascospore formation in *Pyronema domesticum*. Pyatt (1969b) investigated the ultrastructure of the ascospore wall of *Pertusaria pertusa* (Figs. 3 and 4). The ovoid ascospores measure $230 \times 60 \mu\text{m}$ and are surrounded by a well-defined wall $9 \mu\text{m}$ thick laterally and $11 \mu\text{m}$ thick at the poles. The wall is composed of five major layers, one of which is especially rich in regularly

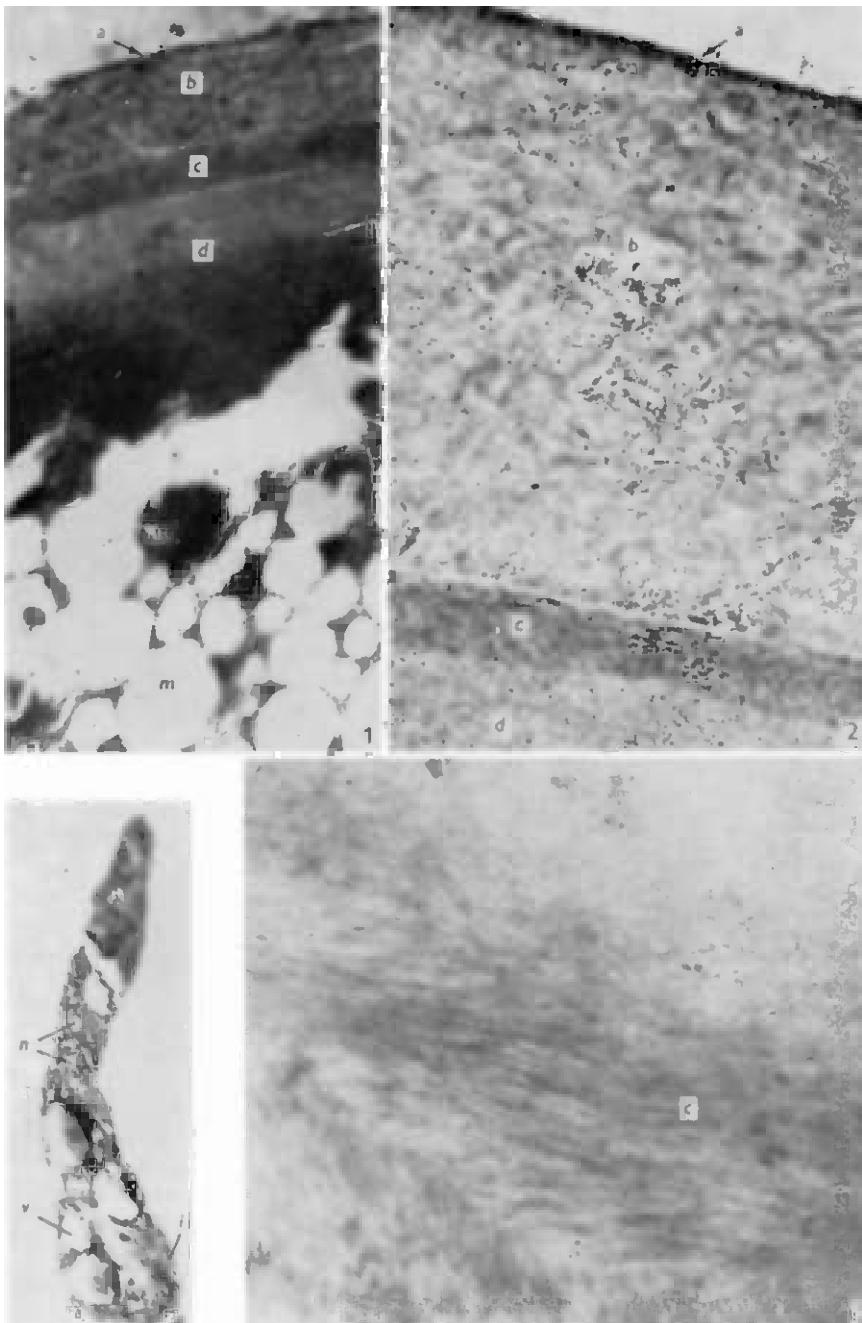


FIG. 4. *Pertusaria pertusa*. (1) Ascospore wall illustrating various layers. $\times 4000$. (2) Outer region of ascospore wall. $\times 16000$. (3) Layer with orientated microfibrils. $\times 22000$. (4) Germ tube. $\times 6000$. m, medulla; v, vacuoles; l, limiting membrane; n, nuclei. From Pyatt (1969b).

orientated, nearly concentrically arranged microfibrils. The germ tubes arise from the inside of the ascospore wall. Initially the germ tube rudiment has a bulbous base in which there is a single nucleus; this nucleus divides into two as the germ tube elongates. One nucleus remains in the base while the other migrates towards the apex of the germ tube where elongation is occurring. Hiltizer (1926) and Kofler (1957) give further accounts of spore discharge and germination.

The medulla of the ascospore is composed of numerous vacuoles associated with cytoplasm containing small and rather ill-defined nuclei.

Although ascomycetes make up the vast bulk of lichens, a few widespread mostly tropical species have a fungal component classified as a basidiomycete. They bear basidiospores exogenously on basidia (see Chapter 2 by Letrouit-Galinou for details).

Finally we have asexual conidia which are produced within pycnidia. Conidia are usually divided into two categories; the very small unicellular microconidia and the much larger, septate, macroconidia.

In all of the above cases the lichen thallus has the potential of producing vast numbers of spores; this spore production and subsequent sporulation must create a high energy demand.

B. Spore Discharge

1. INTRODUCTION

Gregory (1952) recognized two spore types in fungi: (a) sedentary, e.g., chlamydospores, zygospores. These permit the organism to survive periods of adverse conditions. These propagules typically germinate at their site of production; (b) dispersal, e.g., conidia, ascospores, and basidiospores. This is the category with which we are concerned as these are the spores which allow for colonization of new regions.

Gregory (1952) indicates that a variety of factors help to disperse these spores at random and he recognizes four types of dispersal: (i) active dispersal; (ii) dispersal by insects and other animals; (iii) rain dispersal; and (iv) wind dispersal.

In these cases he recognizes two stages of dispersal both of which are energy requiring: separation from parent stratum and transport to a distance.

2. TECHNIQUES

Probably the most useful piece of apparatus which can be used in studies of ascospore discharge is the spore train illustrated in Fig. 5A. This has been used for work on fungi and has been described by Ingold (1933), Walkey and Harvey (1966), and Pyatt (1969a).

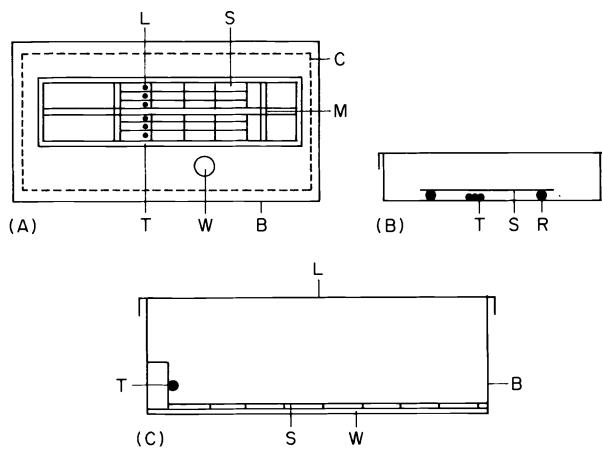


FIG. 5. (A) Spore train. (B) "Static" apparatus. (C) Apparatus to measure distance of spore discharge.

A "tram" (T) of microscope slides (S) is drawn, by a cord (M) leading to an electric motor, at a known rate across the lichen thalli (L) which are held on moist cotton wool. Ascospores are caught on the underside of the slides (S). The apparatus is enclosed within a Perspex box (B) and therefore there is no wind influence; humidity is maintained by the open water surfaces (W) and by moist cotton wool (C) placed around the edge of the box. The apparatus was kept within a growth chamber at a temperature of $14^\circ \pm 1^\circ\text{C}$. A time clock was used to obtain various light regimes. Another method is to make use of an automatic volumetric spore trap as described by Hirst (1952). In this case, spores are "sucked" from the substratum (following discharge) and impact on a microscope slide travelling at 2 mm/hour.

A far simpler "static" set up can occasionally be used (Fig. 5B). A portion of thallus (T) bearing ascocarps is placed into a damp petri dish. By means of a glass rod (R) a microscope slide (S) is suspended above the thallus. The slide is removed periodically and counts are made of the number of spores present.

3. NATURE OF SPORE DISCHARGE

a. DISTANCE. Brodie and Gregory (1953) made use of *Lycopodium* spores to illustrate that funnel or egg-cup-shaped vessels lost spores more readily than rectangular vessels or horizontal slides. They point out that wind passing over the cup creates enough turbulence inside to lift spores into the main airstream.

Bailey and Garrett (1968) found a maximum distance of discharge of

TABLE II
DISTANCE OF ASCOSPORE DISCHARGE^a

Species	Distance (mm)																			
	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39
<i>Xanthoria parietina</i>	41	320	893	2107	2309	2651	1509	983	324	41	17	3	0	0	0	0	0	0	0	0
<i>Enterographa crassa</i>	266	251	420	120	36	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecidea limitata</i>	129	381	397	386	261	209	186	111	59	23	9	3	0	0	0	0	0	0	0	0
<i>Graphis elegans</i>	0	1	11	25	25	28	34	31	42	42	35	19	8	8	0	2	1	2	0	0
<i>Lecanora atra</i>	15	32	128	319	362	845	649	491	287	185	39	35	11	0	2	0	0	0	0	0
<i>Caloplaca heppiana</i>	0	61	209	281	172	92	56	21	4	0	0	0	0	0	0	0	0	0	0	0

^aCalculated from four experiments.

45 mm in the case of *Rhizocarpon umbilicatum*. This figure applies to horizontal discharge in a closed system where there is no wind effect.

Pyatt (1969a) investigated the distance of ascospore discharge in six species using the apparatus illustrated in Fig. 5C. From Table II it can be seen that the maximum distance to which a spore was ejected was 35 mm in the case of *Graphis elegans*. However, maximum spore accumulations were usually found approximately 3–13 mm from the ascocarp. Distances of this order are unlikely to explain the colonization of new areas by lichens but this discharge would be sufficient to carry the ascospores from the substrate on which they were produced into the turbulent air.

Pyatt (1969a) found that the first discharged spores of *Lecidea limitata* travel up to 10 µm farther than later discharged spores. This is not a reflection of ascospore size but possibly the first discharged spores are expelled more forcibly.

b. RATES OF DISCHARGE. Bailey and Garrett (1968) found the rates of discharge to vary and that the total discharge declined rapidly over a period of 7 days from a peak within the first 24 hours. Pyatt (1969a) also found that ascospore discharge reached a peak 1–2 hours after initiation

TABLE III
HOURLY DISCHARGE RATES (AVERAGE OF FOUR REPLICATES)^a

Time	Species							
	<i>Lecanora conizaeoides</i>	<i>Pertusaria pertusa</i>	<i>Lecanora subfuscata</i>	<i>Graphis elegans</i>	<i>Peltigera polydactyla</i>	<i>Lecidea macrocarpa</i>	<i>Usnea florida</i>	<i>Xanthoria parietina</i>
0900	6030	9	4710	1476	3	0	41	0
1000	7220	86	960	977	1200	47	23	95
1100	3094	28	552	94	6310	23	16	390
1200	1753	11	119	112	4119	620	0	123
1300	1756	9	33	78	972	242	33	107
1400	1142	2	61	80	1058	201	23	81
1500	1101	4	24	63	491	163	11	26
1600	1446	14	11	10	422	81	54	12
1700	1060	10	70	21	312	80	41	4
1800	3826	2	460	435	130	90	46	320 ^b
1900	3843	10	330	294	1600	43	28	3109
2000	2305	12	240	79	389	10	21	294
2100	1364	8	136	54	312	8	11	98
2200	703	3	49	28	109	13	9	61

^aFrom Pyatt (1969a).

^bThallus placed in dry petri dishes.

by moistening air-dried thalli. After this the rate of discharge decreased irregularly (Table III). After 9 hours the portions of thalli were transferred to dry petri dishes. In *Lecanora conizaeoides*, *L. subfuscata*, *Graphis elegans*, *Peltigera polydactyla*, and *Xanthoria parietina* there was a significant increase (Table III) in the degree of spore discharge for 1–2 hours and then values fell; this does lend some support for the observation of Ahmadjian (1961) that ascospores are discharged as the ascus ruptures during drying. However, the observations of Bailey and Garrett (1968) and Pyatt (1968a) that there was massive sporulation following the addition of water to thalli casts much doubt on this.

c. RHYTHMS. Quite a considerable amount of work has been carried out on rhythms and periodicity of spore discharge in nonlichenized fungi by various workers, e.g., Manachère (1968), Carpenter (1949), Ingold and Cox (1955), and Gay *et al.* (1959). Walkey and Harvey (1968a, b) investigated the effect of factors such as climate and temperature on the spore discharge rhythms of pyrenomyctetes. Fahim (1966) carried out experiments to determine the effect of light and other factors on the sporulation of *Alternaria porri*. He found a high degree of sporulation after 2 hours exposure to sunlight followed by 48 hours incubation in the dark.

Pyatt (1968a) obtained the following data on rhythms of ascospore discharge in lichens.

Lecidea macrocarpa. With a 12/12 (light hours/dark hours) alternation at $14^\circ \pm 1^\circ\text{C}$, it exhibited a well-defined nocturnal rhythm (Fig. 6). When transferred to conditions of continuous light, the rhythm persists for a while, but eventually is depressed. From Table IV, it can be seen that

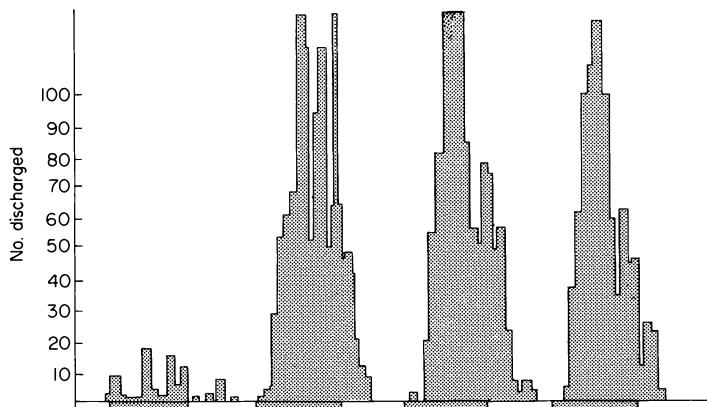


FIG. 6. Rhythm of sporulation in *Lecidea macrocarpa* under conditions of 12/12 light/dark alternation at $14^\circ \pm 1^\circ\text{C}$.

TABLE IV
AVERAGE SPORE DISCHARGE VALUES DURING THE LIGHT AND DARK PERIODS OF A
12/12 LIGHT HOURS/DARK HOURS ALTERNATION^a

Species	Light (hours)	Dark (hours)	Percentage
<i>Enterographa crassa</i>	273	482	(64% in dark)
<i>Lecidea macrocarpa</i>	113	359	(76% in dark)
<i>Pyrenula nitida</i>	112	336	(75% in dark)
<i>Graphis elegans</i>	657	1181	(64% in dark)
<i>Lecanora conizaeoides</i>	2636	1637	(62% in light)
<i>Caloplaca aurantia</i>	752	633	(55% in light)
<i>Protoblastenia rupestris</i>	837	1662	(66% in dark)

^aFrom Pyatt (1969a).

approximately three-quarters of the sporulation occurred during the night period.

Pyrenula nitida. In a 12/12 (light hours/dark hours) regime, no distinct rhythm of sporulation was apparent (Fig. 7).

Enterographa crassa. It shows some degree of nocturnal synchronization, but light was found to be essential for ascospore production and/or discharge.

Graphis elegans. No clear rhythm was found; indeed, it shifts from mainly daylight to mainly nocturnal discharge.

Peltigera canina. This tends to be a daylight species but it is greatly influenced by the availability of moisture.

i. Periodicity. Bailey and Garrett (1968) showed that *Lecanora conizaeoides* tends to discharge spores more readily at low temperatures. This agrees with Werner (1927) who indicated that winter and early spring are periods of maximum discharge in *Xanthoria parietina*.

Scott (1959), however, pointed out that spore discharge is affected by the moistening of apothecia by rain, dew, and mist. In temperate regions the annual distribution of these types of precipitation will, he believes, result

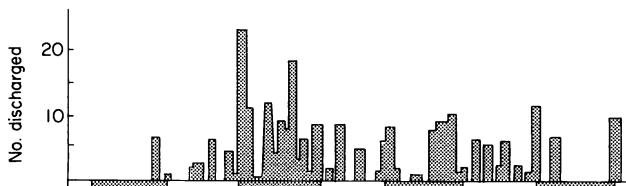


TABLE V
SPORE DISCHARGE IN LICHENS^{a,b}

Species	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Lecanora campestris</i>	P	P	L	L	L	L	L	L	M	P	P	P
<i>Lecanora conizaeoides</i>	R	R	L	L	L	L	M	M	M	P	M	M
<i>Lecanora atra</i>	P	P	M	M	L	L	M	M	M	P	P	P
<i>Lecidea limitata</i>	P	P	L	M	M	L	L	L	M	P	P	P
<i>Lecidea macrocarpa</i>	P	P	P	P	P	M	M	M	M	P	P	P
<i>Baeomyces rufus</i>	—	—	M	M	—	—	M	M	M	M	P	M
<i>Ochrolechia parella</i>	P	P	L	M	L	—	—	—	M	P	P	P
<i>Pertusaria pertusa</i>	P	—	—	L	M	M	—	L	M	P	P	P
<i>Xanthoria parietina</i>	P	P	L	L	M	M	M	M	M	P	P	P
<i>Caloplaca heppiana</i>	P	M	L	L	L	M	M	M	M	M	P	P
<i>Toninia coeruleonigricans</i>	P	L	M	M	P	P	M	M	M	M	P	P
<i>Buellia canescens</i>	N	N	N	N	N	N	N	L	M	P	P	P
<i>Graphis</i> spp.	P	P	L	M	L	M	P	M	L	M	P	P

^aFrom Pyatt (1969c).

^bLetters designate sporulation, i.e., R = 0–50, L = 50–200, M = 200–1000, P = 1000 or more spores discharged. N refers to the absence of ascocarps and a dash (—) to the absence of sporulation.

in maximum sporulation during spring and autumn. Verseghy (1965) agrees that the amount of rainfall undoubtedly influences the spore production of lichens and found that in dry years the asci are sterile in both spring and autumn. Also she noted that in rainy years the spore production is intensive in autumn. Des Abbayes (1951) reached the conclusion that in western Europe sporulation is most pronounced in the spring.

Pyatt (1969c), in a monthly investigation over 1 year, found a fairly distinct annual periodicity of spore discharge (Table V), with peaks generally occurring during the period from October to January inclusive.

ii. *Propagule Types.* Ascospore discharge alone will not lead to the production of new thalli; for this to occur the ascospore must come into contact with a certain specific algal cell. Obviously, it seems most probable that this chance encounter will not always occur. However, in the case of *Pertusaria pertusa* and *Lecidea limitata* (Pyatt, 1969a), and perhaps other species, the fungus–alga association is not always lost even during sporulation.

Sections of verrucae (Fig. 8), taken after exposure of ascocarps to various humidities, reveal that the gelatinous matrix or hymenial gelatin within the apothecium cavity tends to shrink as the humidity decreases. From Fig. 8 it can be seen that there are aggregations of algal cells (*Protococcus*) in the

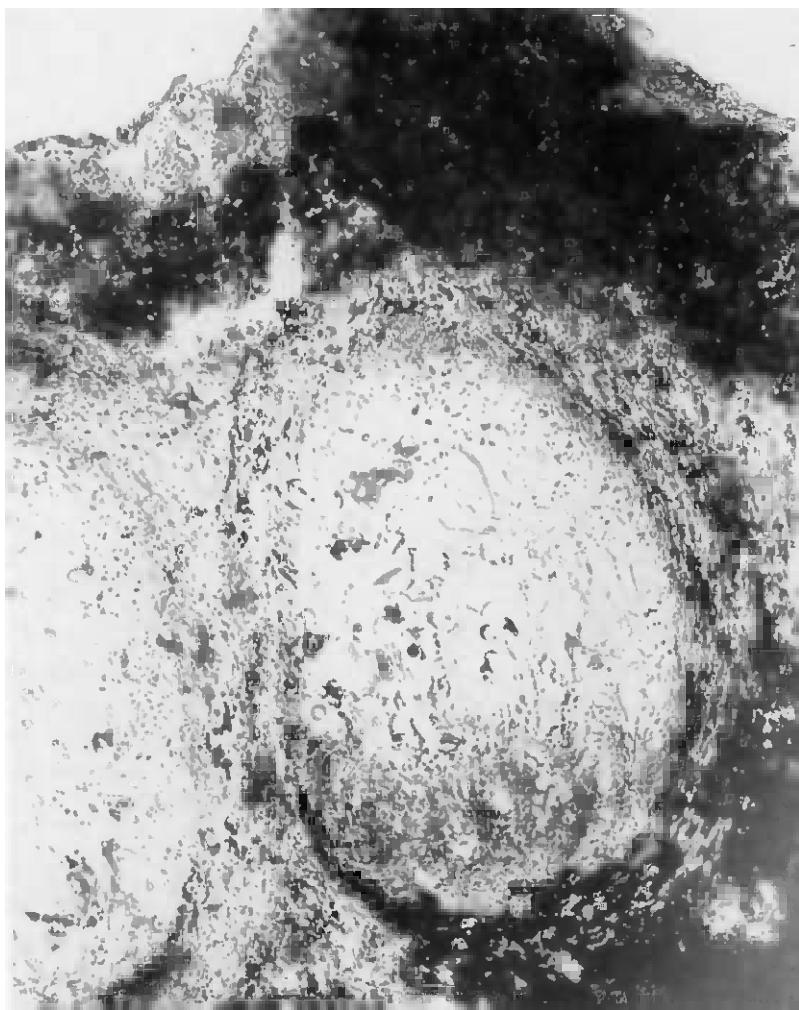


FIG. 8. Section through verruca *Pertusaria pertusa*.

ostiole region. It appears, then, that at high humidity the gelatinous matrix within the apothecium swells and aids in forcing the large ascospores out through the ostiole. As the ascospores escape, they frequently pick up algal cells from the ostiole region. An average of 4% of the ascospores examined on slide deposits carried algae (Fig. 9). Of these ascospores approximately 60% carried one algal cell, 30% carried two, and 10% carried three or more.

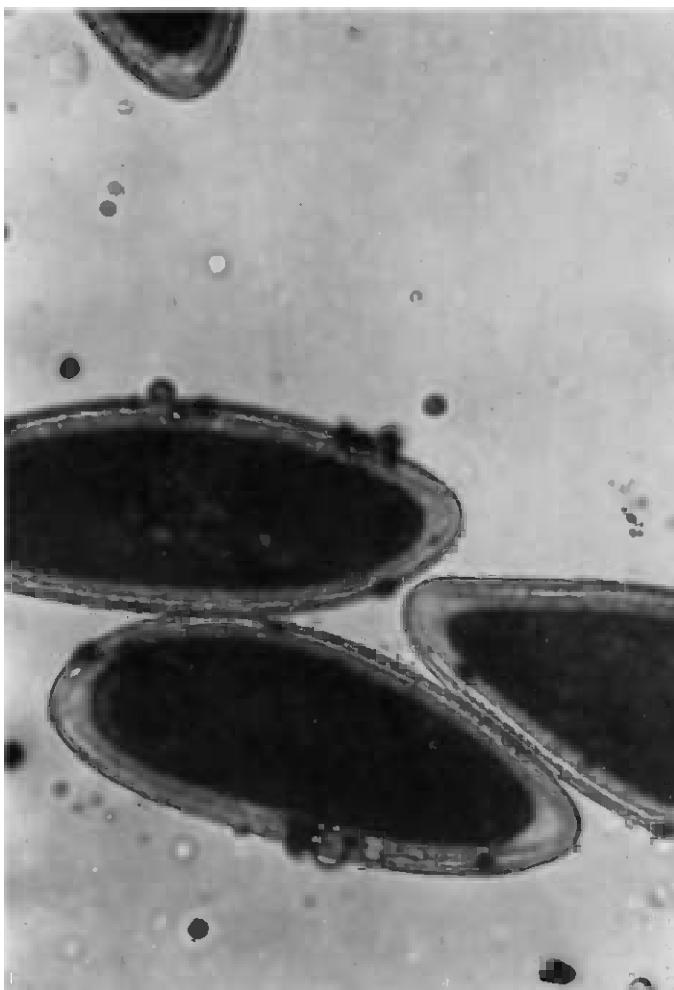


FIG. 9. Ascospore of *P. pertusa* with associated algae.

Thus, in the case of *Pertusaria pertusa*, reestablishment of the composite thallus is likely to occur quite frequently due to the discharge of a joint propagule

F. B. Pyatt (unpublished) has investigated ascospore discharge in *Xanthoria parietina*. A number of spore deposits were collected, in fairly dry petri dishes, and these were investigated to determine the average number of ascospores present in each grouping. From Table VI it is apparent that usually the propagule contains eight ascospores.

TABLE VI
ASCOSPORE GROUPINGS IN *Xanthoria parietina*^a

Number of ascospores associated (on slide deposit)	Percentage occurrence
1	1.0
2	1.2
3	1.2
4	1.0
5	2.2
6	3.6
7	2.2
8	74.2
12	0.6
16	3.4
22	0.2
24	1.6
50	7.6

^aFrom Pyatt (1969a).

Pyatt and Harvey (1973) have studied the fungus *Trichothecium erraticum* which occurs on thalli and apothecia of *Caloplaca heppiana* and *Lecanora campestris*. When infected thalli were enclosed within damp petri dishes, ascospores of both the host and pathogen were discharged within two days. They used the spore-train technique and observed that the spore discharge of both *Caloplaca heppiana* and *Trichothecium erraticum* generally occurred between 10 PM, and 6 AM, i.e., both were nocturnal species.

From an examination of an infected apothecium of *Caloplaca heppiana*, it was found that discharged ascospores of the parasite were present on the hymenial surface of the host. Indeed it does seem possible that the smaller spores of *Trichothecium erraticum* ($2-2.5 \times 6-6.5 \mu\text{m}$) may be picked up by the larger spores of *Caloplaca heppiana* ($10.5-13.0 \times 6.3-8.4 \mu\text{m}$) at the moment of discharge to yield a composite propagule.

iii. *Animal Dispersal.* A somewhat unusual method of ascospore distribution has been described by Pyatt (1968d). Rotifers living on the surfaces of apothecia of *Xanthoria parietina* actively ingest ascospores. Possibly these rotifers may be splashed off and could then continue to move in a water film. It was observed that there was a 15% germination of excreted ascospores in those rotifers which had contained 20 or more spores, whereas in those which contained only 3-8 ascospores the voided spores were broken and did not germinate. Gregory (1952) describes how the spores of coprophilous nonlichenized fungi such as *Sphaerophorus* are deposited on vegetation and are later eaten by animals.

4. FACTORS AFFECTING SPORE DISCHARGE

a. LIGHT. Leach (1962) and Leach and Trione (1965) showed the importance of ultraviolet radiation in inducing sporulation in a number of fungi, and have illustrated that the 230 and 290 nm regions of the spectrum are particularly effective. Table IV, from Pyatt (1969a), illustrates the effect of light on lichen sporulation.

b. TEMPERATURE. Ingold (1960) suggested that in most pyrenomyctes, sporulation is probably arrested below 5°C but increases in rate up to 35°C. He concluded that temperature is probably most important in connection with spore maturation.

However, Hart (1926) found that at any given relative humidity, an increase of temperature generally leads to a decrease in the viability of fungus spores, and the lower temperatures, above 0°C, favor longevity. Pyatt (1969a) found that extremes of temperature repressed spore discharge and that in certain species there was more sporulation at 20°C than at 6°C.

c. HUMIDITY AND RAIN. Verseghy (1965) points out that water, which is absorbed over the entire surface of the thallus, is stored in capillaries mostly among the hyphae and so the substratum is less significant in lichens for the absorption of water; the relative humidity of the environment, she notes, is much more important. Her findings are largely in agreement with those of Blum (1964).

Ascospore discharge has seldom been observed when dry thalli are placed in dry petri dishes. Indeed, Pyatt (1969a) showed that spore discharge rarely occurs in *Pertusaria pertusa* below a relative humidity of 90%.

Pyatt (1968a) showed that when *Pyrenula nitida* and *Peltigera canina* were sprayed with fine water droplets there was a massive liberation of spores irrespective of the time or the light regime. Gregory *et al.* (1959) have shown that fungus spores can be dispersed by splashes.

Fahim (1966), working with *Alternaria porri*, found the optimum relative humidity for sporulation to be less than 100%. His data agree with those of Cochrane (1958), who indicates that most evidence suggests a generalization that in most fungi reproductive processes are favored by a moderate as distinct from a high atmospheric humidity.

Following the work of Ingold and Marshall (1962) on stimulation of spore discharge by reduced humidity in *Sordaria*, Austin (1968) investigated the effects of air speed and humidity changes on spore discharge in *Sordaria fimicola*. She found that a reduction of the relative humidity from 95% to 70% or 35% leads to an immediate but temporary increase in discharge rate (see Table III). A relative humidity of 70% apparently only affected actual discharge, while prolonged exposure to 35% relative humidity interferes as

TABLE VII
EFFECTS OF PRECIPITATION ON SPORULATION^a

Species	Effect of dew	Effect of flooding
<i>Xanthoria parietina</i>	Many spores released	Many spores released
<i>Pertusaria pertusa</i>	Very few released	Very few released
<i>Lecanora conizaeoides</i>	Spores and algae released	Spores and algae released
<i>Lecanora subfuscata</i>	Spores released	Spores released
<i>Ochrolechia androgyna</i>	Spores and algae released	Spores and algae released
<i>Usnea florida</i>	Spores released	Spores released
<i>Lecidea limitata</i>	Spores released	Spores released
<i>Graphis scripta</i>	Spores released	Spores released

^aFrom Pyatt (1969a).

well with earlier stages in spore development. She concludes that the immediate effect on discharge is physical, not biochemical, and that the perithecial wall seems to play some role in the control of spore discharge.

Pyatt (1969a) carried out additional experiments by enclosing lichen thalli in petri dishes containing water to maintain a humid atmosphere. In some cases droplets of water were pipetted and positioned over ascocarps, i.e., synthetic dew formation. In other cases the thalli were flooded. From Table VII it can be seen that these forms of precipitation do encourage sporulation.

d. WIND. Gregory (1952) points out that in a wind tunnel, at wind speeds in excess of 1 meter/second, the effects of gravity on objects as small as spores are negligible.

Kelly *et al.* (1951) used an airplane to trap spores and illustrated that convection and turbulence are sufficient to carry spores to great heights in the atmosphere.

The problem of spore removal by wind can be solved in two main ways: (a) by raising the stalk of the apothecium through the laminar boundary layer into the turbulent air, as in stipitate *Calicium*; (b) by active discharge into the turbulent air. Once this has occurred, long-distance spore dispersal can occur.

Gregory (1952) found at the same time that under normal turbulence conditions, 99.9% of the spores are deposited within 100 meters of the source. Eventually the spore is deposited, and Gregory points out that the principal methods are sedimentation, impaction, boundary-layer exchange, turbulent deposition, and rain washing.

e. pH. This has been investigated by Pyatt (1968a, 1969a) in an initial study using both citric acid and phosphate buffer solutions. With *Xanthoria*

parietina a distinct optimum for sporulation occurred at pH 4–5. This is illustrated by the duration of ascospore production and by the total number of ascospores produced. *Graphis elegans* has a fairly distinct optimum for spore discharge at pH 5–6.

f. SPOROGENIC SUBSTANCES. Trione, *et al.* (1966) found that certain substances affected sporulation. Whittington and Penn (1967) used camphor vapor as it has been shown to increase the frequency of diploidization in vegetative hyphae (Roper, 1952). They also found that exposure resulted in a definite increase in the number of aberrant asci.

g. MISCELLANEOUS. It has been indicated (Pyatt, 1968a) that ascospore discharge is inhibited in some species when the thallus is maintained upon a nutrient rich substrate. Atmospheric pollutants can also have an effect. Pyatt (1969a) exposed lichen thalli to 10 vpm of sulfur dioxide for a period of 10 days in sealed jars. From the results in Table VIII it is apparent that sulfur dioxide inhibited the sporulation of the species investigated.

Aggregation of spores may give spores in groups a degree of protection against certain environmental conditions. This can be shown by collecting spore deposits of *Lecanora conizaeoides* and *L. subfuscata* on microscope slides. As slides dried the spores moved distances of up to 80 μm until large groups of ascospores were obtained. When these slides were placed in a humid atmosphere the spores separated.

C. Spore Germination

1. INTRODUCTION

Schein and Rotem (1965) have demonstrated that the "germinability" of the spores of nonlichenized fungi is inversely proportional to both temperature and atmospheric humidity, and Teitel (1958) indicated that the length of life of spores is affected by environmental conditions.

Fox (1966), working with *Ramalina ecklonii*, found that the ascospores did not germinate in less than 5 weeks of incubation in a wide variety of

TABLE VIII
EFFECT OF SULFUR DIOXIDE ON SPORULATION^a

Species	Thalli exposed to SO ₂	Control
<i>Pertusaria pertusa</i>	No sporulation	Sporulation
<i>Xanthoria parietina</i>	No sporulation	Prolific sporulation
<i>Lecidea limitata</i>	Slight sporulation	Prolific sporulation
<i>Graphis elegans</i>	No sporulation	Prolific sporulation

^aFrom Pyatt (1969a).

cultural conditions. He found no difference in germination rates between cultures incubated in light or darkness.

Recent work on spore germination in lichens has been done by Bailey (1966b), who examined discharged spores to see if germination had occurred. This was also done by Garrett (1968). Pyatt (1968a) showed that ascospore germination in a water film generally commences within 4–5 days after the start of sporulation. He also noted that ascospores of crustose species generally tend to have a higher percentage viability than ascospores of foliose and fruticose types. It is conceivable that in these latter types, the elevation of the thallus into the turbulent boundary layer of the atmosphere favors soredia dispersal and dispersal of thallus fragments. In the case of crustose species, thallus fragmentation is probably less likely to occur and the active discharge of ascospores must be considered an important attribute.

2. RHYTHMS AND PERIODICITY

Pyatt (1969c) found that massive sporulation is not invariably associated with high percentage germination values; see Tables V and IX. The following data can be seen in Fig. 10:

Lecanora campestris. Maximum germination values of 30% occurred in October, December, and January; this is also the period of maximum spore discharge.

Lecanora conizaeoides. Spore germination, within the 10-day test period, was only observed in December.

Lecanora atra. Prolific discharge occurs from October to February; during this period the peak values of germination are 40%. After spore dis-

TABLE IX
ASCOSPORE GERMINATION SHOWING THE AVERAGE PERCENTAGE VALUES
OBTAINED EACH MONTH^a

Species	Percentage germination after 10 days ^b											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Lecanora conizaeoides</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>Pertusaria pertusa</i>	60	—	—	2	5	0	—	2	2	0	26	40
<i>Xanthoria parietina</i>	0	0	0	0	0	0	0	0	0	6	0	0
<i>Caloplaca heppiana</i>	0	0	1	2	0	0	0	0	9	15	20	15
<i>Toninia coeruleonigricans</i>	10	0	0	0	0	0	0	0	0	0	0	10
<i>Buellia canescens</i>	N	N	N	N	N	N	N	5	10	30	30	20

^aFrom Pyatt (1969c).

^bN refers to the absence of ascocarps and a dash to the lack of spore discharge.

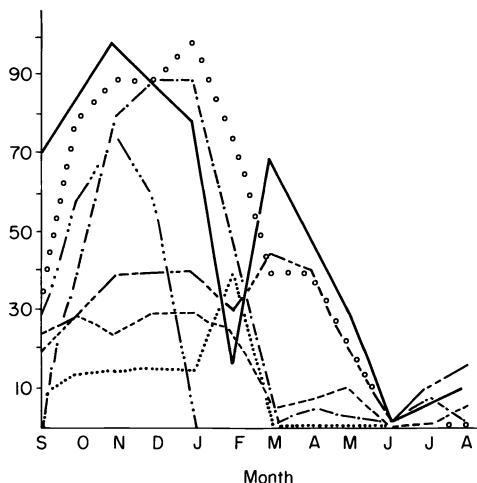


FIG. 10. Percentage of spores germinating after 10 days (monthly figures). The species are *Lecanora campestris* (—), *L. atra* (— — —), *Lecidea limitata* (....), *L. macrocarpa* (—), *Ochrolechia parella* (○ ○ ○), *Graphis* spp. (— · — · —), and *Baeomyces rufus* (— · — · — · —).

charge has decreased in March, spore germination reaches a peak of 45%. During spring, spore discharge and germination drop and then begin to increase in July.

Lecidea limitata. Sporulates from October to May. During November germination is 100%.

Baeomyces rufus. Prolific spore discharge occurs during November and this is when the highest germination values are found (75%). Discharge did occur but with no germination in March and April. Hence it appears that in this species, ascospores produced during March and April undergo a period of dormancy prior to germination.

Ochrolechia parella. Prolific sporulation occurs during October to February; germination increases from 80% in October to 100% in January and then falls. Pyatt (1968b) noted that some germ tubes tend to swell during their passage through the episore.

Pertusaria pertusa. The germination of the ascospore of this species has been described by Pyatt (1968c). Prolific spore discharge occurs during October to January and germination increases from zero in October to 60% in January.

Xanthoria parietina. Germination was only observed in October which is part of the period of maximum discharge.

Caloplaca heppiana. Prolific spore discharge is limited to November, December, and January, and maximum germination (20%) occurs in November.

Toninia coeruleonigricans. There are peaks of sporulation in November to January and May to June. However, there was only one peak value (10%) of germination which occurred during December and January.

Buellia canescens. Peak germination values of 30% occurred during the period of prolific discharge.

Graphis spp. Prolific sporulation occurs from November to February and peak germination values (90%) occur in December and January.

Times for germination have been investigated by various workers and the following are some of the results obtained: 10–24 hours, Ahmadjian (1961); 24–144 hours, Kofler and Bouzon (1961); and 10 days or less, Henriksson (1958).

3. TEMPERATURE AND HUMIDITY

Merek and Fergus (1954) found that the endoconidia of *Endoconidiophora fagacearum* remained viable longest under dry conditions. Groom and Panniseet (1933) noted that lower relative humidities favor retention of viability of specific fungus spores. Cochrane (1958) showed that longevity increases as temperature decreases to 0°C. Cochrane (1945) and Hart (1926) indicate that uredospores of rust fungi have greatest longevity at moderate relative humidities.

4. pH

Pyatt (1968a) found that the pH optimum for ascospore germination was not always the same as that of sporulation and that material collected from different areas showed somewhat different responses.

5. NUTRIENTS AND ALGAE

Scott (1959) found with *Peltigera praetextata* that an aqueous extract of the phycobiont must be present for successful germination.

Lösel (1967), working with the fungus *Agaricus bisporus*, noted that germination was stimulated by vapor diffusing from dilute solutions of various short-chain fatty acids. Lösel suggests that this is not a pH effect, but is due to the entry of the compounds into metabolic pathways.

Pyatt (1968a) investigated ascospore germination on various agars. He found that media with a low nutrient content are more stimulatory to the early stages of ascospore germination than those media with a high nutrient content.

Pyatt (1969a) collected ascopores on agar infused with extracts of the bark on which the lichens were growing. The extracts were prepared by

TABLE X
EFFECT OF BARK EXTRACT ON ASCOSPORE GERMINATION^a

Species	Percent germination	
	Agar and bark extract	Plain agar
<i>Pertusaria pertusa</i>	15	9
<i>Thelotrema lepadinum</i>	100	100
<i>Lecanora subfuscata</i>	92	70

^aFrom Pyatt (1969a).

washing a portion of the bark which was then macerated and finally filtered. The results are shown in Table X.

The condition of sporostasis, where spores fail to germinate on account of inhibitors produced by the parent culture or cultures of another species, has been described by Robinson and Park (1966). This condition is sometimes found to depend upon the continuous production of a volatile substance by mycelium.

Another kind of investigation was carried out (Pyatt, 1969a) to determine whether extracts of a given lichen could suppress the germination of an ascospore of another species (Table XI). Ascospore germination is suppressed in *Xanthoria parietina* by *Parmelia physodes* and *Ochrolechia parella* extracts; in *Pertusaria pertusa* by *Usnea comosa* and *Xanthoria parietina* extracts. It appears from this initial experiment that extracts of lichens from the habitat of the species being tested are able to inhibit the germination of ascospores from such areas.

TABLE XI
EFFECT OF OTHER SPECIES ON ASCOSPORE GERMINATION^a

Species	Extract ^{b,c}						
	C	1	2	3	4	5	6
<i>Xanthoria parietina</i>	10	10	4	4	10	—	—
<i>Pertusaria pertusa</i>	10	—	—	10	—	0	0

^aFrom Pyatt (1969c).

^bExtracts were prepared by washing portions of thalli and then macerating them in water; the ones used are water (C), *Lobaria pulmonaria* (1), *Parmelia physodes* (2), *Ochrolechia parella* (3), *O. androgyna* (4), *Usnea comosa* (5), *Xanthoria parietina* (6).

^cResults are expressed in terms of percentage germination.

TABLE XII
PERCENTAGE VIABILITY AFTER DESICCATION (AVERAGE OF FOUR REPLICATES)^a

Weeks	Species								
	<i>Lecanora conizaeoides</i>	<i>Lecanora subfuscata</i>	<i>Graphis elegans</i>	<i>Lecidea limitata</i>	<i>Peltigera polydactyla</i>	<i>Lecidea macrocarpa</i>	<i>Usnea florida</i>	<i>Pertusaria pertusa</i>	<i>Xanthoria parietina</i>
0	5	20	60	10	0	75	0	16	0
1	0	18	43	10	0	59	0	24	0
2	0	18	39	9	0	29	0	20	0
3	0	14	35	9	0	12	0	19	0
4	0	12	20	8	0	7	0	21	0
5	0	15	18	9	0	7	0	7	0
6	0	13	19	7	0	5	0	8	0
7	0	9	11	4	0	6	0	8	0
8	0	9	4	5	0	5	0	7	0

^aFrom Pyatt (1969a).

6. POLLUTION

Pyatt (1969a) calculated that the average germination of ascospores of *Lecidea macrocarpa*, collected from near the pollution source in the steel producing town of Port Talbot, was 62% after 14 days in a humid atmosphere. The value with material collected from a more distant site was 79%. This does seem to indicate that air pollution probably adversely affects the viability of lichen ascospores and so possibly those lichen species propagated by ascospores are less likely to colonize heavily polluted regions than species propagated by other, perhaps more resistant, means.

7. PERCENTAGE VIABILITY OF ASCOSPORES AFTER DESICCATION

Pyatt (1969a) placed ascospore deposits in dry, silicone grease sealed petri dishes for varying amounts of time. They were eventually transferred to a humid atmosphere and the percentage viability was calculated after 8 days of exposure. From Table XII it can be seen that the viability of ascospores falls with extended periods of desiccation.

Acknowledgments

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Chapter 5

FINE STRUCTURE

Elisabeth Peveling

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I. Introduction

Significant electron microscopic investigations of lichens did not begin until a few years ago. This is late compared with other organisms. The late start was due primarily to the technical difficulties in the preparation of lichen thalli. A lichen thallus is a heterogeneous structure that consists of two different symbionts, each one usually requiring a different preparative treatment. In addition, the fungal hyphae are arranged in layers of different densities and contain intercellular material which may be gelatinous, fibrillar, or crystalline. Despite these obstacles, the progress of lichen ultrastructure has been rapid and our knowledge in this area is approaching that in

lichen taxonomy and physiology. We know many details of the substructure of the vegetative organs of lichens. Of particular interest are cellular structures that are unique to the lichen symbiosis and those which constitute the new morphological entity we recognize as the lichen thallus.

In addition to cellular structures, the different number and composition of layers in the cell wall of the symbionts, the distribution of lichen acid crystals, and the arrangement of the algal cells and fungal hyphae in the thallus provide considerable variation among lichens. The scanning electron microscope (SEM) reveals more variety in the micromorphology of thalli. The SEM gives a good impression of the three-dimensional inner organization of the thallus, its surface structure, and the structures by which thalli adhere to the substrate. The freeze-etch technique has been another method used to examine lichen ultrastructure (Ellis and Brown, 1972).

This chapter summarizes the submicroscopical investigations on lichens. The substructure of phycobionts and mycobionts and their symbiotic relationships are considered as well as changes in substructure due to different ecological conditions. Thallus surface structures are illustrated with the scanning electron microscope.

II. Cellular Structure of the Symbionts

A. Protoplast of the Lichenized Phycobionts

Of the 26 genera of lichenized algae (Ahmadjian, 1967), the common blue-green phycobionts *Nostoc*, *Gloeocapsa*, and *Scytonema* and the most important green phycobionts *Trebouxia*, *Coccomyxa*, *Myrmecia*, and *Stichococcus* have been studied with the electron microscope. A general feature of these phycobionts is their smaller size relative to free-living or cultured forms. An exception to this is *Stichococcus* which enlarges considerably when lichenized.

1. BLUE-GREEN PHYCOBIONTS

Nostoc phycobionts have been studied in *Peltigera polydactyla* (Peat, 1968), *Peltigera canina*, *P. rufescens*, and *Leptogium hildenbrandii* (Peveling, 1969c). *Gloeocapsa* has been examined in *Gonohymenia mesopotamica* and *G. sinaica* (Paran *et al.*, 1971). *Scytonema* was described in *Heppia lutosa* (Ahmadjian, 1967) and *Cora pavonia* (Roskin, 1970).

In the phycobionts studied, a difference between centroplasma and chromatoplasm is visible. The two main components of the protoplast are not restricted completely to the central and peripheral areas of the cells. The chromatoplasm with its thylakoids can extend almost to the

middle of the cell. Thus, the thylakoids show two characteristic positions. One part of the thylakoids has a tendency to run parallel to the cell wall (Fig. 1). Other thylakoids are directed in more or less distinct curves to the center of the cell. In this case four to seven single, long, parallel thylakoids often form layers of lamellae. Sometimes reticulations occur between the lamellae parallel to the cell wall (Fig. 1). In mature cells of *Gloeocapsa* less closely packed thylakoids were described (Paran *et al.* 1971). Also, the thylakoids in the *Nostoc* of *Leptogium hildenbrandii* may be swollen to small vesicles. Such vesicular thylakoids seem to be characteristic of degenerating cells since they appear regularly with vacuoles of different sizes in the protoplast.

In contrast to free-living blue-green algae, the lichenized forms have many osmiophilic globules scattered between the thylakoids. In the symbionts of *Peltigera canina*, *P. polydactyla*, and *P. rufescens* the globules always are single and have a diameter of 60–100 nm. In the *Nostoc* phycobiont of *Leptogium hildenbrandii*, they are in groups of three to eight and their diameter is about 50 nm. *Gloeocapsa* cells have similar osmiophilic bodies with a less regular shape (Paran *et al.*, 1971).

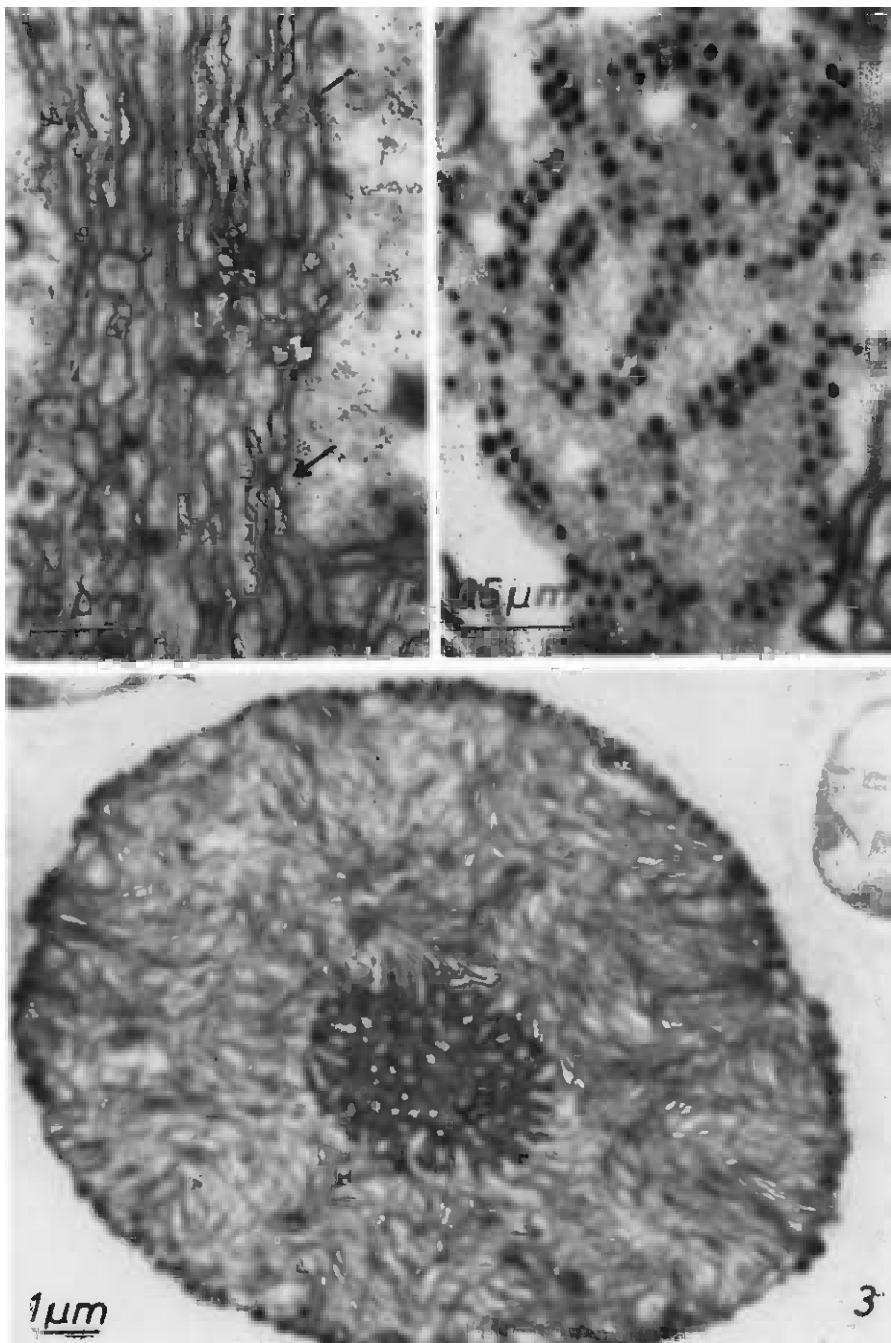
Besides the dense osmiophilic globules there are other positively or negatively stained bodies in the protoplast which appear mainly in the centroplasm. The function and origin of these different cellular inclusions are unknown.

Heterocysts were found occasionally in the *Nostoc* symbionts of *Leptogium hildenbrandii* (Peveling, 1969c). They differ from other cells by a poor differentiation of thylakoids. The lamellae are short and few in number but many osmiophilic globuli are present.

The main difference in protoplast differentiation between blue-green phycobionts and their free-living counterparts is the occurrence of osmiophilic globules.

2. GREEN PHYCOBIONTS

Among the Chlorophyceae, the chlorococcalean alga *Trebouxia* is the most frequent phycobiont. Table I lists those lichens with *Trebouxia* whose ultrastructure has been studied and all other lichens which have been investigated with the electron microscope. *Trebouxia* is a unicellular alga, and its cells are characterized by a large chloroplast surrounded by a small area of cytoplasm. *Trebouxia* has been divided into two groups according to the shape of the chloroplast and its position during division (Ahmadjian, 1960; Jacobs and Ahmadjian, 1968). *Trebouxia* I, which is common in fruticose lichens, has an irregular chloroplast with deep inlets. During division the chloroplast segments take a parietal position against the cell wall. *Trebouxia* II, which occurs mostly in foliose and crustose lichens, has a chloroplast



margin which is smoother than that of *Trebouxia* I and the chloroplast segments at division do not assume a parietal position.

A common feature of the *Trebouxia* chloroplast is the high number of thylakoids (Fig. 3). Examined in detail one can observe many differences in the arrangement of thylakoids. The lamellar system may consist of numerous long thylakoids, which cross the chloroplast as single structures or pile up to form stacks. Typical grana and stroma thylakoids are usually absent in these chloroplasts (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969; Peveling, 1969a; Galun *et al.*, 1970a). A thylakoid arrangement similar to that of higher plants was found in the *Trebouxia* of *Caloplaca aurantia* var. *aurantia* where a granalike organization of five to ten thylakoids was described (Ben-Shaul *et al.*, 1969). In one *Cladonia* species the *Trebouxia* phycobiont has a chloroplast with a distinct differentiation of grana and stroma thylakoids (Peveling, 1969a). Most *Trebouxia* phycobionts have a chloroplast which is filled with many close-lying thylakoids. These lamellae are not long straight structures but always appear rather short and are often swollen to form irregular vesicles. At present, we are unable to say if the differences in the *Trebouxia* chloroplast reflect different species or if they are the result of specific physiological conditions.

All *Trebouxia* chloroplasts have in their center a large pyrenoid which is traversed by thylakoids. The thylakoids may cross the pyrenoid as parallel lines (Galun *et al.*, 1970a), as long curves (Fig. 2) (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969; Peveling, 1969a), or they may widen into broad channels (Fig. 3). Fisher and Lang (1971a) described pyrenoid vesicles in *Trebouxia* of *Ramalina menziesii* and *R. reticulata*. These vesicles show a circular or a flattened profile and surround an electron transparent intra-vesicular space. Serial sections reveal that the vesicles are interconnected.

The pyrenoid matrix also contains dense osmiophilic globules which are aligned along the thylakoids. These globules—ranging in diameter from 40–100 nm—have been observed in all investigated *Trebouxia* cells except those of *Usnea rockii* and *U. pruinosa* (Chervin *et al.*, 1968). The typical dense osmiophilic globular appearance becomes visible only after fixation with osmium tetroxide or glutaraldehyde followed by osmium tetroxide. With permanganate the globules are more or less electron transparent and become somewhat angular (Handley *et al.*, 1969; Fisher and Lang, 1971a). After this fixation, the globules seem surrounded by a single membrane. Usually, the

FIGS. 1–3. Fig. 1, thylakoids parallel to the cell wall between two *Nostoc* cells in *Peltigera canina*. Arrows indicate reticulations of the thylakoids; Fig. 2, pyrenoid of *Trebouxia* cell from *Cladonia fimbriata*. Aligned on the curved thylakoids are pyrenoglobuli which are located directly opposite to each other. Figs. 1–2 are of material fixed with glutaraldehyde–osmium tetroxide; Fig. 3, *Trebouxia* cell from *Cetraria pinastri*. Note the central pyrenoid (P) which contains pyrenoglobuli along the widened thylakoids. Fig. 3 is of material fixed with osmium tetroxide.

TABLE I
LIST OF PHYCOBIONTS AND LICHENS THAT HAVE BEEN EXAMINED WITH THE
ELECTRON MICROSCOPE

Organism	Reference
I. Blue-green phycobionts	
<i>Calothrix</i> sp. in	
<i>Lichina pygmaea</i>	Peveling (1973)
<i>Nostoc</i> sp. in	
<i>Hydrothyria venosa</i>	Jacobs and Ahmadjian (1973)
<i>Leptogium hildenbrandii</i>	Peveling (1969c)
<i>Peltigera canina</i>	Peveling (1969c)
<i>Peltigera polydactyla</i>	Peat (1968)
<i>Peltigera rufescens</i>	Peveling (1969c)
<i>Scytonema</i> sp. in	
<i>Cora pavonia</i>	Roskin (1970)
<i>Heppia lutosa</i>	Ahmadjian (1967)
<i>Gloeocapsa</i> sp. in	
<i>Gonohymenia mesopotamica</i>	Paran <i>et al.</i> (1971)
<i>Gonohymenia sinaica</i>	Paran <i>et al.</i> (1971)
II. Green phycobionts	
<i>Stichococcus diplosphaera</i> in	
<i>Endocarpus pusillum</i>	Ahmadjian and Jacobs (1970)
<i>Trebouxia</i> sp. in	
<i>Alectoria nidulifera</i>	Jacobs and Ahmadjian (1969)
<i>Anaptychia palmatula</i>	Jacobs and Ahmadjian (1969)
<i>Aspicilia</i> sp.	
<i>Caloplaca aurantia</i> var. <i>aurantia</i>	Galun <i>et al.</i> (1970a,b)
<i>Caloplaca erythrocarpa</i>	Ben-Shaul <i>et al.</i> (1969)
<i>Caloplaca flavovirescens</i>	Galun <i>et al.</i> (1971b)
<i>Caloplaca pyracea</i> v. <i>leucostigma</i>	Galun <i>et al.</i> (1971b)
<i>Caloplaca velana</i>	Galun <i>et al.</i> (1971b)
<i>Cetraria islandica</i>	Jacobs and Ahmadjian (1969)
<i>Cetraria pinastri</i>	Peveling (1969a)
<i>Cladonia cristatella</i>	Jacobs and Ahmadjian (1969, 1971a,b); Moore and McAlear (1960)
<i>Cladonia fimbriata</i>	Peveling (1969a)
<i>Cladonia major</i>	Peveling (1969a)
<i>Cladonia</i> sp.	Peveling (1969a)
<i>Cladonia rangiferina</i>	Jacobs and Ahmadjian (1969)
<i>Cornicularia normoerica</i>	Walker (1968)
<i>Lecanora muralis</i>	Peveling (1968a)
<i>Lecanora olea</i>	Galun <i>et al.</i> (1970b)
<i>Lecanora radiosua</i>	Galun <i>et al.</i> (1970c)
<i>Lecanora rubina</i>	Jacobs and Ahmadjian (1969)
<i>Lecanora subplanata</i>	Galun <i>et al.</i> (1970b)
<i>Lecidea decipiens</i>	Galun <i>et al.</i> (1971a)
<i>Lecidea limitata</i>	Griffiths and Greenwood (1972)
<i>Lecidea olivacea</i>	Galun <i>et al.</i> (1971a)

TABLE I (*continued*)

Organism	Reference
<i>Lecidea opaca</i>	Galun <i>et al.</i> (1971a)
<i>Lecidea scalaris</i>	E. Peveling (unpublished)
<i>Lecidea</i> sp.	Moore and McAlear (1960)
<i>Lepraria neglecta</i>	E. Peveling (unpublished)
<i>Parmelia caperata</i>	Jacobs and Ahmadjian (1969); Peveling (1969a)
<i>Parmelia panniformis</i>	Peveling (1968b)
<i>Parmelia pertusa</i>	Fujita (1968)
<i>Parmelia physodes</i>	Peveling (1969a)
<i>Parmelia sulcata</i>	Jacobs and Ahmadjian (1969), Webber and Webber (1970)
<i>Parmelia trichotera</i>	E. Peveling (unpublished)
<i>Physcia aipolia</i>	Brown and Wilson (1968); Peveling (1969a)
<i>Physcia pulverulenta</i>	E. Peveling (unpublished)
<i>Pseudevernia furfuracea</i>	Peveling (1969a)
<i>Ramalina menziesii</i>	Fisher and Lang (1971a)
<i>Ramalina maciformis</i>	Peveling (1970b)
<i>Ramalina reticulata</i>	Handley <i>et al.</i> (1969), Griffiths and Greenwood (1972)
<i>Ramalina siliquosa</i>	Griffiths and Greenwood (1972)
<i>Squamaria crassa</i> var. <i>crassa</i>	Galun <i>et al.</i> (1970a,b)
<i>Usnea ceratina</i>	Peveling (1969a)
<i>Usnea comosa</i>	E. Peveling (unpublished)
<i>Usnea florida</i>	Griffiths and Greenwood (1972)
<i>Usnea pruinosa</i>	Chervin <i>et al.</i> (1968)
<i>Usnea rockii</i>	Chervin <i>et al.</i> (1968)
<i>Usnea strigosa</i>	Jacobs and Ahmadjian (1969)
<i>Xanthoria candelaria</i>	Peveling (1969a)
<i>Xanthoria parietina</i>	Peveling (1968b); Jacobs and Ahmadjian (1969)
<i>Coccomyxa</i> sp. in	
<i>Baeomyces roseus</i>	Jacobs and Ahmadjian (1969)
<i>Baeomyces placophyllus</i>	E. Peveling (unpublished)
<i>Icmadophila aeruginosa</i>	E. Peveling (unpublished)
<i>Peltigera aphtsa</i>	E. Peveling (unpublished)
<i>Myremcia biatorella</i> in	
<i>Dermatocarpon hepaticum</i>	Galun <i>et al.</i> (1971c)
<i>Dermatocarpon miniatum</i>	Peveling (1969b)
<i>Lobaria pulmonaria</i>	Reznik <i>et al.</i> (1968)
<i>Maronella coricius</i>	Ahmadjian (1967)
Unidentified green algae in	
<i>Texosporium sancti-jacobi</i>	Tibell and van Hofsten (1968)
<i>Verrucaria maura</i>	Griffiths and Greenwood (1972)
<i>Verrucaria mucosa</i>	Griffiths and Greenwood (1972)

osmiophilic globules are described as very homogeneous structures. Replicas of freeze-etched cells of *Trebouxia erici* show the globules to be smooth and different from the surrounding pyrenoid matrix (K. A. Fisher, unpublished, according to Fisher and Lang, 1971a). Only at very high magnification and after chemical fixation did Fisher and Lang observe granules and fibrils in the globuli that were similar to those of the pyrenoid matrix.

The globules occur mainly in the pyrenoid although some may be scattered also in the stroma between the thylakoids adjacent to the pyrenoid. In *Squamaria crassa* var. *crassa* the globules were located only around the pyrenoid (Galun *et al.*, 1970a).

The chemical nature of these globules was tested with Sudan IV, which gave both negative (Brown and Wilson, 1968) and positive results (Jacobs and Ahmadjian, 1969). The smooth structure of the globules after freeze-etching was taken as additional evidence of their lipid composition (Fisher and Lang, 1971a). Because these globules are similar in size, contrast, and location with the lipoquinone-containing plastoglobuli of higher plants (Lichtenthaler, 1968), they were termed plastoglobuli (Peveling, 1968b, 1969a). Jacobs and Ahmadjian (1969) later named the globules pyrenoglobuli on the basis of the position of the globules within the cell. The final decision on the name of these structures should not be made until we know more about the composition of the globules both inside and outside of the pyrenoid.

The lipid-containing globules are assumed to be storage products (Jacobs and Ahmadjian, 1969; Peveling, 1968b, 1969a). A study with high-resolution radioautography showed that the globules are later products of photosynthesis (Jacobs and Ahmadjian, 1971b). If so, then these globules probably have to undergo seasonal or, in culture, special physiological changes. Lichens kept dry for several years show a deterioration of the normal lamellae structure and a corresponding increase in the number of plastoglobuli. Short-term desiccation which does not involve thylakoid changes causes a reduction of plastoglobuli (Peveling, 1968a). The degree of desiccation influences not only the number but also the location of plastoglobuli within a chloroplast. According to Jacobs and Ahmadjian (1971a), pyrenoglobuli are concentrated at the periphery of the pyrenoid when a thallus is exposed to prolonged conditions of desiccation. Brown and Wilson (1968) found the opposite to be true. These authors reported pyrenoglobuli at the periphery of the pyrenoid under hydrated conditions.

Starch, the general storage product in plant cells, is found also in lichen algae. The occurrence of starch in lichens was discussed in detail by Geitler (1933) on the basis of light microscope observations. With the electron microscope large starch plates were found in the *Trebouxia* of *Physcia aipolia* (Brown and Wilson, 1968) while Jacobs and Ahmadjian

(1969), in contrast, reported small disks of starch in the *Trebouxia* of *Xanthoria parietina*. In both cases, starch was located around the pyrenoid as well as being scattered in the stroma toward the periphery of the chloroplast. Starch was not present in each investigated thallus. Ahmadjian (1966) reported the maximum activity for starch production to be in the spring and fall. The hydrated state of a thallus seems to be responsible for the occurrence of starch (Brown and Wilson, 1968; Peveling, 1970b; Jacobs and Ahmadjian, 1971a). Jacobs and Ahmadjian (1971a) observed that thalli which were collected dry produced starch when wetted and incubated for 7 days under continuous illumination. When the lichens were dried again the starch disappeared within a short time.

Starch and pyrenoglobuli are considered to be storage products of the phycobionts. While starch is produced only in hydrated and illuminated lichens, the globules are found also in dried thalli. Recently, Jacobs and Ahmadjian (1971a) hypothesized that the lipids of the plastoglobuli may function as a water source. The authors supported their idea by the fact that respiration persists in dry lichens at water contents of about 1% of the thallus dry weight (Ahmadjian, 1967).

The nucleus and several mitochondria are located in the small cytoplasmic region between the chloroplast and plasmalemma. Dictyosomes have been described in the cultured *Trebouxia* of *Cladonia cristatella* (Jacobs and Ahmadjian, 1971a) and *Ramalina menziesii* (Fisher and Lang, 1971a). The dictyosomes were present most frequently during cell division. A typical endoplasmic reticulum could not be identified in these phycobionts. There were a large number of homogeneous globules with a diameter from 100–1000 nm that were considered to be polyphosphate inclusions and cytoplasmic lipid globules.

The ultrastructure of *Stichococcus diplosphaera* in squamules of *Endocarpon pusillum* is very similar to that of *Trebouxia*. *Stichococcus* has a chloroplast which almost fills the cell and contains a central pyrenoid. Ten parallel chloroplast lamellae pass through the pyrenoid. Pyrenoglobuli were found at the periphery of the pyrenoid and in the stroma of the chloroplast. Starch was present also (Ahmadjian and Jacobs, 1970).

Other chlorophycean phycobionts whose ultrastructure has been studied include *Myrmecia biatorella* (Galun *et al.*, 1971c) and representatives of the genus *Coccomyxa* in *Baeomyces placophyllus*, *Icmadophila aeruginosa*, and *Peltigera aphthosa* (Peveling, in preparation). *Myrmecia biatorella* has a centrally located nucleus, few mitochondria, and, in young cells, a parietal chloroplast whose thylakoids are stacked in groups of three to four. The number of plastoglobuli is small in young cells but increases during enlargement of the cells and decreases in aging cells (Galun *et al.*, 1971c). The lichenized *Coccomyxa* has a cup-shaped chloroplast which lacks a pyrenoid

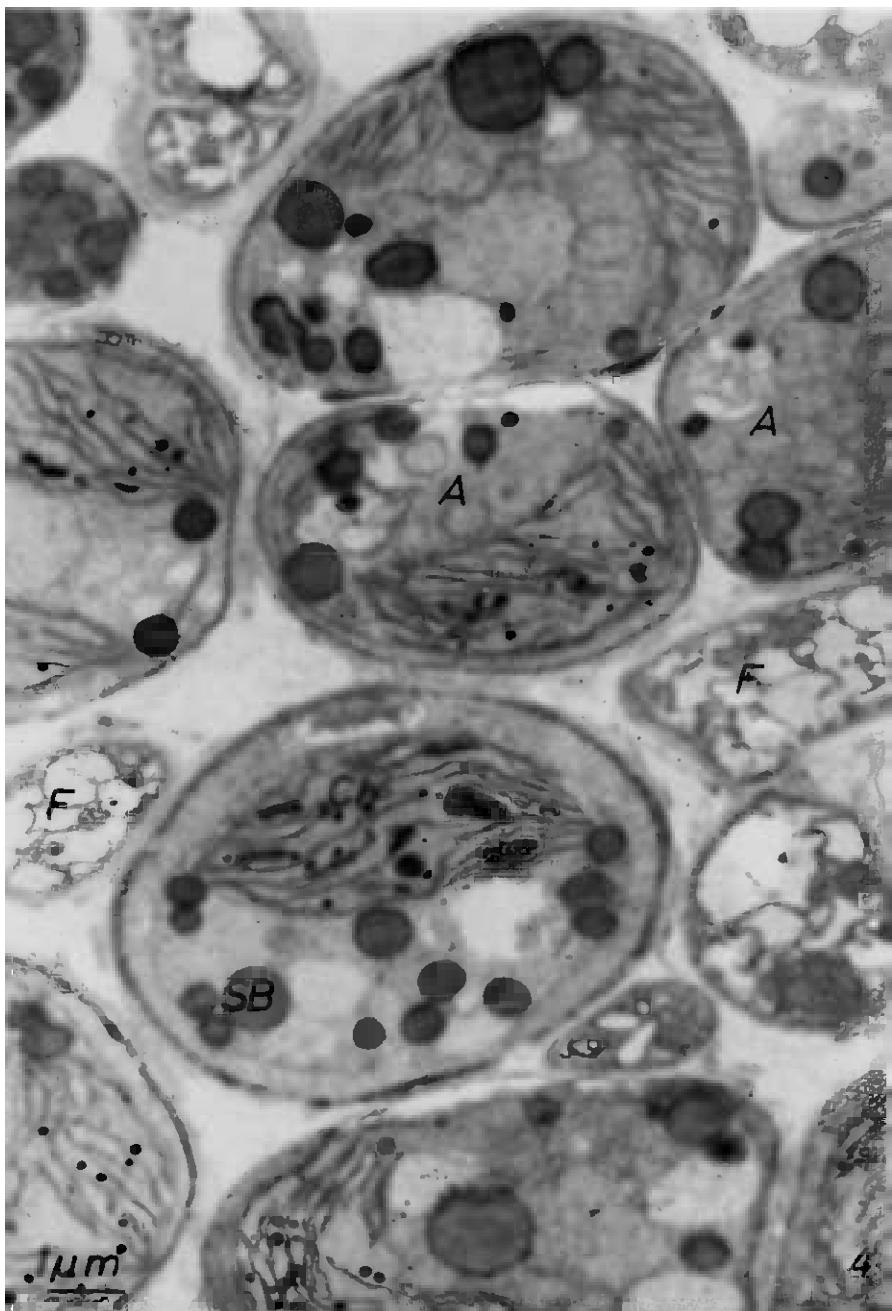


FIG. 4. Part of the algal layer from *Baeomyces placophyllus* showing close contacts between algae (A) and fungi (F). Algal cells (*Coccomyxa*) contain a chloroplast (Ch) and osmiophilic storage bodies (SB). Material fixed with glutaraldehyde–osmium tetroxide.

and which is smaller than the chloroplast of *Trebouxia* (Fig. 4). The chloroplast takes up only half of the protoplast and the cytoplasmic area is greater. The chloroplast has long thylakoids of which three to six are usually piled up in stacks (Figs. 5 and 6). Scattered in the stroma are many plastoglobuli which can reach a diameter up to 300 nm. Starch grains also are present. The cytoplasm contains a nucleus, mitochondria, and dictyosomes. The latter consist of up to six typical disks and show a number of golgi vesicles. Many large globules of more or less dense contrast also are present in the cytoplasm. A single limiting membrane can be observed for many of these bodies, which are considered as storage products.

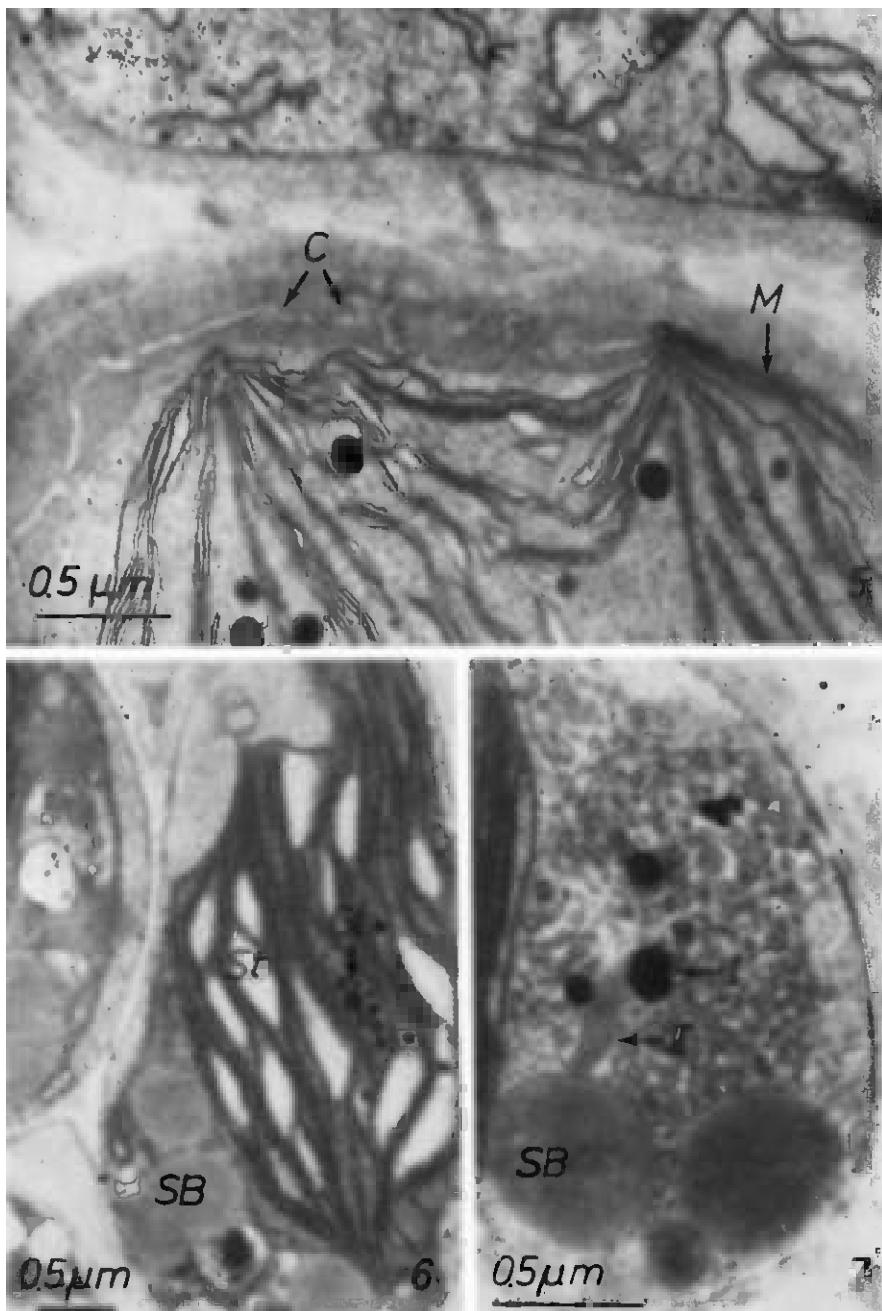
The *Coccomyxa* phycobiont of *Icmadophila aeruginosa* shows another structure which has not been described before. In ultrathin sections of cells one or several areas appear which are 1–2 μm in diameter (Fig. 7). They are round to elliptical with several invaginations of their surrounding single membrane. Inside they possess a number of large vesicles 50–100 nm in diameter in a light ground substance. These vesicles are formed by one or two concentric membranes. Many vesicles show a dark center of the same contrast as the surrounding membranes. Besides the vesicles two different stained inclusions also occur in these zones. One type shows the same reaction to fixation with osmium tetroxide as the plastoglobuli in the chloroplast while the second type in its staining ability can be compared with the large storage bodies of low contrast in the cytoplasm. Often almost all the contents of these areas disappear and only small remnants of the three different structures can be made out.

The unique features of the green phycobionts are as follows: (1) algae with a pyrenoid, i.e., *Stichococcus* and *Trebouxia*, have a large number of osmophilic globules which are aligned along thylakoids; (2) *Coccomyxa* has in its cytoplasm areas which are separated by a single membrane and which include large numbers of small vesicles as well as irregular inclusions of different staining ability.

B. Protoplast of the Lichenized Mycobionts

Most of the mycobionts whose ultrastructure has been studied are ascomycetes. The exceptions are *Cora pavonia*, which is a Basidiomycete (Roskin, 1970), and *Lepraria neglecta*, which is an imperfect fungus (E. Peveling, unpublished).

The ultrastructure of all the observed mycobionts is similar. The hyphae in the three layers of the lichen thalli, i.e., cortex, algal layer, and medulla, are more or less vacuolated. The cytoplasm has numerous ribosomes, often several nuclei, mitochondria with cristae, a rather extensive endoplasmic reticulum and, according to their staining ability, two types of granules which are considered to be storage products. The granules appear mainly



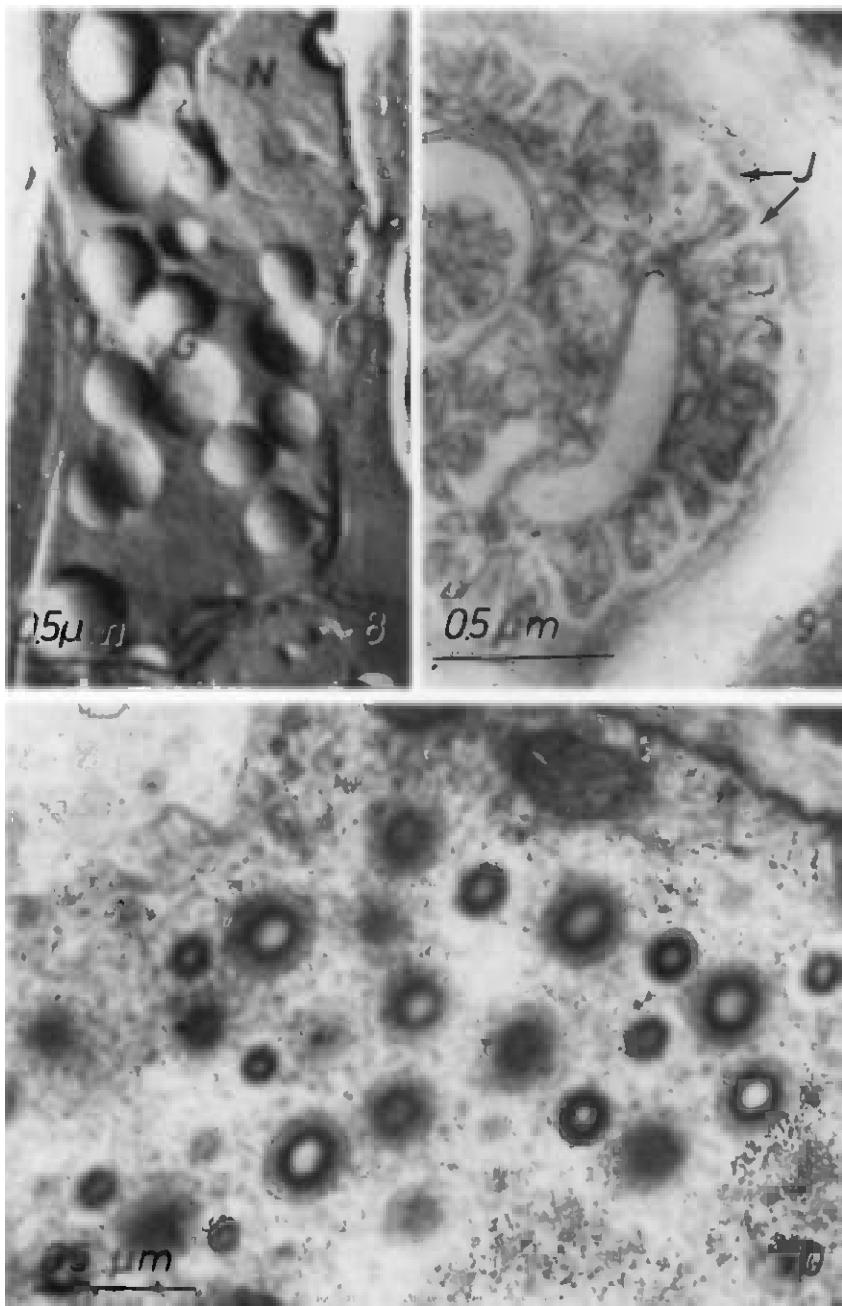
Figs. 5-7. Micrographs of *Coccomyxa* phycobionts. Fig. 5, from *Baeomyces placophyllus* showing hyphal cell and in the algal wall channel-like (C) and myelinlike (M) structures; Fig. 6, from *Icmadophila aeruginosa* showing long stacked thylakoids, plastoglobuli (GL), and

in the medullary hyphae (Fig. 8) but occur also in the cortex and algal layer. Between the cell wall and cytoplasm appear lomasomes whose development in mycobionts was described by Jacobs and Ahmadjian (1969). These organelles are common to most nonlichenized fungi.

There are several structures which are characteristic of lichen fungi. The structure most often described is the ellipsoidal body (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969) or concentric body according to Peveling (1969b). These two terms express one difference between the otherwise similar structures. In the protoplast of *Physcia aipolia* (Brown and Wilson, 1968) and those lichens examined by Jacobs and Ahmadjian (1969; see Table I), these bodies show an elliptical shape whose long axes reach 100 nm. The ellipsoidal bodies consist of an electron transparent core and two surrounding shells. "Fingerlike projections" are associated with the outer shell according to Brown and Wilson (1968). Because of the associations of these projections to surrounding membranes the authors discuss the role of these bodies as membrane synthesizers or organelles concerned with transport of materials. Ellis and Brown (1972) consider these bodies to be the functional equivalents of the Golgi apparatus. The importance of these structures for cell metabolism was discussed also by Peveling (1969b) in relation to a change in the development of the different shells. Peveling described the bodies as round and perfectly concentric structures with a diameter of 300–400 nm (Fig. 10). Fingerlike projections were seen as rays fixed at the inner shell if the outer one did not form. Griffiths and Greenwood (1972) in their study of concentric bodies agreed with Peveling's descriptions of these structures. They reported for the first time the presence of concentric bodies in two nonlichenized ascomycetous fungi and listed 43 lichens in which these bodies have been identified. The bodies are present in the hyphae of the different regions of the thallus, but they have not been found in the ascospores. Jacobs and Ahmadjian (1973) did not find concentric bodies in the aquatic lichen, *Hydrothyria venosa*, and proposed that drying is a factor that influences the development of these bodies.

Another characteristic of mycobionts is the invaginated plasmalemma (Figs. 9 and 11) which has been reported for almost all lichenized fungi (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969, 1971a; Peveling, 1969b). The most extensive invaginations occur in fungal hyphae which are close to the algal cells. Because this differentiation enlarges the protoplast surface it is considered an adaptation that promotes the exchange between the symbionts.

starch (St) in the chloroplast and storage bodies (SB) in the protoplast; Fig. 7, from *Icmadophila aeruginosa* showing an area surrounded by a single membrane that contains many small vesicles and two types (I and II) of storage bodies. In addition, there are large storage bodies (SB) in the cytoplasm. Material fixed with glutaraldehyde-osmium tetroxide.



Figs. 8–10. Hyphal protoplasts. Fig. 8, a medullary hypha of *Dermatocarpon miniatum* showing many storage globules (G) and nucleus (N). Freeze-etched; Fig. 9, invaginations (J).

A third unique structure in mycobionts are crystals. In *Physcia aipolia* they were observed in membranous sacs, but only after fixation in osmium tetroxide (Brown and Wilson, 1968). In *Peltigera canina* crystals were identified in glutaraldehyde fixed material but were not distinct and did not have a membranous sac (Peveling, 1969b). The possible relationship between such crystals and lichen acids has been raised by Brown and Wilson (1968).

Hyphal septa (Fig. 12) have been described in lichenized Ascomycetes (Peat, 1968; Jacobs and Ahmadjian, 1971a; Paran *et al.*, 1971; Peveling, 1968a) and lichenized Basidiomycetes (Roskin, 1970). Septal pores are often plugged with Woronin-like bodies.

C. The Cell Walls of Lichenized Algae and Fungi

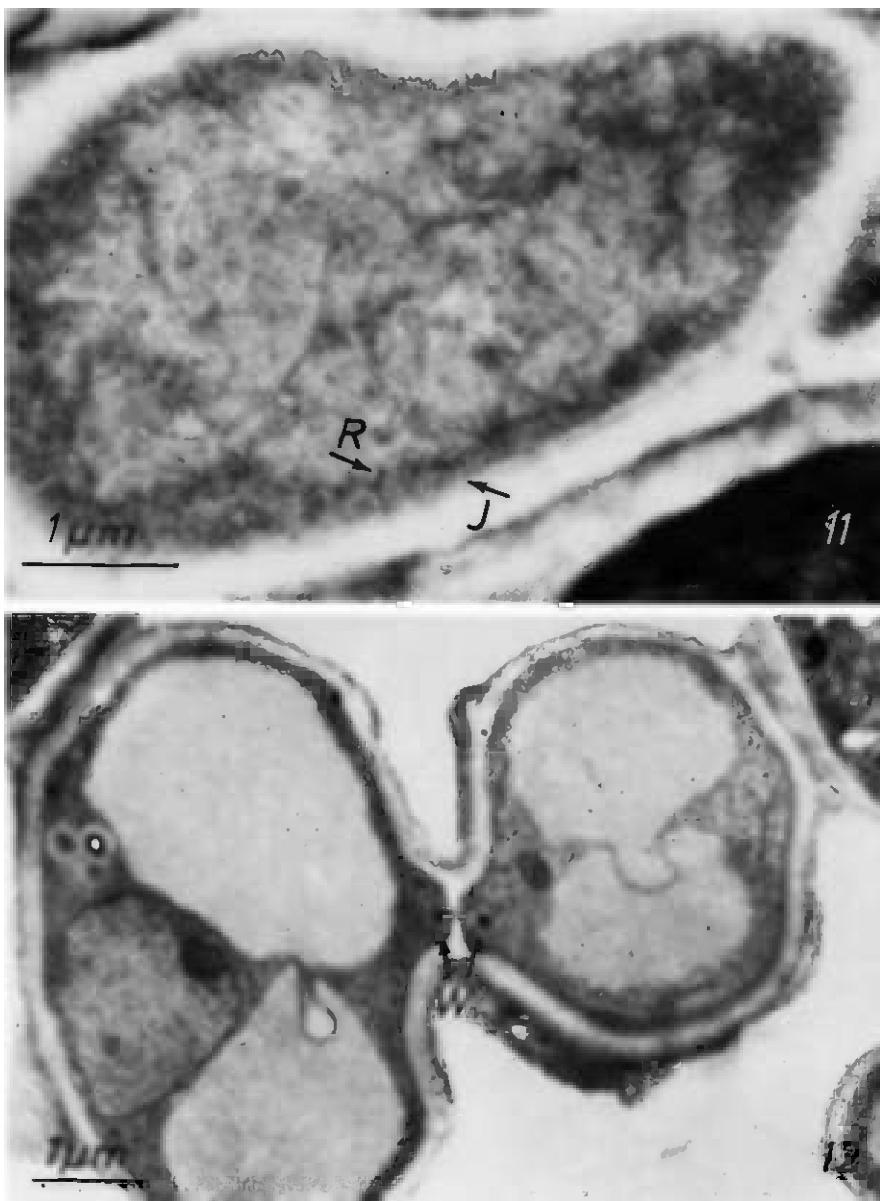
The constitution of the cell walls of the algae and the fungi determines to a certain degree the growth form of a thallus.

1. CELL WALLS OF THE ALGAE

There is a fundamental difference in the cell walls of blue-green and green phycobionts. The blue-green cells have a sheath around their cell wall (Peat, 1968; Peveling, 1969c; Paran *et al.*, 1971). For *Nostoc* cells in *Peltigera polydactyla* only an inner wall and a cell sheath that consisted of a fibrillar substance were described (Peat, 1968). The *Nostoc* cells in *Leptogium hildenbrandii*, *Peltigera canina*, and *P. rufescens* as well as *Gloeocapsa* in *Gono-hymenia mesopotamica* and *G. sinaica* showed a more detailed wall: one that consisted of four small zones of alternate contrast (Peveling, 1969c; Paran *et al.*, 1971). The inner zone may consist of a mucopolymer as shown with cytochemical studies in *Phormidium unicatum* (Frank *et al.*, 1962). In addition to the four layers, there is a fibrillar outer sheath. In *Peltigera*, the *Nostoc* phycobiont has fibers which are packed tightly to form a 100-nm-thick sheath. The *Nostoc* cells in *Leptogium hildenbrandii* have their fibers in the outermost layer much more extended so that their sheaths are 1 μm wide (Peveling, 1969c). The whole sheath seems to be swollen which may account for the gelatinous character of this thallus.

The cell walls of *Trebouxia* vary in thickness from 100–500 nm and usually consist of two layers with different contrast (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969). Occasionally, a small additional layer can be found which surrounds several algal cells (Chervin *et al.*, 1968) or even

of the plasmalemma in *Dermatocarpon miniatum*. Material fixed with osmium tetroxide; Fig. 10, concentric bodies in *Peltigera canina*. Material fixed with glutaraldehyde-osmium tetroxide.



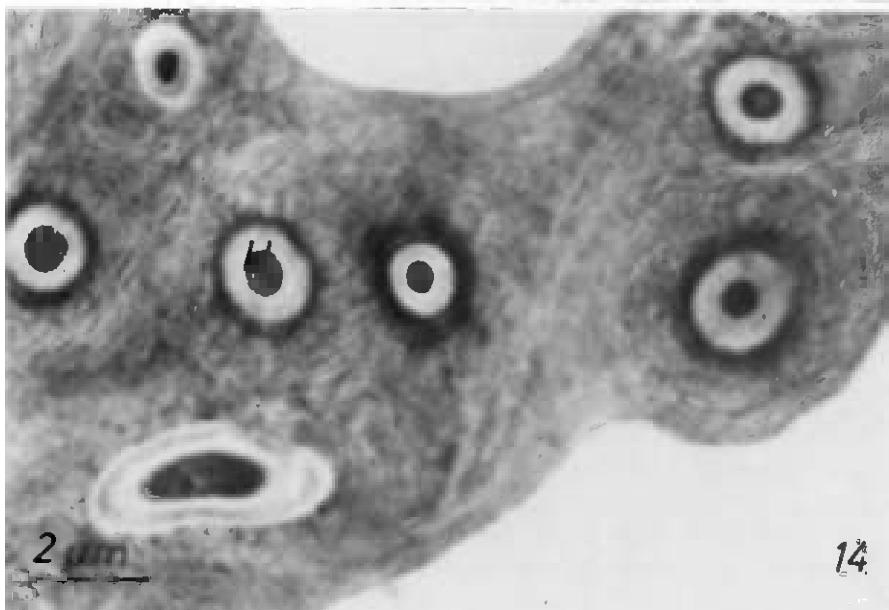
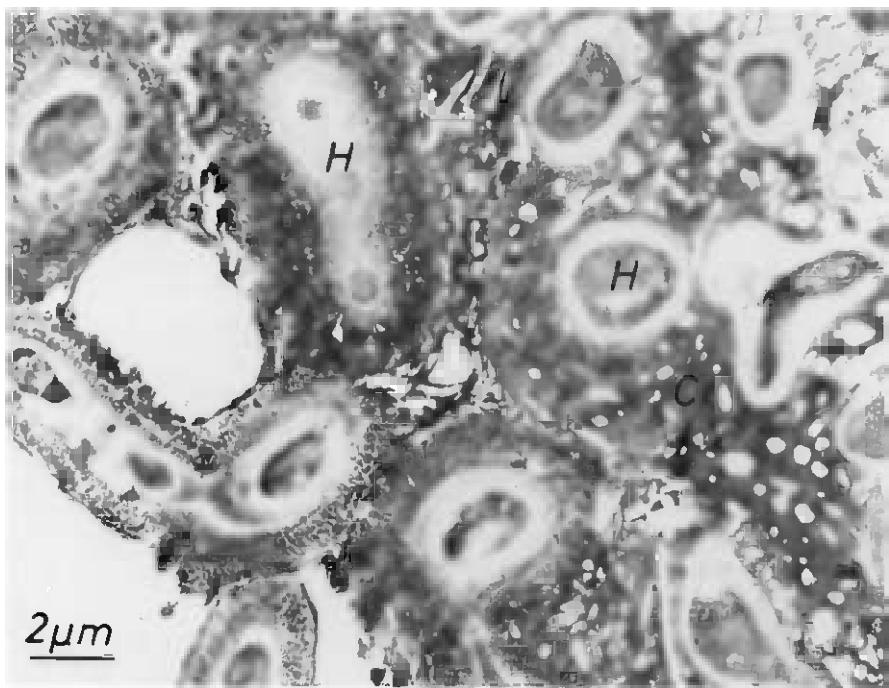
FIGS. 11-12. Fig. 11, ultrathin section of hyphae of *Dermatocarpon miniatum* showing invaginations (J) of the plasmalemma bordered by densely packed ribosomes (R); Fig. 12, two connecting hyphae in *Peltigera rufescens*. Note the Woronin-like bodies (W) close to the septal pore. Material fixed with osmium tetroxide.

algae and fungi (Peveling, 1969a). Probably this layer is a remnant of a sporangial wall.

The *Coccomyxa* cell walls in *Baeomyces placophyllus* and *Icmadophila aeruginosa* show three layers (Fig. 4). The main layer is the middle one. It is 40–100 nm thick and has a high contrast and a fine granular appearance. From this layer to the plasmalemma there is a space which varies in width, structure, and contrast. It has the same appearance as the middle zone and also a branched system of channel-like structures (Fig. 5). The width of such a structure, which is bordered by a membrane like the plasmalemma, varies from 20 to 100 nm. Occasionally, the plasmalemma is connected to the limiting membrane of the channel-like structures. Myelinlike figures and globules also occur in this zone. Finally, there is the outermost layer of the cell wall which is very irregular in width.

2 CELL WALLS OF THE FUNGI

The differentiation of the hyphal walls varies according to the position of the hyphae in the thallus. The simplest and thinnest walls are those of hyphae which are in contact with the algae. These walls have a fibrillar appearance and are only 150–400 nm thick (Chervin *et al.*, 1968; Jacobs and Ahmadjian, 1969; Peveling, 1969b). Hyphae which make up the cortex are similar. In some lichens the hyphae in the cortex are arranged to form a paraplectenchyma. In such a case, only a small layer of extracellular substance separates the hyphae. More often the hyphae of the cortex—those of the upper as well as of the lower cortex—are embedded in a thick extracellular substance that has a microfibrillar nature (Bednar and Juniper, 1964; Brown and Wilson, 1968; Fujita, 1968; Handley *et al.*, 1969). The density differs in a single thallus and also from species to species. Usually scattered between the submicroscopic fibers are crystals of lichen substances. The electron microscope generally shows only the outline of such crystals because they tend to be extracted during preparation (Fig. 13). Hyphae of the cortex can have multilaminated walls but this is more characteristic for the hyphae in the medulla. It is difficult to determine sometimes whether the different layers around a hypha are part of the proper cell wall or if they are extracellular additions to the wall (Fig. 14). All the layers of a single hypha form a structure as wide as 1.5 μm . The basic elements of the multilaminated layers are fibers which can run in concentric circles around the hypha protoplast or are perpendicular to it (Figs. 13 and 14). All the fibers are not packed tightly and crystals of lichenic acids may be deposited between them or on the surface of the multilaminated layers. The thickness of the surrounding layers depends on the number of hyphae in the medulla. If



Figs. 13-14. Fig. 13, part of the cortex of *Usnea ceratina* with hyphae (H) embedded in a thick fibrillar substance. Scattered between the hyphae are the outlines of crystals (Cr) of lichen acids left after fixation; Fig. 14, hyphae in the medulla of *Xanthoria parietina*. Note the fibrillar material arranged concentrically around the hyphal (H) walls. Material fixed with glutaraldehyde–osmium tetroxide.

a medulla consists of a large number of hyphae, the walls of the individual hyphae are thinner than those in a medulla made up of fewer hyphae. By this distribution and differentiation the stability of a thallus is guaranteed.

D. The Cultured Symbionts

There are difficulties in isolating and culturing the symbionts of many lichens. For this reason, it is not possible to make exact comparisons between the fine structure of the lichenized algae and fungi and their isolated counterparts. Nevertheless, we do have data about structures that seem to be common to lichenized and isolated lichen symbionts and other structures that reflect differences between the two forms.

1. ISOLATED PYCOBIONTS

From *Trebouxia*, the most common phycobiont, the following six isolates have been studied: *Trebouxia albulescens* from *Buellia punctata*, *T. anticipata* from *Parmelia ruderata*, *T. decolorans* from *Xanthoria parietina*, *T. erici* from *Cladonia cristatella*, *T. gelatinosa* from *Parmelia caperata*, and *T. impressa* from *Physcia stellaris* (Laudi *et al.*, 1969; Jacobs and Ahmadjian, 1971a; Fisher and Lang, 1971b). These algae were cultured either in organic or inorganic media and either under illumination or in the dark. All the *Trebouxia* cells showed a pyrenoid in the center of a large chloroplast. The arrangement of the thylakoids showed the same variation as already described for lichenized cells. *Trebouxia erici* grown under 1075 and 3600 lux instead of 215 lux showed a decrease in stacked thylakoids (Fisher and Lang, 1971b). *Trebouxia decolorans* and *T. albulescens* were more sensitive to strong light (3000 lux) in their cultured state compared with their lichenized form.

While the lichenized *Trebouxia* usually have a single pyrenoid, several may occur in the cultured cells (Fisher and Lang, 1971b; Jacobs and Ahmadjian, 1971a). The pyrenoids are traversed by thylakoids whose appearance can be different even in one species, a fact that was noted also in lichenized *Trebouxia* (Peveling, 1969a). The pyrenoglobuli, which are aligned along the intrapyrenoid thylakoids, measured 100 nm in diameter in cells of *T. erici* that were grown in inorganic culture. Cells grown in organic media had pyrenoglobuli that were only 30–50 nm in diameter and they were fewer in number (Fisher and Lang, 1971b; Jacobs and Ahmadjian, 1971a). Moreover, *T. erici* grown in organic media at 215 lux showed more pyrenoglobuli than cells cultured at 1075 or 3600 lux. After transfer of *T. erici* from organic to inorganic culture there was a marked increase of pyrenoglobuli (Jacobs and Ahmadjian, 1971a). Starch accumulation varies in lichenized and isolated phycobionts according to differences in the culture conditions.

Fisher and Lang (1971b) found that young cells grown at 215 lux contained larger amounts of starch than cells grown at 1075 or 3600 lux.

The cytoplasm forms a thin rim around the chloroplast and reveals endoplasmic reticulum, mitochondria, and ribosomes. In the isolated *T. erici* more dictyosomes were observed than in the lichenized form (Fisher and Lang, 1971b). This is probably due to the higher division rate in culture. Two types of storage products are apparent in the cytoplasm of the cultured cells. One product is an electron-dense spherical body, alveolate or mottled in appearance, which is commonly present in vacuoles. Such bodies are absent in cells cultured in low phosphate medium. According to Fisher accumulations of polyphosphate were demonstrated in these bodies. The second type of storage body are electron-transparent storage droplets similar to those in lichenized phycobionts.

In addition to allowing for observations of the vegetative cells, cultures present a chance to analyze the structure in dividing cells.

The building of aplanospores and zoospores by *T. erici* is described as very similar (Jacobs and Ahmadjian, 1971a). The division starts with an expansion of the pyrenoid and its fragmentation into smaller parts. Pyrenoglobuli and the osmiophilic globuli outside the pyrenoid fuse. At this stage, at least one dictyosome is evident in each cell. Two daughter chloroplasts move to a parietal position against the cell wall and several mitochondria become aligned along the edge of each chloroplast. These mitochondria then divide into many smaller ones. At this stage the nucleus has assumed a central position in the cell and becomes surrounded by electron-transparent storage droplets. Simultaneously, many dictyosomes are present and appear to be producing large numbers of electron-transparent vesicles, some with electron-dense cores. The daughter chloroplasts then divide successively many times. Nuclear division occurs before cytokinesis. The daughter cells as aplanospores or zoospores are released through an exit pore in the wall of the zoosporangium. A very typical structure of the zoospores is the eye spot. But this differentiation is found only in zoospores which originated from cells in inorganic medium. The eye spot when viewed in longitudinal section consists of one row of ten electron-dense droplets.

The cell walls of isolated *Trebouxia* cells grown in organic or inorganic media possess a fibrillar surface which becomes obvious by carbon replicas or after freeze-etching. This fibrillar component of the cell wall was not detected in lichenized *Trebouxia* (Jacobs and Ahmadjian, 1971a).

2. ISOLATED MYCOBIONTS

Only very few isolated mycobionts (*Cladonia cristatella*, *Cora pavonia*, *Herpothallon sanguinum*, and *Lecanora dispersa*) have been studied to date.

In the case of these few samples, it was observed that in cultured mycobionts the cell wall with its configuration of three layers is similar to that in lichenized mycobionts. The plasmalemma of the free-living mycobiont cells is relatively smooth in contrast to the highly invaginated plasmalemma of the lichenized forms. The ellipsoidal or concentric bodies could not be detected in the cultured mycobionts. The only exception was in pycnidia-producing cultures of the mycobiont *Cladonia cristatella* (Jacobs and Ahmadjian, 1971a). This colony had been soaked in citrate-phosphate buffer solution at pH 2.8 for 5 days and then placed onto a surface of agar made up with the same buffer solution. Whether the concentric bodies found in the hyphae of this colony formed in response to the developmental process, that is to the formation of pycnidia, or to the special conditions of the culture is not known.

One more characteristic feature of isolated mycobionts is their self-infection. Young hyphae penetrate older hyphae by haustoria. During this process the older cells were devoid of cytoplasmic contents or filled with a granular electron-dense material. According to J. B. Jacobs (personal communication), this fact may explain the relatively slow growth rates of lichen fungi. Intrahyphal hyphae also represent a form of autophagy whereby under poor nutrient conditions these fungi can cannibalize themselves as a means of persisting through difficult conditions.

III. Symbiotic Relationship between Fungus and Alga

Contacts between the lichen symbionts are either by closely appressed fungal hyphae or by haustoria. There are two types of haustoria according to light microscope investigations. An intramembranous haustorium penetrates only the algal cell wall and does not extend much beyond the wall. An intracellular haustorium penetrates deeply into the cell. Lichens with a poorly defined thallus usually have intracellular haustoria while those with a well-structured thallus generally have only intramembranous haustoria (Tschermak, 1941; Geitler, 1963; Plessl, 1963).

A. Intracellular Haustoria

Haustoria were the first lichen structures demonstrated with the electron microscope (Moore and McAlear, 1960; Durrell, 1967). These early investigations on lichen ultrastructure confirmed only the existence of haustoria and did not reveal many details of haustorial structure and the infected algal cells. Prominent intracellular haustoria were observed in *Maronella laricina* (Ahmadjian, 1966), *Lecanora muralis* (Peveling, 1968b), *Lecanora rubina*

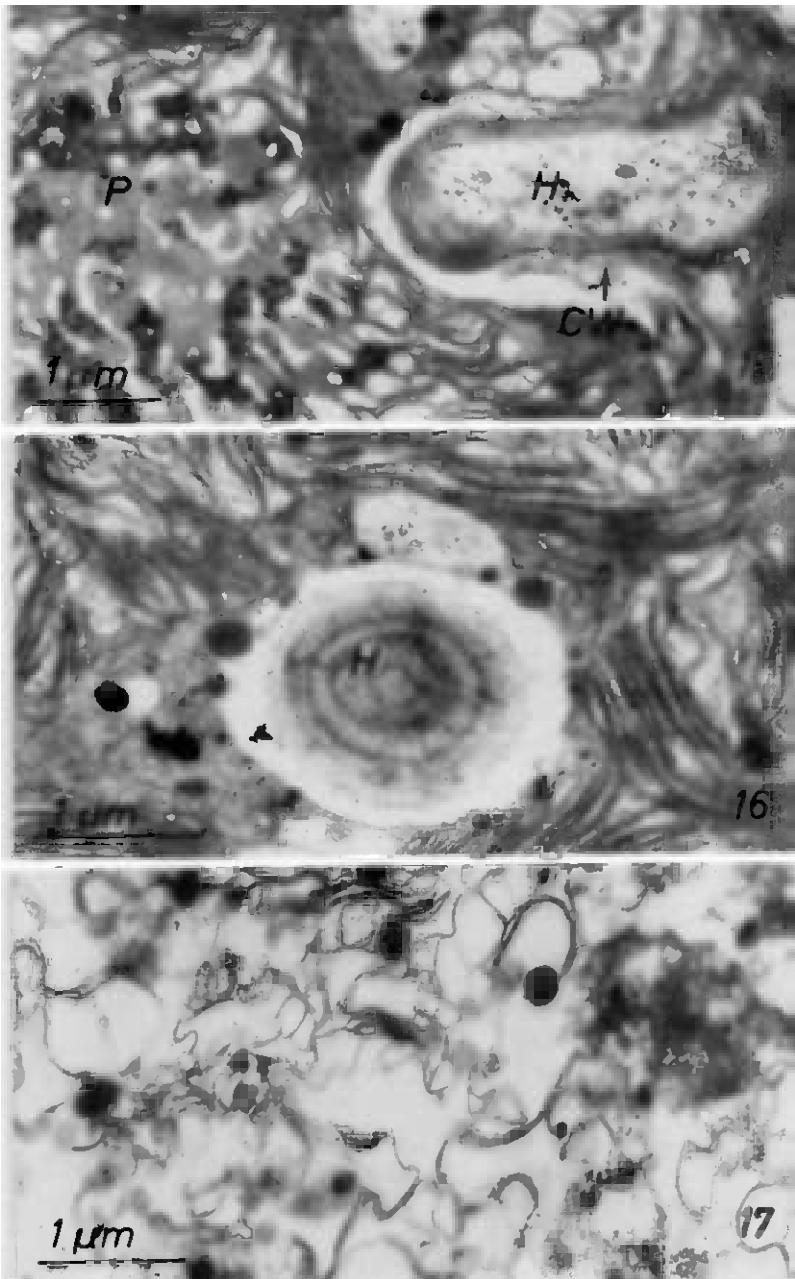
(Jacobs and Ahmadjian, 1969), *Cora pavonia* (Roskin, 1970), *Parmelia sulcata* (Webber and Webber, 1970), *Lecanora olea* (Galun *et al.*, 1970b), *Lecanora radiososa* (Galun *et al.*, 1970c), and occasionally in *Squamaria crassa* (Galun *et al.*, 1970a).

The haustorium is always represented by a fungal tip whose diameter is smaller than that of the main hyphal branch. In one algal cell up to three haustoria were found in a single ultrathin section. The haustoria form a single tip or they become bifurcated. The cell wall of a haustorium is usually thinner than that of the hyphal walls outside the algal cell. While penetrating into the alga the haustorial cell wall remains intact and does not show any visible changes in structure (Figs. 15 and 16). However, the cell wall of the alga is ruptured. In some lichens only the outer layer of the algal cell wall breaks and the inner one is invaginated by the haustorium (Jacobs and Ahmadjian, 1969). In other lichens, the algal wall material forms a "short collar" around the penetrating haustorium (Roskin, 1970). There are only fine fibrillar structures between the cell wall of the haustorium and the plasmalemma of the algal cell. Possibly, these are remnants of the algal cell wall (Peveling, 1968b).

The haustorium contains mesosomes and "lysosomelike organelles" (Webber and Webber, 1970) that may produce lytic enzymes necessary for penetrating the algal cell wall. The other alternative of a mechanical rupture of the algal cell also has been proposed.

Following the penetration of a haustorium, changes in the ultrastructure of the algal protoplast can be observed (Peveling, 1968b). In *Lecanora muralis* the following alterations of the phycobiont were observed. First, the protoplast contracted. The plasmalemma was not penetrated by the haustorium but remained intact until a late stage in degeneration. Myelinlike structures appeared between the algal cell wall and the plasmalemma. The main degeneration of the protoplast began with the dissolution of the pyrenoid in the chloroplast. Then the thylakoids puffed up or adhered together. Plastoglobuli of various sizes and number were scattered between the thylakoid remnants. In a later stage the chloroplast matrix and the cytoplasm became lighter and all the various protoplast structures including the plasmalemma were no longer visible as distinct bodies (Fig. 17). Finally, the cell wall of the alga ruptured and the remaining contents of the alga, i.e., parts of its wall, lamellae, vesicles, and globular particles, were distributed between the intact algae and fungi.

Similar effects of degeneration were observed in uninfected *Trebouxia* of an *Aspicilia* (Galun *et al.*, 1970a). From their observations the authors concluded that the degenerative changes were part of the normal life cycle of the alga and not due to any influence of the haustorium. Therefore, according to our present knowledge, it can be assumed that there are two types of morphological degeneration of the algae: the normal life cycle of an



Figs. 15-17. Haustoria in *Lecanora muralis*. Fig. 15, longitudinal section of an haustorium (H). Note the ruptured algal cell wall (CW) and pyrenoid of the phycobiont (P). Fig. 16, cross section of haustorium (H) within an algal cell; Fig. 17, part of a degenerated phycobiont. Material fixed with glutaraldehyde–osmium tetroxide.

alga with its decaying process and the degeneration caused by fungal haustoria.

A different type of algal cell penetration was described in *Dermatocarpon hepaticum* (Galun *et al.*, 1971c). In the zone of contact between the symbionts an electron lucent area arises after disintegration of the fungal and algal cell walls. The naked fungal protoplast grows into this area while the algal protoplast retreats. The space between the two protoplasts becomes filled with poorly defined material that integrates with the algal wall.

B. Intramembranous Haustoria

Intramembranous haustoria have been observed in *Cladonia cristatella*, *Lecidea sp.* (Moore and McAlear, 1960), *Gonohymenia mesopotamica*, *G. sinaica* (Paran *et al.*, 1971), *Usnea pruinosa*, and *U. rockii* (Chervin *et al.*, 1968). These haustoria show the same thinner walls in the contact region as the intracellular haustoria. The only striking difference observed in these haustoria was an increased number of mitochondria (Chervin *et al.*, 1968). The augmented appearance of mitochondria in a haustorium seems reasonable although they have not been described in haustoria of other lichens.

We can expect that intramembranous haustoria, which are rather difficult to detect with the light microscope, will be found more frequently with the electron microscope. Such observations already have been made with *Endocarpon pusillum* (Ahmadjian and Jacobs, 1970).

C. Contact between Alga and Fungus without Haustoria

Fungi which do not penetrate their symbiotic algal cells are closely attached to them. According to light microscope observations, the hyphal parts lying on the algal cells can develop special shapes (Bornet, 1873). Ultrastructural observations show that the protoplast of such appressed hyphae have an enlarged surface. Such a differentiation can favor the flow of material from alga to fungus which has been demonstrated in labeling experiments to be very rapid (Smith and Drew, 1965; Drew and Smith, 1967a,b; Jacobs and Ahmadjian, 1971b).

Recent observations of *Lichina pygmaea* indicate that special structures of the algae also may favor a transport of metabolic products (Peveling, 1973). The plasmalemma and outer cell wall of the *Calothrix* phycobiont can evaginate. At the same time numerous small vesicles appear in the fibrillar sheath of this blue-green symbiont.

IV. Thallus Layers and Surface Structures as Viewed with SEM

Classification is the main emphasis in lichenology along with studies on the symbiotic way of life. Determination of the large number of lichen

species requires an exact description of thallus morphology and reproductive organs. To a great extent this has been done by macroscopic and light microscope observations. Today, the scanning electron microscope (SEM) allows for a more detailed analysis of thallus structures. This instrument with its high resolution and a resulting image of a marked three-dimensional character shows even the smallest differentiation of lichen morphology. The orientation of phycobiont and mycobiont within a thallus can be studied as well as the different surface bodies and the structures by which a thallus is fixed to the substrate.

A. Thallus Layers

Fungi and algae are either scattered regularly in a thallus (homoiomeric lichen) or they are restricted to special layers (heteromeric lichen). Studying this arrangement with the light microscope may be difficult because of the thin sections needed to obtain good resolution. In such sections the symbionts tend to loosen so that their natural arrangement is no longer evident. With the SEM such difficulties are overcome and the real distribution of the symbionts can be observed (Figs. 18 and 19). A disadvantage of this method is that the protoplasts are not preserved so that details of the symbiont cells are not revealed.

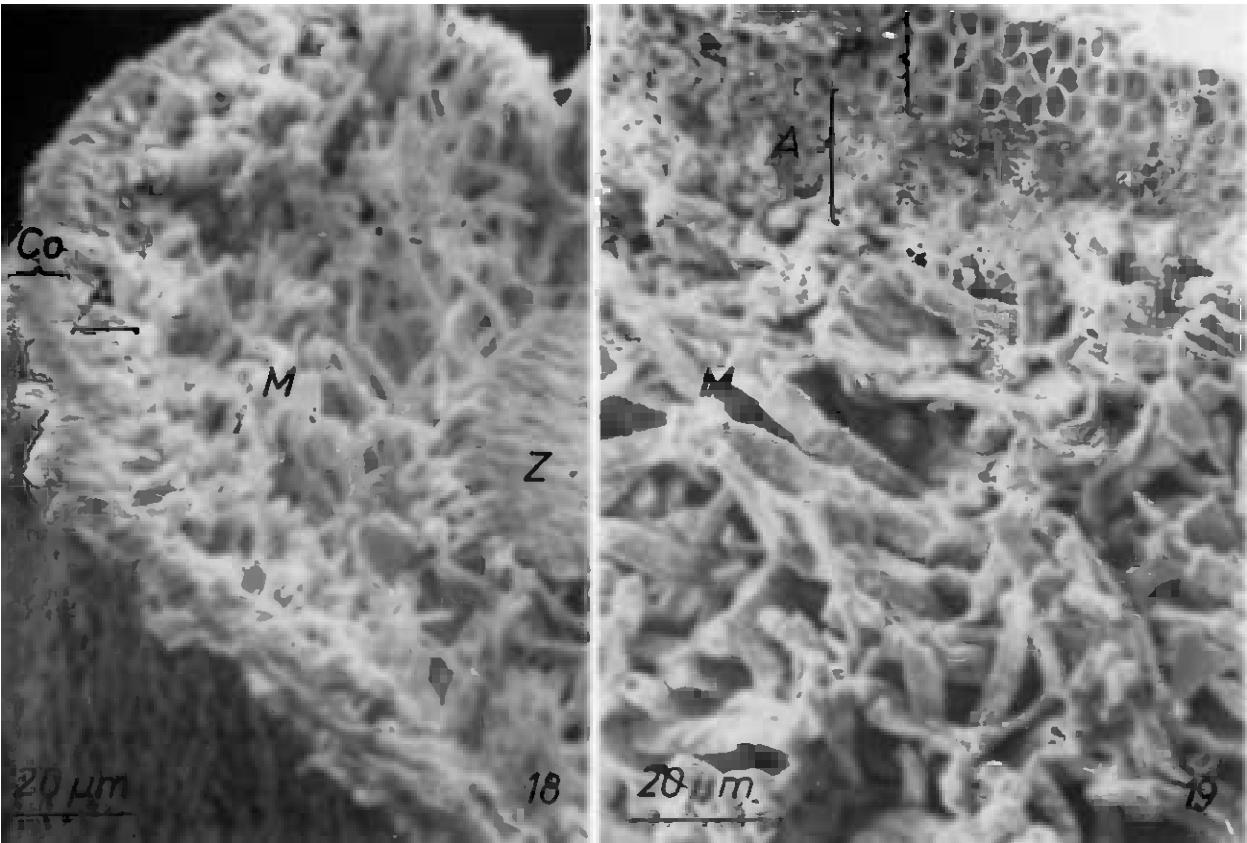
Particularly, the SEM gives an excellent view of hyphal distribution. The cortex can be discerned as a plectenchyma (Fig. 19) or as hyphae embedded in a thick gelatinous substance (compare Figs. 13 and 18). Hyphal orientation in the medulla is clearly evident. Numerous small and tightly interwoven hyphae can be seen as well as hyphae which are thicker in diameter but fewer in number. Within the thalli the distribution of crystals of lichen acids also can be studied with the SEM. The crystals are attached directly to the walls (Figs. 26 and 28) or they are scattered between the symbionts (Fig. 27).

B. Surface Structures

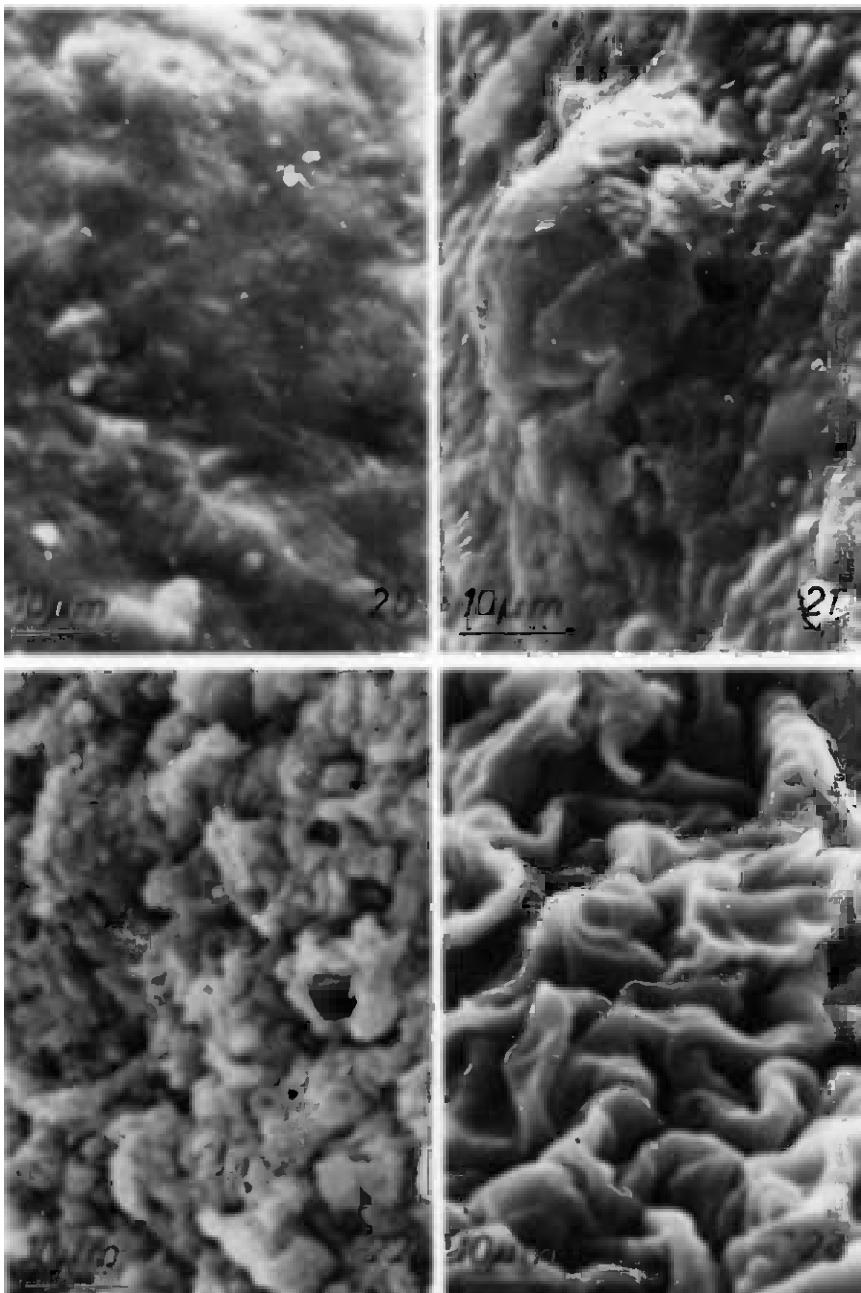
The surface of a lichen thallus consists of the cortical layer and structures which appear above the cortex as asexual and reproductive structures. They are best visible on air-dried thalli. Water-saturated lichens have uniformly swollen surfaces and structural details after freeze-drying are observable only in some soaked objects (Peveling and Vahl, 1968).

1. THE CORTICAL SURFACE

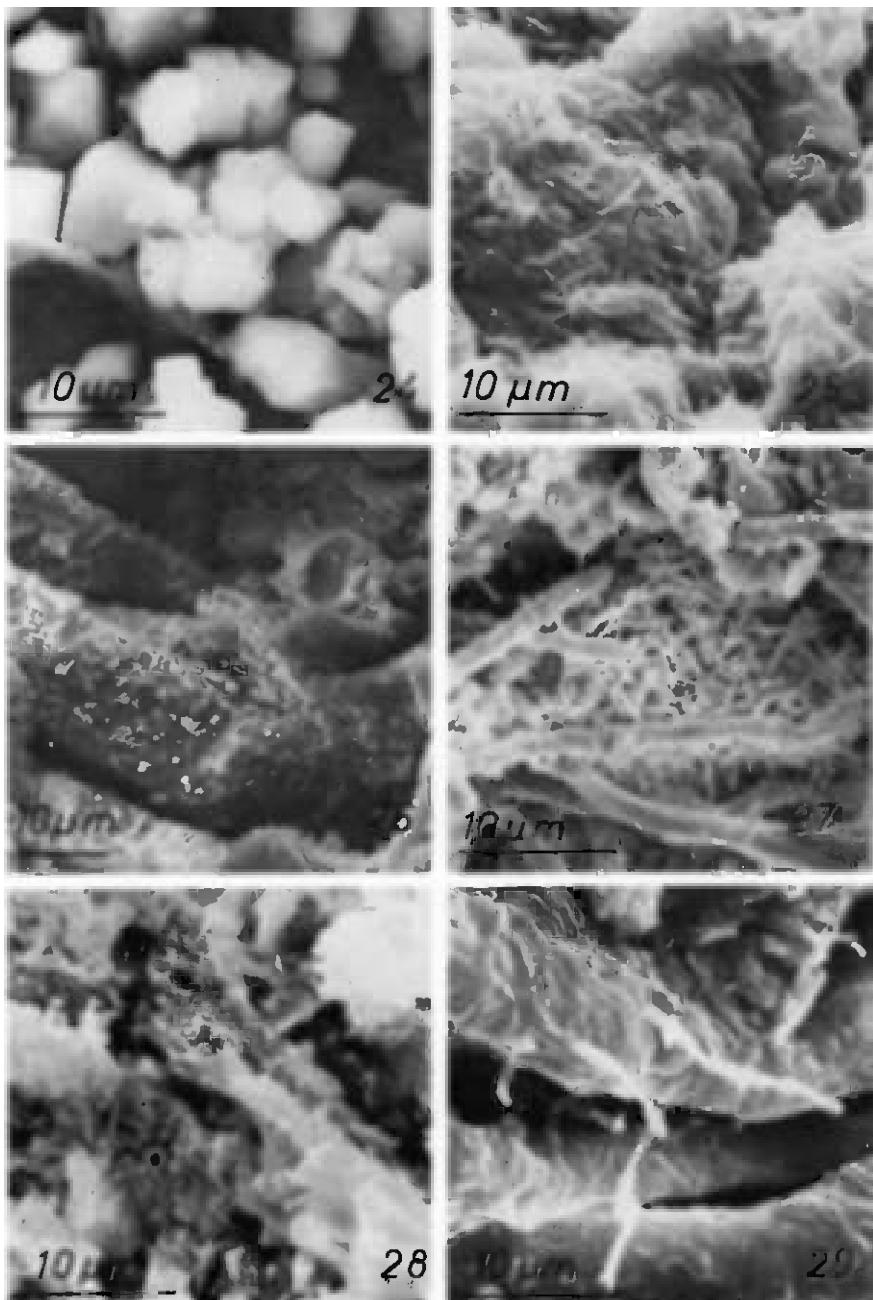
The lichen surface varies from smooth to roughly sculptured (Peveling, 1970a). In the extremely smooth surface not even the orientation of the cortical hyphae can be made out (Fig. 20). Only occasionally do nodulelike



Figs. 18–19. Fig. 18, cross section of one-half the thallus of *Usnea ceratina* showing outer cortex (Co), algal layer (A), medulla (M), and the central cord (Z); Fig. 19, cross section of *Peltigera rufescens* thallus showing plectenchyma (PL), algal layer (A), and the thick interwoven medullary hyphae (M). Material air-dried.



Figs. 20-23. Cortical surfaces of thalli. Fig. 20, smooth surface of *Cetraria islandica*; Fig. 21, parallel hyphae in the cortex of *Usnea ceratina*; Fig. 22, perpendicular hyphae in the cortex of *Roccella* sp.; Fig. 23, furrows and ridges in the thallus of *Collema tenax*. Material air-dried.



Figs. 24-29. Crystals of lichenic acids and other hyphal adherents. Fig. 24, rhombic and cubic-shaped crystals on the surface of *Physcia dimidiata*; Fig. 25, sticklike crystals embedded in the uppermost cortical layer of *Xanthoria parietina*; Fig. 26, small flake-shaped crystals on the medullary hyphae in *Peltigera rufescens*; Fig. 27, needle-shaped crystals between

elevations appear. Moreover, very small apertures are scattered in varying distance all over such a thallus. In some lichens which have a thick external homogeneous layer the direction of the cortical hyphae is still evident. A parallel orientation of the hyphae to the thallus surface is seen typically in the beard lichens where the hyphae also run parallel to the long axis of the thallus (Fig. 21). Apertures of round or elliptical shape occur between the visible hyphae in the thallus surface. Hyphae parallel to the thallus surface were clearly demonstrated in representatives of *Cornicularia* and *Aleatoria* (Hawksworth, 1969).

There are hyphae which are arranged perpendicular to the surface (Fig. 22). This type of orientation can be seen with the light microscope, but to bring out all the particles and their distribution between the ends of the hyphae higher magnification is needed.

Finally, there are lichens which do not have a typical cortex. If such a thallus is dry its surface shows alternating furrows and ridges (Fig. 23).

2. ADHERENTS TO THE CORTICAL SURFACE

Crystals of lichen acids which may be rhombic, cubic, flake, or needle-shaped (Figs. 24–28) cover parts of the thallus or the whole surface. These crystals may be scattered on the surface (Fig. 24) or embedded in the uppermost homogeneous cortical layer (Fig. 25). In addition to the crystals of lichen acids, threadlike forms occur occasionally (Fig. 29). They seem twisted and differ distinctly from crystals.

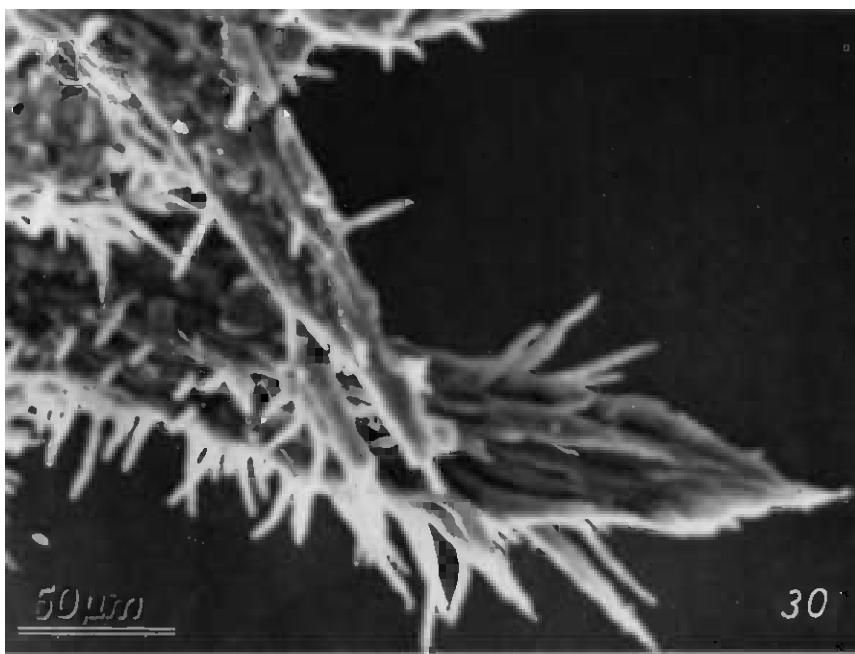
Tiny hairs occur at the brink of a thallus in some lichens. They are very often overlooked and only the SEM shows that they consist of several hyphae which form this brittle structure. At the end of such a hair several hyphae form a sharp tip while along its length short, single hyphal ends come out from the main structure (Fig. 30).

The other surface structures such as cypellae, cephalodia, isidia, soredia, pycnidia, and apothecia have not been explored in detail by the SEM. One illustration of isidia is seen in Fig. 31.

C. Rhizines

Lichens are attached to their substrate by different means. The most common structures of attachment are the rhizines, which are conglomerates of fungal hyphae. On the basis of observations with the SEM, three main forms of rhizines can be described.

hyphae of a soralium in *Cetraria pinastri*; Fig. 28, flake-shaped crystals between medullary hyphae of *Parmelia carporrhizans*; Fig. 29, threadlike adherents to hyphae in the uppermost cortex of *Collema tenax*. Material air-dried.



Figs. 30–31. Fig. 30, small brittle hairs at the end of the thallus of *Anaptychia ulotrichoides*; Fig. 31, isidia on the thallus of *Pseudevernia furfuracea*. Material air-dried.

The first type are those which grow out of the lower cortex and form a tip (Fig. 32). These rhizines become columnlike but still show a tip at their end. After touching the ground the tip enlarges to a footlike structure which consists of very homogeneous material and is adapted exactly to the ground (Fig. 33). The footlike structure of several rhizines can fuse. Such rhizines are especially common in *Parmelia*.

A second type of rhizine is formed by bundles of hyphae which are not closely connected. Single hyphae in these bundles can be recognized and at the end of such a bundle, where it touches the ground, the hyphae radiate outward (Figs. 34 and 35). Cross sections of such rhizines reveal that those which are loosely organized have hyphae with thick hyphal walls while the tightly formed rhizines have hyphae with thin walls and between them are thick layers of a homogenous substance (Reznik *et al.*, 1968).

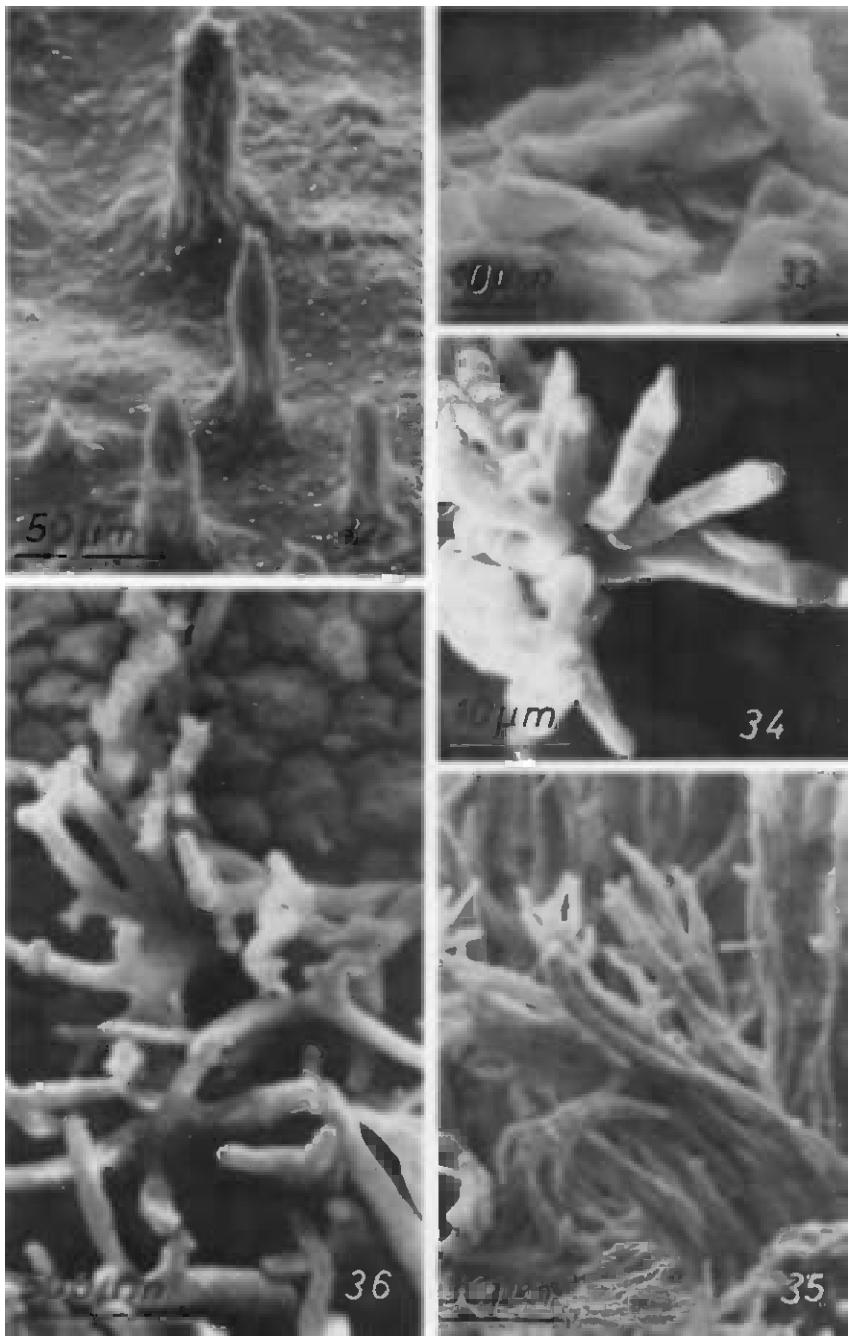
A third type of rhizine is that which appears as a single structure but consists of several hyphae (Fig. 36). The surface of these rhizines is smooth so that they appear as a single structure. At their ends these rhizines can be bifurcated.

V. Some Aspects of Changes in Ultrastructure Due to Ecological Conditions

Lichens as well as higher plants show different ecotypes within one species. The main factors which produce ecotypes are climate and substrate. Ecotypes can differ in their morphological appearance and show specific physiological characteristics.

Concerning lichens, it is of special interest if ecotypes are characterized by different forms in their symbiotic relationship. Ben-Shaul *et al.* (1969) observed ecotypes of *Caloplaca aurantia* var. *aurantia* from the Mediterranean seashore, a mountainous region in the Mediterranean, and from desert locations in the Negev. In the specimens from the Mediterranean area fungi and algae were in close contact, but the authors could not observe any penetration of algal cells by haustoria. Lichens from the desert, however, did have algal cells penetrated by haustoria. Similar results were obtained with *Lecanora radiosa* (Galun *et al.*, 1970c). In thalli collected in the moderate climate of the Mediterranean region haustoria were found only in senescing and already disorganized algal cells and they were rare. Thalli from the desert showed many haustoria in senescing as well as in young algal cells. In two *Gonohymenia* lichens, one from a xeric habitat and the other from a moderate climate, no differences in the fungus–alga relationship were found (Paran *et al.*, 1971).

The reported studies were done with material which was soaked for 2–10 days. Therefore, the question may be raised if under natural environ-



FIGS. 32-36. Different forms of rhizines. Fig. 32, young rhizines on the lower cortex of *Parmelia caperata*; Fig. 33, an older enlarged tip of a rhizine of *Parmelia caperata*; Fig. 34, view of the end of a hyphal bundle from a rhizine of *Lobaria pulmonaria*; Fig. 35, rhizines

mental conditions the same differences are present. It is known that increasing humidity quickly stimulates photosynthetic and respiratory activity (Lange, 1969a,b) as well as starch production (Brown and Wilson, 1968; Peveling, 1970b) and that the development of haustoria depends on the condition of a thallus (Tschermark, 1941).

VI. Conclusions

The recent studies on lichens with the scanning and transmission electron microscopes have revealed new and interesting structures in the symbiotic algae and fungi. These protoplasmic structures, besides providing new aspects of micromorphology, are of special interest because they are not present in the free-living counterparts of the lichen symbionts. These structures are considered to be important for the metabolism of the thalli. In the future, specific labeling of substances followed by electron microscopic autoradiography should reveal the exact mechanism of the cellular and intercellular flow of metabolic products. These studies should lead us to a better understanding of the physiology of lichens and will help to explain the unique characteristics of these mutualistic associations.

Acknowledgment

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made up of hyphal bundles on the lower cortex of *Lobaria pulmonaria*; Fig. 36, rhizines of *Umbilicaria crustulosa*. Material air-dried.

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Part II

PYHSIOLOGY OF THE INTACT THALLUS

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Chapter 6

ABSORPTION AND ACCUMULATION OF MINERAL ELEMENTS AND RADIOACTIVE NUCLIDES

Y. TUOMINEN and T. JAAKKOLA

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I. Introduction

The subject of this chapter is the most imperfectly explored field of lichen biology when compared with the huge amount of experimental facts published on metal cation uptake and translocation in vascular plants. The lack of information in this area can perhaps be attributed to the dual nature of lichens and to the lack of a highly differentiated internal organization similar to that of higher plants. Further, there are experimental difficulties such as the relatively slow rate of thallus growth and the instability of the symbiotic state during long-term experiments in the laboratory. To a certain extent, the small universal economic importance of these organisms has also discouraged investigations in this area. The studies dealing with the uptake, accumulation, and translocation of mineral elements or radioactive nuclides have all used the composite thallus, living or dead. No studies have been made on the mineral uptake properties of phyco- and mycobionts which can be cultured separately.

Although the thallus must be viewed as a functional unit with properties different from those of its components, it nevertheless can be understood as a kind of vector sum of the components, so that the results of studies on fungal and algal physiology are very valuable for the design of theoretical models for experimental verification. On the other hand, the direct general-

ization of results obtained with other plants can be misleading. For example, Gerloff and Fishbeck (1969) have discovered interesting facts on the quantitative cation requirements of some green and blue-green algae. They showed that a critical cell concentration of Ca, i.e., the minimum cell content of Ca which permits maximum or near maximum total growth of an organism, was extremely low. The requirement for Ca of six species was 0.06% from the oven-dry weight. This suggests differences in the physiology and function of Ca in algae and in angiosperms. This observation may be important in lichen physiology for some species of these algal groups are phycobionts of lichen thalli. Another problem has been the imperfect information on the chemically complex composition of fungal cell walls (Aronson, 1965). This has been due in part to a lack of analytical methods and in part to the difficulties in preparing cell-wall samples. Many recent papers have indeed clearly revealed the structural complexity of the cell wall. The significance of the fungal cell wall, which is the main component of a composite thallus, in the early phases of cation uptake has been recognized for a long time. An unexpected fact, also mentioned by Aronson, is that uronic acids, which evidently play an important role in the cation binding of most forms of higher plants and mosses, have not been demonstrated conclusively in fungal cell walls.

The most serious obstacle to the study of models of the uptake of metal cations, in spite of the lively experimental activity, is probably the theoretical difficulties in energetics and molecular structure at the cellular barrier membranes, the chemistry of which seems to be unexpectedly complex. In principle, the uptake of metal cations is divided into the passive, physico-chemical phase and an active phase that depends on metabolic sources of energy. The active uptake has been adequately demonstrated and the sites of input for different groups of cations have been revealed by competitive experiments. In spite of many attempts, however, the active carriers of cations have not been found. It is an appropriate time for new experimental and/or theoretical ideas. For example, the new, developing thermodynamics of irreversible processes has energetically outlined some very interesting possibilities of the stationary state for the coupling of the processes which are worthy of closer experimentation.

There was a new phase in the investigations of mineral accumulation by lichens at the end of the 1950's when it was discovered that the radionuclide contents of lichens and mosses were much higher than those of other plants. At the beginning of the 1960's several investigations of environmental radioactivity in the arctic regions of Scandinavia and Alaska showed high body burdens of strontium-90 and cesium-137 in animals and man due to the food chain that leads from lichen through reindeer or caribou to Lapps and Eskimos. These observations resulted in several studies on lichens, which

are the first link of this food chain. Early investigations determined the radionuclide contents of lichens and compared these to the concentrations found in other plants. Later studies were concerned with the radionuclide distribution in lichen thalli and the retention and elimination rate of radionuclides from thalli. These studies make it possible to predict the future development of radionuclide levels in the biosphere and to evaluate the total radiation dose for man through food chains starting with lichens.

The investigations of radioactive fallout nuclides have produced new information about the interaction between metal ions and lichens, because radioactive fallout acts like an extremely fine tracer under natural conditions. Radioactive nuclides also afford an excellent way to explore in the laboratory or under field conditions the inherent turnover status of metal cations.

II. Metal Content of Lichens

Inactive Nuclides

The determinations of inactive metal nuclides in lichen thalli provide limited information on the physiology of lichens because they are based almost exclusively on total ash analyses. A further drawback is the lack of time dimension. The ash method also is sensitive to the fortuitous contamination of the samples by inorganic compounds. This source of error, however, can be minimized by using the physiologically more natural dry weight as a reference base instead of ash. On the other hand, the use of the ash method can be readily understood from the chemist's point of view, for the aim of these studies has mainly been biogeochemical prospecting without any biological purposes. The published total amounts of metal nuclides in the ash or in the mass unit of dry weight do provide a secure starting point for future studies.

1. ATMOSPHERE AS A SOURCE OF METALS

Jenkins and Davies (1966) showed a close correlation between metal amounts in lichen ashes of *Lecanora gangalooides* and *Parmelia omphalodes* and those in the ash of material deposited from the atmosphere. Their results are based on a detailed statistical examination of the analytical data provided in part from an investigation on the role of saxicolous lichens in the initial stages of pedogenesis and in part from a routine survey of the trace element status of 127 mountain top soils. The concentrations of individual elements in a particular lichen species tended to converge to a "preferred value." Their conclusion was that some constant factor apparently domi-

nates the metal composition of these lichens. For this reason, the correlation of the trace-element content of a lichen with that of its rock substratum was normally poor, except in the cases of rapidly weathering rocks like basic tuffs and ultrabasic rocks. In particular they observed that the correlation of the "preferred values" between different lichen species within one region was better than that obtained between samples of the same species in different regions. Thus, they concluded that the constant factor involved was external and regional in nature rather than physiological. Jenkins and Davies showed that the contents of trace elements in saxicolous lichens and in organic top soils conformed surprisingly well to the trace element distribution of ashed atmospheric deposits. They could not, however, judge how far the trace-element composition of lichens was controlled by atmospheric contamination. They suggested that, because of the slow growth rate and the hydrolability of thalli, the evidence for the dominance of atmospheric sources in the trace element composition was strong.

Until recently, it was generally believed that lichens absorbed mineral nutrients mainly from the poor ion content of the rainwater that passed over the surface of the thalli. However, the substratum has also been shown to function as a source of metal ions.

2. SUBSTRATUM AS A SOURCE OF METALS

Many studies have reported on the ability of lichens to take up and accumulate metals and nutrient substances from the substratum, even through the dead basal parts of the thalli. The accumulation of certain metals is due primarily to the slow growth rate and longevity of lichens. However, these studies have not revealed any clear physiological, taxonomic, or morphological relationships between metal accumulation and lichens. Some indirect evidence exists for such relationships, such as the preference of certain lichens for special substrata and the ability to tolerate unusually high amounts of certain metals. A rare example of the effect of structural properties of thalli on the accumulation of metals is the observation by Hanson *et al.* (1967), as a short comment in their paper, that the ^{137}Cs amounts were the greatest in those species characterized by a greater amount of surface area (*Cetraria richardsonii*, *Dactylinia arctica*, *Nephroma arcticum*) rather than the branching types.

Lounamaa's (1956) large-scale study on the trace-element content of lichens, mosses, ferns, conifers, deciduous trees, shrubs, dwarf shrubs, grasses, and herbs allows for comparative studies of lichens with other plants. According to Lounamaa, lichens differ considerably from higher plants and mosses in trace-element concentrations. Most of the elements he studied occurred in greater amounts in lichens than in any other plants.

Manganese was the only trace element that was found in lower amounts in lichens than in higher plants. Cobalt, Ni, Mo, and Ag occurred in the same amounts in lichens as in other plants from corresponding habitats, whereas Zn, Cd, Sn, and Pb were in much higher concentrations in lichens. Lounamaa also showed that the effect of the substrata on the metal content of lichens was revealed in the trace-element composition of species growing on silicic and ultrabasic rocks. For example, the Cr, Mn, Co, Ni, Zn, Ga, Y, Zr, Sn, and Pb contents of lichens on ultrabasic rocks were higher than those of samples from silicic rocks.

Jaakkola *et al.* (1967) and Jaakkola (1969) reported results very similar to those of Lounamaa (1965) with respect to Fe. They detected a higher content of inactive iron in the dead basal parts of reindeer lichens than in the living tops, i.e. about two to three times that of the top parts. In the case of *Cladonia alpestris*, the thallus of which changes gradually to an organic decomposed substratum, these high amounts of inactive iron apparently arise from the substratum. In addition to this, according to Jaakkola, the fallout iron ^{55}Fe seemed to move relatively rapidly from the top part toward the base of the thallus. This movement seems to be unidirectional, because no comments on possible back diffusion exists. This kind of information on the mobility of metal nuclides within a lichen thallus is rare in the present literature.

The paper by Lange and Ziegler (1963) on the Fe and Cu content of lichens is of interest from a physiological point of view. The lichens investigated grew as extensive communities on the 400-year-old hills of slag in smelting-works. This old industrial area in the Harz Mountains is of interest because old floristic material is available so that the succession of lichen communities can be reconstructed and the tolerance relationships of species with respect to Fe revealed. Besides the usual determinations of Fe and Cu in dry weight, Lange and Ziegler carried out large histochemical studies on the localization of Fe within thalli and compared the Fe and Cu contents of lichens with the results of chemical analyses of the substrata. The amounts of Fe were high and ranged from 6000 to 16,000 ppm in dry weight, the highest value, 55,000 ppm, being found in *Acarospora sinopica*. The same species growing on substrata poorer in Fe showed smaller contents of Fe within its thalli. The results were about the same for Cu. The amounts of Cu were in the range of 37–1100 ppm in dry weight. The highest value, 1670 ppm, found in *Rhizocarpon oederi* was doubtful because of the difficulties in separating the thalli from the substrata, a difficulty that is true for many lichen species. On the other hand, this species, which is known as an "iron-lichen," showed very clearly the usual superficial deposition of Fe on the hyphal cell walls within the thallus. Some of the Fe was bound in a manner that could be detected only by ashing the samples.

Lange and Ziegler noted that these lichens tolerated very high amounts of Fe and Cu. On the other hand, these metals are used only in trace amounts in the culture media of fungi and algae and the Cu tolerance of many algae is especially low. The tolerable amounts of Fe and Cu for fungi and algae were far below the amounts found in the lichens they studied. The following explanations for this observation were suggested: (a) unspecified cytoplasmic resistance of lichenized fungi and algae to metal cations; (b) some kind of immobilization of the ions in the chelated form within the cytoplasm of the symbionts; and (c) active and/or passive transport of the ions to regions external to the plasma and cell wall and localization in the form of insoluble salt on the surface of the thallus.

They considered the third explanation (c) to be the most plausible because large amounts of Fe can be observed frequently on the cell walls, in intercellular spaces, and even as a coating over the outer surfaces of the thalli of *Acarospora sinopica*, *A. smaragdula* f. *subochracea*, and *Rhizocarpon oederi*.

Some authors have given amounts of the rare earths in lichens but the most complete study on the contents of all the rare earths in lichens is that of Erämetsä and Yliruokanen (1971a). To evaluate the enrichment of the rare earths they compared the mass spectrographically measured values with the crustal averages of these elements. The ratio was usually between 0.3 and 2.0, but in samples collected from places with higher than average rare earth contents, the ratio was 2–5. The amounts found are separately summarized in Table I.

3. DIVERGENCES IN METAL AMOUNTS

In general, the amounts of trace elements between different species show high variability. For example, Co, which has a concentration range in lichens of 3–30 ppm in ash (Lounamaa, 1956), occurs in greater amounts in *Umbilicaria pustulata* (10–30 ppm) than in *Stereocaulon paschale* (max. 10 ppm) which both grow on silicic rocks. Lounamaa found exceptionally high values of Mn (10,000 ppm in ash) in *Cladonia alpestris* and *Parmelia saxatilis* while the same species collected and studied in Norway (Solberg, 1967) had values of about 2500 ppm in ash. Presumably, according to Solberg, differences in the composition of the substrata have a decisive role. Solberg's Mn values in *Cladonia deformis* (12,600 ppm), *Evernia prunastri* (6100 ppm), *Usnea dasypoga* (13,750 ppm), and *U. longissima* (8000 ppm) in the ash were similar to those found by Lounamaa.

The results of Zn accumulation studies by Lambinon *et al.* (1964) revealed clearly differences in the physiology and tolerance of several lichen species with respect to Zn. The lichens were divided into four groups: (a) species that displayed high accumulation of Zn without harmful effects, i.e., *Diplo-*

TABLE I
CONTENTS OF RARE EARTHS IN LICHENS (ppm in ASH) DETERMINED BY SPARK SOURCE MASS SPECTROGRAPHY

Species	Ash (%) ^a	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
<i>Cladonia arbuscula</i>	1.17– 1.58	50–87	67–140	8–15	37–91	8–16	2.7–4.6	9.4–25	0.9–1.7	4.8–9	0.9–1.4	2.6–4.8	0.41– 0.45	1.6–5.2	0.27– 0.52
<i>C. alpestris</i>	1.32– 1.33	49–120	77–170	11–27	38–150	11–40	2.2–8.7	8.6–28	1.1–3.3	6.3–18	0.8–3.5	3.0–9.3	0.28– 1.3	3.4–8.6	0.15– 0.60
<i>C. rangiferina</i>	1.06– 1.59	52–75	58–77	7.2–8.7	30–34	5.0–6.7	1.3–1.7	3.8–6.5	0.33– 0.66	2.2–6.1	0.25– 0.63	0.62– 1.5	0.08– 0.28	0.6–2.4	0.07–0.2
<i>Stereocaulon paschale</i>	1.69– 2.68	19–110	28–190	3.8–23	12–100	2.7–19	1.1–4.0	3–21	0.3–2.0	2.5–15	0.2–2.2	0.92– 6.7	0.07– 1.0	0.5–10	0.05–1.0
<i>S. saxatile</i>	3.68	45	75	11	41	12	2.3	13	1.3	8.6	1.4	4.5	0.51	3.4	0.20
<i>Nephroma arcticum</i>	4.35	150	280	31	120	21	5.7	21	1.4	8.6	1.2	3.9	0.48	4.1	0.42

^aAsh values as weight percentages in the dry matter (Erämettsä and Yliruokanen, 1971a).

schistes scruposus var. *bryophilus*; (b) species whose Zn content varied in successive samples (*Stereocaulon nanodes*); (c) species which possessed high amounts of Zn but showed pathological changes, i.e., *Cladonia arbuscula*, *C. fimbriata*, *C. furcata*, *C. implexa*, *C. rangiformis*, *C. tenuis*, *C. verticillata* var. *cervicornis*, and *Stereocaulon dactylophyllum*.

Several species accumulate exceptionally high amounts of Zn. For example, in the saxicolous lichen *Umbilicaria pustulata*, Zn is accumulated up to 1.67% of the ash (Lounamaa, 1965). This indicates an efficient mechanism for the selective uptake of Zn. A careful analysis of thermodynamic data of the Zn binding, compared with the many papers on the combination of proteins with metal ions, might reveal an uptake component based on proteins instead of carbohydrate polymers.

Another example of the differences between lichens is *Thamnolia vermicularis* which accumulates only Ag (Sainsbury *et al.*, 1968). The other elements these investigators found in the lichen ash were less than corresponding ones found in the substrata.

LeRoy and Koksoy (1962) suggested the use of lichens for biogeochemical prospecting. However, because of the differences between lichens in accumulating metals, they emphasized that the final recommendations for the use of certain lichens in prospecting must be based on the total status of metal composition of the species rather than only one or two divergences. The lichen species they studied (*Caloplaca elegans*, *Lecanora rubina*, *Parmelia conspersa*, and *Umbilicaria hyperborea*) in the Turkey Creek in Colorado showed high concentrations of Sr, Ti, V, Y, appreciable amounts of Cu, Cr, Pb, Mo, and Zn, and the presence of Be.

Along with many other authors, LeRoy and Koksoy have drawn attention to a common source of error in the studies of the mineral content of lichens. It is difficult to avoid the contamination of the samples by the substratum, because of the close contact of the species with it and even the decaying of the lower parts of the thallus. Other sources of error that they mentioned are the composition of surface and near-surface water, age of the thalli, the contamination from the atmosphere, and soil that was not removed from the thalli during cleaning for the experiments. Radioactive fallout, also mentioned as a possibility, is of secondary importance with respect to inactive nuclides, because the absolute mass of a certain radionuclide representing a rather high radiation effect is usually vanishingly small compared with the inactive mass of that element.

Czapek (1920) and Boresh (1935) reviewed the earlier investigations and gave amounts of Ca, Mg, K, Na, Fe, Mn, and Al. Tables II and III contain more recent values of published amounts of inactive nuclides in lichens.

III. Concentration and Retention of Radionuclides

A. Artificial Radionuclides

The nuclear weapons tests carried out in the atmosphere, nuclear reactor operations, and space applications of nuclear energy are the main sources of artificial environmental radioactivity. The nuclear weapons tests, due to the widespread environmental radioactivity they produce, have been the most important source of the radionuclide content of lichens.

Most of the radionuclides produced by nuclear detonations are fission products. In addition, radioactive nuclides are formed by the interaction of released neutrons with soil, air, water, and parts of nuclear devices. The amount of different radionuclides produced depends on the detonation yield, the fission to fusion ratio, and the height or depth of burst over sea or land as well as on the elemental composition of the environment of the detonation. Radionuclides enter the atmosphere in a fireball formed by nuclear detonations. All material in the fireball is vaporized because of the high temperature. When the fireball cools, condensation of material as particles of different sizes occurs. When the detonation yield is of kiloton range practically all the radionuclides remain in the troposphere and are deposited in about two months. The deposition of radionuclides depends on the distance from the detonation site, on the size of particles, and on directions and velocities of winds. In the nuclear detonations of megaton range, the fireball rises into the stratosphere. The tropospheric component of megaton surface explosions is only about 5%. The radionuclides in the stratosphere are so finely divided that they do not fall down by gravitation only, but through so-called injections of the stratospheric air. These injections into the troposphere are strongest in spring and between 30°–60° N latitude. The mean residence time of radionuclides in the stratosphere has been evaluated to be about 8 months for radionuclides injected in arctic latitudes and about 18 months for those injected near the equator. Thus, the nuclear detonations of megaton range are the main source of the worldwide contamination by long-lived radionuclides. The dependence of global stratospheric fallout on latitude is a very important factor to consider when comparing the radionuclide contents of lichens collected at different locations. The latitudinal distribution of the deposition of radionuclides has been considered usually on the basis of the distribution of ^{90}Sr by latitude. This is a radionuclide whose behavior in the environment is best known. In 1963, the year with maximum fallout, the average ^{90}Sr deposition in 80°–90° N latitude band was 4.9 mCi/km² (year total). This value increased with the decrease in latitude and reached the maximum of 18.9 mCi/km² in

TABLE II
CONTENTS OF BIOLOGICALLY SIGNIFICANT METALS IN LICHENS^a

Reference	No. of species	No. of samples	Na (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mo (ppm)	Determined in
Bertrand and Bertrand (1947)	3	3	40–900 ^b	900–3000								Dry weight
Yarilova (1947)	3	3	790–1000	2600–8500	170–660	470–9640	34–39	180–470				Dry weight
Prestegge (1955)	3	3				800–1400						Dry weight
Warren and Delavault (1955)	1	3						4–180	26–61			Dry weight
Lounamaa (1956)	16	59				100–10000		100–3000	1000–10000	1000–3–30		Ash
Wöhlbier and Lindner (1959)	1	2	50–70	3100–3200	370–480	2300	170–190	380–620	3–4	80	0.1–	Dry weight
Oborn (1960)	4	4					3180–5020					Dry weight
Maquinay <i>et al.</i> (1961)	2	2							225–3500			Dry weight
Mićović and Stefanović (1961)	4		1.5–2.7	7.1–8.96	0.9–5.04	26–46.6	0.36–0.41	2.25–3.16	0.02	+ ^c		% wet weight as oxides
LeRoy and Koksoy (1962)	4	9							150–1000	200–2000	10–50	Ash
Lange and Ziegler (1963)	10						400–16000	15–1100				Dry weight
Lambinon <i>et al.</i> (1964)	14	41							50–93400			Dry weight
Malyuga (1964)	1	1							1000			Ash
Lounamaa (1965)	20	49				560–4500	11000–78000			1300–16700		Ash

Scotter (1965)	10	25			600– 3800				Dry weight
Mitchell <i>et al.</i> (1966)	3	3		150–970					Dry weight
Paribok and Alekseeva- Popova (1966)	4	8				1000– 6000	30000	80– 200	20
Jaakkola <i>et al.</i> (1967)	4	6		200–360	260– 1000	26– 145	200– 635	2.4–4.4	20– 56
Mäkelä (1967)	1	1	200	2000	300	700			Dry weight
Podkorytov (1967a)	9	9				2.5–27			Dry weight Dry weight
Podkorytov (1967b)	8	8				3.3–15			Dry weight
Solberg (1967)	16	16		600– 5400	100–900	100– 2900	12– 187	37 species	Dry weight
Jaakkola (1969)	1	6					111– 415		Dry weight
Lyon <i>et al.</i> (1970)	1	1					130		Ash
Erämetsä and Ylirukanen (1971b)	6	12						1–20	Ash

^aThe collecting of the table has been valuably supported by Miss Inkeri Ylirukanen's literature review in 1971.

^bThe two figures indicate the limits of usual variation without the highest values observed.

^c+, only qualitatively determined.

TABLE III
CONTENTS OF UNBIOLOGICAL METALS IN LICHENS^a

Reference	Li	Be	Al	V	Cr	Co	Ni	Ga	As	Rb
Kratzmann (1913)				1.76						
				28.2						
Rankama (1940)							790			
Bertrand and Bertrand (1947)									2.4– 24.6	
Yarilova (1947)				19– 286						
Bertrand (1954)	0.02– 0.29 ^b									
Lounamaa (1956)					6– 1000	3– 100	10– 3000	3–30		
Wöhlbier and Lindner (1959)	340– 410				1.47– 1.72			1.4– 1.6		
LeRoy and Koksoy (1962)	1–3		50–70		30– 150			15– 100		
Paribok and Alekseeva-Popova (1966)					80– 1000	30– 80	150– 3000			
Jaakkola <i>et al.</i> (1967)					0.6– 1.6					
Podkorytov (1967a)				0.33– 2.55						
Sainsbury <i>et al.</i> (1968)	+ ^c			+ ^c				+ ^c		
Mićović and Stefanović (1961)										
Erämetsä and Yliruokanen (1971a)										
Erämetsä and Yliruokanen (1971b)										

^aThe collecting of the table has been valuably supported by Miss Inkeri Yliruokanen's literature review in 1971.

^bThe two figures indicate the limits of usual variation observed.

^c+, only relative values.

40°–50° N. The minimum value in 10°–20° S, small maximum in 40°–50° S and values in the latitude band of 80°–90° S were 0.9, 1.8, and 0.2 mCi/km², respectively (Hardy, 1971). In any given latitude the amount of deposition is fairly well correlated with that of precipitation.

In addition to the latitudinal distribution of fallout the sampling date must be taken into consideration when comparing the results of different lichen

TABLE III (*continued*)

Sr	Y	Zr	Nb	Ag	Cd	Sn	Ba	Hf	W	Pb	Values given as
											% ash
											ppm in ash
											ppm in dry
											weight
											ppm in dry
											weight
											ppm in dry
											weight
10– 200	30– 300		1–10 100	10– 100					300– 3000	ppm in ash	
1000– 5000		5–7		10– 30					30– 1500	ppm in ash	
150– 300				30– 50	400– 2000				300– 600	ppm in ash	
0.8– 118					0.16– 0.96 + ^c				1.1– 21 + ^c	ppm in dry weight	
					±					% ash as oxides	
11–82						650– 1500				ppm in ash	
							1–19	1–40		ppm in ash	

studies because the content of a given radionuclide depends on the time that has elapsed between production and radioactivity determination.

1. ^{137}Cs AND ^{90}Sr IN LICHENS

Among the artificial radionuclides, ^{137}Cs and ^{90}Sr are the most harmful long-term contaminants to man. This is due to the high yield of these radionuclides in fission and to long physical half-life (^{137}Cs , 30.2 years; ^{90}Sr , 28.9 years). In addition, they are absorbed efficiently by living organisms because they are chemically related to the physiologically important elements potassium and calcium.

sium and calcium. They are the most studied radionuclides in lichens. The more important is ^{137}Cs because it is readily transported to man by the lichen-reindeer (caribou)-man food chain. Strontium-90 is located almost exclusively in the bones of reindeer. Therefore, a break for ^{90}Sr occurs in this food chain. Large numbers of ^{137}Cs investigations are also facilitated by the ease of its radioassay.

a. THE CONTENT OF ^{137}Cs AND ^{90}Sr . The first ^{137}Cs and ^{90}Sr determinations in lichens were made at the end of the 1950's. In 1959, Gorham reported that the lichens in the English Lake District contained three times more radionuclides than higher plants. Hvinden and Lillegraven (1961) gave an average ^{137}Cs content of 36 nCi/kg dry weight for three lichen samples collected in northern Norway in the autumn of 1959. They also collected all lichens in a small, specified area and calculated the ^{137}Cs concentration of lichen per square meter. Comparing these results to the cumulative amount of ^{137}Cs deposited per the same area unit, they found that lichens have a very high efficiency for the retention of fallout ^{137}Cs .

At the beginning of the 1960's the first values of ^{137}Cs and ^{90}Sr in lichens of the arctic region were published (Liden, 1961; Weichhold, 1962). These investigations have now been going on for over 10 years in the United States (Alaska), Sweden, Finland, and the Soviet Union, and they form the most essential part of the information about radionuclide contents in lichens. Tables IV, V, and VI present the ^{137}Cs and ^{90}Sr contents of lichens, mainly of the genus *Cladonia*, determined in these four countries during 1959-1970. Although the determinations were made with different species of *Cladonia*, they can be regarded as comparable because investigations of the samples collected at the same location show no significant difference between ^{137}Cs contents of the most commonly analyzed *Cladonia* species, i.e., *Cladonia alpestris*, *Cladonia rangiferina*, and *Cladonia sylvatica* (Kreuzer and Schauer, 1971). When the results of different investigators given in the tables are compared it should be noted that the figures are affected by sampling techniques, growth environment, and number of samples used for each value of radionuclide content. From Tables IV and V it can be seen that the maximum ^{137}Cs and ^{90}Sr contents in lichens (30-70 and 10-13 nCi/kg dry weight, respectively) occurred during 1964-1965. The level of ^{90}Sr and especially that of ^{137}Cs has decreased slowly after 1965 when the annual deposition of radioactive fallout has been only a few percent compared with the total amount accumulated in lichens. Thus, lichens act as a substantial reservoir of ^{137}Cs and ^{90}Sr radionuclides for a long period of time. In 1960 the ^{137}Cs and ^{90}Sr contents of lichens in Finland were about five to ten times higher than in vascular plants (Salo and Miettinen, 1964). In the year with maximum fallout the ^{137}Cs concentration in lichens was only three to six times higher

TABLE IV

^{137}Cs CONTENT IN LICHENS FROM FINLAND, U.S.S.R., AND ALASKA (U.S.A.), COLLECTED DURING 1960–1970. SAMPLES OF ANAKTUVUK PASS, ALASKA, ARE *Cladonia-Cetraria* LICHENS, OTHER SAMPLES ARE *Cladonia* LICHENS (ALL RESULTS HAVE BEEN GIVEN AS nCi $^{137}\text{Cs}/\text{kg}$ DRY WEIGHT)

Sampling year/half-year	Finland ^a	U.S.S.R. ^a		U.S.A. (Alaska) ^a	
		Murmansk region ^b	Other arctic regions ^b	Anaktuvuk Pass	Palmer-Anchorage region
1960	28 (6) ^c	—	—	—	—
1961	22 (2) ^d	26±7 ^g	17–19 ^g	—	—
1962/I	—	—	17 ^g	—	—
1962/II	22 (15) ^e	—	—	11 (2) ^h	—
1963/I	—	48±10 ^g	—	—	—
1963/II	37 (3) ^e	—	—	17 (3) ^h	—
1964/I	—	—	74±6 ^g	30 (7) ^h	—
1964/II	64 (3) ^e	—	—	25 (26) ^h	—
1965/I	—	50±1 ^g	43 ^g	26 (2) ^h	—
1965/II	56 (3) ^e	27±4 ^g	24±7 ^g	32 (10) ^h	—
1966/I	—	34±4 ^g	—	30 ^l	—
1966/II	43 (5) ^f	33±7 ^g	—	25 ⁱ	—
1967/I	—	—	—	14–21 ⁱ	—
1967/II	44 (5) ^f	—	21±4 ^g	14–25 ⁱ	50 ^k
1968/I		27±3 ^g	13 ^g	13–17 ^l	—
1968/II	31 (2) ^f	—	—	15–20 ⁱ	33
1969/I	—	—	—	22 ^j	—
1969/II	39 (6) ^f	—	—	15 ^j	—
1970/I	—	—	—	13 ^j	24 ^l
1970/II	36 (5) ^f	—	—	—	—

^aNumber of samples has been given in parentheses.

^bStandard deviation of the mean value (\pm) indicated.

^cPaakkola and Miettinen (1963).

^dSalo and Miettinen (1964).

^eMiettinen and Häsänen (1967).

^fRahola and Miettinen (1971).

^gNiznikov *et al.* (1969).

^hHanson *et al.* (1967).

ⁱHanson (1971).

^jHanson and Eberhardt (1971).

^kKoranda *et al.* (1969).

^lMartin and Koranda (1971).

than in vascular plants. In 1969 the ^{137}Cs content of *Cladonia alpestris* in Finnish Lapland was about 20 times higher than that of *Deschampsia flexuosa*

TABLE V
⁹⁰Sr CONTENT OF LICHENS IN NORTHERN REGIONS, FROM ANAKTUVUK PASS, ALASKA
 (U.S.A.), U.S.S.R., SWEDEN, AND FINLAND DURING 1959–1970. MOST SAMPLES
 ARE *CLADONIA* SPECIES

Sampling time	nCi ⁹⁰ Sr/kg dry weight ^a			
Year/half-year	Alaska (U.S.A.)	U.S.S.R.	Sweden	Finland
1959/II	3.3 (1) ^b	2.3 (1) ^e	—	—
1960/I	—	—	—	9.9 (2) ^g
1960/II	2.0 (5) ^b	4.7 (1) ^e	—	6.8 (5) ^g
1961/I	1.7 (6) ^b	—	4 ^f	—
1961/II	5.3 (8) ^b	12 (1) ^e	—	3.9 (2) ^h
1962/I	—	3–7 (37) ^e	—	—
1962/II	3.5 (2) ^c	3–4 (7) ^e	5.5 ^f	—
1963/I	—	7 (6) ^e	—	—
1963/II	6.7 (3) ^c	—	13 ^f	—
1964/I	11 (7) ^c	9 (5) ^e	12 ^f	—
1964/II	8.3 (26) ^c	—	13 ^f	—
1965/I	7.0 (4) ^c	10 ^e	—	—
1965/II	6.4 (10) ^c	6–17 (11) ^e	11 ^f	—
1966/I	9 ^d	8 (7) ^e	—	—
1966/II	8 ^d	—	6 ^f	—
1967/I	5 ^d	—	—	—
1967/II	9 ^d	—	4 ^f	—
1968/I	6 ^d	—	—	—
1968/II	2.5 ^d	—	4.5 ^f	—
1969/I	5 ^d	—	—	—
1969/II	3.5 ^d	—	4 ^f	—
1970/I	4 ^d	—	—	—

^aThe number of samples are in parentheses.

^bWatson *et al.* (1964).

^cHanson *et al.* (1967).

^dHanson and Eberhardt (1971).

^eRamzaev *et al.* (1967).

^fPersson (1971).

^gPaakkola and Miettinen (1963).

^hSalo and Miettinen (1964).

and *Equisetum sylvaticum* samples (Rahola and Miettinen, 1971). In 1967 the ¹³⁷Cs concentration in *Cladonia* lichens of Alaska (Anchorage Area) averaged 54 nCi/kg dry weight and in perennials and herbs from 9 to 38 and from 0.1 to 3.4 nCi/kg dry weight, respectively (Koranda *et al.*, 1969).

The ¹³⁷Cs/⁹⁰Sr ratio, which in the atmospheric fallout ranges from 1.4 to 2.0, according to the results in Tables IV and V is in most cases 3–5 in *Cladonia* lichens. Thus, the retention of ⁹⁰Sr by lichens is lower than that of ¹³⁷Cs.

Table VI presents the ^{137}Cs content of lichens in Anaktuvuk Pass (Alaska) and Sweden. The results have been calculated as nanocuries per square meter and are in good agreement. This observation and the small variance of results from Sweden (Liden and Gustafsson, 1967) show why this form is preferred in studies of radionuclide concentration in lichens. Furthermore, the results calculated per unit area make it possible to consider the lichens as meters of cumulative fallout.

Two to three times higher ^{137}Cs and ^{90}Sr concentrations have been reported for *Cetraria richardsonii*, *Cornicularia divergens*, and *Nephroma arcticum* than for *Cladonia* species sampled at the same locations (Salo and Miettinen, 1964; Hanson *et al.*, 1967). The higher concentrations of radionuclides of the species mentioned are mainly due to the character of the growing site. For instance, *Cornicularia divergens* grows usually on ridgetops, where winds reduce snow cover and expose the lichen to direct atmospheric deposition almost throughout the year. Lower ^{137}Cs and ^{90}Sr concentrations have been found for *Stereocaulon paschale* than for *Cladonia* species.

The highest radionuclide values were found in *Parmelia conspersa* in the Georgia Piedmont (U.S.A.) by Plummer and Helseth (1965) and Plummer (1969). The ^{137}Cs content was 150 nCi/kg dry weight in 1962. The maximum value, found in 1966, was about 450 nCi. In 1968 the ^{137}Cs content was 230 nCi/kg dry weight. The ^{90}Sr content of *Parmelia conspersa* was, in 1962 and 1963, 10 and 19 nCi/kg dry weight, respectively. The radionuclide content of *Cladonia* lichens was also high at the Georgia Piedmont. The maximum average value of ^{137}Cs in 1964 was 71 nCi/kg dry weight (Plummer, 1969). The amount of fallout in the Georgia Piedmont region, according to the ^{90}Sr determinations in precipitation, has been almost twice as high as

TABLE VI
 ^{137}Cs CONTENT OF LICHENS IN ALASKA, U.S.A. (ANAKTUVUK PASS), SWEDISH LAPLAND,
 AND SOUTHERN SWEDEN DURING 1961–1965 (VALUES ARE GIVEN AS nCi $^{137}\text{Cs}/\text{m}^2$)

Sampling time (year/half-year)	Alaska, U.S.A. ^a (nCi/m ²)	Swedish Lapland ^b (nCi/m ²)			Southern Sweden ^b (nCi/m ²)		
		Mean	No. ^c	Range	Mean	No. ^c	Range
1961/II	—	—	—	—	19.6	(1)	—
1962/II	18	19.7	(5)	18.1–24.2	20.5	(4)	18.0–25.1
1963/II	28	27.3	(4)	25.5–29.9	35.5	(3)	34.8–35.9
1964/I	41	35.9	(4)	29.5–45.8	38.5	(4)	31.6–46.0
1964/II	—	42.0	(5)	33.8–54.8	44.6	(3)	43.4–46.4
1965/II	48	44.1	(4)	34.5–59.7	—	—	—

^aHanson *et al.* (1967)

^bLiden and Gustafsson (1967)

^cNumber of samples has been given in parentheses.

that at most locations of the 40°–50°N latitude belt. The main factor, however, for the high radionuclide concentrations in *Parmelia conspersa* was that this lichen grows on granitic outcrops and rainwater which enters the outcrop flows through the lichen carpet which retains and filters the radionuclides.

b. DISTRIBUTION IN LICHEN THALLI. The distribution of ^{137}Cs and ^{90}Sr in lichen thalli has been the object of radionuclide investigations since the beginning of the 1960's. Usually, the radionuclides ^{137}Cs and ^{90}Sr have been analyzed separately in the upper part of the thallus that consists of green, living tissue and in the lower part of the thallus that contains slimy, grayish, and mostly dead tissue. The first observations showed that the ^{137}Cs content, calculated as nCi/kg dry weight, was two to fourteen times higher in the top part than in the lower part (Paakkola and Miettinen, 1963; Salo and Miettinen, 1964; Hanson *et al.*, 1967). The ^{90}Sr content was about the same in both parts. Nevstrueva *et al.* (1967) divided *Cladonia* lichens sampled during the winter of 1964–1965 into four 1-cm parts. If the radioactivity of the top part was considered to be 100%, the results of these four parts for ^{137}Cs were 100, 56, 37, and 27%. Corresponding results for ^{90}Sr were 100, 91, 82, and 91%. The authors assumed that the higher concentration of ^{137}Cs in the top part was due to contamination during the period of intensive fallout and thus the tendency of the equalization of the ^{137}Cs content along the height of lichens should appear during the years of low fallout. The analyses of ^{137}Cs in samples collected in the same location in 1968 indicated, however, that distribution of ^{137}Cs in the lichens had remained practically unchanged (Niznikov *et al.*, 1969). The relative ^{137}Cs activities of the five parts (1 cm) from top to bottom were 100, 60, 43, 23, and 25%.

Kreuzer and Schauer (1971) reported a smaller difference in the ^{137}Cs content between the upper and lower parts of *Cladonia sylvatica* and *Cladonia rangiferina* than for *Cladonia alpestris*. This is probably due to the different textures of the upper parts and different relations between the algae and fungal cells within the lichens. The factors affecting the distribution of ^{137}Cs and ^{90}Sr in lichen thalli are discussed later in the subsection on translocation.

The investigations on distribution of radionuclides in lichens indicated the tenacious binding of fallout radioisotopes, ^{137}Cs especially, in lichens. However, the seasonal cycling of radionuclides was suggested as a possibility (Longhurst *et al.*, 1967). Hanson and Eberhardt (1971) collected lichens from the same locations in Alaska during summer and winter and divided samples (*Cladonia* species) into upper 6 cm of thallus, lower 6 cm of thallus, 4-cm layer of humus, 6-cm layer of organic-mineral soil, and mineral soil at 30 cm below the soil surface. The ^{137}Cs concentration was about four times higher

in the upper part than in the lower part of lichens. They found a seasonal cycle of radionuclides in lichens. There were maximum values in late summer and minimum values in midwinter. The ^{137}Cs content was relatively stable in the upper part of the lichens and showed cycling between lower portions and the humus layer. Cesium-137 moved more readily into soil layers than ^{90}Sr . According to Niznikov *et al.* (1969), 2% of the ^{137}Cs in soil penetrates into the lichen.

The exchange of ^{90}Sr between humus and the lower portions of lichens resulted in seasonal changes of concentrations (Hanson and Eberhardt, 1971). The ^{90}Sr content of the soil beneath the lichens was very low, which indicated that ^{90}Sr was tightly bound in the humus layer. The total amount of ^{90}Sr increased in the lower part of lichens during the winter period at the expense of amounts above and below the stratum (Hanson and Eberhardt, 1971). Because changes in radionuclide concentrations due to seasonal cycling are relatively slight, the difficulties of evaluating accurately the amount of fresh fallout during investigations may result in considerable errors.

c. THE HALF-LIFE OF ^{137}Cs AND ^{90}Sr IN LICHENS. To predict the radiation exposure to man due to the accumulation of radionuclides along the food chain lichen-reindeer-man it is essential to know the elimination rate of radionuclides in the first link, lichen. The residence time is also important for mineral cycling studies in natural plant communities. The effective half-life (T_{eff}), which is the result of biological and physical loss, has been commonly used as the unit of the elimination rate. For T_{eff} the following equation is valid:

$$T_{\text{eff}} = \frac{T_{\text{phys}} \cdot T_b}{T_{\text{phys}} + T_b}$$

T_b = biological half-life

T_{phys} = physical half-life (30.2 years for ^{137}Cs and 28.9 years for ^{90}Sr)

The first estimations for T_{eff} of ^{137}Cs in lichens at the beginning of the 1960's were about 1 year. Later investigations have indicated this value to be much longer.

Accurate values of deposition of radionuclides near the sampling locations are required to determine the mean residence time or effective half-life of radionuclides in lichens. Liden and Gustafsson (1967) obtained in *Cladonia* lichens the value of 17 ± 4 years for T_b and 11 years for T_{eff} . They made ^{137}Cs determinations in Swedish Lapland in 1962-1965. Near the sampling location there was an observation station where ^{137}Cs has been

measured in precipitation since 1957. Thus, it was possible to calculate the integrated deposition of ^{137}Cs . Miettinen (1967) included in the effective half-life the factor of consumption of lichens by grazing reindeer. The estimated value of T_{graz} was 11 years. Using this value and the value of 17 years for biological half-life obtained by Liden and Gustafsson, he calculated the value of 5.5 years for effective half-life of ^{137}Cs in lichens (*Cladonia* species). Niznikov *et al.* (1969) reported the value $T_{\text{eff}} = 2.5$ years for lichens. This value must be compared with the value $T_{\text{eff}} = 5.5$ years reported by Miettinen, for Niznikov *et al.* calculated this value on the basis of the results of reindeer-meat analysis. They suggested that with increasing time from the moment of fallout T_{eff} of lichens will increase.

Until 1966 the annual fallout has been so significant, compared to the cumulative fallout, that considerable corrections had to be made when the residence time of radionuclides was investigated. These corrections decrease the accuracy of half-life determinations. Martin and Koranda (1971) followed the ^{137}Cs level of lichens at different locations in Alaska during 1967–1970. During this time the fallout was only a few percent from the cumulative fallout. The biological half-life in *Cladonia* lichens collected in central Alaska was 8.1 years ($T_{\text{eff}} = 6.4$ years). For lichens collected in coastal areas of Alaska and on Amchitka Island in the Aleutians the T_b values were 3.7 and 3.0 years, ($T_{\text{eff}} 3.3$ and 2.7 years), respectively. For evergreen vascular plants collected in the central area of Alaska, Martin and Koranda obtained the effective half-life of 1–2.7 years. The shorter half-life found in the coastal and marine regions is probably due to the larger amount of precipitation which may cause some kind of washout in lichens. Another possible reason for the shorter half-life obtained in the coastal area compared to lichens of central Alaska is the higher growth rate of lichens which results in higher production of biomass and thus the dilution of ^{137}Cs concentration.

The effective half-lives of ^{137}Cs and ^{90}Sr in lichens have been studied also by applying radioisotopes to natural lichen communities (Hanson and Eberhardt, 1971) and with laboratory experiments (Witkamp and Frank, 1967).

Hanson and Eberhardt (1971) used ^{134}Cs , ^{137}Cs , ^{85}Sr , and ^{90}Sr isotopes. In an experiment where ^{137}Cs was applied as single droplets to lichen podetia the radioactivity remained practically the same during the first year. During the subsequent three years, the ^{137}Cs content decreased corresponding to the biological half-life of 6.7–9.9 years ($T_{\text{eff}} = 5.5$ –6.9 years). The results from the experiment where ^{134}Cs isotope was sprinkled on lichens and washed by a heavy rain after application showed no significant decrease in ^{134}Cs concentration during the period of four years.

Witkamp and Frank (1967) placed lichen samples (*Cladonia subtenuis*) with and without soil in special plastic boxes (9.5 cm diameter). Cesium-137 was added with simulated rain during 3 months. Then the samples were washed during 10 weeks. They obtained 1.8 years for the effective half-life. However, the presence of soil decreased this value to 0.9 year. This half-life is much shorter than the values of 2.7–11 years given earlier. To determine the contribution of biological uptake to the total retention Witkamp and Frank (1967) sterilized some lichen samples with 12% ethylene oxide (16 hours at 9 psi) before adding ¹³⁷Cs. The retention of the sterilized samples was only 4–8% less than that of living lichen samples. They concluded that the 4–8% represented the biological uptake.

The different results of the studies where radionuclides were applied to lichens indicate that it is difficult to simulate radioactive fallout. In addition, factors like temperature, pH value, and chemical form of applied radionuclides affect the results significantly.

The effective half-life of ⁹⁰Sr is much shorter than that of ¹³⁷Cs and the range of values reported is small. Hanson and Eberhardt (1969) obtained for ⁹⁰Sr the biological half-life of 1.2–1.6 years ($T_{\text{eff}} = 1.2\text{--}1.5$ years) using ⁸⁵Sr and ⁹⁰Sr labelling for natural lichen communities. Persson (1971) has reported for T_{eff} of ⁹⁰Sr the value of 2.5 ± 0.8 years. The shorter half-life of ⁹⁰Sr compared to that of ¹³⁷Cs is in agreement with the earlier mentioned observation of the higher value of ¹³⁷Cs/⁹⁰Sr ratio in lichens than in fallout.

2. OTHER ARTIFICIAL RADIONUCLIDES IN LICHENS

a. FISSION PRODUCTS. In addition to ¹³⁷Cs, many other γ emitting radionuclides produced by fission have been determined in lichens. The most significant ones are ⁹⁵Zr ($T_{1/2} = 65.5$ days), ¹⁰⁶Ru ($T_{1/2} = 368$ days), ¹³⁵Sb ($T_{1/2} = 2.7$ years, and ¹⁴⁴Ce ($T_{1/2} = 284$ days). The percentages of these nuclides in fresh fallout are high. Owing to the short physical half-life compared with ¹³⁷Cs and ⁹⁰Sr and the fact that considerable migration through food chains to man has not been found, these radionuclides are of slight importance. The maximum values in lichens were found during 1963. Hanson *et al.* (1967) reported in *Cladonia-Cetraria* lichens values of 86, 32, and 200 nCi/kg dry weight for ⁹⁵Zr–⁹⁵Nb, ¹⁰⁶Ru, and ¹⁴⁴Ce, respectively. Plummer (1969) found in *Cladonia* species 8 nCi ¹²⁵Sb/kg dry weight in 1964. In 1970 Koranda *et al.* (1971) obtained in *Cladonia* lichens collected in Alaska the values 0.7–4.1, 2.8–7.9, 0.8–1.8, and 7.6–28.3 for ⁹⁵Zr–⁹⁵Nb, ¹⁰⁶Ru, ¹²⁵Sb, and ¹⁴⁴Ce, respectively. Especially high concentrations of these radionuclides were reported again in *Parmelia conspersa* in the Georgia Piedmont, U.S.A. (Plummer, 1969). The maximum value for ¹⁴⁴Ce was 2094 nCi/kg dry weight in

1963. This seems to be the highest radionuclide value found in lichens. At the same time the maximum value of ^{106}Ru in *Parmelia conspersa* was 1061 nCi/kg dry weight. For ^{95}Zr - ^{95}Nb and ^{125}Sb Plummer reported maximum values of 567 and 74 nCi/kg dry weight, respectively, in the autumn of 1962.

Cesium-144 was nearly uniformly distributed in lichen thalli and moved deep into the soil (Plummer, 1969; Hanson and Eberhardt, 1971). The lowest retention occurred for ^{106}Ru which moved rapidly through the lichens into the soil (Hanson and Eberhardt, 1971). This indicates the nonselective nature of lichens for ^{106}Ru .

b. ACTIVATION PRODUCTS. The radioactive activation products found in lichens were produced mainly by nuclear tests. Small amounts of some radionuclides are, however, continuously produced in the atmosphere by interactions with cosmic rays. Iron-55 and ^{54}Mn are the most investigated activation products in lichens.

The accumulation of ^{55}Fe has also been observed in the lichen-reindeer (caribou)-man food chain. Palmer and Beasley (1965) reported 14 nCi/kg dry weight in Alaska in 1964. Persson (1967, 1969) gave the maximum value of 44 nCi/kg dry weight in Sweden in 1965. According to Jaakkola (1969), the maximum value of ^{55}Fe in *Cladonia* lichens in Finland was 96 nCi/kg dry weight in 1964. The maximum level in vascular plants in 1963, the year of maximum fallout, was about the same as the level in lichens at the same time, but one year later the ^{55}Fe content of lichens was 5–20 times higher than in vascular plants and this ratio has increased during the subsequent years. According to Jaakkola, ^{55}Fe was distributed between the upper and lower parts of lichens in 1966 so that the specific activity (pCi ^{55}Fe /mg stable iron) was approximately equal in the two parts. But when calculated in relation to dry weight the ^{55}Fe content of the lower part, which contains considerably more inactive iron, was about three times that of the top part. In 1968 the specific activity of ^{55}Fe was about twice as high in the lower part as in the top part. This means that the behavior of ^{55}Fe in lichens is very different from that of ^{137}Cs . Iron-55 was leached relatively rapidly from the top part toward the lower part. Jaakkola (1969) obtained an effective half-life of 1.4 years for ^{55}Fe in lichens, which corresponds to the biological half-life of 2.9 years. This is very similar to the values obtained for ^{90}Sr .

The maximum ^{54}Mn concentration observed in arctic regions has been 15 nCi/kg dry weight in 1964 (Hanson *et al.*, 1967). In Georgia (U.S.A.) Plummer (1969) in 1963 found maximum concentrations of 406 nCi and 33 nCi/kg dry weight in *Parmelia conspersa* and *Cladonia* species, respectively. According to investigations in Alaska, ^{54}Mn concentrations were slightly higher in the upper part than in the lower part of lichens (*Cladonia* species). During the winter, ^{54}Mn appeared to move from the upper layers of soil into the lower part of the lichens (Hanson and Eberhardt, 1971).

The behavior of ^{65}Zn in *Cladonia* lichens was very similar to that of ^{106}Ru . There was a rapid loss of ^{65}Zn from lichen communities and a rapid movement of this radionuclide into the soil (Hanson and Eberhardt, 1971).

Some values are reported also for ^{22}Na , ^{60}Co , ^{63}Ni , ^{88}Y , ^{110}Ag , ^{134}Cs , and ^{155}Eu in lichens (Jenkins and Hanson, 1967; Hanson *et al.*, 1967; Persson, 1968; Koranda *et al.*, 1969, 1971; Plummer, 1969; Beasley and Held, 1969).

3. THE LICHEN-REINDEER(CARIBOU)-MAN FOOD CHAIN

The determinations of radionuclides in the biosphere have indicated that the exceptionally high concentrations in people of some arctic population groups are not a result of the high local amount of fallout but are due solely to ecological factors. The efficiency of the food chain lichen-reindeer (caribou)-man in accumulating radionuclides is caused mainly by the following factors. First, lichens grow in large populations in cold and nutrient-deficient arctic regions. They grow slowly and have a long lifetime. Lichens have a high capacity to absorb nutrients from the air, rain, and snow. Second, lichens are the major food source of reindeer (caribou) in the winter season (about 8 months long). Third, Lapps and Eskimos eat large quantities of meat and edible organs of reindeer and caribou.

In addition to the previously mentioned artificial radionuclides the accumulation of natural radionuclides of ^{210}Po and ^{210}Pb has been observed in this same food chain. The occurrence of these radionuclides in lichens will be described later.

The maximum radiation dose rate of ^{137}Cs to man, 560 mrad/year, was reported for reindeer herders of the Murmansk region (U.S.S.R.) in 1966 by Niznikov *et al.* (1969). In Finnish Lapland the main source of irradiation for the present generation of reindeer herders is ^{210}Po (3 rem/30 years). The second in importance is the natural external radiation (1.7 rem/30 years) and the third is the fallout nuclide ^{137}Cs (1 rem/30 years). The value of ^{137}Cs was estimated assuming no significant changes in the fallout situation. The radiation dose to the reindeer herders due to the radionuclides transported along the food chain lichen-reindeer-man is about double compared to the radiation dose due to the natural external radiation.

4. LICHENS AS RADIOACTIVE FALLOUT METERS

Investigations that applied ^{137}Cs to *Cladonia* lichens have indicated a nearly quantitative absorption of the isotope (Hanson *et al.*, 1967; Witkamp and Frank, 1967; Plummer, 1969). Plummer used 500 nCi $^{134}\text{Cs}/\text{m}^2$ for *Cladonia* lichens and found no significant loss of radioactive cesium after a heavy rainfall. Svensson and Liden (1965b) and Hanson (1967) compared the ^{137}Cs concentrations of natural lichen communities with the ^{137}Cs deposition values obtained near the sampling locations and found good

agreement. These observations support the use of lichens as meters of cumulative fallout. There are, however, some reports of considerably lower ^{137}Cs concentrations in lichens (nCi/m^2) than the estimated cumulative amount of fallout. These divergent results are probably mainly due to the different effective half-lives of ^{137}Cs in various growing sites and partly due to sampling. The measurement of cumulative amount of radionuclide deposition calls for special care in selecting sampling sites and in collecting samples. Furthermore, to get reliable results it is necessary to have an estimation for the effective half-life of radionuclides in lichens. Uncertainty about this quantity decreases the accuracy in estimating the amount of cumulative fallout.

Because lichens can absorb, in a fairly quantitative manner, some radionuclides, they can be used as indicators of the age of the debris in the case of local or intermediate (tropospheric) fallout. This method is based on changes of ratio of relatively short-lived radionuclides. Svensson and Liden (1965a) obtained good results using the ratios of $^{140}(\text{Ba} + \text{La})/^{95}(\text{Zr} + \text{Nb})$ and $^{103}\text{Ru}/^{95}(\text{Zr} + \text{Nb})$ in lichens. The estimated age of debris varied from 23 to 170 days in their studies and the deviations between test times and dates calculated on the basis of the radionuclide analyses of lichen were from 0 to 7 days.

B. Natural Radionuclides

Although the existence of natural radionuclides has been known for many decades, the determination of these radionuclides in plants was started in the 1960's along with the studies of artificial radionuclides.

One of the first investigations on natural radionuclides in lichens was conducted by Grodzinsky (1959). He stated that lichens and mosses contained high radioactivities compared with other plants. He found also that the natural radioactivity in lichens was mainly due to the accumulation of heavy radioactive elements. The contribution of ^{40}K was only 2–5% of the total β activity.

1. ^{210}Po , ^{210}Pb , AND ^{226}Ra IN LICHENS

Because of the accumulation of ^{210}Po and ^{210}Pb in the arctic food chain, these isotopes are the most commonly studied natural radionuclides in lichens. $^{226}\text{Radium}$, which is formed by radioactive decay of uranium, decays in the soil to gaseous ^{222}Rn . This escapes into the atmosphere and decays there, forming a chain of solid radioactive daughter nuclides. The mean residence time of the atmospheric ^{222}Rn is about 15 days and it is long enough to produce a detectable amount of ^{210}Pb , the long-lived descendant ($T_{1/2} = 22$ years) of ^{222}Rn . ^{210}Pb returns to the earth's surface. Before de-

TABLE VII
THE ^{210}Po AND ^{210}Pb CONTENTS OF LICHENS. THE VALUES ARE GIVEN AS pCi/gm DRY WEIGHT.

Reference	Location	Sampling year	Species	pCi/gm dry weight	
				^{210}Po	^{210}Pb
Hill (1965)	United Kingdom	—	<i>Caloplaca elegans</i>	7.8–10.0	—
Hill (1965)	Arctic region	—	<i>Cl. alpestris</i>	3.5–8.1	—
Holtzman (1966)	Finland	1961	<i>Cl. alpestris</i>	13.1	4.5
Holtzman (1966)	Alaska, U.S.A.	1961	<i>Cornic. divergens</i>	—	26–70
Holtzman (1966)	Alaska, U.S.A.	1961	<i>Cl. sylvatica</i>	3.0	11.5
Jaworowski (1966)	South Spitsbergen	1957–1958	<i>Cl. species</i>	—	1.4–8.0
Jaworowski (1966)	South Spitsbergen	1965	<i>Cl. mitis</i>	—	6.3–13.4
Jaworowski (1966)	Poland	1957–1965	<i>Cl. species</i>	—	1.5–6.7
Jaworowski (1966)	Poland	1955–1964	<i>Cl. species</i>	—	1.0–3.2
Jaworowski (1966)	20°E ^c	1966	<i>Cl. species</i>	—	0.6–13.4
Holtzman (1968)	New Hampshire, U.S.A.	1965	<i>Cl. species</i>	7.3–17	5.1–10
Blanchard (1967)	Alaska, U.S.A.	—	<i>Cl. species</i>	3.6–5.6	3.4–6.7
Blanchard (1967)	Ohio, U.S.A.	—	<i>Cl. species</i>	5.2–11	5.7–12
Blanchard (1967)	Denmark	—	<i>Cl. species</i>	3.9	—
Kauranen and Miettinen (1969)	Lapland, Finland	1961–1966	<i>Cl. alpestris</i>	5.7–9.5	5.6–10.2
Kauranen and Miettinen (1969)	Southern Finland	1964–1967	<i>Cl. alpestris</i>	4.6–6.9	5.1–8.0
Litver <i>et al.</i> (1969)	U.S.S.R.	Before 1900	<i>Cetr. islandica</i>	—	14.7
Litver <i>et al.</i> (1969)	Murmansk, U.S.S.R.	1900–1945	<i>Cetr. islandica</i>	—	7.0–35
Litver <i>et al.</i> (1969)	U.S.S.R.	1958–1966	<i>Cl. alpestris</i>	6.4 ± 0.5^a	8.4 ± 0.8^b

^aAverage value of 20 samples \pm 1 standard deviation of the mean.

^bAverage value of 12 samples \pm 1 standard deviation of the mean.

^cThe samples were collected along the 20th meridian (East) between 41° and 77° N.

position, which occurs mainly with rainfall, some ^{210}Po ($T_{1/2} = 138$ days), a descendant of ^{210}Pb , also is formed. The $^{210}\text{Po}/^{210}\text{Pb}$ ratio in rainwater is about 0.1. After deposition, ^{210}Po continues to grow until radioactive equilibrium is reached.

The results reported for ^{210}Po and ^{210}Pb in lichens are given in Table VII. In general, the concentrations are very similar for all samples collected in various countries. Most analyses have been made in *Cladonia* species. In *Cornicularia divergens* Holtzman observed 26 and 70 pCi of $^{210}\text{Pb}/\text{gm}$ for two samples. These concentrations are much higher than values reported for *Cladonia* lichens. Also, the ^{210}Pb values reported for *Cetraria islandica* are higher than the values for *Cladonia* species. The ^{210}Po and ^{210}Pb concentrations in vascular plants in Finland were about one-twentieth and one-seventh of the contents in lichens. The ratio of ^{210}Po to ^{210}Pb in lichens was 0.92 in Lapland and 0.85 in southern Finland. In vascular plants this ratio was 0.26 (Kauranen and Miettinen, 1969). In lichens Blanchard (1967) reported the value 0.96 for this ratio. Thus, the ratio of ^{210}Po to ^{210}Pb is consistently close to, but somewhat below, one (0.1 in rainwater). This indicates that the residence time of both polonium and lead in the slowly growing lichens is long. Jaworowski (1966) has observed the dependence of ^{210}Pb concentrations on sampling year in lichens collected at Spitsbergen (77°N , 20°E) and in Middle Europe. The ^{210}Pb concentrations were considerably higher after 1957 than during preceding years. He suggested that this enhancement was partly due to the tetraethyl lead introduced into the atmosphere and partly produced by nuclear detonations. The significance of these sources is obviously very low, for according to most investigations there is no systematic increase in the ^{210}Pb concentration with time (Litver *et al.*, 1969; Kauranen and Miettinen, 1969; Persson, 1970). Litver *et al.* (1969) analyzed ^{210}Pb in samples collected before 1900, in 1900–1945, and in 1958–1966 (Table VII).

No increased ^{210}Pb values were found either in sediment and soil samples collected in the close proximity of the U.S. nuclear test sites (Beasley, 1969).

Persson (1970) estimated the effective half-life of ^{210}Pb in lichen carpet to be 7 ± 2 years.

The reported ^{226}Ra concentrations in lichens varied between 0.04–0.5 pCi/gm dry weight, values that are one-tenth to one-hundredth of the contents of ^{210}Po and ^{210}Pb . Consequently, ^{226}Ra cannot be the direct source of these radionuclides.

2. ^{40}K IN LICHENS

Potassium-40, together with ^{238}U and ^{232}Th , is the main source of natural ionizing radiation in the biosphere. The natural potassium contains 0.0118% of radioactive ^{40}K isotope. The total potassium content of lichens varies

from 1 to 8 mg/gm dry weight. The ^{40}K content in lichens in Alaska ranged from 2.2 to 3.6 pCi/gm dry weight for *Cladonia* lichens and 7.9 pCi/gm dry weight for *Nephroma arcticum* (Koranda *et al.*, 1969). The ^{40}K content of lichens is low compared to that in humus and soil layers beneath the lichen. According to Hanson and Eberhardt (1971), the ^{40}K concentrations in soil were 7–10 times higher than in lichens. This indicates the inability of lichens to absorb potassium from the substratum. The efficient manner by which lichens accumulate ^{137}Cs , that is chemically related to potassium, can be explained partly by the tendency of lichens to satisfy their potassium requirements.

IV. Uptake and Translocation of Cations

A. Uptake Mechanism

Only a few systematic studies have been made on the physiology of cation binding by lichens. With all the reservations mentioned earlier, the following assumptions can be considered as the starting point of the studies.

With respect to cation uptake and requirements, the lichenized algae and fungi are at least approximately similar to free-living forms. The results obtained with free-living algae and fungi could be extrapolated to the lichen thallus. Further, the active phase of the cation uptake of all plant forms is a membrane property and evolutionarily so old that it can be considered to be fundamentally similar in all plants. Possibly, the same is true with respect to the passive phase of the cation uptake which is supposed to be based on the physical chemistry of the cell wall. However, the mass of the prevailing mycobiont with its chemically complex cell walls probably will make it difficult to determine the role of the phycobiont in this regard. At present, the experimental facts allow only an attempt to characterize the passive phase of the uptake process.

1. THE MECHANISM OF PASSIVE UPTAKE

The passive phase of the cation uptake of lichens seems to be some kind of cation-exchange process. This idea is supported by experiments describing the inactive Sr and ^{137}Cs binding of *Cladonia alpestris* and *Ramalina reticulata*, respectively.

The results of the experiments on the inactive Sr binding of *Cladonia alpestris* (Touminen, 1967) can be summarized as follows. When the living tops of the thalli were immersed in a dilute solution of Sr, the equilibrium between the thallus and the solution was rapidly established in a few minutes and remained stationary for several hours. The effect of temperature on the

equilibrium was very low. The binding of Sr was reversible and this allowed the results to be interpreted thermodynamically. The Sr binding was strongly dependent on the pH of the solution, the binding being undetectable under pH 3 and then increasing to its highest value of about 0.3 mEq/gm dry weight at pH 6–7. Further, the Sr binding seemed to follow the law of mass action. The H⁺ dissociation of the *Cladonia alpestris* thallus also was reversible. The cation selectivity of the thallus is similar to that of ordinary cation exchangers, i.e., the thallus prefers bivalent ions to monovalent ones. All these observations support as a first approximation the model of cation exchange for the uptake mechanism.

Tuominen attempted to estimate the standard changes of heat, free energy, and entropy of the binding process with the following results: $\Delta H^0 = +2$ kcal/mole, $\Delta G^0 = -4$ kcal/mole, and $\Delta S^0 = +26$ eu, respectively, at the normal temperature of 20°C. Because of the lack of other similar estimations of these quantities for plant material, the significance of these figures can not be judged. However, in comparison with published data for a typical chelate (Sr chelate of diaminotetraacetic acid = EDTA) and for a typical cation exchanger (Ca and Dowex 50), the above values of *C. alpestris* resemble more closely those of the Sr-EDTA chelate. ΔS^0 is similar to that of the chelate, +26 eu instead of +2 eu of Dowex. ΔG^0 is about half the value of the chelate being also negative, but ΔH^0 of *C. alpestris* is positive instead of the negative value of the chelate. ΔG^0 and ΔH^0 of Dowex 50 are much smaller than those of *C. alpestris*, being about -0.4 kcal/mole and +0.2 kcal/mole, respectively. In spite of the positive ΔH^0 of *Cladonia alpestris*, binding is still possible but very weak. This is advantageous because the binding must be understood as a first catch of the cations at which the further transport is possible without any great loss of energy. This similarity between the *C. alpestris* thallus and the Sr-EDTA chelate is probably not incidental for it seems to be possible to design a binding model based on the assumption that every binding site within the thallus consists of a pair of carboxyls or other acidic groups (Tuominen, 1968, 1971).

The interesting study on the uptake of carrier-free ¹³⁷Cs by *Ramalina reticulata* (Handley and Overstreet, 1968) supports the uptake model outlined above. Cesium-137 was employed at a concentration of 1.0 μ Ci/liter and the living tissue samples were washed in running water for 5 minutes just before immersion in the solutions. The uptake was determined by counting the material with a well-type scintillation detector. It was shown that the uptake of ¹³⁷Cs was not directly linked to metabolism. The authors also observed both the rapid establishment of the equilibrium and the low temperature dependence of the uptake. The process was 70% complete within half an hour. This time for the establishment of the equilibrium, in contrast to Tuominen's few minutes, is obviously due to the use of a radioisotope with a higher accuracy of detection compared with the inactive Sr.

However, Tuominen began the measurements always after 2 hours from the beginning of the experiments, so that this discrepancy in the equilibration time hardly has any effect on the results.

Especially noteworthy is the statement by Handley and Overstreet that the ^{137}Cs uptake pattern of *R. reticulata* differs greatly from that displayed by excised barley roots, the uptake rate of which remained constant for more than 2 hours and was strongly temperature dependent. Further, their data showed that monovalent ions (Li, Na, K, and Rb) reduced the ^{137}Cs uptake in accordance with their position in the lyotropic series. At concentrations below 10.0 $\mu\text{Eq/liter}$ neither Na nor Li had any significant effect on the uptake of ^{137}Cs , while 2.5 μEq of Rb and stable Cs per liter were sufficient to reduce the uptake 70–80%, resembling the behavior of excised barley roots. However, the barley roots evidently have sites which strongly prefer K and Rb to other monovalent cations and can bind Cs as well. They could not observe this kind of effect in *Ramalina*.

Calcium seems to have a special role in the uptake of ^{137}Cs by *Ramalina*. First, its effect on the uptake was very small in spite of hints about the presence of a barrier to entry of ^{137}Cs , which seems to be stabilized by Ca. Removal of Ca by pretreatment of the tissue in monovalent salt solutions increased the uptake of ^{137}Cs . Uptake under anaerobic conditions was also greater than normal, but in this case Ca displaced from the barrier membrane was not lost from the tissue and thus the normal permeability was rapidly reestablished when aerobic conditions were restored. For the relatively small competitive ability of Ca they suggested that the spacing of negatively charged exchange sites may prefer two monovalent cations to a single one at each pair. Possibly this statement can be considered to be equivalent with Tuominen's binding sites composed of two acidic groups.

Handley and Overstreet (1968) found Sr to be more effective than Ca in competitive experiments. This observation is also supported by the separation factors of *Cladonia alpestris* (Tuominen, 1967): $\text{Sr/Ca} = 1.5$ compared, for example, with $\text{Cs/K} = 1.0$. Further, the values $\text{Sr/K} = 4.0$ and $\text{Sr/Cs} = 3.0$ of *C. alpestris* show the preference of divalent cations to monovalent ones. One possibility to explain these preference relations can be based on the differences in the radii of the hydrated ions and the three-dimensional structure of the binding site. For example, the Sr ion as a smaller one compared with Ca could fit closer to the site, the structure of which has possibly been solved by Sterling.

2. THE NATURE OF BINDING SITES

The titration curves of some lichen thalli (Tuominen, 1967) show that there are acidic groups within the thalli. This was also postulated by Handley and Overstreet. These groups have different pK values. The groups of pK

3.3 resemble the pectic carboxyls. According to Tuominen, there seems to be a moderate correlation, with respect to certain species, to the uronic acid content of the thalli hydrolysate determined by the carbazole method. This method is well known and widely used, although it has drawbacks because of the interferences. The early authors assumed that fungal cell walls contained pectic substances on the basis of results obtained primarily with ruthenium red staining. However, these results are at present questionable and uronic acids seem never to have been demonstrated conclusively in fungal cell walls (Aronson, 1965). Aronson suggested the chromatographic detection of uronic acids in cell-wall hydrolysates, but according to him this has not been accomplished. Y. Tuominen has failed in this attempt with lichens (personal communication). As a result there were only uncertain spots on the chromatograms in spite of many attempts. Now we can think that these spots were traces of uronic acids of the phycobiont so that the lichenized fungi also do not seem to contain pectic substances. Thus, the above identification of the pK 3.3 sites as pectic carboxyls is uncertain.

On the other hand, Y. Tuominen (personal communication) observed that the lichen thallus is readily stained, reversibly, by ruthenium red, which has been believed to be a specific indicator for polyuronic acids, but which was later found to stain other substances. Now Sterling (1970) has possibly found a new method to characterize the binding sites or at least to eliminate some possibilities. He solved the crystal structure of ruthenium red ($-$ ammoniated ruthenium oxychloride) and revealed what exactly is being stained by it. Using X-ray diffraction techniques he showed that the staining groups of ruthenium red consisted of the ruthenium ion lying in the middle of a square planar complex of four ammonia molecules. The stained sites in the macromolecules have two negative charges, 4.2 \AA apart. The staining group takes the position with its plane perpendicular to the axis between the negative groups. In pectic acids such sites occur between each monomer unit and its next adjacent neighbor. Sterling's work supports the above-mentioned model with the sites consisting of a pair of acidic groups.

Puckett *et al.* (1973) have also confirmed the ion exchange model of the cation uptake of lichens with a very interesting extension using eleven species of *Cladonia*, *Umbilicaria*, and *Stereocaulon*. Employing both dilute and concentrated solutions of Fe, Pb, Cu, Ni, Zn, and Co for the determination of the uptake capacities and the competition studies for the cation selectivity patterns of the thalli, they demonstrated that the ion exchange is modified by metal complex formation and possibly chelation, even for alkali and alkaline cations. This result supports the earlier assumption in this chapter that the functional groups are composed of a pair of binding sites. According to Puckett *et al.*, the binding sites seem to be oxygen and oxygen–nitrogen donors and the ion exchange is the strong-field type. The conclusions of the

authors, based on the observed selectivity patterns, is further supported by the remarkable similarity of the selectivity coefficients between the thalli and the chelating resin Dowex A-I with the groups mentioned above. As an interesting detail they again take up the possibility that the pectic substances could be the chemical basis of the binding sites in the thalli, because they are potential oxygen binding sites.

The experiments on the kinetics of the exchange reaction clearly support the prediction of two functional binding groups with different reaction rates, the slower one setting in when the uptake has reached 60–70% of its equilibrium value. The metabolic inhibitors had no effect on the metal uptake, in spite of the fact that the samples were apparently alive, since they were dried only at + 25°C and used within 48 hours.

3. THE EPIPHYTIC LICHENS

According to Tuominen (1967), of the lichen species he studied the epiphytic ones had the greatest capacity of Sr uptake. Bearing in mind the well-known sensitivity of some members of this ecological group, for example *Usnea* sp., to atmospheric pollution, it seems worthy to study whether the epiphytic species in general have a greater uptake capacity than species growing on the earth. Clymo (1963) also observed high uptake values for *Usnea*. Further, the results of studies (Mićović and Stefanović, 1961) dealing with the chemical composition of some epiphytic lichens (*Evernia prunastri*, *Ramalina farinacea*, and *Usnea hirta*) compared with the ash analyses of *Cetraria islandica*, which grows on soil, revealed that the ashes of these epiphytic species differed widely from the ash of *C. islandica*. The chemical compositions of the ash of epiphytic species are rather similar to one other but differ from the oak bark substratum on which they grew. Also, Lounamaa (1965) observed differences in chemical composition between epiphytic and epilithic species. Finally, of the four lichens in which Solberg (1967) found an unusually high content of Mn, three were epiphytic.

These scattered observations show clearly that epiphytic lichens are an interesting group with regard to metal uptake.

B. Translocation

It is well recognized that the substratum has a certain effect on the metal content of lichen thalli. However, thus far this conclusion is based on the comparison of the analyses of both the lichens and the substrata. This subsection consists of more direct studies on the mobility of metal cations from the substratum to the thalli and within it. In this discussion, simple diffusion seems to have a central role.

Barashkova (1963) studied to what extent the connection between the living tops of *Cladonia rangiferina* thalli and the substratum is maintained through the dead basal portion of the thalli. She conducted water-culture and field experiments. The growth of thalli was faster on substrata rich in nutrients and quantitative microscopic measurements revealed that the enhanced growth was directly proportional to the amount of the phyco-biont. So there must exist translocation of nutrients within the thalli from the base to the top.

A more detailed picture of the mobility of cations within the thallus was obtained by Nevstrueva *et al.* (1967), who immersed the green tops of *Cladonia rangiferina* in an aqueous solution of ^{137}Cs or ^{90}Sr chlorides of 2 nCi/ml at pH 7.0. After 4–11 days the distribution of the nuclides became constant within the thalli. Strontium-90 was distributed in the lichen evenly, measured per unit dry weight, both in the part which was immersed in the solution and in the older part which was left in the air. On the contrary, ^{137}Cs was concentrated predominantly at the part of the thallus which was in the solution. Only about 5% of the ^{137}Cs was translocated to the older part. Similar distribution was observed if the old part of the thallus was dipped into the solution. When the whole lichen was immersed into the solution, the two nuclides were distributed relatively evenly in the thallus with a tendency to accumulate in the older part. All this means that Cs and Sr can migrate relatively freely in the thallus from top to base and backward. Strontium-90 can also be washed out of the thallus more rapidly than ^{137}Cs .

Without any suggestion for the binding mechanism, Subbotina and Timoféeff-Ressovsky (1961) showed that certain crustaceous lichens treated with ^{89}Sr lost about 60% of the radioactivity during immersion in pure lake water, whereas the loss of ^{137}Cs was smaller, about 13%. So, ^{137}Cs seems to be bound more strongly than ^{89}Sr . They studied the uptake of radionuclides of Fe, Co, Zn, Sr, Ru, Cs, and Ce from dilute aqueous solutions using different lichen species. After 11 days they checked the balance of radioactivity and determined the distribution of the radioactivity between lichens, the bacterial film formed on the surface of the solutions, and the water. A portion of the radioactive lichens was transferred to pure lake water for 8 days for the determination of the percent deactivation. In general, the uptake was about 50–62%, except for Zn and Ce, which displayed nearly total absorption.

Hanson *et al.* (1967) performed similar experiments on the effect of seasonal snow cover on the distribution of fallout radionuclides in *Cladonia alpestris* and the movement of artificially applied radionuclides within the thalli. Strontium-90 concentrations increased during summer months when the lichens were exposed to atmospheric fallout deposition, stabilized at

the onset of snow cover, and then decreased at the half-life rate of 120–220 days during winter periods. At the same time, the concentrations of ^{137}Cs in all the lichens investigated increased with time while no apparent decreases occurred during periods of snow cover. This indicated that lichens bind ^{90}Sr less strongly than ^{137}Cs .

Hanson *et al.* artificially applied ^{85}Sr and ^{134}Cs to the lichens both by drops and sprinkling. There was practically no difference between the calculated and measured amounts of ^{85}Sr and ^{134}Cs applied as single drops but the amounts of $^{134}\text{Cs}/\text{m}^2$ measured in the sprinkled areas were about half the amount calculated to have been applied. One obvious difference between the treatment methods was the pH of the solutions, about 5.0–6.0 in the sprinkled solutions and 3.0 in the solutions added dropwise. Unfortunately, the experiment was complicated by a heavy rainfall for several hours immediately following application of the solutions. However, the greater mobility of ^{85}Sr compared to ^{134}Cs was again illustrated by their loss of 65 and 22%, respectively, during 1 year after application.

The most interesting feature of all these experiments is that the Sr ions applied to lichens were more mobile than the Cs ions similarly applied, because the diffusion experiments described by Tuominen (1968, 1971) display exactly the reverse order of the mobilities, i.e., ^{137}Cs seems to move rather freely within the thallus of *Cladonia alpestris* compared with ^{90}Sr , which displays rather strong interaction with the binding sites of the thallus. The important difference between these experiments is that the previous ones were performed in free-air using living thalli, whereas the later diffusion experiments were carried out in the closed test tubes in the laboratory with dead thalli. We can try to explain the discrepancy as follows.

Possibly the lower mobility of Cs within the thallus is due to immobilization based on the active uptake by cells of both the myco- and phycobiont. However, it seems plausible that the mass of the mycobiont masks the effect of the algal component. According to Rothstein (1965), the fungi follow the common physiological fact that the uptake of monovalent cations is much more efficient than that of bivalent ones. This effect is very clear between K and Ca, which are the chemical homologues of Cs and Sr. The input site of the K ions can also transport other alkali cations. For example, the relative affinity of the K carrier of yeast cells for Cs is 1:15. This same carrier can also transport Mg but the affinity is very low. In addition to this, the small size of the Cs ion in spite of hydration possibly has a special effect. The physiology of fungi shows further that the maximal rate of input of the alkali cations is very strongly affected by pH, and possibly just on the region of 3–6 which appeared in the above experiments, if we have courage to generalize the results of the yeast studies. Thus, the mutual ratio of mobilities of Cs and Sr can possibly have different values depending on the

experimental conditions, because the intrinsic mobility of Cs within the thallus of *C. alpestris* is high when the sites of input into the cells are out of function. We see that it is useful to try the separation of the active and passive phase of the uptake by using dead thalli. This seems to be reasonable, especially in the case of lichens, in order to study the diffusive movement of cations within the thallus, because both the appearance and the anatomy of lichens appear to be independent of the physiological state of the thallus.

This physiological immobilization is supported by the results of the experiments made by Subbotina *et al.* In spite of the bacterial films in the flasks, which indicated the decomposition of the thalli during the long experimental periods, Cs ions were still strongly bound by the thalli. This fact can only mean that Cs ions are actively translocated deep into the cells, obviously using the energy of metabolism, and bound by the living cytoplasm. The suggestion of the metabolic immobilization of Cs is also supported by Kreuzer and Schauer (1971) who favor the assumption that *Cladonia* possesses a specific affinity for the ^{137}Cs binding, this confirmed also by the more general observation that fungi are specifically able to accumulate ^{137}Cs .

The reason that Tuominen (1968, 1971) carried out the diffusion experiments in closed test tubes was that the thalli are totally hydrolabile. It was impossible to maintain a constant water content of the thalli for diffusion experiments in the free-air of the laboratory. The only way was to close them in an atmosphere saturated with water vapor. However, he simulated the effect of the wind by applying certain amounts of oven-dried silica gel into the test tubes. The larger amounts of silica gel clearly affected the rates of Cs and Sr diffusion, especially in the case of Cs. After 96 and 192 hours the accumulation of ^{137}Cs and ^{90}Sr could be detected, respectively, at the tops of the 7-cm-long thalli. The form of this "saturation curve" of ^{137}Cs seems to be theoretically predicted if the movement of ions is supposed to be diffusive. In this case the form of the curve can be derived assuming the reflection at the top of the thallus and the reflected curve superposed on the first one. This is in accordance with the observation (Nevstrueva *et al.*, 1966) that cations can move within the thallus to the opposite directions, even as trace amounts. With respect to ^{137}Cs , Tuominen (1968) demonstrated this reflection phenomenon by performing a model experiment with the cotton-wool simulation of the thallus. The cotton wool simulates the thallus rather well for it has a similar "hyphal structure" and it possesses acidic groups. The different shape of the reflection diffuse curves of ^{90}Sr shows the close interaction between Sr ions and the fixed sites within the thallus. Further, this interaction is also seen from the different shapes of ^{137}Cs and ^{90}Sr diffusion curves, the later ones possessing characteristic convexity compared with the ^{137}Cs curves with normal diffusive shape. This convexity seems also to be theoretically predictable using the diffusion

model based on the reversible binding of Sr ions at the sites of a pair of acidic groups (Tuominen, 1971).

The rate of diffusion is dependent on the total ionic strength of the solution. The decreasing ionic strength will cause a decrease in the rate of diffusion. However, in spite of the marked decrease of diffusion at lower ionic strength, the trace amounts of cations can move in the thalli as mentioned above. Of course, the diffusion is a slow process when a "new cation" begins to diffuse, being proportional to the square root of time, but it is very plausible that the diffusion equilibrium has already been established at the beginning of thallus growth and then maintained during the slow growth of the thallus. Thus, the small additional amounts of cations are adequate to restore the cation status in the intermicellar spaces of cell walls even at the top region of the thalli. In addition, it seems possible to postulate, on the basis of the silica-gel effect described above, some kind of pumping effect of wind when the thalli are wet.

Hanson and Eberhardt (1971) found the models of the cation binding and diffusive translocation developed by Tuominen to be applicable to their observations in natural lichen communities, when they tried to simulate behavior of ^{137}Cs in the lichen-caribou-Eskimo food chains. They postulated a very interesting seasonal cycle of ^{137}Cs concentration in lichens as described in more detail in the subsection on the radioactive nuclides. The radionuclide cycling within natural arctic lichen communities seems to be a more dynamic process than previously noted.

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Chapter 7

PEDOGENETIC SIGNIFICANCE OF LICHENS

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I. Introduction

A. Historical

The literature relating to the importance of lichens in the disintegration and decomposition of rocks (commonly referred to as weathering) and in soil formation (pedogenesis) is replete with contradictory statements. While it may be true that the role of lichens in rock weathering and pedogenesis was exaggerated by eighteenth- and nineteenth-century naturalists, it appears that this role has been underestimated by many twentieth-century ecologists.

Linnaeus (1762) discussed the ability of crustose lichens to colonize unweathered rocks and to accumulate windblown material. According to Linnaeus: "Crustaceous lichens are the first foundation of vegetation."

Lindsay (1856) considered lichens to represent a group of plants:

... humble and insignificant though it appear to be, are of infinite importance as handmaids of Nature in operating her changes on the face of our globe, in softening down the pointed crags of our mountains, in covering with fertile soil alike the bare surface of the volcanic lava and the coral islet, in a word they are the basis of soil and consequently of vegetation.

Based largely on the observations of Salter (1856), Goeppert (1860), and Bachmann (1911), which will be discussed in the appropriate sections, Smith (1921) also considered that lichens were important agents in rock weathering and soil formation.

More recently, there has been much controversy concerning the pedogenetic significance of lichens. Cooper and Rudolph (1953) questioned the classical role of lichens in soil formation, and Beschel (1965) stated that: "Ecesis on rock surfaces . . . is so insignificant . . . that only crass ignorance can propagate the fairy tale of lichens being important pioneer plants." In contrast, Schatz and Martin (1960) considered that lichens were among the primary biochemical weathering agents involved in the conversion of bare rock into soil.

That the role of lichens in rock weathering and soil formation should evoke such discordant statements is at first surprising. Evaluation by early workers of the role of lichens in the weathering of rocks was based on field observations, whereas in the latter part of the nineteenth century major emphasis was placed on the ability of carbon dioxide and oxalic acid produced by lichens (Uloth, 1861) to function as "corrosive agents" of the substratum. Although the mechanical action of lichen hyphae and rhizines was recognized by early workers (Smith, 1921), subsequent studies (Fry, 1922, 1924, 1927) provided a rational explanation of the mechanisms involved and the effects on the substratum. More recently, the ability of many lichen compounds to function as metal-complexing agents and thus promote chemical weathering of minerals and rocks has received attention (Schatz *et al.*, 1956; Syers, 1969; Iskandar and Syers, 1972). Because evaluation of the role of lichens in soil formation has been based largely on field observations, the interpretations of research workers have tended to be influenced by the physical nature of the substratum on which the lichen is growing.

B. General Review

At this stage it is necessary to define "weathering" and "soil formation." Weathering may be loosely defined as the total effect of all processes involved in the disintegration (physical processes) and decomposition (chemical processes) of rocks. Soil formation is defined as the transformation of rock material into soil. The fact that the soil is a product of weathering

modified in a particular way by "living nature" was emphasized by Nikiforoff (1935) who considered that the biosphere was the dominant factor in soil formation. Joffe (1949) maintained that although there was no distinct demarcation between weathering and soil formation, they should be examined in their historical sequence, namely, weathering as the first phase and soil formation as the second phase. If the term weathering is restricted to processes brought about by inorganic agencies, then the onset of biological weathering could be regarded as constituting soil formation. Since this review is concerned largely with the effect of lichens on their substrata, it is more convenient to evaluate the pedogenetic significance of lichens in terms of the physical (biogeophysical) and chemical (biogeochemical) processes involved in the weathering of rocks. In addition, the role of lichens in plant succession and soil development will be discussed.

II. Biogeophysical Weathering

The physical weathering of the substratum on which saxicolous lichens grow can be described conveniently in terms of the mechanisms involved. Of these, rhizine penetration and thallus expansion and contraction are the most important.

A. Mechanisms

1. RHIZINE PENETRATION

Rhizines are bundles of fungal hyphae that can penetrate the rock on which the lichen is growing. The extent (depth) of penetration is thought to be influenced by the chemical and physical composition of the rock (Smith, 1921) and by the nature of the thallus (Syers, 1964). The ability of rhizines to penetrate the substratum and cause mechanical disintegration was recognized by several early workers. Guembel (1856) considered that the disintegration of granite was caused by the mechanical action of rhizines, and Goeppert (1860) suggested that rhizine penetration caused the disintegration of rock below several foliose species. Similar observations were reported by Polynov (1945).

Many crustose lichen species that grow on limestone, particularly the obligate calcicoles, have thalli which are partly or wholly embedded in the substratum (Fry, 1922). Rhizines, originating from the medulla, may penetrate down to 15 mm in the limestone (Smith, 1921). The high solubility of calcium carbonate, compared to that of the minerals of other rocks, facilitates this deep penetration (Fry, 1922; Syers, 1964). Dissolving the underlying limestone in dilute hydrochloric acid revealed a network of fine

rhizines of different density, the length of individual hyphae varying according to the species of lichen (Fry, 1922). The length of the rhizines ranged from 400 μm to 2.8 mm in the three species investigated. Using thin sections and the staining technique proposed by Jones (1959), Syers (1964) reported that the depth of penetration of the rhizines of a range of lichens growing on limestone varied from 300 μm to 16 mm. This study also showed that thalli of endolithic and pioneer species had poorly developed rhizines. The thicker thalli of epilithic crustose lichens had a more extensive network of rhizines which achieved a deeper penetration.

The penetration of rhizines into the substratum does not seem to occur in a random pattern. Bachmann (1904) found that rhizines penetrated mica crystals in granite and followed the lines of cleavage. In contrast, feldspar or quartz crystals in granite were not penetrated by the rhizines. Similarly, Yarilova (1950) reported that lichen hyphae penetrated and disintegrated plagioclase feldspar crystals in syenite; chlorite and feldspar were attacked to a much smaller extent. Rhizines penetrated the cleavage planes in calcitic fossil debris in limestone (Syers, 1964) and caused mechanical disintegration of glass surfaces (Mellor, 1923).

2. THALLUS EXPANSION AND CONTRACTION

Fry (1927) has indicated that epilithic crustose lichens growing on rocks have a medullary zone that is attached directly to the substratum. According to this study, when the cortical tissue at the marginal fringe of the thallus contracts during drying it creates a pulling strain which may tear the thallus and leave the extreme margins attached to the substratum. In species with thicker thalli, the hyphal tissue may be torn from the substratum detaching rock fragments. Thus, small-scale disintegration of the substratum occurs at the margin of thalli. Lichen thalli contain a high proportion of gelatinous or mucilaginous substances which expand and contract on wetting and drying, as does gelatin when subjected to the same conditions (Smith, 1921; Fry, 1924). The development of a laminated structure in the shale substratum, particularly below highly gelatinous apothecia, and the incorporation of shale fragments into the hyphal tissue of thalli were demonstrated in detailed studies of the mechanical action of crustose lichens on their substrate (Fry, 1927). Schist, gneiss, and obsidian showed less disintegration below lichen thalli and fewer mineral fragments were incorporated into the thalli.

The role of rhizines and haptera (suckerlike sheaths) in the attachment of the thalli of foliose and fruticose lichens to the substrate was described by Smith (1921) and by Poelt and Baumgärtner (1964). Rhizines are abun-

dant but not universal in foliose species and are either scattered or confined to special areas of the lower thallus surface. A mucilaginous enlarged tuft of loose hyphae at the apex provides a strong attachment to the substratum. Following attachment to the substratum, the hapterum increases in size and strength (Smith, 1921). Fry (1924) showed that the haptera in *Xanthoria parietina* were concentrated closely behind the growing margin of the thallus. The greater contraction of the upper surface of the thallus on drying, coupled with the firm attachment of the haptera to the substratum, caused either (1) detachment of particles of the substratum, (2) removal of a very thin film of substratum, or (3) tearing of the thallus. This work also showed that expansion of the thallus occurred on rewetting. The presence of shale fragments in the haptera of *Xanthoria parietina* growing on shale (Fry, 1924) and on glass (Mellor, 1922) suggests that haptera can reattach themselves to the surface. Detachment of particles of the limestone substratum by haptera and the separation of the thallus from haptera which remained in the limestone rock were reported by Syers (1964). Limestone fragments were occasionally observed in the interior of the haptera.

B. Effect on Substratum

The disintegration of the rock surface below the thalli of saxicolous lichens has been reported frequently in the literature (Lindsay, 1856; Goepert, 1860; Smith, 1921; Fry, 1922, 1924, 1927; Levin, 1949; Syers, 1964). The extent to which this disintegration is the result of biogeophysical as compared to biogeochemical processes is not clear. Perez-Llano (1944) concluded that mechanical action, resulting from drying, was more important than the effect of chemical reactions. Mechanical disintegration, according to Fry (1927), precedes chemical decomposition.

The effect of the surface area of the solid phase on the rate of chemical weathering is well established. It is probable that the chief contribution of biogeophysical processes is to increase the surface area of the mineral or rock and thereby render it more susceptible to biogeochemical weathering. Brammall and Leech (1943) found that the mechanical disruption of crystals along weakly bonded planes in the structure, as reported when lichen rhizines penetrate minerals (Bachmann, 1904; Yarilova, 1950; Syers, 1964), accelerates chemical decomposition. In addition, the detachment of fragments of the substratum (Fry, 1924; Levin, 1949; Syers, 1964) is a direct and readily observable example of biogeophysical weathering brought about by lichens.

The extent of biogeophysical weathering below lichen thalli appears to be influenced strongly by the nature of the thallus and by the chemical and

physical composition of the rock substratum. It is possible that mechanical disintegration of the substratum below crustose lichens is caused largely by rhizine penetration and expansion and contraction of the thallus because haptera are invariably absent (Smith, 1921). Because of the nature of attachment and the greater freedom of movement of the thallus, foliose species are probably more effective in the biogeophysical, but not in the biogeochemical, weathering of the substratum. Expansion and contraction are considered to be more pronounced at the periphery of the thallus, where rhizines or haptera are more abundant (Fry, 1924). The work of Fry (1924, 1927) suggests that the physical composition of the substratum, particularly the hardness and degree of cleavage, determines the extent of biogeophysical weathering. Fry showed also that the extent of disintegration and incorporation of mineral fragments decreased in the order, shale > gneiss > obsidian, although some fracturing of obsidian was noted. Because of the comparatively high solubility of calcium carbonate, calcareous rocks are more susceptible to biogeochemical weathering than siliceous rocks (Syers, 1964), and it is possible that biogeophysical weathering is of lesser importance.

III. Biogeochemical Weathering

Lichens produce a variety of chemical compounds which are of potential importance in the biogeochemical weathering of minerals and rocks. Of these compounds, carbon dioxide (CO_2), oxalic acid, and a large group of substances frequently referred to as lichen acids, but called lichen compounds herein, are the most significant.

Because water is essential for chemical reactions to take place, a knowledge of the ability of lichens to absorb and retain water is important to an understanding of the role of lichen compounds in biogeochemical weathering. Lichens are able to absorb water from either the liquid or vapor phase and can withstand extremes of desiccation (Smith, 1962). Early work by Bachmann (1923) indicated that crustose thalli and, in particular, endolithic species absorb more water, on a weight-for-weight basis, than foliose thalli. Ried (1960) showed that the saturated water content of most lichens ranged from 100 to 300% of dry weight. The suggestion (Smith, 1961) that the medulla of a thallus may act as a primitive water reservoir for the metabolically more active algal layer is particularly significant. The medulla in crustose lichens is in direct contact with the substratum, and this increases greatly the possibility of chemical dissolution reactions. Because lichen thalli can retain water against drying, chemical weathering reactions can proceed for longer time periods than on rock surfaces without a lichen cover.

A. Mechanisms

1. CARBON DIOXIDE

The CO₂ produced by lichens frequently has been implicated in the decomposition of minerals and rocks (Guembel, 1856; Uloth, 1861; Smith, 1921; Paine *et al.*, 1933; Kononova, 1966; Jackson and Keller, 1970), although the precise role of biogenic CO₂ as a chemical weathering agent has not been evaluated. Interest in the role of CO₂ in biogeochemical weathering arises from the fact that this compound dissolves in water and furnishes hydrogen ions (H⁺) according to the following equations:



where (g) is gaseous and (aq) is aqueous. The ability of H⁺ to promote the decomposition of minerals and rocks is well established in the literature relating to mineral weathering (Keller, 1957). In particular, the solubility of carbonate minerals is highly dependent on the H⁺ concentration (pH) of the aqueous system.

The relative rates of respiration and photosynthesis of lichen thalli are dependent on temperature and water content (Smith, 1962; Lange, 1969). Lichen tissue usually has an appreciably lower photosynthetic rate per unit surface area than the leaves of higher plants although the respiration rates may be of a comparable order (Smith, 1962). Consequently, lichens are characterized by a very low net assimilation rate. The work of Gannutz (1970) suggests that high respiration rates may be obtained at night in early spring following photosynthesis during the previous daylight period. According to Adams (1971), *Cladonia rangiferina* showed a net CO₂ loss at all levels of hydration at 40°C and at low levels of hydration at 30°C. In a study of the aftereffects of drought upon the rates of respiration and photosynthesis of lichens, Ried (1960) concluded that at the end of a drought period the rate of respiration increased rapidly whereas the rate of photosynthesis gradually increased to the normal rate, resulting in a net loss of CO₂. The CO₂ produced by respiration, when dissolved in water, would furnish H⁺ ions which could participate in chemical reactions with minerals of the substratum. The importance of biogenic CO₂ in chemical weathering is not known, but it probably is of much less significance than lichen compounds which act as metal-complexing agents.

2. OXALIC ACID

The importance of oxalic acid in the chemical weathering of minerals and rocks was emphasized by several early workers. Salter (1856) suggested

that the oxalic acid produced by lichens was the principal agent in the disintegration of rocks, and Zopf (1907) considered that this compound was an "... efficient solvent of argillaceous earth and iron oxides." Other workers (Uloth, 1861; Smith, 1921) indicated that oxalic acid was important in rock decomposition.

The association of oxalic acid with calcium in lichens, particularly in species inhabiting limestone, has long been recognized (Braconnot, 1825; Zopf, 1907; Smith, 1921). The fact that calcium oxalate occurs on the outer surface of the fungal hyphae in the lichen thallus or on the surface of the upper cortex (Smith, 1921) suggests that oxalic acid is excreted and that calcium oxalate forms as an insoluble, extracellular deposit (Syers *et al.*, 1967). Appreciable amounts of calcium oxalate, up to 60% of the dry weight of *Rhizocarpon calcareum* (Mitchell *et al.*, 1966) and 66% of the dry weight of *Lecanora esculenta* (Euler, 1908), have been reported. Results of a recent study (Syers *et al.*, 1967) suggest that the production of appreciable amounts of calcium oxalate (more than 5% of dry weight) may be characteristic of obligate calcicolous lichens, rather than all species growing on limestone. It would be expected that the formation of calcium oxalate would decrease the concentration of calcium in solution in immediate contact with a lichen thallus growing on limestone, thus facilitating further dissolution of the calcium carbonate. The fact that calcium oxalate has a very low solubility in water, however, implies that this compound is largely immobile in the biogeochemical weathering environment. In addition, water in contact with and flowing over a limestone surface is probably rich in calcium, and the removal of a small amount of calcium from solution due to calcium oxalate formation could have only a small effect on the overall soluble calcium level in the system. Calcium oxalate formation is, consequently, of relatively minor significance as a factor in biogeochemical weathering.

3. COMPLEXING ACTION OF LICHEN COMPOUNDS

a. WATER SOLUBILITY OF COMPOUNDS AND COMPLEXES. It is commonly considered (Smith, 1921; Smith, 1962; Haynes, 1964; Culberson, 1970) that lichen compounds are insoluble in water. Because of this, the role of lichen compounds in biogeochemical weathering has largely been discounted. Many of these compounds have high molecular weights (Asahina and Shibata, 1954; Culberson, 1969), which would argue against their solubility in water. The presence in many of the compounds of polar groups such as —OH, —CHO, and —COOH (Fig. 1), however, would argue against complete water insolubility. The ability of lichen compounds to function as metal-complexing agents and thus promote biogeochemical weathering ultimately depends on whether these compounds are soluble in water under natural conditions.

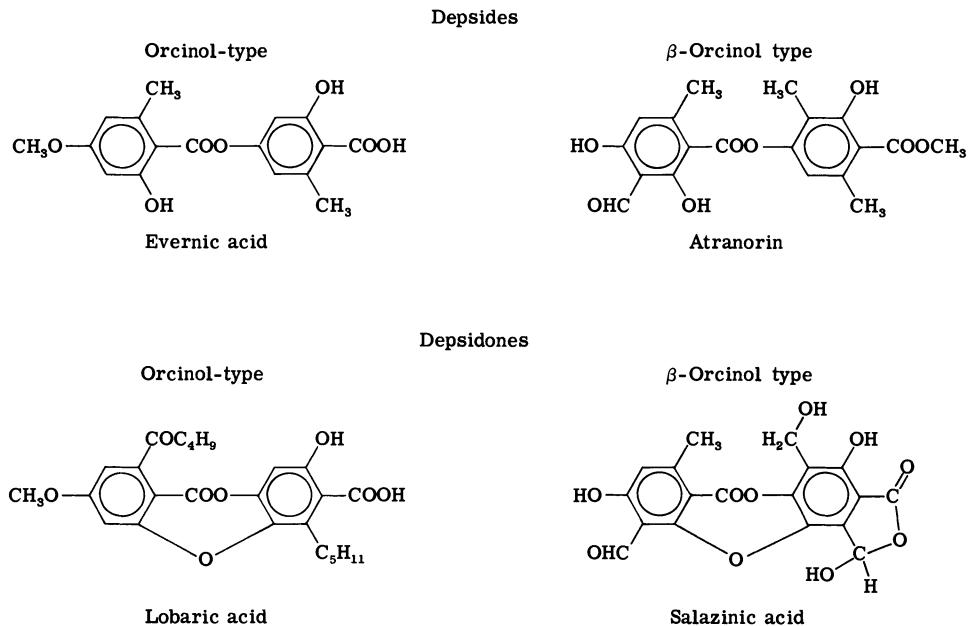


FIG. 1. Structural formulas of depsides and depsidones of the orcinol and β -orcinol type. (From Culberson, 1969.)

Several lines of evidence indicate that lichen compounds are sufficiently soluble in water to behave as metal-complexing agents. The antimicrobial properties of many lichen species are well established (Burkholder *et al.*, 1944; Stoll *et al.*, 1947; Bustinza, 1951; Hennigsson and Lundström, 1970); usnic acid has been implicated frequently as the active component. Laakso *et al.* (1952), however, isolated several lichen compounds which showed antimicrobial properties and concluded that all lichen species which did not give a color reaction with ferric chloride (a qualitative test for the phenolic group) had low antimicrobial activity. The fact that lichen compounds exhibit antimicrobial properties suggests that they are not insoluble in water. It is probable that the antibacterial action involves a metal-complexing mechanism in view of the remarkable correlation which exists between antibacterial properties and metal-complexing ability for synthetic organic compounds (Martell and Calvin, 1952).

The formation of soluble complexes when solid lichen compounds (Schatz, 1963; Syers, 1964; Syers, 1969; Iskandar and Syers, 1972) or water solutions of lichen compounds (Iskandar and Syers, 1972) are allowed to react with suspensions of minerals and rocks shows that lichen compounds are to some extent soluble in water. Recently, the water solubility of four depsides and six depsidones, commonly occurring lichen compounds, was determined by microgravimetric and spectrophotometric techniques (Iskandar and Syers, 1971). This study showed that the lichen compounds had a low but significant solubility in water; values ranging from 5 to 57 mg/liter were obtained. There was no obvious relationship between water solubility and the structural classification of the lichen compounds. Water solubility was influenced by the nature and number of polar groups in the molecule. The solutions of all compounds absorbed radiation in the ultraviolet region and the absorbance was related to the water solubility determined by microgravimetric analysis. The finding that lichen compounds are slightly soluble in water is consistent with the work of Malicki (1965), who showed that usnic acid could be extracted from *Cladonia* spp. by water sprayed in the field or by soaking thalli in water for 18 hours in the laboratory. The more water-soluble phenolic units from which depsides and depsidones are synthesized (Wachtmeister, 1958) could be expected to occur in lichens under field conditions. According to Henriksson (1957), ammonia and other alkaline nitrogenous products derived from lichen phycobionts could increase the water solubility of lichen compounds.

The fact that soluble metal complexes are formed when lichen compounds react with minerals and rocks in the laboratory is particularly significant. If these complexes are formed under field conditions, and there is no obvious reason to believe that they should not, then being soluble, the products of biogeochemical weathering can be removed from the site of weathering.

Kononova (1966) emphasized the importance of the formation of water-soluble complexes in the biogeochemical weathering of rocks.

b. RATE AND EXTENT OF COMPLEX FORMATION. Only in recent years has metal-complex formation been recognized as a biochemical weathering factor in pedogenesis (Bloomfield, 1951; Swindale and Jackson, 1956; Davies *et al.*, 1960). Additional studies have suggested that microbial products (Duff and Webley, 1959; Henderson and Duff, 1963; Kononova, 1966), particularly lichen compounds (Schatz *et al.*, 1954, 1956; Schatz, 1963; Syers, 1969; Iskandar and Syers, 1972), can function as metal-complexing agents.

Rapid formation of soluble colored complexes when lichen compounds or ground lichen thalli were shaken with water suspensions of minerals and rocks was reported by Schatz (1963) and Syers (1969); no information was obtained, however, for the amounts of cations complexed. Of the six lichen compounds investigated by Iskandar and Syers (1972), four formed soluble, colored complexes with biotite. The extract obtained from the interactions of lecanoric acid, a depside of the orcinol type, and biotite was reddish-yellow in color. The formation of a reddish-yellow complex when lecanoric acid is allowed to react with calcium salts is used as a specific color test for this acid (Culberson, 1969). Because a complex may be colorless or adsorbed by the silicate phase, the fact that a lichen compound does not form a colored complex does not necessarily indicate that complex formation has not occurred. Iskandar and Syers (1972) showed that significant amounts of Ca, Mg, Fe, and Al were complexed by lichen compounds. In general, greater amounts of divalent than trivalent cations were complexed. For a particular cation, a similar amount was released from the silicates by solutions of the lichen compounds and by solid lichen compounds. These findings suggest that lichen compounds are sufficiently soluble in water to form soluble metal complexes.

Lichen compounds frequently contain polar donor groups in ortho (adjacent) positions (e.g., —OH and —COOH in evernic and lobaric acids; —OH and —CHO in salazinic acid and atranorin, Fig. 1) which favor the complexing of cations (Syers, 1969). Ginzburg *et al.* (1963) commented on the importance of ortho —OH and —COOH groups in the decomposition of nepheline, chlorite, and kaolinite by organic compounds. In addition, water-soluble phenolic units may be important in metal-complexing reactions (Culberson, 1969). The low solubility and weak acidity of lichen compounds largely preclude their effectiveness as biogeochemical weathering agents if these compounds were to function solely as acids (Hale, 1961). Several studies (Schatz, 1963; Syers, 1969; Iskandar and Syers, 1972) have shown that the release of cations is not caused by reactions directly involving

hydrogen ions. Metal-complexing reactions provide a more satisfactory explanation for the decomposition of minerals and rocks by lichen compounds in laboratory studies.

A metal-complexing action may be involved in the accumulation by lichens of radioactive cations such as ^{90}Sr . Subbotina and Timofeev-Resovkii (1961) reported high accumulation coefficient values for crustose lichens suspended in aqueous solutions of the radioactive isotopes of several metals. Schulert (1962) suggested that a chelation mechanism was involved in the accumulation of ^{90}Sr by lichens and Tuominen (1967) showed that the uptake of ^{90}Sr by *Cladonia alpestris* was a physicochemical process and was not metabolically controlled.

It is dangerous to extrapolate experimental findings obtained in the laboratory to the conditions which exist in the field. As pointed out by Smith (1962), no evidence has yet been presented to show that lichen compounds form soluble complexes in the field. Lichen compounds are extracellular and are usually present in the medulla (Smith, 1921), which in crustose lichens is in direct contact with the substratum and may act as a primitive water reservoir (Smith, 1961). Depsides and depsidones, similar to the compounds used by Schatz (1963), Syers (1969), and Iskandar and Syers (1971, 1972), are the most abundant lichen compounds (Smith, 1962). Thus in crustose lichens, a reserve of slightly soluble lichen compounds that may form soluble complexes with cations is in direct contact, or in close proximity, to the rock substratum.

B. Effect on Substratum

Smith (1962) suggested that lichens cause little change to their substrata after initial colonization. The results of several studies, however, indicate that chemical and mineralogical changes do occur in the composition of the rock below lichen thalli and that these changes could not be accomplished in the relatively short time period required for colonization. Because the calcium carbonate of limestones is slightly soluble in water, the effects of biogeochemical weathering are readily seen below the thalli of many lichen species growing on limestone. Many calcicolous lichen species become immersed in the limestone substratum and develop an endolithic thallus; several early workers considered that a chemical reaction between the lichen and the limestone was involved (Sollas, 1880; Smith, 1921; Fry, 1922, 1924; Bachmann, 1928; Schmid, 1929). The development in limestone of perithecial pits, sometimes referred to as "foveolae" (Smith, 1921), provides further evidence of biogeochemical weathering caused by lichens. A thin section of the endolithic thallus of *Verrucaria sphinctrina* with a flask-shaped perithecium immersed in the limestone substratum is shown in Fig. 2a.

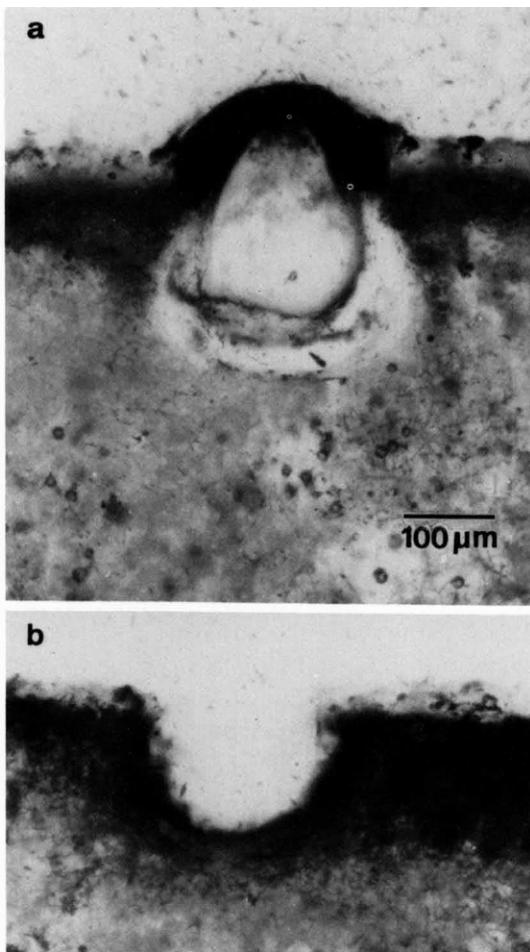


FIG. 2. (a) Thin section of the endolithic thallus of *Verrucaria sphinctrina* growing on limestone showing a partly immersed perithecioid with a black carbonaceous lid. (b) Thin section of a pit in the limestone substratum previously occupied by a perithecium. Both sections stained with chlorazol black. (From Syers, 1964).

When the perithecium dies, a hemispherical pit is left in the limestone (Fig. 2b). Various explanations have been offered to account for the origin of these pits, but other than to suggest that a solution process is involved, none is convincing. Carbon dioxide dissolved in water (Fry, 1922) and lichen compounds (Smith, 1921; Syers, 1964) have been implicated.

Jackson and Keller (1970) found that the weathering crust of lichen-covered basalt was thicker than that of lichen-free basalt which indicates

that chemical weathering is more intense in the presence of lichens. They showed also that the lichen-covered weathering crust was much richer in Fe and poorer in Si, Ti, and Ca, as compared to the lichen-free weathering crust whose composition was much closer to that of the unaltered basalt. The authors concluded that lichens played an important role in the chemical weathering of basaltic lava flows in Hawaii.

Information on the synthesis of new mineral phases as a result of biogeochemical weathering caused by lichens has been presented in the literature. Russian workers have pioneered this field (Jacks, 1953, 1965). Polynov (1945) suggested that authigenic (secondary) quartz, montmorillonite, and illite formed during the mineralization of lichen residues by synthesis from the elements absorbed by the lichen. The colloid fraction of the weathering crust below lichens, referred to as lichen dust by Russian workers, was found to have a cation exchange capacity of 70–110 mEq/100 gm, similar to that of montmorillonite (Aidinyan, 1949). The identical silica:sesquioxide ratio (1.3) of the colloid fraction and of the lichen ash, which was about half that of the original rock, was indicative of a biological origin for the colloid fraction (Aidinyan, 1949). The layer of lichen dust resulting from the action of lichens apparently may be several millimeters in thickness (Polynov, 1945), suggesting that lichens are active agents in the accumulation of soil-forming materials (Jacks, 1953). A rapid weathering of plagioclase feldspars and the slight weathering of chlorite below lichens on syenite have been reported by Yarilova (1950), who concluded that acid excretions from the hyphae were largely responsible for the decomposition of the rock. Bachmann (1904, 1907, 1911) and Jackson and Keller (1970) have demonstrated the formation of authigenic oxides and hydrous oxides of Fe below lichen thalli. Amorphous Fe compounds were formed by the decomposition of the garnet of micaceous shales by *Rhizocarpon geographicum* (Bachmann, 1904). Whereas hematite, a crystalline Fe oxide, was thought to be the only form of Fe oxide present in a lichen-free weathering crust on basalt, a very poorly crystalline Fe oxide gel was identified in lichen-covered weathering crusts (Jackson and Keller, 1970). The fact that the latter mineral was associated exclusively with lichens implies that it is biogenic.

In discussing the chemical and mineralogical changes in the substratum caused by lichens, it is important to realize that dust particles from external sources can be trapped by lichen thalli (Emerson, 1947), thus complicating the interpretation of the data. Sufficient evidence has been presented, however, to indicate that, in certain situations, lichens have a considerable effect on the substratum and that this is due largely to biogeochemical weathering. This process probably is accelerated by biogeophysical weathering which increases the surface area of the substratum.

IV. Plant Succession and Soil Development

A. Plant Succession

The classical concept of the role of lichens in plant succession envisages the colonization of bare rock surfaces by crustose species which are replaced by foliose species (Linnaeus, 1762; Clements, 1916; Braun, 1917; Plitt, 1927; Weaver and Clements, 1938) and/or by mosses (Cooper, 1912; Braun, 1917; Weaver and Clements, 1938; Kubiena, 1943). Emerson (1947) has asserted that:

The crustose forms are the world's greatest pioneers. No organism other than a crustose lichen can maintain itself on a perfectly plane, clean rock surface. . . . Without the pioneering activities of crustose lichens other plants could become established slightly or not at all in many places.*

Other investigators, however, have concluded that lichens do not play a significant role in plant succession (Oosting and Anderson, 1937; Keever *et al.*, 1951; Cooper and Rudolph, 1953; Winterringer and Vestal, 1956; Palmer and Miller, 1961; Tezuka, 1961). Keever *et al.* (1951) indicated that crustose and foliose lichens were always the first plants to colonize granite, but in no case were they found to be essential to further plant development. Similarly, Cooper and Rudolph (1953) reported that the presence of lichens does not always indicate the beginning of plant succession and concluded that the importance of lichens in plant succession has been exaggerated.

Many of the workers who consider that lichens play an insignificant part in plant succession (and often soil development) have been concerned with unconsolidated, transported geological material, such as volcanic ash, glacial moraine, and talus or scree. Cooper and Rudolph (1953) indicated that volcanic ash from Mt. Ngauruhoe, New Zealand, could possibly act as a substratum for vegetation "... without the necessary lichen-moss stage." Palmer and Miller (1961) also found that gravel deposited by the recession of the Rotmoos Gletschen, Austria, was colonized by dwarf willow after one year of exposure of the gravel, whereas lichens were absent for 13 years. Similar findings have been reported by Leach (1930) for the colonization of unstable talus slopes. Because the physical nature of unconsolidated materials is more favorable for the root development and growth of higher plants than is a plane rock surface, it is not surprising that lichens do not initiate plant succession on these materials (Syers, 1964). In a study of the vegetation of the Faroe Islands, Ostenfeld (1906) distinguished

*From "Basic Botany" by F. W. Emerson. Copyright 1947, Blakiston. Used with the permission of McGraw-Hill Book Company.

between a lithophyte sere on bare rock surfaces, initiated by lichens and mosses, and a chomophyte sere in rock crevices and scree, pioneered by certain angiosperms. These two distinct habitats are often confused in the literature relating to the role of lichens in plant succession and soil development. Although the role of lichens in plant succession remains a controversial issue, consideration of the physical and perhaps the chemical nature of the substratum may help to resolve much of the contradictory information reported in the literature.

B. Nutrient Accumulation

The ability of saxicolous lichens to accumulate nutrients is well established in the literature (Lindsay, 1856; Smith, 1921; Polynov, 1945; Jacks, 1953, 1965; Smith, 1962), although the extent to which these nutrients are derived from the substratum, rainwater, or atmospheric dust is not always clear. Smith (1961) suggested that, because lichens frequently live in barren habitats where the supply of nutrients is expected to be poor, the nutrition of lichens must be a particularly important part of their physiology. He also pointed out that it would not be surprising to find that lichens possess highly efficient mechanisms for the accumulation of a range of elements from dilute solutions. Available data indicate that lichens may accumulate large amounts of several major (Polynov, 1945; Jacks, 1953, 1965; Syers 1964) and minor (Lounamaa, 1956; Maquinay *et al.*, 1961) essential elements and radioactive fallout cations (Gorham, 1958, 1959; Tuominen, 1967, 1968) (see Chapter 6).

Although the chemical composition of the substratum exerts considerable influence on the lichen species, the concentration by lichens of "organogenic" elements, such as P, S, and K, was found to occur on acidic, basic, and calcareous rocks (Bobritskaya, 1950). According to Smith (1962), lichens may obtain nutrients from water that passes over the thallus. The greater accumulation of radioactive fallout by lichens, compared to that by mosses and angiosperms, was attributed (Gorham, 1959) to the greater surface area per unit dry weight of lichen thalli.

Nitrogen fixation has been demonstrated in a few lichen species (Bond and Scott, 1955; Scott, 1956; Millbank and Kershaw, 1969, 1970) which contain blue-green algae. Because most lichens do not contain blue-green algae, it is reasonable to assume that N is obtained from sources other than N fixation (Smith, 1962). An early study (Salomon, 1914) showed that several lichens could absorb both ammonium and nitrate N from culture media, and Smith (1960) reported that simple organic N compounds were absorbed rapidly by *Peltigera polydactyla* (see Chapter 9). Lichens may provide a

TABLE I
ACCUMULATION OF NITROGEN (N), PHOSPHORUS (P), POTASSIUM (K), AND IRON (Fe) BY LICHENS GROWING ON LIMESTONE^a

Lichen species	N (%)	P (μg/gm)	K (μg/gm)	Fe (μg/gm)
<i>Verrucaria sphinctrina</i>	0.72	486	1140	810
<i>Caloplaca citrina</i>	2.42	2000	3990	5020
<i>Aspicilia calcarea</i>	1.31	695	1530	2680
<i>Physcia caesia</i>	1.90	1800	3250	4140
<i>Xanthoria parietina</i>	1.98	1320	3950	3210
Limestone rock	trace	48	120	230

^aData of Syers (1964).

habitat for a group of oligonitrophile microorganisms involved in the N cycle in primitive soils (Evdokimova, 1957; Stebaev, 1963). Shields (1957) reported an average value of 4312 μg/gm for the total N content of lichen crusts from volcanic soils, the bulk of the N being in the amino form. Values for the total N content of several lichen species growing on limestone are given in Table I.

The finding that lichens accumulate P and transform the primary calcium phosphate mineral, apatite, into forms which are available for the growth of subsequent colonizing plant species has been reported by several Russian workers (Bobritskaya, 1950; Lazarev, 1945; Polynov, 1945). Lazarev (1945) studied the accumulation and transformation of P on miaskites and gneissic granites and obtained a 400-fold increase in the P content of *Parmelia* spp. over that of the unweathered rock. The accumulation of P by lichens growing on limestone ranged from 10- to 400-fold relative to the unweathered substratum (Table I). Lazarev (1945) also reported about 90-fold enrichment of phosphorus in the fine-earth fraction below lichen thalli and concluded that P in this fraction occurred mainly as organic and Fe-bound phosphate. In the "series of biological absorption" of nutrients constructed by Polynov (1945), P, and possibly S, were regarded as being absorbed predominantly during the lichen stage of plant succession and soil development. Russian workers (Jacks, 1953, 1965) have indicated that other elements, such as Mg, Ca, K, and Fe are accumulated by lichens and converted to forms which are available to higher plants. These studies have assumed that the elements are derived from the substratum. The ability of lichens to accumulate K and Fe is evidenced by the data in Table I. Bobritskaya (1950) also found that *Parmelia* spp. accumulated large amounts of Fe.

The occurrence of high concentrations of Zn, Cd, Pb, and Sn in lichens is particularly interesting since higher plants have a low tolerance of these

elements. Lounamaa (1956) found exceptionally high concentrations of these elements in several lichens but less Mn and B than in higher plant species collected from the same area. The unusually high content of Zn (3300 $\mu\text{g/gm}$) in *Stereocaulon nanodes* (Maquinay *et al.*, 1961) suggests that this metal exists in a complexed, less toxic form within the thallus.

Whether or not nutrients are derived from the substratum, rainwater, or atmospheric dust, it is apparent that lichens can accumulate several elements that are probably essential for the growth of mosses and higher plants. The accumulation of P by lichens is significant, because of the elements present in soil organic matter (C, H, O, N, S, and P), P frequently limits organic matter accumulation during soil development (Walker, 1965).

C. Soil Development

The accumulation of primitive or "lithomorphic" soils below saxicolous lichens is well documented (Linnaeus, 1762; Lindsay, 1856; Clements, 1916; Plitt, 1927; Weaver and Clements, 1938; Polynov, 1945; Emerson, 1947; Targul'yan, 1959). Microorganisms and insects feed on living and dead lichen thalli and utilize the solar energy stored by the lichens during photosynthesis (Jacks, 1965). Organic acids produced during the decomposition of the organic material are probably also involved in the attack of primary minerals (Kononova *et al.*, 1964; Ilyaletdinov, 1969) and this accelerates biogeochemical weathering. The ability of lichens to trap atmospheric dust has been observed by several workers (Salomon, 1914; Trümpener, 1926; Weaver and Clements, 1938; Emerson, 1947). It has been suggested that the atmospheric dust which lodges on the surface of the thallus becomes mixed with organic matter produced by decomposition of the thallus (Emerson, 1947) and with particles of the underlying rock which are detached by biogeophysical and altered by biogeochemical weathering processes (Syers, 1964). Polynov (1945) discussed the formation of organomineral particles below lichens; a process which, according to Jacks (1965), may be the first manifestation of the unique and most characteristic feature of soil formation.

The formation of a primitive soil below lichens has several significant consequences. Nutrients, particularly P, S, Mg, Ca, and K, which are frequently essential to other plants that may replace lichens, are stored in an available or potentially available form (Syers, 1964; Jacks, 1965). The development of cation exchange capacity and the production of exchangeable cations, such as Ca, has been reported by Aidinyan (1949). The retention of cations and anions by the exchange complex should retard losses by leaching. Water-holding capacity should increase because of the accumulation of organomineral material and this may provide a more favorable habitat for the development of plants such as mosses.

The slow growth rate and longevity of lichens are frequently held as objections to their role in soil development (Cooper and Rudolph, 1953;

Hale, 1961). If it is held that lichens initiate plant succession and soil development in certain situations, such as plane rock surfaces, the slow rate of growth of lichens does not preclude their ability to function as biogeophysical and biogeochemical weathering agents, to accumulate nutrients, and to be responsible for the accumulation of organomineral material. In such situations soil formation is itself a rather slow process.

V. Conclusions

Lichens can be important agents in the biogeophysical and biogeochemical weathering of minerals and rocks and, in certain situations, may play an important role in plant succession and soil development.

Rhizine penetration and thallus expansion and contraction cause mechanical disintegration of the substratum. Biogeophysical processes influence biogeochemical weathering by contributing to an increase in the surface area of the substratum.

The significance in biogeochemical weathering of hydrogen ions furnished by the dissolution of CO_2 in water is unknown but is expected to be small. Similarly, oxalic acid produced by lichens is probably of minor importance in biogeochemical weathering. Lichen compounds have a low but significant solubility in water, consistent with the antimicrobial properties of certain compounds. Soluble metal complexes are formed when lichen compounds are allowed to react with minerals and rocks in the laboratory. The formation of such complexes does not appear to have been demonstrated in the field. Chemical and mineralogical changes in the substratum below lichen thalli indicate that biogeochemical weathering occurs under field conditions. These effects are readily seen below lichens on limestone.

Consideration of the physical nature of the substratum is essential to an evaluation of the contradictory information in the literature on the role of lichens in plant succession and soil formation. Lichens accumulate several elements, frequently in large amounts. The accumulation of N, P, and S is particularly significant because these elements are stored in an available or potentially available form and can be used by mosses and higher plants which may replace lichens during soil development.

The mixing of organic matter from the decay of the thallus, mineral particles detached from the substratum, and atmospheric dust trapped by the thallus may produce a primitive or lithomorphic soil.

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Chapter 8

PHOTOSYNTHESIS AND CARBOHYDRATE MOVEMENT

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I. Introduction

Probably the first study on the physiology of lichens was carried out by De Candolle (1798). He was concerned mainly with the various ways by which lichens absorb water, but noted also that "if one places a Pixide lichen (=pyxy-cup lichen *Cladonia pyxidata*) under water in the sun, one sees a few air bubbles cover the superior surface of the leaves but in the interior of the cup a bubble is formed which eventually surpasses the edge of the cupule. This little phenomenon seen in the sun makes a charming spectacle." In 1867, Schwendener stated that lichens were composed of two separate entities, a fungus and an alga. Since that time it has generally been assumed

that the major part of the organic material used by the lichen fungus for growth and metabolism comes from the photosynthetic products of the symbiotic alga. It is now possible to show experimentally that carbohydrate fixed by the alga does pass to the fungus, but it is also conceivable that organic materials are absorbed by the fungus from water which flows over the lichen. This energy input may be important in terricolous lichens such as *Peltigera* sp. which grow beneath deciduous trees. The rotting leaves leached by rain provide organic, nutrient-rich solutions from which lichens could derive benefit. The importance of this energy input to lichens has still to be assessed experimentally.

II. Photosynthesis by the Intact Lichen

A. Rates of Carbon Fixation in Lichens

1. METHODS

Most of the studies that have assessed the rates of carbon fixation in lichens have used manometric techniques (Jumelle, 1892; Smyth, 1934; Stålfelt, 1936; Butin, 1954; Ried, 1960a,b). A colorimetric technique was used by Lange (1956) to measure gas exchange, while Baddeley *et al.* (1971) employed an oxygen electrode for this purpose. The advantages of the latter method are that only a small amount of lichen is required and the results are gained rapidly. Also, the method is useful for studies on the relation of light intensity and photosynthetic rates (K. A. Kershaw, personal communication). Recently, it has been possible to examine gas exchange in lichens in the field with an infrared gas analyzer under conditions that approximate those of the natural microenvironment (Bliss and Hadley, 1964; Lange, 1969, 1970; Lange *et al.*, 1970a,b). This apparatus enables a measure of the amount of carbon dioxide fixed, per unit area of lichen-covered ground, to be calculated accurately. However, it is essential that adequate monitoring of temperature and moisture be done in the aerial environment and in the lichen (inside and outside the experimental cuvette) before such data may be used to interpret what occurs in the field.

In many experiments that measure photosynthesis the amount of carbon dioxide absorbed from the air around the specimen is measured. This disregards the simultaneous respiration that is occurring and the results obtained are designated as *apparent* or *net* photosynthesis. In higher green plants, rapidly photosynthesizing tissues have a rate of photosynthesis which is 10–20 times greater than the rate of respiration. Thus the “net” photosynthetic rate, although an underestimate, is not appreciably less than the “true” rate. Values for the “true” rate of photosynthesis are obtained by

correcting the net rates for the quantity of carbon dioxide released, during the measurement period, by samples placed in complete darkness.

In lichens, where the autotrophic algae make up only a small fraction (by weight or by volume) of the complete thallus, net photosynthesis is significantly less than the true rate. Thus,

$$\begin{aligned}\text{Amount CO}_2 \text{ assimilated by lichen} = & \text{ amount of CO}_2 \text{ removed from air} \\ & + \text{ amount of CO}_2 \text{ produced by fungal respiration} \\ & + \text{ amount of CO}_2 \text{ produced by algal respiration}\end{aligned}$$

The second term on the right-hand side of the equation can be large, particularly under laboratory conditions where the water content of a thallus is high and the temperature between 18°–25°C. Thus, measurements of net photosynthesis can greatly underestimate the amount of carbon assimilation by lichen algae.

The third term on the right-hand side of the above equation is probably small compared with the second but there is no experimental evidence to prove this. It should be noted that respiratory rates of green plants in the dark are not always the same as their respiratory rates in the light when photosynthesis occurs simultaneously. In many plants, under light conditions, photorespiration occurs and this may be greater or less than dark respiration depending on conditions and the plant involved. Photorespiration takes place via the glycolate oxidase pathway and sufficient amounts of glycolate are produced only during active photosynthesis to support this type of respiration (Jackson and Volk, 1970). In order to learn the true rates of carbon fixation by lichens several corrections should be applied to the measured net photosynthesis. These corrections have not been applied in the past.

In many experiments on lichen material, net photosynthesis is measured as mg of CO₂ fixed per hour per gram dry weight. It is questionable whether this is the best way to express results since the proportion of alga to fungus varies from lichen to lichen and the dry weight changes during the year. If photosynthesis were expressed in terms of unit area the results could be compared with higher plant leaves where net photosynthesis is usually between 10 and 20 mg CO₂ per square decimeter of leaf area per hour. Also, by knowing the area covered by a particular lichen on a tree trunk or other habitat, one could calculate the amount of energy fixed by lichens in a particular situation. Another index of apparent photosynthesis is the gain in dry weight. In higher plants under conditions favorable to photosynthesis this gain is usually between 0.50 and 2.0 gm per square meter per hour (Meyer *et al.*, 1960). Perhaps the most satisfactory way to express the rate of photosynthesis is per milligram of chlorophyll because the amount of alga in

lichens varies. However, the practical difficulties of extracting the pigments completely and an inability to compare such results with data available for other plants dictates that data in published work should be expressed on the basis of at least two parameters.

2. RESULTS

The maximum rate of net photosynthesis by a range of lichens was calculated by Ried (1960a) to be between 0.34–3.2 mg/CO₂/50 cm²/hour. Bliss and Hadley (1964) reported optimal rates of 0.30–0.38 mg/CO₂/gm dry weight/hour for three alpine lichens. Many estimates of photosynthetic rate have failed to take into account a seasonal variation in photosynthetic rate due to a change in the physiological activity of lichen algae or number of algae within a given area of thallus. Schulze and Lange (1968) gave comparative figures for higher plants and a lichen expressed as mg CO₂ assimilated per square decimeter per hour. (Table I). The lower figure for the lichen was explained in terms of its lower chlorophyll content, i.e., from one-fourth to one-tenth that of leaves (Wilhelmsen, 1959). In addition, the upper fungal cortex of lichens is more opaque than the epidermis of leaves and absorbs 26–43% of the incident light instead of 4–13% (Ertl, 1951). Further, the opacity of the upper cortex increases by up to 30% as the thallus dries out. Bednar (1963) estimated that the algae in *Peltigera aphthosa* made up only 3–5% of the total volume.

There is considerable variability in the number of algae per square centimeter of lichen thallus. Harris (1971) found that there were 0.9–4.2 × 10⁶

TABLE I
A COMPARISON OF THE MAXIMUM NET CO₂ ASSIMILATION OF LEAVES
OF SEED PLANTS AND THE LICHEN *Hypogymnia physodes* IN LATE WINTER
UNDER CONDITIONS OF LIGHT SATURATION, OPTIMAL TEMPERATURE,
GOOD WATER SUPPLY, AND NORMAL ATMOSPHERIC CO₂
CONCENTRATION^a

Organism	mg CO ₂ /dm ² /hour
Herbaceous plants of economic importance	20–24
Sun plants	12–24
Shade plants	4–16
Deciduous broad-leaved trees (sun leaves)	10–20
Deciduous broad-leaved trees (shade leaves)	around 6
Evergreen conifers	4–8
<i>Hypogymnia</i> at 2.8°C and 30,000 lux	3.8
<i>Hypogymnia</i> at 0°C and 12,000 lux	3.5
<i>Hypogymnia</i> et -6°C and 12,000 lux	0.44

^a From Schulze and Lange (1968).

algae in *Parmelia* sp. Hill and Woolhouse (1966) estimated a mean chlorophyll content of $3.0\text{--}4.8 \times 10^{-6}$ mg chlorophyll per algal cell in *Xanthoria aureola*.

B. Ecological Factors Affecting the Photosynthetic Rate

1. MOISTURE CONTENT

Lange (1969) found that the rate of net photosynthesis increased rapidly with hydration up to 60% saturation in *Ramalina maciformis*. A hydration compensation point was found at 20% of the water-holding capacity at 10°C and 10,000 lux. In this species hydration above 60% saturation had no significant change in gas exchange as measured with an infrared gas analyzer. Kershaw and Rouse (1971), however, found that *Cladonia alpestris* from moist shaded habitats in the Canadian low arctic had an optimum rate of net photosynthesis at 52% saturation and assimilation fell off rapidly when the thallus was wetter or drier. They suggested that this characteristic could explain the absence of this species from wet fens and bogs. Samples of *Cladonia alpestris* from open habitats in the Canadian arctic showed an adaptive response to drier habitats—the maximum net photosynthesis rates were at 30% saturation and assimilation fell only slowly as thallus saturation was reduced to 10%. In a more recent study Kershaw (1971) found that the optimum net assimilation rates occurred between 35 and 70% of thallus saturation in different species. A close relationship was found to exist between the ecology of a species and the percentage saturation at which maximum assimilation occurred. The interpretation and comparison of data from different studies is difficult unless it is realized that percent saturation is calculated in two ways. Zero percent saturation may be the oven-dry weight or the weight obtained by drying the thallus over calcium chloride for 24 hours. The first method removes about 10% more water but perhaps is ecologically less valid.

The effect of the water content of a thallus on net photosynthesis has been noted earlier. Ried (1960b) showed that for *Umbilicaria cylindrica* the optimum rate was at 65% saturation but this was reduced by half when the thalli were fully saturated. He found, however, that in more loosely organized thalli, i.e., those without lower cortices (*Peltigera*) or with cypellae (*Sticta*), photosynthesis was most rapid at 90% saturation with only a small decline above this level. He felt the decline was due to the difficulty of gas exchange in fully saturated leathery thalli such as *Umbilicaria*. However, the findings of Kershaw and Rouse (1971) with *Cladonia alpestris*, which has a hollow tubular thallus, casts doubt on this.

Below a critical moisture content most lichens assume a state of suspended animation in which no carbon assimilation occurs and the respiration rate is

very low. Some lichens from moist or aquatic habitats, e.g., *Verrucaria elaeomelaena*, are damaged by as little as 24 hours of drought (Ried, 1960a). Other lichens from desert habitats, e.g., *Ramalina maciformis*, can withstand 51 weeks of drought with a thallus water content of only 1% and quickly regain their initial photosynthetic activity when rewetted (Lange, 1969). Short-term drying did not influence subsequent gas exchange but prolonged drying inhibited photosynthesis in desert lichens. The longer the drought the more time was required for reactivation of photosynthesis.

The amount of water required to induce minimum net photosynthesis varies considerably. Lichens from mesic habitats usually have to be wetted with water before photosynthesis can begin. However, lichens from deserts can absorb enough water from the humid night air to enable brief periods of photosynthesis. Lange (1969) found that *Ramalina maciformis* could absorb sufficient moisture to reach the water compensation point when it was in equilibrium with air at 80% relative humidity (-287 atm water potential). The desert lichens exhibited 90% of maximum rate of net photosynthesis when they were in equilibrium with saturated air. Under natural conditions, *Ramalina maciformis* and *Teloschistes lacunosus* were moistened by nightly dew and photosynthesized for 3 hours after sunrise. As the lichens dried the moisture compensation point was crossed and carbon dioxide was emitted for a short period. No gas exchange was detected for the rest of the day until the thallus was moistened again in the evening. The CO₂ balance of lichens over any 24-hour period with a nightly dewfall averaged 0.54 mg/CO₂/gm dry weight. Crustose and foliose lichens from the Negev desert showed similar characteristics to the fruticose species mentioned above (Lange *et al.*, 1970b). Lange (1970) calculated that the annual photosynthetic gain would allow for a thallus growth of 5–10% and that the dewfall in the Negev desert contributed decisively to this production.

2. LIGHT

Some lichens species are found on sun-exposed rocks and others grow only under shaded rock overhangs; corticolous lichens have a similar range of habitats. These ecological preferences have been explained in terms of different compensation points. For example, *Usnea dasypoga*, which grows on tree trunks, reaches the compensation point at 400 lux while *Ramalina fraxinea*, which is typical of sunlit branches, has a compensation point at 2000 lux. The light intensity that resulted in half maximum photosynthesis was found to be 2000 lux in the first example and 7000 lux in the second. Thus Barkman (1958) found a fairly good relationship between light requirements and habitat preference (see Haynes, 1964). Hosokawa and Odani (1957) found that treetop species of lichen had a higher 24-hour compensation

point than species growing on the base of the tree. Harris (1971) confirmed this but felt that the variation in respiration and photosynthetic rates will reflect the temperature and variation in algal numbers with time of year and habitat. Thus, the 24-hour compensation point may change for any lichen species from month to month and habitat to habitat. He showed that thalli of *Parmelia caperata* from the lower parts of a tree had a lower rate of net photosynthesis (under laboratory conditions) than specimens from the top of the tree. This was explained in terms of the number of algal cells per square centimeter of thallus. Thus, in thalli at the top of the tree were 34.2×10^5 cells/cm² whereas those from the base of the tree only contained 24.0×10^5 cells/cm². Harris also found that algal numbers increased by 1.2–1.7 times between January and September in species of *Parmelia* growing in England.

In a detailed study, Lange (1969) found that the desert lichen *Ramalina maciformis* reached light saturation at 20,000 lux at 2°C. The light compensation point of fully saturated thalli of this species varied with temperature. At low temperatures (–5° to + 2°C) it was 200–300 lux but at 27°C it was 8000 lux. This is hardly surprising since the algae have to fix much more carbon dioxide at the higher temperature to balance the greatly increased respiratory activity of the fungal portion of the thallus.

3. LOW TEMPERATURES

"Lichens are among all plants those which can most easily tolerate very low temperature. They are found in abundance at high altitudes and in polar regions where no other vegetation could exist" (Jumelle, 1890). The research by Jumella pioneered a facet of lichenology which still attracts interest.

The lowest temperatures at which lichens have assimilated are shown in Table II. Photosynthesis at these temperatures is surprisingly efficient. For example, Lange (1965) found that in *Letharia vulpina*, an alpine lichen, carbon dioxide uptake was maximal at 7°C, only slightly depressed at 0°C, and still half the optimum at –5°C. However, at temperatures between –5° and –10°C, Atanasiu (1969) found that photosynthesis of lichens collected during winter months in Bucharest, Rumania was very low, about 2 mg CO₂/100 gm fresh weight of lichen. As the temperature rose above –5°C at the end of February, photosynthesis increased noticeably.

The ability to withstand adverse conditions is necessary for lichens growing in an arctic environment. *Umbilicaria arctica* and *Umbilicaria lyngei* colonize rock surfaces which are blown free of snow in winter and they must be able to withstand prolonged temperatures of around –40°C and 3 months of darkness each year in the Canadian High Arctic. Of eight lichens tested, only *Umbilicaria vellea* showed damage after exposure to –196°C as mea-

TABLE II
THE LOWEST TEMPERATURES AT WHICH LICHENS SHOW NET PHOTOSYNTHESIS

Lichen	Minimum temperature (°C)	Worker
Alpine lichens	-20	Henrici (1921)
<i>Anaptychia leucomelaena</i> ^a	-1	Lange (1962)
<i>Cladonia alcicornis</i>	-24	Lange (1962)
<i>Hypogymnia physodes</i>	-6	Schulze and Lange (1968)
<i>Lobaria pulmonaria</i>	-7	Atanasiu (1969)
<i>Neuropogon</i> sp.	-18.5	Gannutz (1967)
<i>Parmelia shimpéri</i> ^a	-3	Lange (1962)
<i>Stereocaulon alpinum</i>	-24	Lange (1962)
<i>Usnea ceratina</i>	-10	Atanasiu (1969)
<i>Usnea submollis</i>	-10	Atanasiu (1969)

^aTropical lichens.

sured by respiratory activity. However, as measured by photosynthetic ability, only five survived slow cooling to -196°C and subsequent slow thawing. In *Caloplaca elegans* and *Rinodina frigida* normal photosynthesis rates were observed within a day after cold treatment. *Xanthoria mawsonii* took 2 days to recover and *Umbilicaria decussata* 7-8 days. Rapid cooling resulted in a slower return to normal photosynthesis, i.e., 21-26 days. Some of the lichens showed more permanent damage; thus *Buellia* sp. respired but did not assimilate (Lange and Kappen, 1972; Kappen and Lange, 1972). These authors concluded that the lichen alga is more sensitive to low temperature damage than the lichen fungus. As noted by Jumelle (1890) and Scholander *et al.* (1953), dehydration increased the cold resistance of lichens—probably because less intracellular ice was formed.

4. HIGH TEMPERATURES

Lichens are found in habitats which are subjected to high temperatures, but the effects of light and water regime on photosynthesis at these temperatures has not been examined in detail. Lange (1953) recorded temperatures of 53°-69°C within or below thalli. One specimen of *Cladonia pyxidata* remained at 62.5°C or above for 4½ hours. Lange defined the limit of heat resistance of lichens as the temperature which caused normal respiration to be reduced by one-half. Dry lichen thalli could withstand a 30 minutes exposure to temperatures from 70°C (*Alectoria sarmentosa*) to 101°C (*Cladonia pyxidata*). However, moist thalli had a much lower limit of heat resistance which varied from 35° to 46°C.

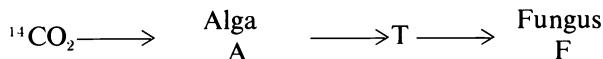
In a recent study, Lange (1969) subjected the desert lichen *Ramalina maciformis* to high temperatures, then moistened the samples and measured gas

exchange using infrared gas analysis. He found that this lichen was unaffected by 30-minute exposures to 65°C. Heating to 67.5°C for the same time resulted in a 50% depression of net photosynthesis but the lichen partially recovered during the period of gas-exchange measurements. Above this temperature *Ramalina maciformis* was permanently damaged and, after exposure to 85°C, samples showed no "real" photosynthesis while parts of the thalli showed red discolorations. At these very high temperatures there was a clear correlation between the amount of damage to the lichen (as measured by gas exchange) and the length of the high temperature exposure period.

Ramalina maciformis proved to be less tolerant to high temperatures when fully water saturated. An exposure to 36°C led to a severe but reversible depression in "net" photosynthesis but heating to 38°C or higher resulted in irreversible damage to the lichen. Thus, even desert lichens are required to be dry to avoid damage at the temperatures prevailing in the middle of the day (highest recorded air temperature being 46.4°C).

III. Interactions between Lichen Symbionts

Since the advent of radioactive tracer techniques and their first application in lichen physiology by Smith (1961), much information has accumulated on the interaction between alga and fungus. About 35 lichen species with 12 different types of algal partners have been studied to determine the nature of the substances which pass from the autotrophic alga to the fungus. When a lichen is allowed to photosynthesize in the presence of radioactive $^{14}\text{CO}_2$ (or $\text{NaH}^{14}\text{CO}_3$ solution) the following events occur.



Carbon-14 is incorporated first into algal photosynthetic products (A), then transferred in some form (T) to the fungus where it finally accumulates in fungal products (F). Each of these steps will be considered in the succeeding sections, but first it is important to consider the various methods that have been used to examine these steps and the kind of information and limitations obtained from each method.

A. Methods for Studying the Interaction

Most experiments done by Smith and his co-workers (Bednar, Drew, Richardson, Hill, and Green) incubated washed lichen samples in liquid media with ^{14}C -labeled sodium bicarbonate for a few minutes to several days. A light intensity of 5000 lux and temperature of 18°–20°C were regularly

employed. Since different lichens have different ecological preferences, the conditions were obviously not optimum for all. Also, as discussed in the previous section, many lichens show a reduced net photosynthesis when they are fully saturated. Thus, the tracer techniques did not give a direct measure of the gross amount of carbon supplied by the alga to the fungus under natural conditions. Further, the specific activity of the compounds that incorporated ^{14}C was seldom measured to determine how much carbon passed between the symbionts under laboratory conditions. The failure to measure specific activity was due to technical difficulties but these are now reduced because of the development of gas-liquid chromatography (Drew and Holligan, 1971).

Although it was desirable that conditions for photosynthesis of lichens in laboratory experiments should be optimum, limited availability of fresh material and the difficulty of preparing samples resulted in the application of standard conditions. This enabled qualitative differences between lichen species to be measured on small amounts of material. After exposure to ^{14}C -labeled sodium bicarbonate solutions in the light, various methods were used to study the fate of fixed ^{14}C .

1. DISSECTION

The algal layer and upper fungal cortex of lichens can be separated from the underlying, purely fungal medulla by dissecting thallus disks into two parts with a fine scalpel under a binocular microscope. The arrival of photosynthetically fixed ^{14}C in the medulla therefore can be studied. Interesting results have been obtained with this technique. It has been demonstrated, for example, that ^{14}C moved to the medulla more rapidly in some lichens than in others (Table III). The disadvantages of this method are that few species have thalli thick enough to dissect and it does not measure the rate or amount of movement from the algae to the adjacent fungal hyphae. However, it does show the rate and amount of transfer between different regions of a thallus.

2. DIRECT ISOLATION OF THE ALGA

In many lichens relatively clean preparations of the phycobiont can be obtained by centrifuging thallus homogenates and thereby separating the fungal fragments from the algal cells. The latter are then washed. The products which these washed algae release into the medium during photosynthesis in ^{14}C -labeled sodium bicarbonate solutions can then be studied. Immediately after the algae are isolated, the major part of the ^{14}C they release is in one simple carbohydrate. The disadvantage of this method is that the algae change physiologically as soon as they are separated from the lichen thallus. If directly isolated algae are cultured, or allowed to age for 24 hours by being

TABLE III
THE RATE OF MOVEMENT OF FIXED ^{14}C FROM THE ALGAL LAYER TO THE
MEDULLA OF VARIOUS LICHENS

Lichen	Alga	Percent ^{14}C that moved from algal layer to medulla	Reference
<i>Dermatocarpon miniatum</i>	<i>Hyalococcus</i>	19.9 in 24 hours	Richardson <i>et al.</i> (1968)
<i>Lobaria amplissima</i>	<i>Myrmecia</i>	2.9 in 3 hours 7.5 in 24 hours	Richardson <i>et al.</i> (1967)
<i>Peltigera polydactyla</i>	<i>Nostoc</i>	40 in 4 hours	Smith and Drew (1965)
<i>Lobaria scrobiculata</i>	<i>Nostoc</i>	40 in 3 hours	Richardson <i>et al.</i> (1967)
<i>Roccella fuciformis</i>	<i>Trentepohlia</i>	3.5 in 24 hours	Richardson <i>et al.</i> (1968)

placed in distilled water, they show a marked and progressive change in their photosynthetic pattern.

Significant differences have not been found in the amount or pattern of ^{14}C fixation between directly isolated algae from marginal thallus lobes and algae from the center of the thallus in *Xanthoria aureola* (Green, 1970). The algae in both young and old parts of the thallus were physiologically active and could be used in experiments. This raises the question as to whether algae in the center of a lichen thallus have a reduced rate of senescence or are replaced progressively during growth of the lichen.

3. ENTRY OF ^{14}C INTO FUNGAL PRODUCTS

In most lichens the soluble carbohydrates of the fungus are different from those of the algae. For example, mannitol is exclusively a fungal product in the great majority of lichens so that the accumulation of ^{14}C in this compound gives information about movement and accumulation of ^{14}C by the fungus under particular experimental conditions. In *Peltigera polydactyla*, Drew and Smith (1967b) directed [^{14}C]mannitol after 2 minutes of photosynthesis by thallus samples, while in *Xanthoria aureola* it was detectable after 3 minutes (Bednar and Smith, 1966). The main limitation of this technique is that many intermediary metabolites are common to both alga and fungus and therefore cannot be used to examine ^{14}C transfer. In addition, it is not certain how fast lichen fungi convert the mobile algal carbohydrate into mannitol. Thus, in some lichens the delayed appearance of [^{14}C]mannitol could reflect slow rates of conversion to this compound rather than slow rates of carbohydrate transfer.

This technique is useful but the results require interpretation in the light of certain exceptions. In lichens containing *Trentepohlia* it has been suggested that both the alga and the fungus contain mannitol (B. Feige, personal communication). Also, de Lestang Laisné (1966) found mannitol in *Rivularia bullata* which she considers is closely related to the *Calothrix* phycobiont of *Lichina pygmaea*.

4. THE INHIBITION TECHNIQUE

Experiments using the methods described in the previous sections showed that glucose moves from alga to fungus in *Peltigera polydactyla* and is rapidly converted to mannitol. Drew and Smith (1967b) devised an "inhibition technique" whereby lichen samples were able to photosynthesize in solutions of 1% [¹²C]glucose which also contained sodium [¹⁴C]bicarbonate. Under these conditions [¹⁴C]glucose appeared in the medium but [¹⁴C]-mannitol could not be detected in the thallus. At some stage during the passage of photosynthate from alga to fungus, the [¹²C]carbohydrate competed successfully with the [¹⁴C]carbohydrate formed by the alga. The [¹⁴C]-carbohydrate, unable to move to the fungus, diffused into the medium. Chromatography and autoradiography of the medium showed that the radioactive sugar released by the alga and the nonradioactive form used to induce inhibition were closely similar and usually the same. This technique was specific in that sugars different from the mobile one could not cause inhibition. Exceptions to this were the glucose analogues, 3-methylglucose and 2-deoxyglucose and to some extent mannose. Similar experiments with *Xanthoria aureola* found that the three pentitols, ribitol, arabinol, and xylitol, caused inhibition but ¹⁴C in the medium appeared only as ribitol. Appreciable inhibition in this lichen was evident only after 12–24 hours of incubation whereas in *Peltigera* there was considerable inhibition after 3 hours.

Using this technique, the carbohydrate that moves between the symbionts can be identified as follows: lichen samples are incubated in solutions of [¹⁴C]sodium bicarbonate with individual sugars that are suspected of moving from alga to fungus. For each sugar solution the amount of radioactivity released into the medium is determined as a percentage of the total ¹⁴C fixed. The sugar which stimulates the greatest release of ¹⁴C is probably the mobile carbohydrate. This can be confirmed by chromatography and autoradiography of the medium to see whether the [¹⁴C]sugar that is released is identical to the [¹²C]sugar used for inhibition. Various lichens have been tested in this manner. The results with *Xanthoria aureola* are shown in Table IV.

The disadvantage of this technique is that a 1% carbohydrate solution is not a natural medium for photosynthesis. In many of the experiments the

TABLE IV
**EFFECT OF EXTERNALLY SUPPLIED 1% ^{12}C CARBOHYDRATES
 ON THE RELEASE OF FIXED ^{14}C FROM *Xanthoria aureola*
 (PHOTOSYNTHESIZING) ON ILLUMINATED $\text{NaH}^{14}\text{CO}_3$
 SOLUTIONS^a**

[^{12}C] Carbo-hydrate	Percent total fixed ^{14}C released to medium in 24 hours	Total ^{14}C fixation
None	1.1	75
Disaccharide		
Sucrose	1.4	98
Trehalose	1.7	57
Hexose		
Glucose	1.8	93 ^b
Hexitol		
Mannitol	3.6	58
Pentose		
Ribose	0.7	70
Pentitol		
Arabitol	20.2	71
Ribitol	23.6	77
Xylitol	10.5	64

^aSample size, 100 mg fresh weight, incubated on 3 ml distilled water with 10 Ci carrier-free $\text{NaH}^{14}\text{CO}_3$ at 18°C and 5000 lux for 24 hours. Total ^{14}C fixation given as thousands of counts per minute.

^bData from separate similar experiment.

total fixation was less than that of control samples incubated on distilled water. In particular [^{12}C]glucose suppressed the net fixation of ^{14}C . This was due possibly to stimulated respiration by the lichen samples resulting in $^{12}\text{CO}_2$ which lowered the specific activity of the sodium [^{14}C]bicarbonate in the sample tubes.

B. Photosynthetic Products of Lichen Algae

In experiments aimed at understanding the roles of alga and fungus in a lichen, the photosynthetic products of the lichen alga can be investigated by examining it in pure culture as though it were a free-living form. However, lichen algae have a number of unusual characteristics which make studies of this type questionable. First, they usually require organic compounds such as glucose in the culture medium to grow reasonably quickly and they can grow heterotrophically in darkness. Second, the pigment systems are unusual as they form chlorophyll in complete darkness and are very sensitive to

strong light. Some strains of *Trebouxia*, at light intensities above 2000–5000 lux, will bleach irreversibly.

Finally, these algae show a progressive change in photosynthetic pattern after isolation from the thallus. Therefore, results of studies with the cultured algae should be used together with studies on algal cells directly isolated from the thallus and short-term experiments on the intact thallus.

Experiments using all these techniques have shown that lichen algae incorporate ^{14}C during photosynthesis on $\text{NaH}^{14}\text{CO}_3$ into sugar phosphates, simple sugars, amino acids, and insoluble compounds. Depending on the genus of algae, one particular sugar or sugar alcohol incorporates most of the ^{14}C in algae which are examined immediately after isolation from the thallus.

1. BLUE-GREEN SYMBIOTS

Studies on *Nostoc* from *Peltigera polydactyla* showed that the photosynthetic carbohydrate was glucose (Drew and Smith, 1967a) and further studies suggest that this is true for most other lichens with blue-green algal symbionts (Richardson *et al.*, 1968). B. Feige (personal communication 1971) found that mannisidomannitol is an important additional photosynthetic product in *Lichina pygmaea* which contains *Calothrix*. He suggested that this compound acts as an osmoregulator like the galactosidoglycerols of the red algae and Chrysophyceae. Feige (1969) also identified a pentitol as a short-term photosynthetic product of *Scytonema*, the blue-green symbiont of the tropical basidiolichen *Cora pavonia*, but this has yet to be confirmed:

2. GREEN SYMBIOTS

During photosynthesis of directly isolated algae most of the ^{14}C accumulates in sugar alcohols. The type of sugar alcohol depends on the genus of alga, i.e., ribitol in *Coccomyxa*, *Myrmecia* and *Trebouxia*; erythritol in *Trentepohlia*; and sorbitol in *Hyalococcus*. In all cases, virtually nothing is known about the biochemical pathway from the initial fixation of $^{14}\text{CO}_2$ to the formation of radioactive sugar alcohol. This is in contrast with related free-living algae such as *Chlorella pyrenoidosa* where both the pathways and probable mechanisms controlling the rates of the various steps are well known (Bassham, 1971).

3. LICHEN ALGAE ON SEPARATION FROM THE INTACT THALLUS

a. CHANGES WITHIN THE CELLS. Green (1970) found that when algal symbionts are isolated from the lichen thallus, aspects of their physiology change very rapidly. For example, in the cells of newly isolated *Trebouxia*,

large amounts of radioactivity occurred in ribitol and sucrose. However, after 24 hours of isolation the proportion of ribitol to sucrose diminished and much more radioactivity was found in ethanol-insoluble compounds. In addition, the algae in pure culture incorporated only a trace of ^{14}C into ribitol during photosynthesis. The reason for the small incorporation of ^{14}C into ribitol may be because the glucose, peptone, and growth substances that are normally added to the culture medium enhance protein synthesis and growth. Green (1970) found that algae cultured in a medium without glucose incorporated more ^{14}C into ribitol while Hill (1970) noted a similar situation in cultured cells after a period of drying.

Hyalococcus, a green phycobiont of *Dermatocarpon* sp., was studied by Green who found that immediately on isolation some 70% of the fixed ^{14}C in the ethanol-soluble fraction appeared in sorbitol, 18% in sucrose and the remainder in other compounds. Of the ^{14}C fixed, 26% was released into the medium. If the algae were suspended for 24 hours in distilled water before photosynthesis, the distribution of radioactivity in the soluble fraction was about the same. However, there was an increased incorporation of ^{14}C into ethanol-insoluble compounds and less ^{14}C released from the cells (Table V).

In newly isolated cells of *Coccomyxa*, 50% of the fixed ^{14}C occurred in ribitol but after 72 hours on distilled water radioactivity could not be detected in ribitol within the cells.

b. SUBSTANCES RELEASED BY THE CELLS. It has been observed generally that directly isolated symbiotic algae release considerable amounts of photosynthate immediately after isolation from the thallus. If algae are allowed to age for several hours in distilled water in the light, the release of ^{14}C during photosynthesis diminishes considerably. Moreover, the re-

TABLE V.
THE DISTRIBUTION OF FIXED ^{14}C BETWEEN THE MEDIUM AND
CELL FRACTIONS OF *Hyalococcus*^a

Time from isolation (hours)	% distribution of fixed ^{14}C		
	Medium	Ethanol soluble	Ethanol insoluble
<i>Directly isolated algae</i>			
0	26.1	44.3	29.6
24	6.2	53.6	40.2
<i>Cultured algae</i>			
	1.3	48.4	50.3

^aIncubated at 5000 lux, 20°C, 6.25 $\mu\text{Ci}/\text{ml}$ for 3 hours. Algae kept on distilled water prior to incubation. Total fixed ^{14}C similar for all treatments. (From Green, 1970).

leased compounds have a low chromatographic mobility and are not the sugars or sugar alcohols that are released immediately after isolation (Drew and Smith, 1967a; Richardson and Smith, 1968b; Green, 1970). The nature of the compounds which remain close to the origin of chromatograms is not known but they probably are glucose polymers of low molecular weight. The type and proportion of the carbohydrates released from cells directly after their isolation from a thallus and after 24 hours of aging is shown in Table VI. Qualitative and quantitative changes occur in the products released by the lichen algae as time elapses after isolation. Apparent anomalies in some of these results are discussed in the following paragraphs.

Nostoc has been directly isolated from two lichens. The isolate from *Peltigera polydactyla* released principally glucose (Drew and Smith, 1967a) while the isolate from *Peltigera canina* released other substances as well (Green, 1970) (Table VI). This may be due to a different strain of alga, more sensitive detection techniques, or differences in treatment of the thallus homogenate. Green's preparations were washed more frequently with distilled water so that the suspension of algae contained fewer fungal fragments or fungal substances that could influence the release of carbohydrates. However, washing with distilled water, a hypotonic solution, may have had deleterious effects on the membrane systems in the cells resulting in the release of several types of organic compounds.

Directly isolated algae, even after 24 hours, released more ^{14}C than algae grown in pure culture. Drew and Smith (1967a) found that cultured *Nostoc* from *Peltigera polydactyla* had a different pattern of carbohydrate fixation and a different morphology from directly isolated cells. The cultured alga formed a thick mucilagenous sheath which was vestigial in the intact thallus.

Richardson *et al.* (1968) found that *Coccomyxa* newly isolated from *Peltigera aphthosa* released ^{14}C in the form of ribitol when allowed to photosynthesize on solutions of sodium [^{14}C]bicarbonate. However, Green (1970) in similar experiments found that other compounds also were released. After 72 hours of separation from the fungus, ribitol could not be detected either in the algal cells or in the medium. The algae which had been separated from their fungal partners for 3 days showed a pattern of carbohydrate distribution similar to strains grown in pure culture for many generations. The different results obtained by Richardson and Green can most likely be explained by slightly different extraction techniques and experimental conditions.

It would be interesting to know in some of these experiments whether the absolute amount of fixation changes in comparable suspensions of algal cells as time elapses from the moment of isolation. It is possible that the algae have a decreased photosynthetic rate after separation from the thallus because the production of the mobile carbohydrate is no longer being stimulated by the

TABLE VI
THE NATURE AND AMOUNT OF CARBOHYDRATE RELEASED BY ALGA IMMEDIATELY AFTER ISOLATION FROM THE LICHEN THALLUS AND AFTER
INCUBATION ON DISTILLED WATER

Genus	Species	Alga	% Released on isolation	Nature of CH (carbohydrate)	% released after 24 hours	Nature of CH	Reference
<i>Peltigera</i>	<i>polydactyla</i>	<i>Nostoc</i>	46	Glucose 100%	12 ^a	Baseline CH	Drew and Smith (1967a)
<i>Peltigera</i>	<i>canina</i>		15	Glucose 22%, baseline CH 44%, organic acids 25%, unidentified CH 9%	7	Baseline CH 100%	Green (1970)
<i>Dermatocarpon</i>	<i>miniatum</i>	<i>Hyalococcus</i>	26	Sorbitol 91%, baseline CH 9%	6	Sorbitol 15%, baseline CH 85%	Green (1970)
<i>Peltigera</i>	<i>aphthosa</i>	<i>Coccomyxa</i> (thallus alga)	20	Ribitol 83%, organic acid 16%. baseline CH 1%	4	Ribitol 1%, organic acids 9%. baseline CH 80%	Green (1970)
<i>Xanthoria</i>	<i>aureola</i>	<i>Trebouxia</i>	8	Ribitol 80%, sucrose 5%, organic acids 4%, baseline CH 9%	1	Ribitol 15%, baseline CH 85%	Green (1970)

^a36 hours of incubation.

TABLE VII
RELEASE AND INCORPORATION OF ^{14}C INTO
INSOLUBLE COMPOUNDS BY ALGAE FROM
Xanthoria aureola

Alga	^{14}C in insoluble compounds (%)	^{14}C released from alga (%)
Thallus alga	2	40 ^a
Freshly isolated alga	21	8
Cultured alga	58	2.5

^aData from inhibition experiments (Green, 1970).

lichen fungus. This might be an alternative way to explain changes in the percentage of ^{14}C in the ethanol-soluble and -insoluble fractions.

In summary, as time elapses after separation from the fungus, lichen algae (a) synthesize less simple sugar or sugar alcohol, (b) form more ethanol-insoluble compounds (c) develop cell sheaths not observed in directly isolated cells; e.g., in *Trebouxia* (Ahmadjian, 1959) and *Nostoc* (Drew and Smith, 1967a), (d) release less photosynthate into the suspending medium. Studies on isolated lichen algae are interesting and valuable but experiments on the intact thallus suggest that lichen algae release a greater proportion of ^{14}C to the fungus than they do to the medium after being isolated (Table VII). Thus, there is limited value in extrapolating results from studies on isolated algae to explain what is happening in the intact thallus.

IV. The Mobile Carbohydrate and Its Release

A. Nature of the Transferred Carbohydrate

Information gained from inhibition experiments and studies on directly isolated algae have indicated that glucose is the typical mobile carbohydrate in lichens containing blue-green algae and sugar alcohols in those lichens containing green algae. More than thirty lichens have now been examined and the results are summarized in Table VIII. Inhibition experiments have shown that the movement of carbohydrate is substantial and in many cases amounts to some 40% of the ^{14}C fixed by the alga in a 3- to 24-hour period. Most of this movement is as a single carbohydrate although the methods used would not detect small amounts of amino acids, vitamins, or other substances.

B. Mechanism of Carbohydrate Release

Studies on the algal symbionts of animals showed two ways of inducing the algae to release carbohydrate to the host. Cernichiari *et al.* (1969) de-

TABLE VIII
CHARACTERISTICS OF CARBOHYDRATE MOVEMENT BETWEEN THE
SYMBIOTS OF LICHENS^a

	Lichen species	Algal symbiont	Mobile carbohydrate	Rate of ¹⁴ C movement between symbionts ^b
Lichens with green algae	<i>Dermatocarpon hepaticum</i>	<i>Myrmecia</i>	Ribitol	Slow
	<i>Lobaria laetevirens</i>	<i>Myrmecia</i>	Ribitol	Slow
	<i>L. pulmonaria</i>	<i>Myrmecia</i>	Ribitol	Slow
	<i>Lecanora conizaeoides</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Lepraria chlorina</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Lepraria incana</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Parmelia surfuracea</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>P. saxatilis</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Sphaerophorus globosus</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Umbilicaria pustulata</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Xanthoria aureola</i>	<i>Trebouxia</i>	Ribitol	Slow
	<i>Gyalecta cupularis</i>	<i>Trentepohlia</i>	Erythritol	Very slow
	<i>Lecanactis stenhammarii</i>	<i>Trentepohlia</i>	Erythritol	Very slow
	<i>Roccella fuciformis</i>	<i>Trentepohlia</i>	Erythritol	Very slow
	<i>R. montagnei</i>	<i>Trentepohlia</i>	Erythritol	Very slow
	<i>R. phycopsis</i>	<i>Trentepohlia</i>	Erythritol	Very slow
	<i>Porina lectissima</i>	<i>Phycopeltis</i>	Erythritol	Slow
	<i>Verrucaria hydrella</i>	<i>Heterococcus</i>	Sorbitol	Intermediate
	<i>Dermatocarpon fluviale</i>	<i>Hyalococcus</i>	Sorbitol	Intermediate
	<i>D. miniatum</i>	<i>Hyalococcus</i>	Sorbitol	Intermediate
	<i>Polyblastia hencheliana</i>	<i>Trochiscia</i>	Sorbitol	Slow
Lichens with blue-green algae	<i>Lichina pygmaea</i>	<i>Calothrix</i>	Glucose glucan	Slow
	<i>Collema auriculatum</i>	<i>Nostoc</i>	Glucose	Fast
	<i>Leptogium</i> spp.	<i>Nostoc</i>	Glucose	Fast
	<i>Lobaria scrobiculata</i>	<i>Nostoc</i>	Glucose	Fast
	<i>Peltigera canina</i>	<i>Nostoc</i>	Glucose	Fast
	<i>P. horizontalis</i>	<i>Nostoc</i>	Glucose	Fast
	<i>P. polydactyla</i>	<i>Nostoc</i>	Glucose	Fast
	<i>Sticta fuliginosa</i>	<i>Nostoc</i>	Glucose	Fast
Lichens with both green and blue-green algae	<i>Sticta</i> spp. (<i>Cyanicaudata</i> group)	<i>Nostoc</i>	Glucose	Fast
	<i>Coccocarpia</i> spp.	<i>Scytonema</i>	Glucose	Slow
	<i>Lobaria amplissima</i>	<i>Myrmecia</i> with <i>Nostoc</i> in cephalodia	Ribitol and glucose	Slow
	<i>Peltigera aphthosa</i>	<i>Coccomyxa</i> with <i>Nostoc</i> in cephalodia	Ribitol and glucose	Intermediate
				Fast

^aAdapted from Richardson *et al.* (1968) and Hill (1970).

^bThe adjectives used to describe rate of ¹⁴C movement are defined in terms of the percentage of total fixed ¹⁴C released in "inhibition" experiments after 3 or 24 hours as follows: "fast," approximately 20–40% released in 3 hours; "intermediate," approximately 10–15% released in 3 hours; "slow," approximately 2–4% released in 3 hours and 20–40% released in 24 hours; "very slow," approximately 1–2% released in 3 hours and 5–2% released in 24 hours.

monstrated the probable occurrence of a pH sensitive surface enzyme in *Chlorohydra viridissima*. They suggested that the host controlled the release of fixed ^{14}C by varying the pH in its cells. Muscatine (1967) and Trench (1971) induced zooxanthellae from *Tridacna* spp. and *Pocillopora damicornis* to excrete glucose, alanine, and glycerol using homogenates of the host tissue. The mechanism of release was thought to be a "factor" which increased the permeability of the algal cell membrane. However, since the pattern of distribution of ^{14}C between intracellular and extracellular compounds was very different, it was suggested that the host factor only affected the plasmalemma of the algal cell and not the membranes bounding the chloroplast and other organelles so that only some of the intracellular compounds were released.

Experiments have been conducted on lichen algae to see whether they were sensitive to the two mechanisms that stimulated carbohydrate release in animal symbionts. In addition, a range of substances have been applied to induce renewed release of carbohydrate from algal cells which have been separated from the fungus for a period of time. These experiments are described in the following sections.

TABLE IX
THE EFFECT OF pH ON THE RELEASE OF FIXED ^{14}C BY THE
DIRECTLY ISOLATED ALGA *Trebouzia*^a

pH	Radioactivity in various fractions			Total ^{14}C fixed (counts/minute $\times 10^3$)	^{14}C in medium (%)
	Soluble	Insoluble	Medium		
3	116	112	99	327	30.3
4	245	69	72	386	18.6
5	330	157	20	507	4.0
6	428	209	23	660	3.5
7	1116	304	29	1449	2.0
8	1386	492	131	2009	6.5
Control distilled water (pH 5.8)	767	270	35	1072	3.3

^aMaterial: 0.5 cm wet-packed volume algae per sample; period of photosynthesis, 18 hours; temperature, 18°C; light, 5000 lux; vessels, 2 × 1 inch specimen tubes; radioactivity 20 Ci NaH $^{14}\text{CO}_3$; medium; 3 ml distilled water buffered with McIlvaine buffer.

1. CHEMICAL FACTORS

a. EFFECTS of pH. Richardson (1967), using *Trebouxia* from *Xanthoria aureola*, found that the release of fixed ^{14}C was stimulated by media buffered to pH 3 and pH 4 but reduced by media at higher pHs. (Table IX). However, it was not certain to what extent the phosphate-citrate buffer affected the results. For example, both sucrose and ribitol were released from the samples incubated on buffer, especially at high pH levels. In contrast, control samples incubated on distilled water released ^{14}C predominantly as [^{14}C]ribitol. Further, the proportion of ^{14}C fixed into sucrose within the cells was greater in samples incubated on buffer than those on distilled water.

Green (1970) examined the effects of pH on directly isolated cells of *Nostoc* and found a marked pH optimum of pH 5.9 for release of fixed

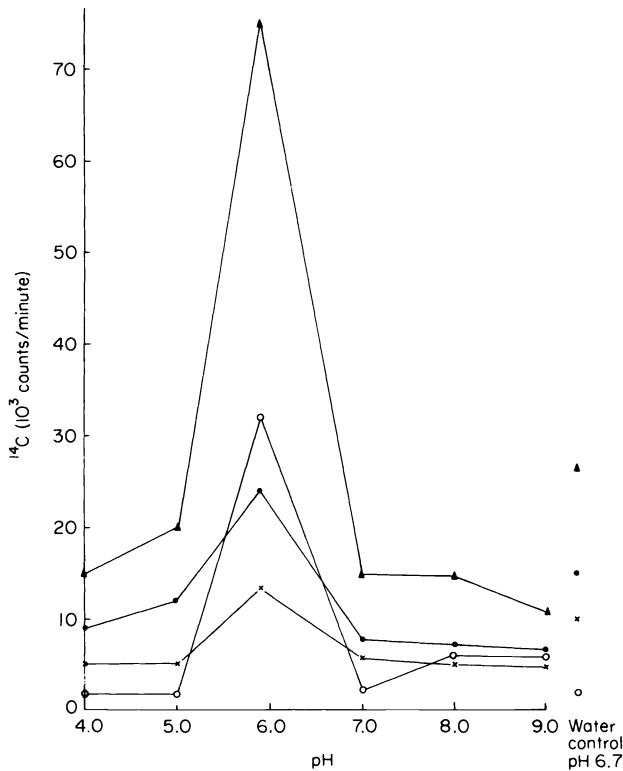


FIG. 1. The effect of pH on the fixation and release of ^{14}C by *Nostoc* from *Peltigera polydactyla* immediately after isolation. Period of photosynthesis 3 hours, temperature 20°C, light intensity 5000 lux, 6.25 μCi of $\text{NaH}^{14}\text{CO}_3$ used per ml of medium. ▲—▲, total ^{14}C fixed; ○—○, ^{14}C in medium; ●—●, ^{14}C in ethanol soluble fraction; ×—×, ^{14}C in ethanol insoluble fraction. (From Green, 1970).

carbohydrate (Fig. 1). However, even at this pH, the ability to release carbohydrate was lost if the algae were aged in distilled water. Thus, factors other than pH must be involved in the release of carbohydrate. Directly isolated cells of *Coccomyxa* did not show a pH optimum for carbohydrate release when they were tested at pH 4.2–9.0.

Green (1970) made further studies on the effect of pH on directly isolated algae of *Xanthoria aureola*. He used the buffer devised by Clark and Lubs (1916) rather than the citrate-phosphate buffers (McIlvaine, 1921) which had been shown to affect the pattern of carbohydrate fixation. Green found that at any of the pH levels he used, very little fixed ^{14}C was released as sucrose in the former buffer system. He compared the directly isolated algae with cells grown in pure culture. The directly isolated algae were treated as follows: (1) allowed to photosynthesize at once on solutions of sodium [^{14}C]bicarbonate buffered at various pH levels; (2) maintained on distilled water for 24 hours before being placed on sodium [^{14}C]bicarbonate buffered to various pH's; (3) placed on buffer at various pH levels at once for 24 hours and then sodium [^{14}C]bicarbonate was added and photosynthesis was studied. The results showed that although net fixation was little affected by the pH of the buffer, the pH did affect the release of ^{14}C by the cells. However, the pure culture of *Trebouxia* released very little ^{14}C under any pH conditions (Table X). It was argued that low pH maintained the ability of the algae to

TABLE X
THE PERCENTAGE OF TOTAL ^{14}C RELEASED IN 3 HOURS BY THE
Trebouxia PHYCBIONT OF *Xanthoria aureola* IN MEDIA BUFFERED TO
VARIOUS pH LEVELS^a

pH	Placed on distilled water for 24 hours and then on buffer	Placed on buffer for 24 hours	Directly isolated photosynthesis at once	Cultured algae
4.0–4.2	17.7	25.0	26.6	1.1
4.9–5.2	6.3	11.7	8.3	1.0
5.8–6.0	3.0	4.5	3.1	1.0
6.3	—	6.7	—	—
7.0–7.2	2.2	—	3.1	1.1
8.0	1.9	—	4.1	1.2
9.0	2.8	—	4.5	0.8
Distilled water	5.7	5.9	3.6	1.1

^aAdapted from Green, 1970.

release carbohydrate but could not induce algae which have lost this ability to regain it. An interesting experiment was done by Green (1970) in which samples of *Xanthoria aureola* were given a pulse of sodium [¹⁴C]bicarbonate in the light for 3 hours and then the algae were isolated directly. The algae were placed on buffer at various pH levels and the release of ¹⁴C was determined over an 18-hour period. At pH 4.2, 32% of the initial pulse was released into the medium and at pH 5.9 very little was released.

In the foregoing experiments where stimulation of carbohydrate release occurred, the carbohydrate was found as one or two substances and not as a spectrum of the labeled compounds which occurred in the ethanol-soluble fraction within the algal cells. Thus, the release was selective and Green suggested that in lichens containing *Trebouxia* there was a pool of ribitol in the cytoplasm which could be lost to the medium. He suggested, however, that the cell organelles contained pools of sucrose, other substances, and possibly a second ribitol pool which were not released to the medium or to the fungus when the alga was in the intact lichen. Finally, although pH levels between 3 and 4 stimulated carbohydrate release in lichens, it seems unlikely that this is the release mechanism in the intact thallus. No information is available about the pH at the algal/fungal interface or within the algal layer of lichens, but Tuominen (1967) did measure the pH of thallus suspensions of *Cladonia alpestris* and found it to be between 3 and 4. However, such a pH within the intact lichen would seem remarkable.

b. THALLUS HOMOGENATES. Green (1970) retained the supernatant produced during centrifugation of thallus homogenates and added it back to the washed, directly isolated, algae. In one experiment there was a significant increase in carbohydrate release by the algae of *Xanthoria aureola*. However, he was unable to verify these results. Water extracts of powdered lichen thalli had no effect. Thus, mechanisms for photosynthate release that are analogous to those found in algal symbionts of animals have not been found in lichens.

c. LICHEN ACIDS AND OTHER SUBSTANCES. Lichen substances have increased the permeability of the protoplasts of *Allium cepa*, *Elodea canadensis*, and *Spirogyra juergensii* (Follmann and Villagrán, 1965) and affected the germination of higher plant seeds (Pyatt, 1967). Lichen acids were not effective in influencing the release of carbohydrate from lichen algae possibly because of their insolubility in water. Atranorin and usnic acid were tested. The latter affected release but it seemed to be due to pH since buffered solutions of usnic acid did not significantly increase the release of carbohydrate. The effects of lichen acids on higher plants may not be relevant since in lichens there is a selective rather than generalized increase in permeability of algal cell. Indeed, lichen acids may play a number of other

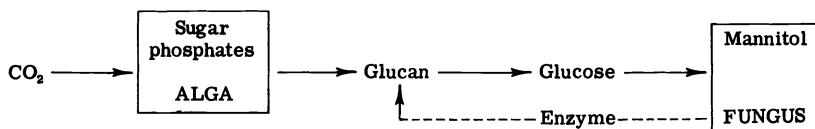
roles in the lichen thallus (Hale, 1967, p. 118; Kinraide and Ahmadjian, 1970).

A number of other substances have been tested and found to be ineffective in influencing the release of photosynthate by algae. These substances including benzylaminopurine, kinetin, coconut milk, and a range of sugars and sugar alcohols (Green, 1970).

d. DILUTION. Many free-living algae release a proportion of photosynthate into the culture medium. For example, Fogg (1966) found that *Chlorella* sp. released glycolic acid into the medium until an equilibrium was established between the internal and external concentration of this substance. Dilution of algal suspension resulted in a further release of glycolic acid until the equilibrium was reestablished. By analogy, Green (1970) wondered whether *Trebouxia* released ribitol until a similar equilibrium was established. He supposed that within the thallus the lichen fungus removed the ribitol and thus maintained a low external concentration and a continuous flow to the fungus. Green investigated this possibility by measuring the proportion of fixed ^{14}C which appeared in the medium at different dilutions of the directly isolated alga from *Xanthoria aureola*. He found that dilution increased the percent of the radioactive photosynthate released from 5 to 21%. If the cells were aged for 24 hours, dilution still increased the amount of fixed ^{14}C that was released but to a lesser extent, i.e., from 0.8 to 9.9%. In the dilute suspensions Green noted that the same amount of radioactive sucrose was released but more radioactive ribitol than in the dense suspensions. However, dilution of pure cultures of *Trebouxia* only produced an increase in photosynthate release of 1.1–1.7%. Thus, other factors must at least initiate the observed release. These results could also be explained in terms of end-product inhibition. Ribitol in sufficient concentration in the medium could inhibit the release mechanisms in the algal cell.

e. POLYSACCHARIDE BREAKDOWN. Hill (1972) recently exposed discs of *Peltigera polydactyla* to the light for 10 minutes in the presence of sodium $[^{14}\text{C}]$ bicarbonate solutions and then studied the redistribution of this pulse in the light on media without radioactivity. He found that within an hour, concomitantly with the rise in ^{14}C in mannitol, there was a fall in the ^{14}C in (1) the insoluble (glucan) fraction, (2) an unknown substance which is probably sugar phosphate, and (3) glucose. Hill further showed that feeding of $[^{14}\text{C}]$ glucose to the algal layer or fungal medulla resulted in $[^{14}\text{C}]$ mannitol being produced by the fungus. Little ^{14}C was found in the sugar phosphate or glucan regions of chromatograms during this experiment. Since it is known that the alga of this lichen provides the fungus with $[^{14}\text{C}]$ glucose, the ^{14}C in sugar phosphate and glucan observed during photosynthesis must be in algal products. Because of these experiments it is suggested that the alga

does not release glucose directly from the cells but forms a glucan outside the plasmalemma. This is then hydrolyzed by an extracellular enzyme produced by the fungus.



The resulting free glucose is rapidly absorbed by the fungus. The principal evidence for this theory is the rapid turnover of ^{14}C in the ethanol-insoluble fraction during transfer of ^{14}C to the fungus. Also, the fact that Drew and Smith (1967a) found that directly isolated *Nostoc* cells, which initially released glucose into the medium, formed a glucose polysaccharide (glucan) after 24 hours and the release of glucose ceased.

In the foregoing section several theories have been advanced to explain carbohydrate release by lichen algae. None of these theories is satisfactory for all lichens and work is needed to determine if there is one mechanism for all lichens or several mechanisms dependent on the type of algal symbiont. The fact that some lichens contain more than one type of alga indicates that there is still value in searching for a single mechanism.

V. Fate of the Transferred Carbohydrate

A. Mannitol

Mannitol is one of the compounds into which the mobile sugar from the alga is converted. Mannitol has been identified in all of the 80 lichens examined by various workers (Lewis and Smith, 1967). Experimental evidence confirming that sugars such as glucose were converted into mannitol was first published by Smith (1963). Disks of *Peltigera polydactyla* incubated on [^{14}C]-glucose for 24 hours incorporated approximately 45–50% of the radioactivity in mannitol. Of the remainder, some 20% was converted into insoluble compounds, 20% was released as $^{14}\text{CO}_2$, and 5% incorporated into a glycoside.

In photosynthetic studies on samples of *Peltigera polydactyla* incubated in the light on sodium [^{14}C]bicarbonate solutions, Drew and Smith (1967b) found that in 45 minutes over 60% of the radioactivity was incorporated into mannitol and about 10% in insoluble compounds. Similar results were obtained with other lichens although the rate of incorporation into mannitol varied, e.g., in *Xanthoria aureola* it took 24 hours of photosynthesis before the

same proportion of radioactivity was found in mannitol. Thus, mannitol can be regarded as one of the principal soluble storage carbohydrates of lichens.

The fate of this product depends on conditions. If the lichen continues to photosynthesize, then some of the mannitol is converted into insoluble compounds. Jacobs and Ahmadjian (1971) investigated the accumulation of these compounds in both the alga and fungus of lichens during photosynthesis. They used a combination of radioautography and electron microscopy to trace the accumulation of radioactivity within the thallus of *Cladonia cristatella*. They found that after 24 hours of photosynthesis in solutions of sodium [¹⁴C]bicarbonate most of the radioactivity was in insoluble compounds within the algal chloroplasts. Radioactivity was indicated by silver grains which were found over starch grains but not within the pyrenoid or over the pyrenoglobuli. This observation suggested that the pyrenoid might not be the center for starch formation as is generally believed. Silver grains were found also in the fungus but were few in number and mainly restricted to the cell-wall area.

If lichens are kept moist in the dark then the mannitol within the fungus is used as a respiratory substrate. Drew (1966) found that in samples of *Peltigera polydactyla* maintained in the dark at 18°C the mannitol content dropped by 40% during the first 48 hours. In another dark experiment where the mannitol pool was labeled with [¹⁴C]mannitol there was a redistribution of ¹⁴C so that approximately 20% was incorporated into ethanol-insoluble compounds, 45% was converted to other soluble substances such as organic and amino acids, 15% was respired, and only 20% of the initial radioactivity remained in mannitol. Similarly, when *Xanthoria aureola* was starved in the dark its mannitol content fell to 50% of its initial concentration after 5 days (Richardson and Smith, 1966).

B. Other Sugar Alcohols

Sugar alcohols other than mannitol or those which have been found to be algal products do occur in lichens. The most common of these is arabitol which was found in 55 of 60 lichens examined (Lindberg *et al.*, 1953). Arabitol is found in *Xanthoria aureola* and its role was suggested by photosynthetic studies on this lichen over 24 hours (Richardson and Smith, 1968a). Samples of lichen were placed on sodium [¹⁴C]bicarbonate solutions for 2 hours. The lichen was then removed, washed, and replaced in the light on distilled water. After intervals of 4–48 hours samples were analyzed. A study of the soluble products (Fig. 2) showed that initially most of the radioactivity was in pentitol but as time progressed the amount of ¹⁴C in mannitol rose. After 48 hours the amount of activity in the soluble fraction declined as the pulse of radioactivity was respiration or converted to ethanol-insoluble compounds. A detailed study of the pentitol area showed that at first this

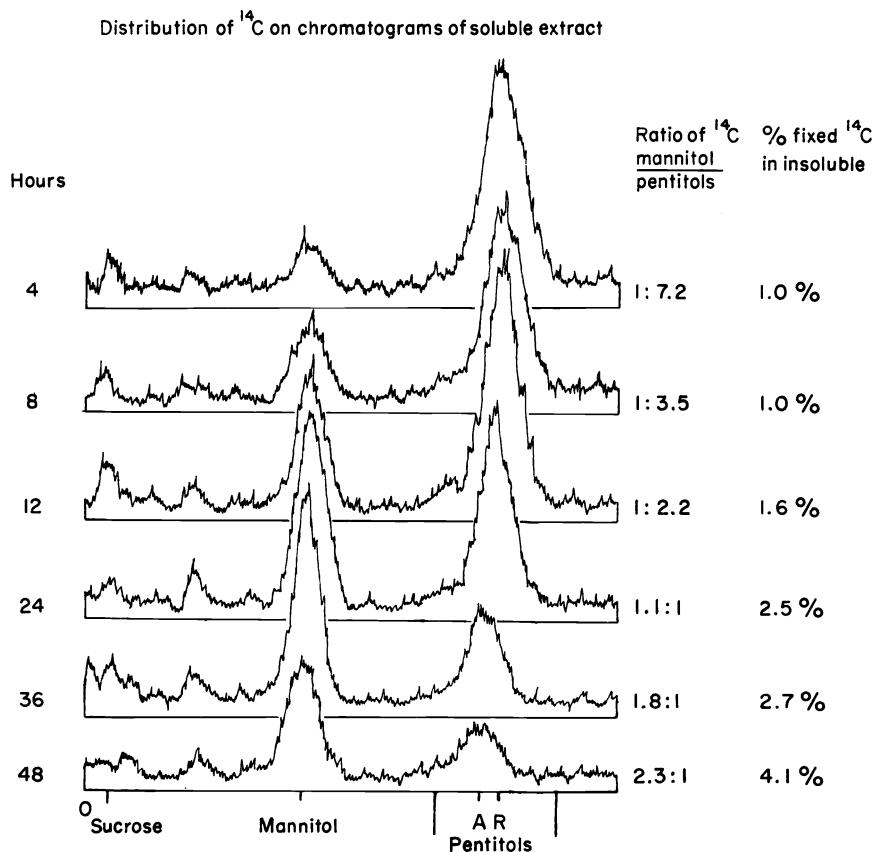
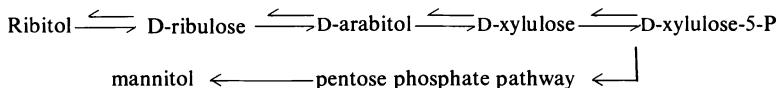


FIG. 2 Chromatogram scans illustrating redistribution of a pulse of fixed ^{14}C in the soluble fraction of *Xanthoria aureola* during 48 hours photosynthesis on distilled water. O, Origin; A, arabitol; R, ribitol. Sample size, 200 mg fresh weight; incubated on 3 ml distilled water at 18°C; light intensity 5000 lux. Preliminary incubation of each sample before start of experiment for 2 hours in 20 μCi $\text{NaH}^{14}\text{CO}_3$ (carrier-free). Solvent system, ethylmethyl ketone: acetic acid: water saturated with boric acid (9:1:1). (From Richardson and Smith, 1968a.)

radioactive peak was composed almost exclusively of [^{14}C] ribitol (the main photosynthetic product of the alga *Trebouxia*). As time elapsed both radioactive ribitol and arabitol were found. After 48 hours the pentitol peak consisted principally of [^{14}C] arabitol. Arabitol may be formed in the fungus during conversion of ribitol to mannitol probably by a pathway similar to that suggested by Lewis and Smith (1967). This has not been confirmed.



Since some 90% of lichens contain ribitol-synthesizing algae, the common occurrence of arabitol is not surprising if the above pathway is operative.

Volemitol has been found in *Dermatocarpon* spp. and *Endocarpon adscendens* (Lindberg *et al.*, 1953) but the exact role of this sugar alcohol in the physiology of these lichens is unknown.

The carbohydrate transferred to the fungus by the lichen alga, at least in part, ends up as structural components of the growing thallus. This is principally in the form of the glucose polymers lichenin and isolichen (Hale, 1967) and some protein. No experimental studies have been done on the rate of production of these components. The importance of the sugar alcohols may, however, be indicated by the fact that these substances accumulate in the thallus. Mannitol was found to comprise approximately 2.5–4% of the dry weight of *Peltigera polydactyla* in England (Hill, 1972) and Pueyo (1960) in France found that the polyol content of eleven species varied from 1.7 to 4.9%. This contrasts with the fact that free sugars which are important in higher plants are only found in minute amounts in lichens (Solberg, 1970).

VI. Carbohydrate Movement between the Symbionts

A. Amount of Movement

A question which remains to be answered is "How much carbohydrate does the fungus receive from the alga to account for the observed thallus growth rates of approximately 2–0.2 cm radial increase per year?" Hill and Smith (1972), using 2-deoxyglucose to inhibit carbohydrate transfer in *Peltigera polydactyla* during photosynthesis on sodium [^{14}C] bicarbonate, have estimated (using gas chromatography which is able to separate the [^{14}C] glucose produced by the alga from the 2-deoxyglucose used for inhibition) that the amount of [^{14}C] glucose available to the fungus is 0.7–1.1 mg/gm dry weight of thallus per hour. A study of the rate of accumulation of $^{14}\text{CO}_2$ into mannitol under as nearly natural conditions as possible would also provide information on the amount of carbohydrate passed between the symbionts. Thus, samples could be exposed to $^{14}\text{CO}_2$ and the amount of radioactivity in mannitol plus the absolute amount of mannitol could then be determined. By assaying samples over a time period one could determine the change in specific activity and also the amount of photosynthate being converted into this soluble storage carbohydrate. The same might be done to the ethanol-extracted residue of the sample when hydrolyzed. Thus, it should be possible to follow quantitatively the incorporation of radioactivity into glucose polymers such as lichenin. The dissection technique could be used to partially distinguish incorporation into fungal as opposed to algal insoluble products.

B. Rates of Movement

The rate of movement between lichen symbionts is still not fully determined. Carbohydrate moves from the alga to the medulla region of the thallus at different rates in various lichens (Table III). However, the rate at which carbohydrates move from the algal cells to the fungal hyphae immediately adjacent are not known. Some indication of this rate can be obtained during inhibition experiments where lichens are given a short pulse of sodium [^{14}C] bicarbonate in the light before being washed and placed on a solution of an appropriate [^{12}C] sugar. The proportion of the pulse which is released into the medium in 24 hours may indicate the rate of movement of the pulse out of the alga (uptake by the fungus being inhibited by the [^{12}C] sugar). Experiments with different lichens showed that there was a considerable variation in the proportion of a pulse released into the medium over a fixed period (Table XI). However, the interpretation of these results may be complicated by the varying pool size of the mobile carbohydrate.

Another way in which the movement of carbohydrate from alga to fungus may be followed is by a further modification of the inhibition experiment technique (Richardson *et al.*, 1968). Samples of each lichen were given preliminary short exposure to $\text{NaH}^{14}\text{CO}_3$ in the light. They were then washed with distilled water and allowed to resume photosynthesis on dis-

TABLE XI
THE PROPORTION OF PULSE OF ^{14}C RELEASED DURING "INHIBITION" IN
11 SPECIES OF LICHEN^a

Lichen	Algae	Carbohydrate released	Proportion of pulse released (%)	Time allowed for ^{14}C release (in hours)
<i>Porina lectissima</i>	<i>Phycopeltis</i>	Erythritol	31	24
<i>Roccella montagnei</i>	<i>Trentepohlia</i>	Erythritol	9	48
<i>Coccocarpia</i> sp.	<i>Scytonema</i>	Glucose	30	24
<i>Peltigera polydactyla</i>	<i>Nostoc</i>	Glucose	33	3-4
<i>Dermatocarpon hepaticum</i>	<i>Myrmecia</i>	Ribitol	26	19
<i>Lepraria chlorina</i>	<i>Trebouxia</i>	Ribitol	61	24
<i>Lepraria incana</i>	<i>Trebouxia</i>	Ribitol	40	10
<i>Peltigera aphthosa</i>	<i>Coccomyxa</i>	Ribitol	65	24
<i>Sphaerophorus globosus</i>	<i>Trebouxia</i>	Ribitol	57	24
<i>Xanthoria aureola</i>	<i>Trebouxia</i>	Ribitol	40	24
<i>Verrucaria hydrella</i>	<i>Hyalococcus</i>	Sorbitol	68	24

^aFrom Hill (1970).

tilled water without radioactive bicarbonate. At increasing time intervals samples were then transferred to 1% solutions of either glucose (for *Peltigera polydactyla*) or ribitol (for *Xanthoria aureola*) and replaced in the light. It was assumed that the residual ^{14}C available for transfer would then diffuse out of the tissues. The total ^{14}C available for transfer was assumed to be equivalent to the amount released by lichens transferred directly from [^{14}C] bicarbonate to [^{12}C] carbohydrate solutions. The amount of ^{14}C moving from alga to fungus during a particular time on distilled water will be equivalent to the total amount available for transfer minus the amount released subsequently in [^{12}C] carbohydrate. From these measurements it was possible to estimate the rate at which an initial pulse moved between the symbionts. It appears that the pulse of fixed ^{14}C passes more rapidly between the symbionts of *Peltigera polydactyla* than between those of *Xanthoria aureola* (Fig. 3). Under this experimental procedure the rate of ^{14}C movement is related both to the overall rate of carbohydrate movement and to the size of the pool of mobile carbohydrate that has to be labeled. Under identical conditions, the pulse of ^{14}C moved about twelve times more quickly between the symbionts in *Peltigera polydactyla* (containing *Nostoc*) than in *Xanthoria aureola* (contain-

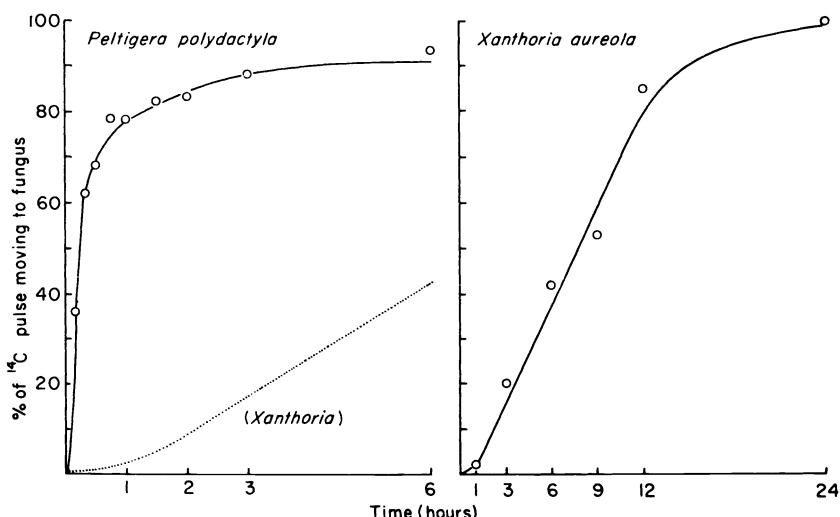


FIG. 3. Comparison of the rate of transfer of a pulse of ^{14}C from alga to fungus in *Xanthoria aureola* and *Peltigera polydactyla* during photosynthesis. Sample size, 100 mg fresh weight for *Xanthoria*, ten disks (7 mm diameter) for *Peltigera*. All material incubated at 18°C at 5000 lux light intensity on 3 ml media per sample. Preliminary exposure to $\text{NaH}^{14}\text{CO}_3$ (10 μCi carrier-free per sample), 10 minutes for *Peltigera*, 2 hours for *Xanthoria*. Percentage of total fixed ^{14}C subsequently moving to fungus in 24 hours, 41% for *Peltigera*; 23% for *Xanthoria*. (From Richardson *et al.*, 1968.)

ing *Trebouxia*). However, the pool of glucose in *Peltigera polydactyla* is very small, amounting to 0.01 mg/gm dry weight, and is scarcely detectable in the thallus (Hill, 1972); by contrast, ribitol may comprise up to 1% of the dry weight of *Xanthoria aureola* which means that the pool of ribitol may be large and take a long time to saturate with ^{14}C in photosynthesis experiments.

This experiment needs to be repeated with the modification that the lichens are first allowed to photosynthesize for a period of a few hours on distilled water without added ^{14}C . This would allow the various carbohydrate pools to fill and attain equilibrium with one another. A pulse of $^{14}\text{CO}_2$ would then be given and followed by the inhibition procedure outlined above. If this were done then the rates of ^{14}C movement observed should be related to the rate of total carbohydrate movement and not depend on the pool size of the mobile sugar produced by the alga. If the specific activity of the released carbohydrate were measured then the amount of carbohydrate transferred could also be determined.

C. Factors Affecting Carbohydrate Transfer

Very little is known about the factors which affect the rate or amount of carbohydrate movement between lichen symbionts. A considerable amount of work could be done in this area using the techniques outlined in Section III.

1. LIGHT

Although some carbohydrate movement occurs in the dark, it is much more rapid in the light (Smith, 1961; Smith and Drew, 1965). Samples of *Xanthoria aureola* and *Peltigera polydactyla* when given a pulse of $^{14}\text{CO}_2$ in the light and then subjected to the inhibition technique showed a rate of [^{14}C]-carbohydrate movement in the dark which was only approximately 10% of that in the light (D. J. Hill, unpublished).

2. pH

This can influence movement by affecting both the rate of release from the algal cells (see Section III) and fungal uptake. For example, in *Xanthoria aureola* low pH stimulated algal release but partly inhibited fungal uptake so that carbohydrate was released from the tissues into the incubating medium. At high pH release from the algal cells seemed to be reduced although fungal uptake appeared normal (Richardson, 1967). In *Peltigera polydactyla* a marked reduction in carbohydrate uptake was noted at low pH (Harley and Smith, 1956). Movement of carbohydrates in lichens therefore seems to be reduced at extremes of pH.

An interesting observation is that lichen algae when directly isolated from the thallus show a sharp reduction in fixation at low pH (Richardson, 1967). However, intact lichens, especially those growing in areas adjacent to industrial areas, e.g., *Cladonia deformis* and *Stereocaulon paschale*, show little or no reduction in fixation when incubated on media buffered to pH 3 and 4 as compared with distilled controls (K. J. Puckett, unpublished). It is possible that these lichen fungi may moderate the effects of low pH. It may be significant that Harley and Smith (1956) and D. J. Hill (unpublished) noted that when samples of *Peltigera polydactyla* and *Xanthoria aureola* were incubated in weak buffer solutions they tended to change the pH of the medium towards the region 5–6 and not to more acid levels.

3. THE SYMBIOTNS

One problem is to decide which symbiont controls the rate and amount of carbohydrate movement. The type of mobile carbohydrate depends on the genus of alga within the thallus (Table XI). There is also an obvious correlation between the rate of movement (as measured by inhibition experiments) and the type of alga present in a particular lichen.

In a series of experiments, Richardson *et al.* (1967) showed that it was the alga rather than the fungus which controlled the rate of ^{14}C movement between the symbionts. The authors examined several species of *Lobaria*. In this genus the fungal components of the lichens are closely related but the algal symbionts are different. *Lobaria scrobiculata* contained *Nostoc*, *Lobaria laetivirens* contained the green alga *Myrmecia*, and *Lobaria amplissima* had a thallus containing *Myrmecia* and upright-growing cephalodia containing *Nostoc*. The cephalodia could be excised and studied separately.

Experiments carried out on samples of these lichens gave the following results. With the thallus of *Lobaria scrobiculata* and the cephalodia of *Lobaria amplissima*, inhibition could be affected using $[^{12}\text{C}]$ glucose in the medium. With this sugar present, $[^{14}\text{C}]$ glucose was released from the samples in considerable amounts after 3 hours and little $[^{14}\text{C}]$ mannitol was found within the thallus or cephalodia. The presence of ribitol in the medium had no detectable influence except in the case of the cephalodia of *Lobaria amplissima* where there was a slightly increased release of ^{14}C probably due to attached fragments of thallus that contained *Myrmecia* (Table XII).

In *Lobaria laetivirens* and the thallus of *Lobaria amplissima* inhibition was brought about only by the presence of $[^{14}\text{C}]$ ribitol in the medium and was substantial only after 24 hours. $[^{14}\text{C}]$ glucose had no effect of ^{14}C release into the medium although a reduction in total ^{14}C fixation was noted. The rate at which inhibition could be affected in these lichens was also reflected in the rate at which ^{14}C moved from the algal layer to the medulla as measured by

TABLE XII
RELEASE OF PHOTOSYNTHETICALLY FIXED ^{14}C TO MEDIUM BY VARIOUS LICHENS IN
THE PRESENCE OF [^{12}C] GLUCOSE AND [^{12}C] RIBITOL (RESULTS EXPRESSED AS PERCENT
IN MEDIUM OF TOTAL SOLUBLE FIXED ^{14}C)^a

Lichen: Contained alga:	<i>Lobaria</i> <i>laetivirens</i> <i>Mymecia</i>	<i>Lobaria</i> <i>amplissima</i> (thallus) <i>Myrmecia</i>	<i>Lobaria</i> <i>amplissima</i> (cephalodia) <i>Nostoc</i>	<i>Lobaria</i> <i>scrobiculata</i> <i>Nostoc</i>
3 hours, 18°C				
Water	0.2	1.6	2.3	0.5
Glucose ^b	0.2	0.6	22.6	21.3
Ribitol ^b	1.7	1.7	1.3	0.6
24 hours, 18°C				
Water	0.2	0.9	2.3	0.7
Glucose ^b	1.1	4.2	56.0	56.8
Ribitol ^b	45.1	39.1	15.3	1.4

^aAll material as samples of 5 × 7 mm thallus disks except *Lobaria amplissima* thalli (5 × 5 mm disks with no cephalodia) and cephalodia (200 mg fresh weight). All samples incubated in shaker bath on 3.05 ml distilled water with 5 μCi carrier-free $\text{NaH}^{14}\text{CO}_3$ in 2 × 1 inch specimen tubes covered with 1-inch diameter cover glasses sealed on with petroleum jelly. Light intensity 5000 lux; controls showed dark fixation to be not greater than 5% light fixation, and no significant release of fixed ^{14}C from lichen material in any medium in the dark.

^bGlucose and ribitol as 1% (w/v) aqueous solutions.

dissection experiments. In *Lobaria scrobiculata* (containing *Nostoc*) some 40% of the fixed ^{14}C moved to the medulla in 3 hours. In the thallus of *Lobaria amplissima* (containing *Myrmecia*) only 2.9% moved in this time. Therefore, the rate of ^{14}C movement depended on the alga and not the fungus. This was exemplified by the situation in *Lobaria amplissima* where the same fungal component is symbiotic with two different algae. These experiments, however, only examined the rate of movement. Hence, although the speed at which the fungus receives ^{14}C is dependent on the type of alga, the absolute amounts of carbohydrate which pass to the fungus over a long period may be similar.

VII. Conclusions

In nearly all studies on photosynthetic rates of lichens, the results are expressed in the same way as those from photosynthetic studies on higher plants. Thus, net photosynthesis has been determined under various conditions. While this enables a comparison with other groups of plants it does not elucidate the effect of such conditions on the two symbionts which constitute the intact lichen. If the true photosynthetic rate were calculated by taking

fungal respiration into account, then it would be possible to explain variations in net photosynthesis or compensation points in terms of either reduced photosynthesis by the alga or increased respiration by the fungus. When the relationship between these parameters and moisture content or temperature has been determined, it will be possible to assess the likely effect of a set of naturally occurring conditions on a particular lichen. At the moment this basic information is not available but such studies could be conducted even in large cities using growth chambers fitted with a simple air-filtration system. Samples of lichen can be kept and grown for several months in this sort of apparatus (Kershaw and Millbank, 1969; Dibben, 1971). It is now evident that fluctuating conditions are necessary for the growth and maintenance of lichens in the laboratory. When the optimum conditions are determined for a particular species, it will enable the preparation of lichen material with as little physiological variability as is found in higher plant material used in photosynthetic studies (see Harris and Kershaw, 1971).

Particular attention should be directed to measuring the microclimate in physiological studies on lichens. Lichenologists appreciate the importance of this in the field but rarely is critical monitoring of experimental chambers of cuvettes done in the laboratory. Thermocouples placed inside thalli or incubating solutions often show that conditions in the lichen are significantly different from those recorded by temperature sensors in the growth chamber or water bath. Insufficient data are usually collected on the light quality of sources employed to illuminate apparatus for photosynthetic studies. Light meters, calibrated in calories per square centimeter, which measure red, far red, and blue portions of the spectrum separately are now available.

The effects of low temperature on net photosynthesis by lichens has been studied extensively and the results show that these plants are remarkably adapted for living in cold conditions. Although there is an equally rich tropical lichen flora, it is not known whether these lichen are specially adapted to photosynthesize at high temperatures. Studies of tropical lichens would be especially valuable since many of them contain *Trentepohlia* and little is known about the physiology of lichens that contain this alga.

Much of the foregoing chapter has been concerned with carbohydrate transfer in lichens. Most of the work has been done in D. C. Smith's laboratory at Oxford. The techniques which have been developed are not difficult and it is hoped that carbohydrate transfer will be studied in many lichen genera in different continents. In most studies standard conditions have been applied but detailed investigations on the effects of different temperatures, light regimes, and moisture content on the rate of carbohydrate transfer in lichens from particular geographical areas would provide interesting information.

The difficult problem as to what causes the alga to release carbohydrate to the fungus has been the subject of much study. It is still not certain whether the role of the fungus is active or passive. It is possible that the fungus (1) maintains a low pH around the alga whose membranes are more permeable under these conditions, (2) produces an enzyme hydrolyzing products which would normally form part of the algal wall, (3) absorbs photosynthate which would be excreted until a particular external concentration had been developed, (4) produces a factor or enzyme causing a direct increase in algal permeability. None of these suggestions have been fully confirmed by experimental findings. D. C. Smith (personal communication) has recently suggested that movement out of the alga may be induced simply by physical contact with the fungus and has suggested the following theoretical model to explain how this might occur.

In the nonsymbiotic condition, the general direction of membrane transport of neutral or weakly ionized molecules is inwards. In symbiosis, physical contact results in either the elimination of this tendency to inward movement or even a positive reversal of direction to produce an outflow from the cell. It is assumed also that in the nonsymbiotic condition the feature which determines the generally inward direction of molecule transport is the membrane potential which operates in a manner analogous to that proposed to explain ion transport (see Robertson, 1968). The maintenance of membrane potential by electron and/or proton flow is necessary for molecule transport across the membrane.

Fungi in general have much higher rates of uptake of molecules such as glucose than algae. This higher rate of uptake would be reflected in a higher membrane potential (e.g., the membrane potential of *Neurospora crassa* has been measured at 200 mV, and *Chlorella* at 40 mV). If the fungal membrane was placed in direct contact with the algal membrane, the potential of the latter might be eliminated or even reversed. This would result in an outflow from the alga. Of course in actual practice the membranes are separated by cell walls. For the model to operate it would be necessary for the charge to be conducted across the cell walls between the membranes. Although such conduction might occur by a flow of charged particles across this space, it is proposed that there is a definite physical contact between the membranes possibly in the form of protein or glycoprotein bridges. Such connections have never been seen but appropriate techniques have not been applied to show them in electron microscopic studies. Fungi and algae have an appreciable protein content in their walls, usually about 7–15%. The existence of these connections is postulated to explain (a) the relative insensitivity to medium pH of movement between the symbionts in the intact thallus, and (b) the absence of any movement for a prolonged period during the initial stages of synthesis from isolated symbionts in laboratory experiments (Hill and

Ahmadjian, 1972), a period when such protein connections were being slowly established.

This theoretical model is now being studied experimentally as there are areas of particularly close association between the fungus and alga in lichens. In most foliose and fruticose forms the fungus develops an appressorium on the algal cell wall but does not seem to penetrate further. In many crustose lichens, at least a proportion of cells have haustoria which invaginate the algal membrane. These might be areas where protein or glycoprotein connections are numerous. However, not all lichens show the structures described above, e.g., lichens containing blue-green algae. In general, lichen thalli kept in the dark or growing in unfavorable situations contain a greater proportion of algae with haustoria; indeed, the fungus under conditions of starvation may attempt to induce maximum flow of metabolites via an increased number of close contact areas.

In most lichens the transferred carbohydrate is rapidly converted to mannitol. This presumably maintains a concentration gradient facilitating the outflow of the mobile carbohydrate. The fungus benefits further by this conversion. Under fluctuating conditions, if the hypothetical model proposed above operates, a reversal in direction of flow to the alga could occur for a period, but if the alga were not able to utilize the polyol the fungus would not be at disadvantage. Such flow reversals could be the time when symbiotic algae obtain vitamins or growth factors necessary for growth and this might explain why fluctuating conditions are necessary for the health of both symbionts in a thallus.

Sugar alcohols have other roles in plants. They form valuable storage products as they contain more reducing power than the corresponding hexoses. They can function also in coenzyme regulation, the reversible synthesis of polyols to sugars being coupled with coenzyme oxidoreduction.



Finally, these compounds may function as osmoregulators as they frequently accumulate in large amounts in plants subject to desiccation or living in environments of high osmotic pressure. Polyols are nonpolar and relatively inert and so may be preferable to other substances such as hexoses. The various roles of polyols in plants and symbiotic associations are discussed in detail by Lewis and Smith (1967) and Smith *et al.* (1969). In lichens experiments have yet to show whether sugar alcohols have all these functions. In particular, the biochemistry and enzymology of the glucose to polyol conversion, or polyol to polyol conversion have yet to be examined. The final fate of much of the transferred carbohydrate in lichens is as protein and polysaccharides such as lichenin. These form the structural com-

ponents of the thallus but here again virtually no experimental work has been published.

In summary, lichens have proved interesting in every aspect of their physiology that has been studied. The large number of gaps in the present state of knowledge of just one aspect of lichenology is evident. It is hoped that biochemists, enzymologists, and physiologists will be enticed to study these plants, which have no obvious economic significance but which are part of any natural plant community and are of increasing importance in conservation and pollution studies.

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Chapter 9

NITROGEN METABOLISM

J. W. MILLBANK AND K. A. KERSHAW

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I. Introduction

Metabolic studies of the intact thallus have been largely neglected by biologists probably because of the supposed biological insignificance of lichens as well as the belief that they are difficult to use experimentally and that reliable and consistent results are hard to obtain. Nitrogen metabolism has suffered particularly because of the absence of a suitable radioactive isotope. The combination of slow growth (and, therefore, presumed sluggish metabolism) with the cumbersome and not very sensitive heavy isotope technique was a particularly discouraging situation. It now seems that lichens have, in some cases, surprisingly active metabolic processes and outstanding powers of recovery after periods of severe environmental stress. Much recent work on nitrogen metabolism has resulted from the availability of the acetylene reduction technique for nitrogenase assay, a much more straightforward procedure. Only those features of nitrogen metabolism peculiar or relevant to the lichen mode of life will be considered in this chapter. This will comprise the absorption and metabolism of nitrogen

by the intact thallus, together with aspects of the physiology of the isolated symbionts where they are important in an understanding of the events in the thallus.

II. Chemical and Biochemical Analyses of Whole Thalli

A. Total Nitrogen Content

Those reports of thallus total nitrogen which are available are summarized in Table I. Lichens with a blue-green phycobiont have significantly higher

TABLE I
THE TOTAL NITROGEN CONTENT OF LICHEN THALLUS

Lichen	Nitrogen content (% dry weight)	Reference
<i>Lichina confinis</i> ^a	3.6–3.9	Hitch (1971)
<i>Lobaria laetevirens</i> ^a	2.2	Goas and Bernard (1967)
<i>Lobaria pulmonaria</i> ^{a,b}	2.7	Goas and Bernard (1967)
<i>Peltigera aphthosa</i> var. <i>leucophlebia</i> ^{ac}	1.9–3.4	Hitch (1971)
<i>Peltigera aphthosa</i> var. <i>leucophlebia</i> ^{a,c}	3.0	Millbank and Kershaw (1969)
<i>Peltigera canina</i> ^a	3.3	Millbank (1972)
<i>Peltigera polydactyla</i> ^a	3.6–4.5	Smith (1960a)
<i>Peltigera praetextata</i> ^a	4.7	Scott (1956)
<i>Placopsis gelida</i> ^a	0.9–1.3	Hitch (1971)
<i>Sticta sylvatica</i> ^a	4.0	Goas and Bernard (1967)
<i>Anaptychia fusca</i>	0.90	Hitch (1971)
<i>Candelariella corallizza</i>	4.2–5.0	Massé (1966)
<i>Cladonia foliacea</i>	0.65	Hitch (1971)
<i>Cladonia impexa</i>	0.33	Hitch (1971)
<i>Cornicularia aculeata</i>	0.38	Hitch (1971)
<i>Evernia prunastri</i>	0.84	Hitch (1971)
<i>Lecanora atra</i>	0.69	Hitch (1971)
<i>Lecanora muralis</i>	6.2–9.24	Massé (1966)
<i>Ochrolechia parella</i>	0.61–0.70	Hitch (1971)
<i>Parmelia physodes</i>	0.49	Hitch (1971)
<i>Parmelia sulcata</i>	0.96	Hitch (1971)
<i>Physcia ascendens</i>	1.0–1.3	Hitch (1971)
<i>Physcia tribacia</i>	3.9–4.7	Massé (1966)
<i>Ramalina siliquosa</i>	0.93	Hitch (1971)
<i>Usnea subfloridana</i>	0.58	Hitch (1971)
<i>Xanthoria candelaria</i>	4.2–4.4	Massé (1966)
<i>Xanthoria parietina</i>	1.21–1.71	Hitch (1971)

^aPhycobiont a member of the Cyanophyceae.

^bPrimary phycobiont *Myrmecia*, *Nostoc* in cephalodia.

^cPrimary phycobiont *Coccomyxa*, *Nostoc* in cephalodia.

nitrogen contents than lichens with green phycobionts. Exceptions are those individuals that grow on substrata rich in nitrogen, e.g., *Candelariella coralliza* (Massé, 1966). This is not surprising, but the supposition that these higher nitrogen contents have a direct bearing on the growth rate and thallus size must be viewed with caution. The fact that *Peltigera aphthosa* benefits greatly from a supply of combined nitrogen (Kershaw and Millbank, 1970) is a case in point. Similarly, the large size of some *Parmelias* (e.g., *Amphigymnia*) and *Umbilicarias*, which do not have blue-green phycobionts, points to a more complex control of growth rate.

B. Intracellular Nitrogenous Compounds

Goas and Bernard (1967) analyzed the soluble nitrogen fraction of *Sticta sylvatica*, *Lobaria laetevirens*, and *L. pulmonaria*. Glutamic acid was by far the most abundant amino acid found in all cases. The authors later reported the presence of glutamic decarboxylase and three transaminases concerned with the metabolism of glutamic acid (Bernard, 1969; Bernard and Goas, 1969). Glutamic-oxaloacetic transaminase (GOT) was the most active in all cases. Optimal conditions for activity of all the enzymes were established and GOT was assayed in five lichens, i.e., the three mentioned above plus *Sticta fulginosa* and *S. limbata*. Ramakrishnan and Subramanian (1964a,b, 1965, 1966a,b) have made qualitative investigations of the amino acids in the soluble nitrogen fraction and hydrolysates of the following lichens: *Cladonia rangiformis*, *C. gracilis*, *Dermatocarpon moulinii*, *Lobaria isidiosa*, *L. subisidiosa*, *Parmelia nepalensis*, *P. tinctorum*, *Peltigera canina*, *Ramalina sinensis*, *Roccella montagnei*, *Umbilicaria pustulata*, *Usnea flexilis*, *U. orientalis*, and *U. venosa*. Fabian-Galan *et al.* (1966) reported the synthesis of several amino acids during photosynthesis in species of *Cladonia*, *Collema*, and *Parmelia*.

Methylamines were found to be abundant in the thallus of *Lobaria laetevirens* (Bernard and Goas, 1968), and Bernard and Larher (1971) demonstrated the important position of glycine in the biosynthesis of mono-, di-, and trimethylamine by this lichen. These authors have proposed a metabolic scheme that summarizes the interrelationships of glycine, the methylamines, sarcosine, and choline and studies are in progress to confirm their proposal.

III. Assimilation of Combined Nitrogen

Most lichens live in barren habitats where their nutrient supply is poor. Thus, absorption from any solution in contact with the thallus is an important aspect of their physiology and the rates observed are extremely rapid. This is reflected in the high rates of accumulation of radioactive material from atmospheric "fallout" (Gorham, 1959) and equally in that lichens are particularly sensitive indicators of toxic atmospheric pollutants

(Hawksworth and Rose, 1970). Ahmadjian (1966) summarized the sparse early work which indicated that due to seasonal low rates of photosynthesis, the organic nutrient requirements of the thallus are quite unable to be met by the phycobiont, and suggested that the deficiency had to be met by direct absorption from the substratum or from liquids in contact with the thallus surface. While this may well be true in many instances, recent work by Lange, Schulze, and Koch (1970) has shown that *Ramalina maciformis* can achieve a net annual gain in assimilated carbon sufficient for a thallus growth of 5–10%.

In certain instances organic nitrogen derived from the older decaying parts of the thallus may be mineralized by epiphytic microorganisms and the derived ammonium ions absorbed by the remainder of the thallus, giving rise to a circulation of nitrogen (Shields *et al.*, 1957). This concept is supported by the observation that some lichens secrete extracellular enzymes that act upon nitrogenous components of their substrate (Galinou, 1956; Moissejeva, 1961). Of the enzymes reported, asparaginase, allantoicase, allantoinase, "lichenase," and uricase all have nitrogenous substrates. The above authors also reported the secretion of enzymes with polysaccharide or other carbohydrate substrates that could promote the availability of nitrogenous components of decaying plant and animal tissue.

Massé investigated the total nitrogen content (1966) and uricase activity (1969) in several lichens, including species that colonize habitats rich in bird excrement. Uricase was not found in the four lichens with blue-green phycobionts and the activity was variable in the others. There was not a clear pattern of activity between "ornithocoprophiles" and "ornithocoprophobes," but *Candelariella*, a characteristic coprophile, had a very active uricase. The level of uricase among the coprophobes was generally feeble or zero, but there were exceptions. Ornithocoprophilic lichens with green alga phycobionts had exceptionally high nitrogen contents (Table I), presumably because of the high N content of the substrate.

Recent investigations on the uptake and assimilation of combined nitrogen are reported by Smith (1960a,b) using disks of *Peltigera polydactyla*. The technique used was to analyze 7-mm disks of thallus for nitrate, nitrite, ammonia, amide, α -amino and total nitrogen. Many disks (up to 450) were used in each experiment and this reduced considerably any metabolic variation that would occur among different thallus specimens. Soluble nitrogen formed about a quarter of the total and of this soluble fraction about 25% was ammonia, 25% α -amino, 3–5% amide, and the remainder was uncharacterized. The amounts of nitrate and nitrite nitrogen were negligible.

When the disks were shaken in ammonium chloride solution, with and without glucose, some striking effects were observed (Fig. 1). Without glucose, absorption virtually ceased after 12 hours; but in the presence of

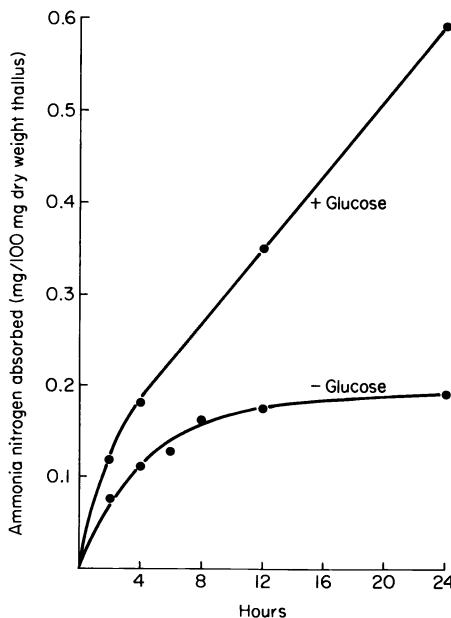


FIG. 1. Ammonia absorption by *Peltigera polydactyla* thallus in 5 mM NH_4Cl with and without 14 mM glucose. (After Smith, 1960a.)

glucose the enhanced rate of uptake continued throughout the experimental period. The absorbed nitrogen produced large increases in the ammonia and amino nitrogen content of the disks, and in the presence of glucose the increases were about threefold greater. There was no significant change in the amide fraction and amides did not seem to be compounds of metabolic importance. The difference between these results and the effects of ammonia uptake on free-living fungi was particularly notable. Thus, there was almost no shift of nitrogen from the soluble to the insoluble fraction in *Peltigera*, even in the presence of glucose, and evidently protein synthesis was not stimulated. About 90% of the ammonia uptake was accounted for in the soluble fractions, even in the presence of glucose. Protein synthesis was apparently limited by factors other than carbohydrate supply.

When disks were incubated in water or buffer without nitrogen for 6 days, the soluble nitrogen fraction of the thallus increased slightly, equivalent to about 5–10% of the total protein nitrogen. Protein breakdown seemed minimal, unlike what is observed in higher plants. The presence of glucose in the medium reduced the level of thallus ammonia nitrogen, indicating a promotion of ammonia utilization. Release of ammonia to the medium was never observed with or without the presence of glucose in the medium.

The second part of Smith's investigation (1960b) dealt with the uptake and utilization of the acid amides asparagine and glutamine and the corresponding amino acids, aspartic and glutamic. This study revealed that asparagine was absorbed more rapidly and in larger amounts than the other substances, which is difficult to explain since the earlier study found that acid amides apparently were of only slight importance in the nitrogen metabolism of the disks.

The absorption from asparagine solutions by disks of *Peltigera* over 24 hours is shown in Fig. 2. Absorption was rapid and ranged from 15 to 22.5 $\mu\text{g N/mg dry weight thallus}/24$ hours; ammonia was released into the medium, and it may be assumed that asparagine entered the disks as the complete molecule since both amide and amino nitrogen diminished equally. The evidence for this assumption was a trial in which equimolar mixtures of aspartic acid and ammonia were assimilated much more slowly than an equivalent amount of asparagine. The asparagine appeared to be stored upon absorption, since large increases in amide nitrogen, roughly equivalent to the losses from solution, were demonstrated. Analyses after absorption showed that the rate of assimilation was very slow and at least 50%

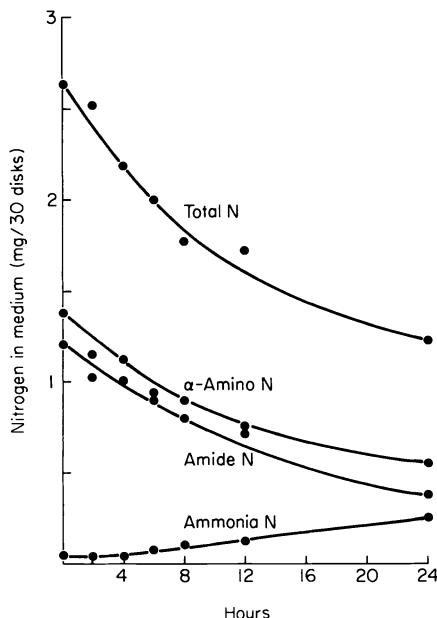


FIG. 2. Changes in the nitrogen fractions of 5 mM asparagine solutions during absorption by disks of *Peltigera polydactyla* thallus at 20°C. (After Smith, 1960b.)

of the amide nitrogen originally absorbed still remained after 72 hours. Synthesis of complex soluble or insoluble compounds during this period was negligible. The only apparent conversion that occurred was deamidation to ammonia; some deamination may also have taken place but this was not supported by experimental evidence. After absorption the asparagine could not be washed out. Smith considered that absorption was an active process since it was inhibited by 25 mM sodium fluoride and accompanied by an increase in the respiration rate approximately equal to that caused by an equivalent number of glucose molecules.

Glucose inhibited the absorption of asparagine, an effect that is opposite to that found in free-living fungi. When free-living fungi were incubated in asparagine media without glucose, deamidation was promoted, i.e., amide nitrogen rapidly diminished, and large amounts of ammonia were released. The amino nitrogen diminished slowly. The addition of glucose to the solution increased the rate of utilization of both amino nitrogen and the released ammonia. Thus, the effect of asparagine plus glucose caused changes in the nitrogen fractions of free-living fungi similar to those brought about in *Peltigera* by asparagine in the absence of glucose. These enigmatic findings, together with the observations that not only was asparagine absorption depressed in the presence of glucose but glucose absorption was depressed in the presence of asparagine, may indicate two uptake mechanisms that are controlled by an unknown common limiting factor. Glucose not only retarded the rate of entry of asparagine but promoted deamidation of that which did get in and also promoted the conversion of ammonia to amino nitrogen and thus retarded its loss to the medium by leakage.

Glutamine and glutamic and aspartic acids were absorbed much more slowly than asparagine (Table II). In all cases, ammonia was released into the medium and the disks also showed significant increases in ammonia—and amino nitrogen. It appeared that glutamine, unlike asparagine, was readily deamidated by the thallus tissue and some of this deamidation was extracellular. Glutamic acid was absorbed at a similar rate to glutamine and aspartic acid least rapidly.

Although rates of absorption are high relative to the slow rates of growth the utilization of the absorbed material is consistent with the slow growth observed.

Smith has also shown (1961) a seasonal variation in the rate of asparagine absorption by *Peltigera polydactyla* as well as the total thallus nitrogen content. There was a period of maximum metabolic activity in late winter and early spring when field conditions were moist and cool, and the light intensity was high. The carbohydrate and nitrogen reserves built up during this period were depleted in the summer when conditions for assimilation

TABLE II
 CHANGES IN THE NITROGEN FRACTIONS OF THE INCUBATION
 MEDIA AND OF THE THALLUS OF *Peltigera polydactyla*
 AFTER INCUBATION FOR 24 HOURS AT 20°C
 AND AT pH 5.7^a

Nitrogen fraction	Nitrogen source ($\mu\text{g N}/100 \text{ mg dry weight of thallus}$)			
	Asparagine	Aspartic acid	Glutamine	Glutamic acid
Amide				
Medium	-1004	0	-524	0
Thallus	+669	-10	-28	-3
Amino				
Medium	-1004	-490	-879	-1042
Thallus	+874	+314	+492	+493
Ammonia				
Medium	+95	+74	+469	+79
Thallus	+202	+67	+322	+239

^aData from Smith (1960b).

were poor. The poor conditions persisted until autumn when a short favourable period recurred. During the winter the lichens became covered by litter and again drew on their reserves. Asparagine-absorbing activity followed a similar pattern, as did nitrogen content, but the latter lagged behind the other parameters, the seasonal effect being delayed.

This work by Smith has been described in detail since it was the first and is still the only detailed study of the uptake and utilization of combined nitrogen by lichens. It was also the first physiological study in which a serious attempt was made to overcome variability problems, and in which detailed chemical analysis formed a major part.

Of the findings, the most significant were that the rapid uptake of combined nitrogen was not followed by protein synthesis, that amide metabolism is of minor importance, and that protein breakdown in periods of starvation is slight. Whether algal products are the direct cause of the major changes in the physiology of the "lichenized" fungus is a problem that will only be resolved when methods are available for studying the isolated symbionts in an undamaged condition and for the *in situ* demonstration of metabolites. At present, cultures of lichen fungi and blue-green algae are necessarily considerably removed from the "symbiotic" condition, having undergone several generations of independent growth.

Nevertheless, Smith's overall findings are clear enough—a biological system has developed in which vigorous powers of nutrient and moisture absorption without special organs have been allied to restrained abilities for synthesis and growth. Thus, every opportunity for absorption of food

material of virtually any kind can be taken, and a steady rate of utilization maintained. This is of great biological advantage in exacting and inhospitable habitats.

IV. Fixation of Elementary Nitrogen

Nitrogen fixation has been demonstrated only in those lichens with blue-green phycobionts. Of the eight genera of blue-green algae listed by Ahmadjian (1967) and Duncan (1970) as phycobionts, only lichens with *Nostoc* and *Calothrix* have so far been shown to fix nitrogen. The lichens in which fixation has been shown either by means of $^{15}\text{N}_2$ or the acetylene reduction technique (Dilworth, 1966; Hardy *et al.*, 1968) are listed in Table III. The phycobionts, of *Peltigera virescens* (Watanabe and Kiyohara, 1963) and *Collema tenax* (Henriksson, 1951), have been shown to fix nitrogen when isolated and fixation can be assumed to take place in the lichen. The first definitive demonstration of fixation by an intact lichen thallus was by Bond and Scott (1955) who used $^{15}\text{N}_2$ and tested *Collema granosum* and *Leptogium lichenoides*.

TABLE III
ESTABLISHED NITROGEN-FIXING LICHEN SPECIES

Lichen	Phycobiont genus	Technique used	Reference
<i>Collema coccophorus</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Rogers <i>et al.</i> (1966)
<i>Collema crispum</i>	<i>Nostoc</i>	C_2H_2	Hitch (1971)
<i>Collema granosum</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Bond and Scott (1955)
<i>Collema pulposum</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Fogg and Stewart (1968)
<i>Collema tunaeforme</i>	<i>Nostoc</i>	C_2H_2	Henriksson and Simu (1971)
<i>Leptogium lichenoides</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Bond and Scott (1955)
<i>Lichina confinis</i>	<i>Calothrix</i>	$^{15}\text{N}_2$	Stewart (1970)
<i>Lichina pygmaea</i>	<i>Calothrix</i>	$^{15}\text{N}_2$	Stewart (1970)
<i>Lobaria pulmonaria</i>	<i>Nostoc</i> (cephalodia) <i>Myrmecia</i> (thallus)	C_2H_2	Millbank and Kershaw (1970)
<i>Peltigera aphthosa</i> var. <i>leucopblebia</i>	<i>Nostoc</i> (cephalodia) <i>Coccomyxa</i> (thallus)	$^{15}\text{N}_2$	Millbank and Kershaw (1969)
<i>Peltigera canina</i>	<i>Nostoc</i>	C_2H_2	Millbank and Kershaw (1970)
<i>Peltigera polydactyla</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Watanabe and Kiyohara (1963)
<i>Peltigera praetextata</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Scott (1956)
<i>Peltigera pruinosa</i>	<i>Nostoc</i>	$^{15}\text{N}_2$	Watanabe and Kiyohara (1963)
<i>Peltigera rufescens</i>	<i>Nostoc</i>	C_2H_2	Henriksson and Simu (1971)
<i>Placopsis gelida</i>	<i>Stigonema/Nostoc</i>	C_2H_2	Hitch (1971)
<i>Stereocaulon</i> sp.	<i>Nostoc</i> (cephalodia) <i>Trebouxia</i> (thallus)	$^{15}\text{N}_2$	Fogg and Stewart (1968)

The existence of nitrogen-fixing bacteria in or on lichens has been referred to frequently; Cengia-Sambo (1923) reported their presence in the cephalodia of *Peltigera aphthosa* and postulated the theory of polysymbiosis. The cephalodia were thought to be analogous to the root nodules of legumes by both Cengia-Sambo and Goebel (1926), although Goebel considered that the *Nostoc* in the cephalodia were responsible for nitrogen fixation. Henckel and Yuzhakova (1936) reported *Azotobacter* in epiphytic lichens and these findings were extended in 1938 by Henckel and Iskina, independently, to include other groups of lichens, including *Cladonia*, which was originally reported as not possessing *Azotobacter*. Scott (1956) refers to the unpublished findings of Dr. G. Metcalfe, in which *Azotobacter* was isolated in many British lichens. However, Walenkamp (referred to by Quispel, 1945) and Krasilnikov (1949) did not detect *Azotobacter* in the 40 species of lichens examined. More recently Panosyan and Nikogosyan (1966) failed to find *Azotobacter* in a number of Armenian lichens they examined and considered this to be due to the lack of any respirable substrate. Bond and Scott (1955), while admitting that *Azotobacter* could have been present epiphytically on the thallus samples they studied, concluded that they made no significant contribution to the fixation observed. They based this conclusion on the lack of the necessary respirable substrate and the absence of anything approaching the number of bacterial cells needed.

In 1956, Scott reported on his studies with *Peltigera praetextata*, extending his earlier work with Bond (Bond and Scott, 1955). Nitrogen fixation was taking place, brought about by the *Nostoc* phycobiont, any contribution by epiphytic *Azotobacter* being considered insignificant. He also showed that some of the nitrogen fixed by the *Nostoc* was transferred to the mycobiont. Results of trials with *Cladonia impexa* were also reported. This lichen did not fix nitrogen, the phycobiont being a green alga, but as *Azotobacter* had frequently been associated with it, it was desirable to clear up the question unequivocally. More recent evidence, from electron micrography of *Peltigera canina*, showed the total absence of a third symbiont (Griffiths *et al.*, 1972). *Azotobacter* can only be a casual epiphyte and the hypothesis of Cengia-Sambo (1923) must be rejected.

A. Release of Fixed Nitrogen by the Phycobiont

Henriksson (1951) showed that between 19 and 28% of the nitrogen fixed by the *Nostoc* phycobiont of *Collema tenax* was released into the growth medium. Release of N is a normal feature of the metabolism of blue-green algae (see, e.g., Fogg, 1962). She inferred that the same thing is happening within the lichen thallus, and this was confirmed by Scott (1956).

Millbank and Kershaw (1969) working with *Peltigera aphthosa* and using $^{15}\text{N}_2$ showed that fixation in this lichen was confined to the cephalodia, which

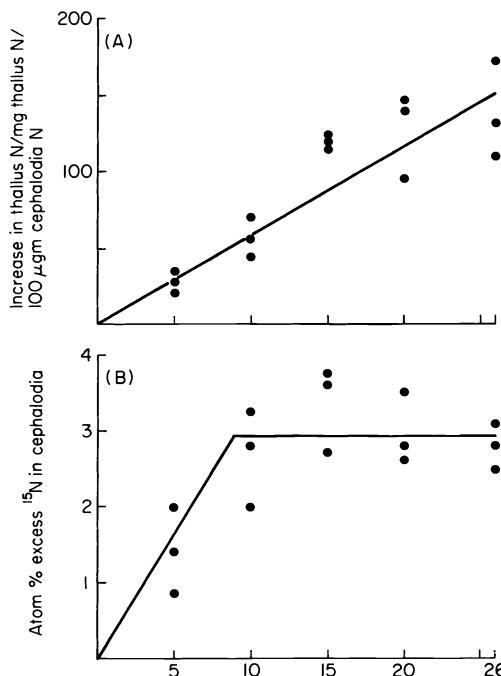


FIG. 3. (A) Uptake of elementary nitrogen by *Peltigera aphthosa* thallus at 12°C. (B) $^{15}\text{N}_2$ enrichment of *Peltigera aphthosa* cephalodia at 12°C when exposed to nitrogen gas enriched to 30% $^{15}\text{N}_2$. (After Millbank and Kershaw, 1969.)

contained the *Nostoc* phycobiont. Thallus from which the cephalodia had been removed by dissection showed no evidence of fixation. Experiments carried on for 25 days showed that incorporation of nitrogen from the atmosphere into the thallus was linear (Fig. 3) but that the level of heavy nitrogen labeling in the cephalodia became constant after 10 days only. It was concluded that there was a continuous throughput of nitrogen via the *Nostoc* and that virtually all the nitrogen fixed by the *Nostoc* was being released to the mycobiont.

The authors, although considering the mycobiont to be controlling the metabolism of the nitrogen-fixing phycobiont, were not able to supply any evidence as to what form the control took. They tended to favor some form of chemical control of the alga, affecting either nitrogen utilization or permeability. As yet, no substances or extracts from lichen thalli have been demonstrated to influence the rate of nitrogen fixation or nitrogen loss from blue-green algal cells. The possibility that the effect is connected with the oxygen tension in the thallus is at least equally likely, and in view of later work to be described below, is at present favored by the writers.

B. The Rate of Nitrogen Fixation of Lichens

The studies referred to so far have mostly dealt with nitrogen fixation either qualitatively or on a "whole thallus" basis. Millbank and Kershaw (1969) attempted to relate the fixation of nitrogen in lichens to the population of the nitrogen-fixing symbiont. They found that the total nitrogen content in *Peltigera aphthosa* was of the order of 3% of the dry weight, and that the algal contribution to the total nitrogen was 7.5% in the thallus and 5.5%* in the cephalodia. Thus, the phycobiont was far less abundant than Scott reported (1956) for *P. praetextata*. Scott estimated the algal content by visual observation of sections, a simple and straightforward technique, but liable to rather large errors and uncertainties. Millbank and Kershaw further showed that the total nitrogen in the cephalodia represented only about 2–4% of that in the entire thallus, so clearly the *Nostoc* nitrogen formed a tiny fraction (5.5% of, say, 3%) of the total thallus nitrogen. Thus, although the total amount of nitrogen fixed per unit of thallus was small, the rate of fixation by the *Nostoc* was rapid and, had it been entirely used for growth, would have permitted a generation time of 18 hours* at 25°C and 24 hours* at 12°C, the latter a representative noon temperature for the natural habitat in spring. This compares with a figure of 19.5 hours for *Nostoc muscorum* in shake culture at 25°C given by Kratz and Myers (1955).

Later studies by Millbank (1972) on *Peltigera canina*, using the acetylene reduction technique for estimating nitrogenase activity, confirmed that fixation was rapid, at least in *Peltigera*. The *Nostoc* nitrogen was again found to be a small proportion of the total, about 2.7% (3×10^6 cells/cm² thallus). The cells were found to be very much larger than when grown in the free-living state, having mean dimensions of 8.3 × 6.7 μm, compared with 4.0 × 3.7 μm. The rate of acetylene reduction was examined in a large number of specimens of thallus, and, as expected, was found to be rather variable. However, the most important finding was that the rate was very rapid. At 10 nmole C₂H₄ evolved/minute/mg *Nostoc* protein the average rate was about three times as fast as was usual in free-living material. Furthermore, heterocysts, although present, were rather infrequent, representing about 3.3% of the total algal cells (Griffiths *et al.*, 1972). This confirmed Peat's observation (1968), and incidentally probably accounts for Drew and Smith's (1967) inability to observe any at all when they examined homogenized thallus under the light microscope.

If nitrogen fixation were confined to the heterocysts as has been recently supposed (Fay *et al.*, 1968), their nitrogenase activity becomes equivalent to 300 nmoles C₂H₄ evolved/minute/mg heterocyst protein. Data on the

*Amended from the figures given in 1969 in the light of later work (Millbank, 1972).

specific activity of pure nitrogenase are quoted by Hardy *et al.* (1971) and, taking a figure of 900 nmole/minute/mg as representative, the conclusion is that about 33% of the total heterocyst protein is nitrogenase. This seems improbable, and fixation in lichens may perhaps be taking place in the vegetative cells. This could well be feasible as a consequence of a possible low internal pO_2 brought about by fungal activity, and is currently under active investigation. If so, it accords with a general hypothesis concerning the site of nitrogen fixation proposed by Stewart (1971) and supported by the results of van Gorkom and Donze (1971).

Henriksson and Simu (1971) have expressed their results for nitrogenase activity in *Collema tunaeforme* and *Peltigera rufescens* on a quantitative basis, but unfortunately relative to the dry weight of the entire thallus. If a number of assumptions are made, the nitrogenase activity per milligram *Nostoc* protein can be shown to be of the same order as found by Millbank (1972). It therefore seems that rapid rates of fixation are a characteristic of lichen *Nostoc* under good conditions.

Study of the nitrogenase activity over the thallus area shows the most rapid rate of fixation, on both an area and a cell-number basis, to be taking place in the mature part of the thallus, about 3 cm behind the growing edge (Millbank, 1972).

C. The Effect of Environmental Factors, Moisture, Light, and Temperature on Nitrogen Fixation

From their study of ten tropical and nineteen arctic lichens, Scholander *et al.* (1952) concluded that "the metabolic rate of a lichen is highly variable, depending largely upon its water content and temperature." Their concern was respiration, but nitrogen fixation is as dependent, with light intensity an additional factor.

Stewart (1965) showed that *Calothrix* sp. and *Nostoc* sp. were capable of some dark fixation of $^{15}N_2$ over a 24-hour period, but it was considerably less than in the light. He considered that nitrogen fixation was not critically light dependent as long as supplies of carbon skeletons, reducing power, and energy were available.

Studies of temperature effects are very sparse. Millbank and Kershaw (1969) reported that the fixation rate of *Peltigera aphthosa* under laboratory conditions exhibited a Q_{10} of approximately 2 over the temperature range 12°–25°C; and Fogg and Stewart (1968) reported that nitrogen fixation by lichens under antarctic conditions "showed some relation to temperature." They suggested that "although appreciable fixation occurs in the vicinity of 0°C, the rates increased rapidly with rise in temperature, and it is probable that the bulk of fixation is accomplished in brief periods when the micro-environment reaches temperatures of 10°C or more."

Thallus moisture content is very critical, especially as the uptake and loss of water by lichen thalli is largely a physical process. The moisture content and rates of absorption and loss have been quite extensively studied (see Chapter 11). Henriksson and Simu (1971) reported the ability of *Collema tunaeforme* and *Peltigera rufescens* to recover their nitrogenase activity after long periods of desiccation. Storage was for periods of up to 30 weeks in plastic containers in the dark, at 12°C. After storage for periods longer than 4 weeks, the nitrogenase activity upon rewetting was about 50% of that observed with material stored for 1 day only; but these rates were achieved after a recovery period of only 24 hours, and in the case of *Collema* a recovery period of 1 hour only was sufficient to restore 50% of the original activity after 22 weeks of storage, and 25% after 30 weeks. These storage conditions were very exacting and show the lichen's remarkable capacity to withstand severe environmental stress.

Hitch (1971) studied the environmental factors affecting nitrogenase activity in lichens, and has reported diurnal and seasonal variation in *Collema*, *Lichina*, and *Peltigera*. When samples collected from the field in a desiccated state were wetted, nitrogenase activity started after lag periods of 60, 20, and 35 minutes, respectively. The thallus moisture contents, however, changed from the original level of 20% oven-dry weight to 200% within 5 minutes. In these lichens, when the moisture content fell below 80–90% of the oven-dry weight, acetylene reduction stopped. If these results hold true for other species, under field conditions with fast-drying rates of the thallus, little or no fixation may be possible in exposed sites and the significance of nitrogen fixation must be questioned.

Nitrogenase activity was maximal at light intensities above about 400 ft-c in *Peltigera* and *Lichina*. In the dark, there was a progressive loss of nitrogenase activity, the rate of the loss probably reflecting the size of the carbon reserves in the thallus. Under constant light intensity (700 ft-c) and saturated moisture content *Lichina confinis* showed optimum fixation at 20°C with lower and upper limits of –3° and 35°C, respectively. *Peltigera rufescens* had a very similar temperature range, –3° to 46°C with an optimum of 31°C. The diurnal variation in fixation rate of *Peltigera polydactyla* and *Lichina confinis* was correlated directly with light intensity and temperature when the thallus was adequately moist. When long-term seasonal effects were considered, the pattern of activity (measured at midday) was again related to temperature, but completely overridden by moisture content. Thus, in temperate northern latitudes, nitrogenase activity could be greater in February than in a dry May, and lowest of all in June to August.

These results all fit with what is beginning to emerge as the fundamental finding of laboratory and field studies, i.e., that metabolism of lichens is essentially opportunist; when conditions are right, absorption, assimilation, synthesis, or whatever the process may be becomes active for as long as

possible and then reverts to a state of quiescence when conditions deteriorate. Nitrogen fixation is no exception. If it is available to the lichen it is utilized; but other sources of nitrogen are as avidly accepted and no one process is uniquely significant.

V. Translocation of Nitrogen Compounds between the Symbionts

Isolated lichen algae are known to release a variety of organic substances into their growth media; and it is assumed that this characteristic is continued in the symbiotic condition although there is little direct evidence so far. Henriksson (1957, 1960, 1961) has shown that a *Nostoc* from *Collema* released about 5% of the nitrogen it fixed into the medium, together with thiamine, biotin, riboflavin, and nicotinic and pantothenic acids. It also released unknown compounds which inhibited the growth of its (cultured) mycobiont. Bednar (1963) showed that the *Coccomyxa* phycobiont of *Peltigera aphthosa* released biotin and thiamine into the medium. Henriksson again, in 1958, reported that an isolated strain of *Nostoc* released substances which can support the growth of the mycobiont, though the nature of the substances was not established. Many lichen fungi are deficient in biotin and thiamine and the release of these compounds by the phycobiont could be of vital significance to the maintenance of healthy growth of the combination.

Kershaw and Millbank (1970) investigated the movement of $^{15}\text{N}_2$ in the three component lichen *Peltigera aphthosa* over periods of up to 55 days, and found that the labeled nitrogen, which had been fixed and then released from the *Nostoc* in the cephalodia, was overwhelmingly present in the thallus mycobiont. After considering the proportions of fungal and green algal cells in the thallus, only about 3% of the expected amount of nitrogen was found in the green algal (*Coccomyxa*) cells. The lichen, when maintained in a suitable enclosure (Kershaw and Millbank, 1969), benefited greatly by the regular addition of combined nitrogen and it seemed that the cephalodia were of minimal value as a source of nitrogenous nutrient to the green algal component; the greater proportion of blue-green algae per unit of thallus in *P. canina* may well provide a more adequate supply of nitrogen, but the observation of Stewart (1966, p. 68) that the majority of lichens contain non-nitrogen-fixing Chlorophyceae as phycobionts and do as well on inhospitable substrates as the nitrogen-fixing ones is nevertheless true.

VI. The Relationship of Lichens to Other Nitrogen-Fixing Symbiotic Systems

Scott (1969) arrived at a definition of symbiosis as a "state of equilibrated physiological interdependence of two or more organisms involving no per-

manent stimulation of defensive reaction mechanisms." This is a synthesis of six criteria, the possession of any four of which he considers adequate to constitute a symbiotic association. These criteria are that the association should (a) be a permanent feature of the organisms' life cycles; (b) involve physical contact between the participants; (c) involve unilateral or bilateral movement of metabolites; (d) ameliorate environmental status, thus giving rise to an extension of ecological range; (e) give rise to morphogenetic effects; and (f) provide opportunity for the production of metabolites not formed by either of the organisms separately.

The other nitrogen-fixing symbiotic systems comprise: the legume-*Rhizobium* association; the nonleguminous angiosperm-root nodule systems (endophytes unidentified); the *Klebsiella-Psychotria* leaf nodule association; and the relationship between blue-green algae with liverworts (*Blasia*, *Cavicularia*), ferns (*Azolla*), Gymnosperms (*Cycas*, *Stangeria*, *Encephalatos*, *Macrozamia*), and Angiosperms (*Gunnera*).

Since the endophyte(s) in the nonleguminous nodule system have not been identified as yet it is not possible to compare their physiology and attributes to the other systems. Leaving them on one side, therefore, it would seem that the nitrogen-fixing lichens are in one respect, much more closely related to the *Blasia*, *Azolla*, *Cycas*, and *Gunnera* association than to the strict nitrogen-fixing symbiosis as typified by the legume-*Rhizobium* system. Thus, although all the associations possess many or all of Scott's criteria, only the legume-*Rhizobium* association is a strict nitrogen-fixing symbiosis, since in all the other systems the process is carried on by an organism able to fix nitrogen in pure culture or free-living in nature. Furthermore, the nitrogen-fixing organism's faculty of release of combined nitrogen is also normal, and is enhanced rather than initiated by the specialized environment. However, the morphological distinctiveness of lichens, in which there is little or no resemblance between the product of the combination and any of the constituent partners, is one of the most outstanding features of the symbiosis, and distinguishes it completely from the green-plant associations. We have evidently an intimate relationship giving rise to profound morphological and biochemical changes but retaining at least some of the fundamental biochemical attributes of the individual partner. Other biochemical features, such as the production of lichen acid and antibiotics and the release of glucose and other carbohydrates by the phycobionts are, however, apparently associated only with the lichenized state and thus qualifies the association under criterion (f) above as well. Given that mutual advantages are features of a symbiotic relationship, it seems most appropriate to consider that the major benefit to the phycobiont(s) in the lichen association is a considerable extension of ecological range, for which they pay the price of greatly reduced rate of growth and what may be termed a state of controlled parasitism by the mycobiont.

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Part III

ENVIRONMENTAL RESPONSE AND EFFECTS

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Chapter 10

RESPONSE TO EXTREME ENVIRONMENTS

L. KAPPEN

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I. Introduction

The question of what constitutes an extreme environment is difficult to answer. It could mean that habitat conditions are extreme for all organisms or it could mean that the conditions are extreme only for lichens. How lichens respond to a particular environmental condition sometimes is difficult to determine because of our incomplete knowledge of their physiology and ecology.

The response of a lichen to an environmental condition may be positive or negative. In a positive sense, extreme habitats may be well-suited for lichens which are known as pioneers of vegetation. Their growth in extreme environments involves adaptations of their physiology and morphology as well as their ecology and vegetational dynamics (synecology). A negative

response, the fact that lichens are absent in a given environment or appear strongly damaged, indicates how far a particular environment is adverse for the survival of lichens.

Physiological and morphological properties enable an organism to respond directly or indirectly to environmental conditions. To what extent lichens can physiologically tolerate environmental stress or avoid it because of their special makeup has yet to be determined. Whether an adaptation to stress is common to all lichens or restricted to only selected taxa will be discussed later.

This chapter will review the physiological and morphological responses of lichens to the most important environmental factors and consider the relationship of these factors to lichen distribution.

II. Determination of Viability and Resistance

Determining viability of a lichen either in the field or under controlled conditions is perhaps the most difficult factor in studies of survival under extreme environments. The reactions of lichens are not pronounced and thus not measured readily within a short period of time. Because of these difficulties, many different kinds of measurements and observations have been used to determine the lichen's responses to extreme environments.

Field observations include mapping the occurrence of lichens, investigating their density or absence in a given habitat, or long-term inspections of the development of damage caused by some special environmental factor. Because of the large complex of natural environmental factors, it is not possible to interpret exactly the results obtained. Only when the tested factor is a dominant one, such as radioactive radiation or air pollution, are such methods significant. The survival of transplanted epiphytes in a test area also gives a valid indication of resistance (Brodo, 1966).

To obtain reliable results for responses to certain environmental factors, it is necessary to test the lichen under controlled conditions. The various methods of subjecting the lichens to extreme temperatures, drought, immersion, and illumination, in the laboratory, cannot be discussed fully here. Although Gannutz (1969) cautioned about the influence of air pollution in areas where many laboratories are located, there are botanical departments located in areas of low pollution that could not affect significantly the measurements (Lange, 1953, 1969; Lange and Kappen, 1972). An appropriate way to avoid this problem is to conduct the experiments in the natural environment of the lichens (Gannutz, 1969). This has become easier to do since we can now control environmental conditions by means of transportable conditioning chambers (Fig. 1) for measuring the CO₂ exchange (Koch *et al.*, 1971; Lange *et al.*, 1968, 1970a,b).



FIG. 1. Part of the field equipment with a conditioned chamber (cf. Koch *et al.*, 1971) as used by Lange *et al.* (1968, 1970a,b) to measure CO_2 exchange of plants. *Ramalina maciformis* is in the chamber (A). The natural conditions, e.g., temperature and air humidity, can be simulated in a wide range or artificially varied and controlled. (B) temperature sensor (outside), (C) temperature sensor (inside), (D) light sensor (outside), (E) light sensor inside, (F) balance for measuring the water intake of the lichens by dew or air humidity. (Courtesy of O. L. Lange).

It is rarely possible to see at a glance whether a lichen is alive or dead. Thus, visual investigation of the viability of lichens is unreliable (Smith, 1962). A cytological examination of small cells with small vacuoles, as are found in lichens, cannot be easily carried out. Staining with neutral red (Stocker, 1927; Siegel and Daly, 1968; Kappen and Lange, 1972) can be applied successfully to many species as an indication of life, but, surprisingly, for the phycobionts of many lichens it indicates death (Schmid, 1933). Stress-induced discoloration and bleaching of the chlorophyll (Rao and LeBlanc, 1966) are conspicuous if the pigment of the lichen is not intense. The viability of the symbionts, especially of the phycobionts, can be determined by their growth in culture after stress treatment (Lange, 1953). The occasional observation of the dissolution of the symbiosis after stress reveals

the damaging effect on the whole lichen. Visual observations generally do not reveal any subtle or immediate effects that may be caused by moderate stress.

Physiological responses, even if reversible, offer more valuable information about the ability of a lichen to survive. Since Jumelle (1890), the measurement of CO₂ exchange has proven to be a quantitative indicator for the response of lichens to any environmental influence. The photosynthetic rate can be determined either by apparent CO₂ intake or by O₂ release. The different methods of measurement are described by Henrici (1921), Stocker (1927), Lange (1956, 1965a, 1969), Ried (1960b), Lange and Kappen (1972), and others. In earlier studies frequent overheating of the gas exchange chambers could not be prevented and thus some measurements are doubtful. The immediate reactions of lichens to different natural environmental conditions and their rates before and after a stress treatment are measured in airstreams of conditioned CO₂ exchange chambers in the laboratory and in the field (Lange *et al.*, 1968, 1970a,b) with simulated natural temperature and humidity conditions.

The return to a normal net photosynthetic rate after stress treatment usually indicates resistance of the tested plants. However, CO₂ exchange of lichens represents the responses of at least two organisms. A lowered apparent CO₂ intake after stress may be the result of the stress-stimulated respiration of one or both symbionts or it may be due to a decrease in the rate of gross photosynthesis of the phycobiont, or even the result of both responses together. Highly stimulated respiration is usually reversible within several days or even hours. Considering the relative volumes of both symbionts, one would assume that most of the CO₂ released by a lichen comes from the fungus. But, since only a small part of the mycobiont is active, its respiration rate may not exceed that of the algal mass (Smith, 1962).

Experiments on the separated and cultured symbionts of *Cladonia rangiferina* and *Lobaria pulmonaria* (Kappen and Lange, 1972) showed that almost two-thirds of the total dark respiration of the lichen was due to the mycobiont. Additionally, it was shown that in the light (10 klux) the mycobiont respired at a reduced rate ($\frac{1}{2}$ – $\frac{2}{3}$). As far as is known from other experiments, the algae have little or no photorespiration. Thus, gross photosynthesis, the action of the phycobiont itself, can be calculated. The algal symbiont responds very sensitively to environmental stress by the lowering of its photosynthetic rate, either reversibly within several hours or days, or irreversibly to a level that is correlated to the number of damaged algal cells (see Fig. 2).

Respiration of lichens responds to moderate or sublethal stress (e.g., desiccation or freezing) with a violent increase in rate, more than four times the normal rate (Ried, 1960b; Lange, 1965a; Kappen and Lange, 1972). After a few hours, or in some cases days, when conditions again become favorable, respiration usually returns to normal. In the event of death, respiration

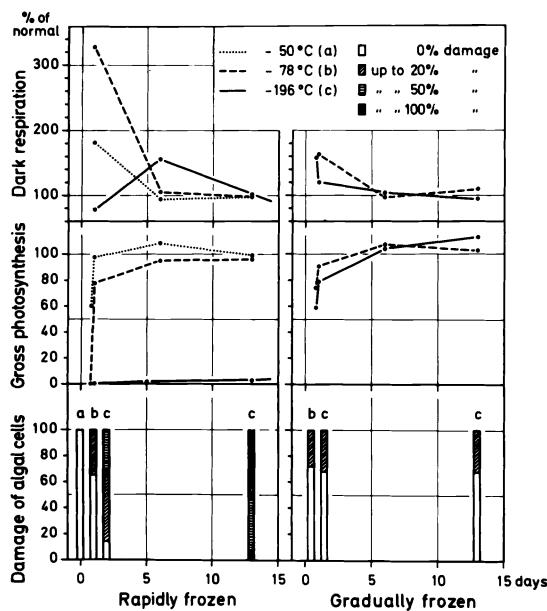


FIG. 2. Vitality of *Cladonia rangiferina* after exposure to -50° , $\sim -78^{\circ}$, and $\sim -196^{\circ}\text{C}$, as indicated by rates of dark respiration and gross photosynthesis (calculated) (see Fig. 8) and by survival (damage) of the phycobionts. The cooling and rewarming rates were either gradual (temperature drop $15^{\circ}\text{C}/\text{hour}$ to -50°C and then after 30 minutes to the end temperature and vice versa after 24 hours or 6 hours) or were rapid. The decreases of the photosynthetic rates can be correlated with the extent of injury to the phycobionts (observed in cross sections of the thallus). Each column represents the amount (%) of algal groups partially or totally damaged. (From Kappen and Lange, 1972.)

usually decreases gradually; of course, the CO_2 release can be raised again by the presence of microorganisms through infection. As a rule, respiration is permanently affected under a far stronger environmental stress than is tolerable for photosynthetic activity. Consequently, the phycobiont is generally much more sensitive than the mycobiont. This was demonstrated clearly by comparing the responses of the symbionts to radiation (Gannutz, 1969), SO_2 (Rao and LeBlanc, 1966), and low temperatures (Kappen and Lange, 1972). Thus, resistance that is determined only by examination of respiration is generally overestimated (cf. Smith, 1962).

A single viability test performed immediately after stress involves the risk of misinterpretation because of different after-effects. Thus, in order to obtain significant results, lichens must be observed repeatedly both preceding and for a longer period after the term of stress.

This chapter will concentrate on the responses of lichens to extreme temperatures, extremely high or low humidity, visible or ionizing radiation,

and mechanical influences. To date about 150 species of lichens from most parts of the world, except the central tropical zone and only a few from Asia, have been tested experimentally with regard to their tolerance to one or more of these stress factors.

III. Responses to Environmental Stresses

A. Response to Drought and Desiccation

The most striking characteristic of lichens is their water relations. Being constituted like a "sponge," the lichens are subjected to a discontinuous change of imbibition and desiccation (Goebel, 1926; Stocker, 1956). The water balance of lichens depends to a great extent on the water conditions in the substrate such as soil surface, tree bark, or other materials (Culberson, 1955) and on the aerial environment—precipitation, dew, or air humidity (cf. Bertsch, 1966a). Fries (1831) has already mentioned *lichenes ab humiditate aeris magis pendeant*.

Natural water loss has been observed repeatedly and measured by many authors (Neubauer, 1938; Scofield and Yarman, 1943; Lange, 1953). The minimum water content of lichens generally is extremely low and ranges between 9 and 2% of dry weight. The remaining water is bound tightly in the protoplasm (cf. Ahmadjian, 1967). Organisms which tolerate very wide amplitudes of moisture content can be defined as "eurypoikilohydric" (Walter, 1960).

Galun (1963) concluded that the homoeomerous thalli of *Psorotrichia numidella* and *Collema tenax* in the Negev desert act like succulent higher plants and have great capabilities of accumulating water and only slowly giving way to desiccation during the dry season. The occurrence of superficial layers on many heteromerous desert lichens also may represent protection against rapid water loss (Zukal, 1896; Galun, 1963). This idea has some support in the fact that thalli protected in this way grow sunken in the ground which offers them shelter (cf. Vogel, 1955). Follmann (1965b) also feels that some soil lichens of the very dry Atacama desert are not completely "hydrolabile." Six hours after a dewfall the crustose thalli still retained 44% of their original water content, although the soil had been warmed up to 62° C by sun radiation. Blum (1965), on the other hand, having investigated the water consumption of twelve lichen species from "xerotic" habitats, tends to reject the significance of xeromorphic adaptations for protection against evaporation, thus confirming earlier observations of Stocker (1927). Thus, in general the lichens tend to get into water potential equilibrium with the ambient medium (See Chapter 11).

1. RESISTANCE AS MEASURED BY VISUAL OBSERVATION AND STUDIES OF THE SYMBIOTNS

Since Hofmeister (1868), it has been shown in many experiments that lichens can tolerate desiccation for several months (Schroeder, 1886; Ewart, 1897; Becquerel, 1948) or years (Thomas, 1921). *Xanthoria parietina*, for instance, was kept dry for 1.5 years in permanent light and afterwards for 1.5 years in permanent darkness. But after 8 days of cultivation on agar the phycobionts were able to produce aplanospores (Rao and LeBlanc, 1966). On the other hand, *Teloschistes flavicans* from South Africa lost the ability to resaturate its thallus when dried for only 19 days in air (Cuthbert, 1934). Already these results suggest that drought resistance of lichens is more a matter of the length of the period of desiccation rather than the intensity.

Thomas (1939) carried out drying tests with cultivated symbionts of ten European lichen species. The mycobionts of seven species tolerated air-drying for 6 weeks without injury, those of the other species 5 weeks. Lichen fungi have many small vacuoles and thick cell walls so they must be very resistant to deformation of the hyphae (cf. Stocker, 1956). According to Thomas' experiments, eight phycobionts of the ten tested species tolerated desiccation for 6 weeks. Similar resistance was found by Lange (1953) with phycobionts from five scotophilous lichen species. *Trebouxiae* of two *Cladonia* species were resistant even to a storage over P_4O_{10} in a desiccator for 41 weeks. The high drought tolerance of *Trebouxia*, according to Ahmadjian (1967), is due to a highly viscous protoplasm, small vacuoles, and very low water content. Such traits were observed by Shields and Durrell (1964) with the soil algae *Nostoc* and *Chlorococcum* which still grew after a desiccation period of 73 years.

The high drought tolerance can be diminished by long periods of moist cultivation. *Coccomyxa* from *Solorina saccata* cultivated in water for 9 weeks already was slightly damaged when subsequently desiccated for only 5 weeks instead of the 23 weeks which they could normally withstand (Lange, 1953). A loss of drought tolerance of *Hypogymnia physodes* as a result of keeping the thallus moist before the test period was also mentioned by Enzgraber (1954).

2. RESISTANCE AS MEASURED BY CO_2 EXCHANGE

From the separate studies elaborated above and from the different methods used, it cannot be definitely discerned whether drought resistance of lichens is uniformly high or to what extent it varies according to species. More comparable and significant data are obtained from measuring CO_2 exchange although methodological differences still must be taken into consideration. Jumelle (1892) was the first to measure the CO_2 exchange of a

lichen thallus as a response to stress. He noted a cessation of the photosynthetic rate and only negligible respiration of *Cladonia rangiferina*, *Ramalina farinacea*, and *Usnea barbata* after a desiccation period of about 12 weeks. According to Stålfelt (1939b), photosynthesis of *Usnea dasypoga* ceased after a desiccation period of about 25 weeks while that of *Ramalina farinacea* and *Cetraria islandica* did not fully recover after around 15 or 19 weeks (cf. also the experiments of Ensgraber, 1954).

Ried (1953, 1960b,d) systematically studied the aftereffects of drought by observing CO₂ intake and dark respiration over extended periods of time. Apart from a characteristically intense initial stimulation of dark respiration, the photosynthetic activity was either reversibly or irreversibly depressed. Ried tested six crustaceous species of the markedly zoned lichen communities on rocks bordering springs and creeks. Already, after desiccation at 40–60% relative humidity that lasted 6 to 12 days, he found characteristic differences in the initial levels of their net photosynthesis. The saxicolous lichen *Rhizocarpon geographicum* was fully active immediately after the desiccation. The amphibic species *Aspicilia lacustris*, *Dermatocarpon fluvatile*, and *Porina lectissima* recovered slowly from an initial 50% depression of their photosynthesis within 20 days. The aquatic lichen *Verrucaria silicea* (= *elaeomelaena* after Ried) was unable to recover to more than 30% of its normal photosynthesis and was irreversibly damaged after only 24 hours of drought. All these results show a clear relationship between the lichens' resistance and the moisture conditions of their natural habitats. Nevertheless, with the exception of the last-mentioned species, these differences in the metabolic responses were no longer perceptible after 20 days. Thus, in this case damage is primarily due to a reduction of substance production. *Dermatocarpon fluvatile*, still undamaged after 4 weeks, finally ceased to photosynthesize after 14 weeks of desiccation (Ried, 1960b). The thalli of *Rhizocarpon geographicum* were still able to recover after 26 weeks of desiccation.

The sensitivity of amphibic lichens is confirmed by the observations of Clement (1950) with lichen communities of an *Aspicilium lacustris* which were damaged when they were exposed from the stream for extended periods of time. The marine lichen *Verrucaria mucosa* was severely damaged after 4 weeks and could not recover its photosynthesis after a desiccation period of 1 week (Ried, 1969). Additionally, Ried (1960d) found that the drought resistance of aquatic lichens decreased along with lower air humidities in the desiccation experiments.

For terrestrial lichens Lange (1953) already had made an extensive exploration of the drought resistance of more than 25 species from various areas and lichen communities in Europe. He placed the thalli over P₄O₁₀ in

a desiccator, or as with the experimental conditions of Ried (1960d), subjected them to desiccation in open air at 50–75% relative humidity. However, the only criterion he used to indicate the degree of damage suffered by the lichens was the less sensitive dark respiration rates before and a few days after the desiccation treatment. Lange had to desiccate some species up to 94 weeks in order to obtain drought stress. He was able to show the characteristic relationship between resistance, length of the desiccation period, and habitat. Thus, epiphytic species usually exposed to fog were very sensitive compared to lichens from xeric stands on rocks. For example, *Usnea florida* withstood only 8 weeks of drying as compared to 54 weeks for *Umbilicaria cylindrica*.

Even within a single species ecotypes may vary in their resistance to desiccation. Thus, after 54 weeks of desiccation over P₄O₁₀ specimens of *Peltigera canina* from a shady forest recovered less well than specimens from a sunny pasture (Lange, 1953).

3. ADAPTATIONS TO LONG PERIODS OF DROUGHT

The adaptation of lichens to desert conditions was demonstrated by Pearson and Skye (1965) with *Lecanora rubina* from Idaho, which showed normal patterns of photosynthesis after being dried for 24 weeks in the laboratory, and by Lange (1969) with *Ramalina* from the Negev desert (Fig. 3). Similarly, antarctic lichens, that exist also under drought conditions, can regain their full photosynthetic capacity after several weeks of desiccation (Lange and Kappen, 1972). On the other hand, the marked limits of distribution of the south Australian lichen, *Chondropsis semiviridis*, which corresponds to the isohyete of 150 mm/year, can be explained by the comparatively low desiccation resistance of this lichen (Rogers, 1971). *Roccella fucoides* from the Mediterranean coast already showed a strong irreversible depression of its rate of CO₂ intake after 2 weeks of desiccation at 0.9% relative humidity (Kappen and Lange, 1972). However, it recovered its normal respiration after a short period.

Lange (1969) demonstrated that *Ramalina maciformis* was significantly more sensitive to a higher than to a lower air humidity (Fig. 3). Its initial level as well as the recovery of the relative rate of apparent CO₂ intake corresponded directly to the different degrees of desiccation. After being desiccated for 27 weeks, the thalli died when they retained a relative water content of 15% but remained alive when they only retained a water content of 1%. Becquerel (1948) recorded a similar effect on the mycobiont *Xanthoria parietina*, by comparing samples which had been desiccated for 6 years in normal air with those kept under vacuum in a desiccator with CaCl₂. From the data of Lange (1953) it can be deduced that several species

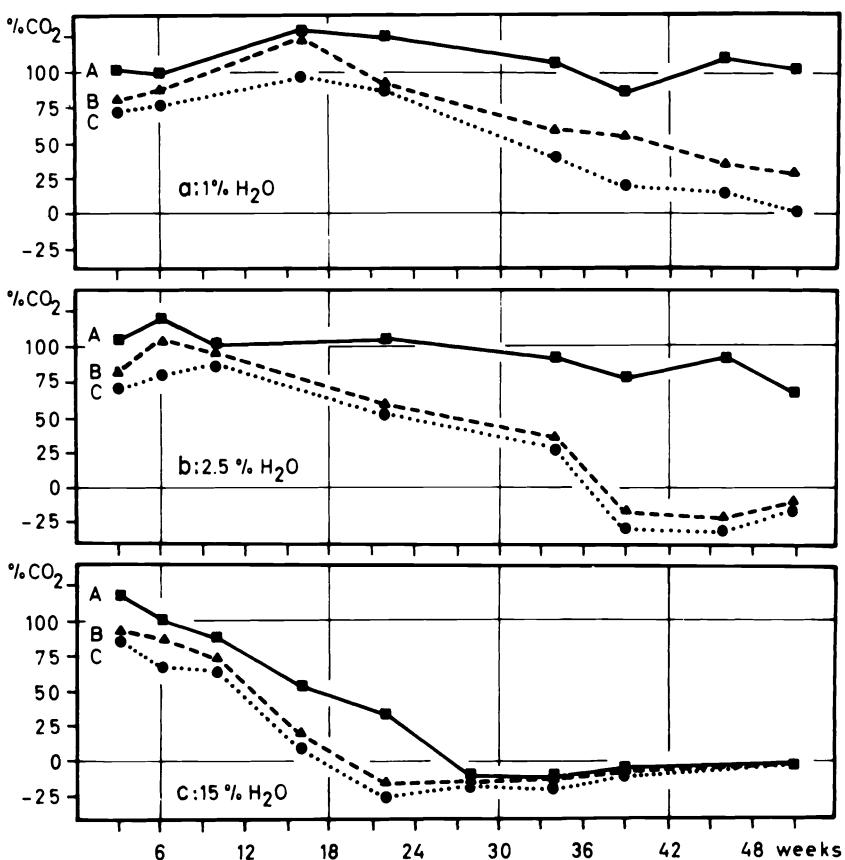


FIG. 3. Carbon dioxide exchange rates in light in percent of normal (ordinate) thalli of *Ramalina maciformis* after different periods of desiccation (abscissa) under different humidity conditions. (a) the water content of the thalli remained at 1%; (b) at 2.5%; (c) at 15% of the dry weight. Photosynthesis was measured after 1.5 hours imbibition (C) and after 4 hours imbibition (B) subsequent to the desiccation period. (A) represents maximum values within the time of the post culture (From Lange, 1969).

appeared to be more sensitive to desiccation in open air than in the lower relative humidity over P₄O₁₀ in the desiccator. The latter treatment was never significantly more deleterious than open air. Injury to lichens which are not fully subjected to desiccation is apparently caused by a continuation of respiration and by metabolic destruction within the chloroplasts (cf. Wilhelmsen, 1959).

Ried (1960d) found that drought resistance is directly correlated to the intensity of desiccation (relative humidity) as well as to the length of the desiccation period. This was evident with *Lecidea soredizoides* (Ried, 1953,

1960d) and *Verrucaria silicea* (= *elaeomelaena* after Ried). Ried did not, however, compare aftereffects of different air humidities over longer periods of time. From an ecological viewpoint we can hypothesize that different types of adaptation exist. The hydrophilous species cannot tolerate intensive desiccation (cf. also Klement, 1950). Species which are more mesophilous may react similarly either to severe or to slight desiccation (cf. Lange, 1969), but are dependent on the length of the desiccation period. Xerophilous species are only sensitive to low humidity which still induces respiration.

4. INFLUENCE OF DESICCATION ON THE METABOLISM OF LICHENS

A temporary depression of the photosynthetic rate after desiccation reduces the dry-matter production of a lichen and leads to a loss of vitality. The effect of desiccation on carbohydrate metabolism was studied by Nifontova (1967) with *Cladonia alpestris*, *C. amaurocraea*, *Hypogymnia physodes*, and *Parmelia caperata*. After being in a desiccator with CaCl_2 for 1 year, the photosynthetic activity of the lichens was reversibly depressed. Hydroxy acids, sucrose, and fructose had accumulated in the cells. Mannitol, a common constituent of lichen fungi, was absent which indicated that its synthesis is very sensitive to drought. The stimulation of respiration after desiccation stress causes considerable loss of carbohydrates. Enzgraber (1954) suggested that this high respiration rate indicates that the substrate of respiration is fatty oils that are produced by conversion of carbohydrates during desiccation. Accumulation of fats subsequent to drying was observed in moss cells by Plantefol (1927) and in algae by Fogg (1953). However, Pueyo (1960) did not detect significant changes in the composition of soluble carbohydrates of *Cladonia impexa* and *Lasallia pustulata* over a period of 3 months of desiccation nor did Peveling (1970) find any change in the amount of starch of *Ramalina maciformis* after desiccation for 4 years.

5. METABOLIC ACTIVITY UNDER LOW WATER CONTENTS

Like aerial algae and fungi (cf. Bertsch, 1966b) most tested lichens show activity and growth even without imbibition of liquid water (Butin, 1954). Lichens are able to respire under very dry conditions. According to Cuthbert (1934), respiration was still measurable in *Teloschistes flavicans* when it had only 0.4% of its total water content. Recently, Büttner (1971) showed with several species of *Cetraria* and *Cladonia* that respiration starts very quickly when the thalli are exposed to humid air.

Photosynthesis is generally activated at higher water potentials but has a steeper gradient than respiration as the air humidity increases (Lange, 1969). At a water content of 25–30% of dry weight several species of *Evernia* and

Ramalina still showed a positive apparent photosynthesis (Bertsch, 1966a; Lange, 1969; Lange *et al.*, 1970a). This level of water content was achieved in the desert lichen *Ramalina maciformis* solely by water-vapor uptake at about 80% relative air humidity (10°C; 10 klux), which corresponds to a water potential of nearly -300 atm (Lange, 1969; see also Butin's (1954) results with the water-repellent lichen *Biatora lucida*). Photosynthesis increased spontaneously with water-vapor intake at a relative humidity well below 100% (Lange and Bertsch, 1965). At maximum water-vapor intake *R. maciformis* reaches nearly 80% of the photosynthetic rate of thalli wetted with liquid water (Fig. 4). For some species, water-vapor intake is probably more profitable for photosynthetic gain than strong imbibition of liquid water, because respiration is higher in the imbibed thalli (cf. Büttner, 1971). After a desiccation period of 16 weeks, *R. maciformis* was able to reactivate its normal photosynthetic rate up to 50% within 2 days, solely by water-vapor intake (Lange, 1969).

It is evident that it is this ability which helps the lichens to survive in a desert environment. Measurements of CO₂ exchange in natural habitats in

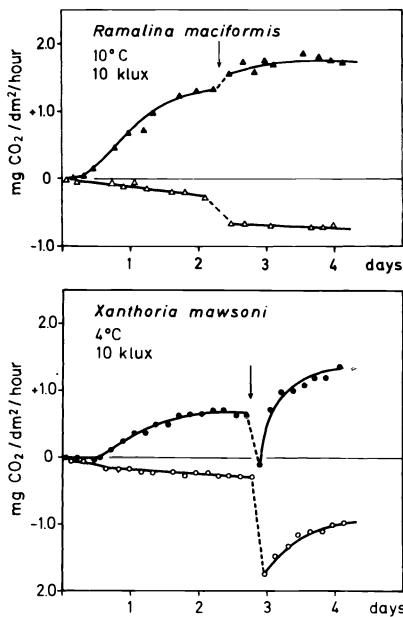


FIG. 4. Reactivation of net photosynthesis ($\blacktriangle \bullet$) and dark respiration ($\triangle \circ$) subsequent to moistening of the thalli with water vapor and then (\downarrow) spraying with water. Illumination and temperatures are indicated. Above: *Ramalina maciformis* in the Negev desert (calculated on surface area after the experiments of Lange, 1969). Below: *Xanthoria mawsoni* from Antarctica. (From Lange and Kappen 1972.)

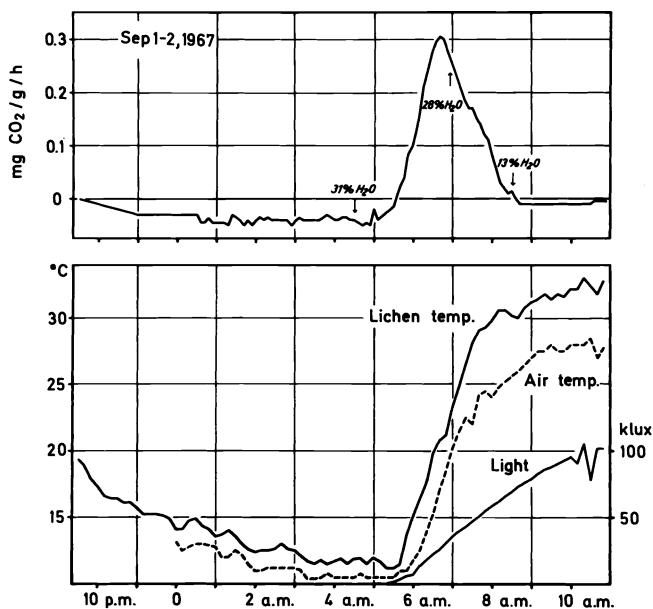


FIG. 5. Carbon dioxide exchange of *Ramalina maciformis* subsequent to intake of water vapor from the air under natural conditions. The water content in percent of the dry matter of the samples is indicated above. The temperatures of the air and of the thalli and illumination are shown below. (From Lange *et al.*, 1970a.)

the Negev desert (Lange *et al.*, 1968, 1970a,b) demonstrated that *Ramalina maciformis*, *Teloschistes lacunosus* and six additional species of *Caloplaca*, *Diploschistes*, *Squamaria*, and *Xanthoria* were able to photosynthesize by means of dew-water condensation in the thallus and by water-vapor intake (Fig. 5).

Antarctic lichens like *Xanthoria mawsoni* can also reactivate their photosynthetic rate up to a high degree through water-vapor intake (Fig. 4). This confirms the field measurements with the same species by Gannutz (1970). Reactivation of photosynthesis of *Buellia frigida* and *Lecanora melanophthalma** was significant but not as pronounced (Lange and Kappen, 1972).

It is possible that the more densely structured lichens, which occur close to melting snow covers in antarctic environments, are less well adapted to using air humidity instead of liquid water (Ahmadjian *et al.*, 1967). Schofield and Rudolph (1969) suggest a possible new way that antarctic lichens

**Omphalodina spilei* (Dodge and Baker) Dodge ad int., according to Follmann.

acquire water, i.e., by a type of temperature regulation. The thallus of *Caloplaca elegans* var. *pulvinata* reflects a high proportion of near infrared wavelengths. The thalli were found to be consistently cooler than the dark rocks of their environment. Consequently, water condenses on the cooler thallus. *Chondropsis semiviridis* is not able to reactivate photosynthesis by air humidity (Rogers, 1971). It unrolls only by means of liquid water but then has immediate high photosynthetic rates with no increased respiration. This is in contrast to lichens from moderate regions and shows an adaptation to semiarid conditions.

Osmotic potentials of -84 to -115 atm, induced predominantly by salt incrustation (Follmann, 1967), do not drastically decrease the vitality of coastal lichens in Chile. In fact, these coastal lichens may profit by the hygroscopic nature of their salt crust. Until recently there have not been any studies on the effect of salt on water uptake and photosynthesis of lichens. Siegel and Daly (1968) found that the respiration of *Cladonia rangiferina* was unaffected by a 1 N KCl solution and even showed some reactivation after being immersed in saturated LiCl solution (osmotic potential about -1000 atm).

For terrestrial species it depends on whether their slow growth as a consequence of long-lasting inactivations enables them to compete with homoiohydric plants on dry, open stands. On the other hand, periods of slight desiccation will exclusively induce respiration because its moisture compensation point (Lange, 1969) is lower than that of photosynthesis. This would occur on warm nights with high air humidity. Smith's remark (1962, p. 561) that "a succession of short and intense droughts, as might occur when hot sunny days are interspersed with dew each night, may be much more damaging than a much longer, unbroken period of drought" may only be relevant for very sensitive lichens, as can be seen from the results of Lange (1969) and Lange *et al.* (1970a). At dawn, dew-moistened lichens immediately begin to photosynthesize. The continuation of the dew imbibed state in the morning, even in many places of the desert, is long enough to allow the lichens to achieve a considerable substance production.

It can be concluded from the present data that desiccation resistance markedly varies according to species. Even the most drought-tolerant lichens need a relatively high water potential to reactivate their photosynthesis (Bertsch, 1966a; Lange, 1969). Consequently, areas in which air moisture rarely exceeds 75% will be hostile for lichens. This humidity limit would be of more importance than the occurrence of a few heavy showers that yield high annual precipitation. Thus, besides high desiccation tolerance the specific ability to yield substance production under poor water conditions plays a decisive role for the existence of a lichen species.

B. Response to Wetness and Inundation

Apart from a few field reports that suggest the wetting factor plays an important role for many lichens, there are only a few investigations about the resistance of lichens to high water contents or inundation. Green algae live in water as well as in air but several aerial species are hydrophobic and will die within 1 week if they are kept submerged in water (Schmid, 1927).

1. EFFECT OF WETNESS ON THE SYMBIOSIS

Dughi (1939a) pointed out that the stability of the lichen symbiosis is delicate and depends predominately on the water condition of the habitat. The symbiosis of *Xanthoria parietina* broke down after 10 days of continuous imbibition, and the same thing happened with *Collema* species where the *Nostoc* symbionts grew out from the thallus (Dughi, 1939b).

The influence of water on lichenization on the surfaces of rocks was observed by Jaag (1945). At places with almost permanent water irrigation, within the center of the so-called *Tintenstriche*, several species of blue-green algae lived in abundance but no lichens were present. Only at the less moist periphery of the irrigated zone did thalli of *Collema* develop. Jaag feels that in the intermediate, slightly more moist zone fungal hyphae that cannot exist in the central wet zone usually overwhelm the algal colonies. Thus, he concluded that lichen synthesis in the natural habitat occurs only when balanced water conditions induce an equilibrium between the fungal and algal virulence (cf. also Martelli, 1890).

Those who try to cultivate lichens or to achieve lichen synthesis must maintain an optimal moisture content of the substratum (Quispel, 1959; Ahmadjian, 1965). Scott (1960) cultivated disks of *Peltigera praetextata* and found that they did not survive when placed on a thick layer of glass wool in petri dishes and kept moist with a nutritive solution. In tubes connected to a reservoir with nutrient solution the growth of the disks was minimal but an abundant outgrowth of the algae occurred when the substrate was kept saturated at a high level. Only when the substrate was saturated intermittently or when specimens were set on filter paper with a lower water-holding capacity could the asymbiotic growth be prevented and the lichen disks develop normally.

2. RESISTANCE TO SUBMERSION IN WATER

The comparative analyses of resistance to submersion shown by terrestrial and aquatic lichens clearly demonstrate the ecological importance of the moisture regime for the distribution of lichens. Ried (1960b) successfully

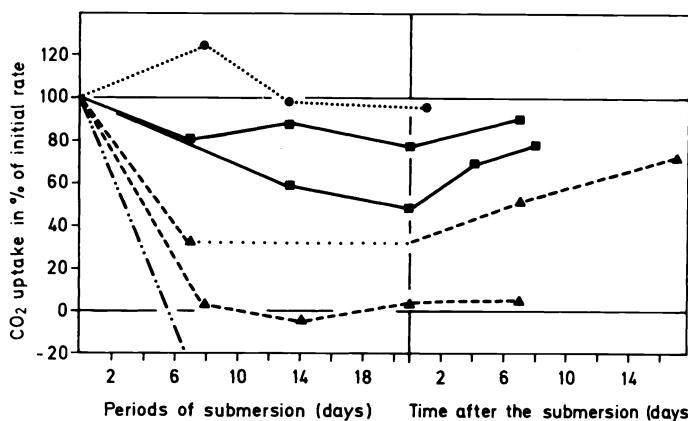


FIG. 6. Relative rates of apparent CO₂ intake after different periods of submersion of terrestrial and amphibic lichens. The samples were submerged in fresh water at 15.5°C and illuminated for 14 hours a day (2000 lux). After the submersion period, the CO₂ intake rates were measured at 17°C and 10,000 lux. ▲—▲, *Rhizocarpon geographicum*; ■—■, *Lecidea soredizodes*; ●···●, *Verrucaria silicea*; ---, *Parmelia saxatilis*. (After Ried, 1960b.)

maintained amphibic species in fresh water for 1–2 months (i.e., *Aspicilia lacustris*, *Dermatocarpon fluviale*, *Porina lectissima*, *Staurothele fissa*). However, terrestrial species (*Rhizocarpon geographicum*, *Parmelia saxatilis*) were very sensitive to inundation (Fig. 6). After artificial submersion for only 1 week, the vitality of *Parmelia saxatilis* was strongly affected. This was indicated by a twofold dark respiration and an irreversible decrease of CO₂ intake in light. The photosynthetic rate of thalli of *Rhizocarpon* recovered to only 10% and respiration decreased to 40%, after 3 weeks of immersion. Even permanent high air humidity resulted in a drop in the photosynthetic rate (O₂ evolution) of *Hypogymnia physodes* (Pearson and Skye, 1965).

The reasons for the markedly different resistance of lichens are to a large extent unknown. The thalli of some sensitive forms became infiltrated and slimy after treatment. It is remarkable that even the algal cells died since in separate culture they can grow without difficulty in liquids. *Rhizocarpon* and others were found to be more tolerant to submersion in cold water. This suggests a change in temperature-dependent metabolic processes, e.g., a strongly increased respiration that leads to injury and also may explain Jaag's (1945) observation that *Collema* species are found at moister places with increased elevation in the mountains.

3. MORPHOLOGICAL ADAPTATIONS TO MOIST HABITATS

Heavy soaking of the thalli caused an immediate lowering of the photosynthetic rate of several epiphytic lichens. Stocker (1927) with *Lasallia*

pustulata and *Lobaria pulmonaria* and Stålfelt (1939b) with *Ramalina farinacea* and *Usnea dasypoga* observed depressions of net photosynthesis above 70–75% of their maximum water contents. Neubauer (1938) also reported a depression of the CO₂ intake of *Pseudevernia furfuracea* which resulted from heavy soaking of the thalli; this was not the case with *Parmelia caperata* and *P. olivacea*. This feature obviously depends on the structure of the lichen thallus (Ried, 1960c). *Umbilicaria cylindrica* (with optimum photosynthesis at a water content of 65%), *U. polyphylla* (73–80%), and *Rhizocarpon geographicum* (approximately 70%) have either a very dense cortex or a very compact thallus which when saturated hinder CO₂ diffusion to the phycobiont. Consequently, very moist conditions can lead to a depression of growth.

Because *Peltigera canina* and other species of *Peltigera* (Smyth, 1934; Ellée, 1938; Butin, 1954) and *Parmelia caperata* (Neubauer, 1938) have a loose thallus structure, CO₂ exchange can occur when they are fully soaked. Scott (1960), however, found that in fully soaked thalli of *Peltigera praetextata* gas diffusion was reduced and only took place between the loosely woven hyphae of the medulla, where actual growth was observed. The aquatic lichens, *Verrucaria silicea* and *Porina lectissima*, have a dense structure but the phycobionts are only covered by a thin cortex. Thus, gas exchange cannot be hindered through soaking of the thallus. The difference in CO₂ intake between *Rhizocarpon geographicum* and *Lecidea soredizoides* at strong water soaking cannot, however, be explained by differences in thallus structure (Ried, 1960c). Büttner (1971) found that the net photosynthesis depression by heavy soaking in lichens was due to an increase of respiration.

With *Ramalina maciformis* Lange (1969) could not detect any influence of strong soaking on the gas exchange. He interpreted this property as advantageous for a desert lichen. Dughi (1939b) stated that lichens from more or less humid places in arid regions must be as tolerant to heavy soaking as they are to strong drought. This characteristic would be important also for lichens living under very changeable water conditions as in granite kopjes in South Africa (Scott, 1967).

Epiphytic lichens such as members of the Usneaceae and *Pseudevernia* are able to avoid heavy soaking by rainwater to a certain degree by repelling it from their sorediate surfaces or incrustations and letting the water quickly run off from the hanging thalli (Zukal, 1896; Klement, 1955). Thus, these lichens are well adapted to live in extremely wet, rainy regions. Water repellency was observed also with crustose lichens on tree bark and on rocks (Schindler, 1935; Butin, 1954). Leprose lichens like *Lecidea orosthea*, *Biatora lucida*, *Chaenotheca arenaria* and other species of the Calicium viridis alliance are apparently ombrophilous because they grow under overhanging rocks. This behavior must, however, be interpreted as reflecting their requirement for the high air moisture that is found in such habitats (Schade, 1913; Schindler, 1935; Kalb, 1970).

C. Response to Low Temperatures and Freezing

From habitat analysis it has been concluded frequently that lichens are very tolerant to low temperatures. Sensitivity, however, was presumed in some *Cladina* species (Ahti, 1961). Nevertheless, for a long time there was little experimental data to support the view that lichens are resistant to cold (Steiner, 1965). Freezing injury in living plants is due less to a temperature drop than to the freezing of water in the tissues. If vascular plants are cooled in spring and summer, when they are highly active, water easily freezes inside the cells, with lethal effects. Dehydration by freezing also is of immediate harm (cf. Levitt, 1966). In autumn perennial plants undergo a hardening process which enables them to prevent ice formation in the cells. The plants gradually supply water to intercellular spaces where its freezing does not affect the cytoplasmic structure. Presumably, plants tolerant to severe desiccation are extremely resistant to freezing. However, this assumption cannot be generalized because some vascular plants with considerable desiccation resistance are relatively sensitive to frost in winter (Kappen, 1966).

1. RESISTANCE IN THE DESICCATED STATE

If plants are desiccated their frost resistance increases considerably. In this sense resistance to cold is a function of desiccation tolerance. Becquerel (1936) cooled samples of *Xanthoria parietina* to slightly above absolute zero for 2 hours after the thalli were dried over CaCl_2 and under slight vacuum. He found no damage 3 hours after the treatment when he compared, microscopically, the tested thalli with the controls. This result agrees with those obtained with seeds and with other poikilohydric organisms such as fungi, bacteria, moss protonemata (Lipman, 1936, 1937), free-living algae (Becquerel, 1950), and the phycobiont *Trebouxia erici* from *Cladonia cristatella* (Basa and Hawrylewicz, 1962). After cooling of the desiccated thalli to the temperature of liquid nitrogen (-196°C), *Ramalina maciformis*, *Umbilicaria vellea*, and *Roccella fucoides* showed normal CO_2 intake almost immediately after rewetting at 10°C and remained active for several weeks thereafter (Kappen and Lange, 1972).

2. RESISTANCE IN THE HYDRATED STATE

Free-living fungi and algae show dissimilar cold resistance if they are frozen while hydrated and active. The aerial alga *Protococcus* dies at -15°C and is replaced by the more resistant (lower than -20°C) *Prasiola crispa* on its natural stand (Knebel, 1936). Populations of *Chlorella pyrenoidosa* were strongly affected by -30°C (Holm-Hansen, 1963; cf. also Siegel *et al.*, 1969). Mycelia from asco- and basidiomycetes and soaked fungal conidia

were killed by temperatures between -10° and -30°C (Bartetzko, 1910; Lindner, 1915; Mazur 1966). Kärcher (1931) reported that cultures of two green algal species and ten fungi survived cooling in liquid nitrogen. But she gave no information about the water relations of the cultures (cf. Lipman, 1937). The mycobiont *Lasallia papulosa* survived a temperature of around -196°C while in a liquid (protective?) substratum (Mish, 1953, cited by Ahmadjian, 1967).

In spring the arctic lichens *Cetraria delisei* and *C. richardsonii* respiration almost normally, after the fresh thalli had been exposed to liquid oxygen (-183°C) for 18 hours. *Dactylina arctica* and *Thamnolia vermicularis* showed respiration reduced to one-third (Scholander *et al.*, 1953). This may reveal a very high resistance at least of the two *Cetraria* species. However, the authors were not sure whether their means of determining the viability of the lichens was sufficient.

Lange (1966) found that thalli of *Cladonia alcicornis* collected in central Europe in the summer and frozen in the fully soaked state to -15°C , withstood continuous deep-freeze storage at this temperature for nearly 2 years

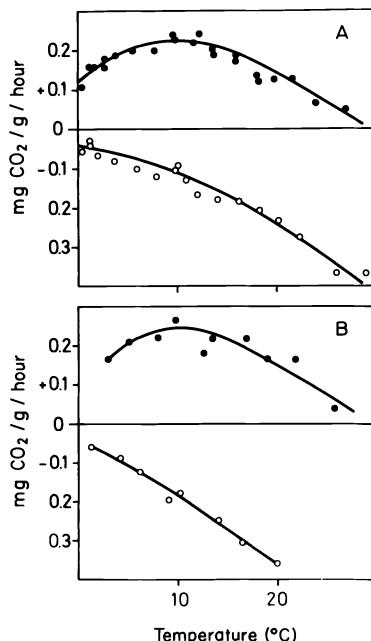


FIG. 7. CO_2 exchange in the light (●—) and in the dark (○—) of *Cladonia alcicornis* in relation to the temperature of the thalli before (A) and after storage at -15°C for 96 weeks (B). Each measurement was carried out after the thalli had been conditioned at 10°C for 20 hours. (From Lange, 1966.)

(Fig. 7). Gannutz found that antarctic lichens already were damaged after a ten-day period at -17°C at Palmer station (cf. Ahmadjian, 1970).

According to an extensive study of rates of the calculated gross photosynthesis and dark respiration before and for a few weeks after cold treatment, several species were found to be resistant to gradual ($15^{\circ}\text{C}/\text{hour}$) freezing of the soaked thalli even to the temperature of liquid N_2 (see Fig. 2). Care was taken to see that they were actually frozen and not just super-cooled. High tolerance was evident with species from Antarctica, showing that they are well adjusted to that environment (Lange and Kappen, 1972), but also with species from Europe (Fig. 2) and even with *Ramalina maciformis* from the Negev desert where temperatures seldom drop below -7°C (Kappen and Lange, 1972). It must be pointed out that these lichens were mostly collected and tested in spring or autumn, when they are probably the most active (cf. Smith, 1962). During these seasons they could hardly be cold-hardened, a state which enables higher plants to withstand temperatures around -10° to -30°C , irrespective of prefreezing processes (cf. Sakai, 1958; Krasavtsev, 1961).

These results corroborate earlier findings with 23 lichen species from various habitats in the world (Kappen and Lange, 1970a). After a cold treatment at around -78°C (being slowly frozen, $15^{\circ}\text{C}/\text{hour}$, finally transferred to solid CO_2 , and then slowly rewarmed), fifteen of them showed normal res-

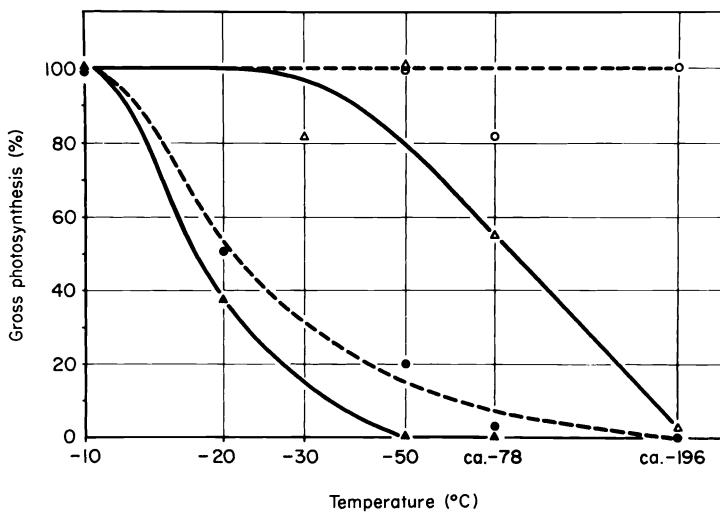


FIG. 8. Freezing tolerance of soaked thalli of *Umbilicaria vellea*, indicated by maximum restoration of gross photosynthesis in the postculture (% of normal). The thalli were cooled and rewarmed rapidly (—), and gradually (---) in September (● ▲) and in December (○ △). Gross photosynthetic rates were calculated by summing up the rates of apparent photosynthesis and 50% of the dark respiration. (From Kappen and Lange, 1972.)

piration and no difference in growth between algae of the tested thalli inoculated on agar and those of controls. Among the resistant lichens there were five species from tropical mountain forests (1100–1600 m) in Argentina. Several lichens from temperate regions were less tolerant, i.e., *Aleurotricha sarmentosa* and *Usnea dasypoga*, which require environments of high humidity and are sensitive to long desiccation periods. In addition, *Cladonia convoluta*, *Roccella fucoides*, and *Sticta marginifera* collected in regions with mild climates showed abnormal respiration and no full recovery of their photosynthesis when exposed to temperatures lower than -50°C (Kappen and Lange, 1970b, 1972). *Umbilicaria vellea*, which comes from an exposed mountainous habitat in Central Europe, is surprisingly tender (Fig. 8). Repeated tests in December showed a strong increase in resistance, which at that time was as high as that of other European lichens. This species is the only one which has shown a seasonal change in freezing tolerance. According to Kallio and Heinonen (1971), in the Arctic this species lives at places where temperature minima of -50°C in winter can occur. In the vegetation period, however, after chilling to -28°C , *U. vellea* respiration strongly and did not recover within 2 days.

A more sensitive reaction of the lichens was generally found when the soaked thalli were exposed immediately to -10° , -20° , ..., or -196°C for 24 or 6 hours (Kappen and Lange, 1970b, 1972). The photosynthetic rate of most of the lichens was irreversibly depressed at -196°C (see Fig. 9) with the exception of *Xanthoria elegans* and *Lecanora melanophthalma* from Antarctica. Fresh thalli of *Cladonia rangiferina* collected from New York

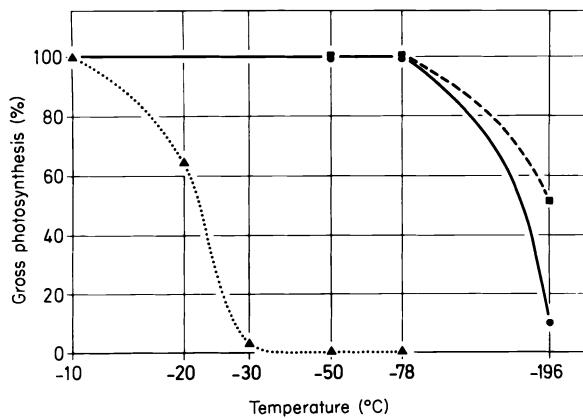


FIG. 9. Freezing tolerance of soaked thalli of 3 lichen species, indicated by maximum restoration of gross photosynthesis in the postculture (% of normal). The thalli were cooled rapidly. ●—●, *Cladonia rangiferina*; ■---■, *Ramalina maciformis*; ▲...▲, *Roccella fucoides*. (After Kappen and Lange 1972.)

State were damaged by immersion in liquid N₂ (Siegel and Daly, 1968), but after this cold treatment the thalli were immediately put into boiling water, thus complicating interpretation of the results.

3. FACTORS UNDERLYING THE FREEZING TOLERANCE OF LICHENS

The reason why many lichens are tolerant to freezing is still a matter of speculation. Gannutz (1970) feels that antarctic lichens remain unfrozen in their natural habitat at -10°C because of their high content of chemical compounds. The high content of carbohydrates and polyols (Hale, 1967) also may be considered. However, Lewis and Smith (1967) doubt whether these substances are effective in this way because lichens accumulate them more during the summer and less in winter. *Roccella fucoides*, one of the species most sensitive to freezing, is rich in sugar alcohols (Shibata, 1963; Feige, 1970).

The reason why lichens were damaged at -196°C when rapidly cooled, but were not damaged when gradually cooled, was investigated with *Ramalina maciformis* by different combinations of cooling and rewarming gradients (Table I). Gradual cooling combined with rapid or gradual rewarming was always harmless. Rapid cooling was deleterious combined with slow as well as with rapid rewarming to +10°C. Rapid cooling and rewarming to +34°C (by quick transfer into a water bath) was harmless. This means that by fast transition of tissues to the temperature of liquid N₂ the water in the cells becomes undercooled or more likely vitrified during the 6 hours of exposure and has no damaging effect. Also, it is necessary that the rewarm-

TABLE I
RATES OF GROSS PHOTOSYNTHESIS AND DARK RESPIRATION IN % OF NORMAL VALUES
AFTER EXPOSURE OF SOAKED THALLI OF *Ramalina maciformis* TO -196°C,
COMBINATIONS OF COOLING AND REWARMING RATES ARE VARIED^a

Cooling rate	Rewarming rate	Gross photosynthesis (% of normal)	Dark respiration (% of normal)
Gradual	Rapid to +10°C	102	132
Gradual	Gradual to +10°C	93	93
Rapid	Rapid to +34°C (water thermostat)	100	~120
Rapid	Rapid to +10°C	42	104
Rapid	Gradual to +10°C	2.3	130
Rapid	Rapid to -10°C and after 1/2 hour to +34°C	1.2	48

^a After Kappen and Lange (1972).

ing velocity is fast enough to pass the temperature range between – 100 and 0°C where ice crystals are formed which disrupt the fine-structure of the cells. Direct cooling to – 78°C and gradual cooling of 15°C/hour may still allow extracellular freezing in the lichens. Obviously, they can more easily release water out of their cells than higher plants when they freeze. This must be due to the lichens' higher membrane permeability. The question arises as to what extent the permeability is caused by factors such as lichen acids (Follmann and Villagran, 1965). The fact that symbionts have small vacuoles and elastic cell walls may also be a reason for the high freezing tolerance of lichens.

4. METABOLIC ADAPTATIONS TO COLD ENVIRONMENTS

The metabolism of most lichens investigated to date seems to be adapted better to cool or temperate conditions rather than to very warm conditions. Extremely cold-resistant lichens have a photosynthetic capacity (as indicated by the gross photosynthetic rate) that is fully reactivated within a few hours after a severe cold stress (Kappen and Lange, 1970b, 1972; Kallio and Heinonen, 1971). Lichens from various habitats in the world are able to photosynthesize at temperatures far below zero (Lange, 1962, 1965a). *Usnea livida* from the tropical rain forest in the Congo showed apparent CO₂ intake down to – 8°C and *Parmelia africana* from the same stand down to – 12°C. *Cladonia alcicornis* took in CO₂ at the lowest studied temperature (– 24°C). This temperature corresponds to an osmotic potential of about – 290 atmospheres. The ability to take in CO₂ was comparatively less in species from Antarctica (*Neurolepon acromelanus* to – 18.5°C; Lange and Kappen 1972) or from the Alps (*Cladonia elongata* to – 13°C). Some species from the Arctic showed apparent CO₂ intake down to temperatures of – 5° to – 15°C, *Cetraria nivalis* even down to – 20°C (Kallio and Heinonen, 1971). The results confirm earlier uncertain and methodologically unreliable results of Jumelle (1892) with *Evernia prunastri* (photosynthesis still at – 40°C) and of Henrici (1921), who reported activity of assimilation and respiration in several species down to – 16°C. The fact that such CO₂ intake reading is reliable was tested by incorporation with ¹⁴CO₂ (Lange and Metzner, 1965). At – 11°C chemical fixation of ¹⁴C in the light was observed with *Cladonia alcicornis* and *Stereocaulon alpinum*. Biosynthesis of chlorophylls and β-carotenes was observed with *Hypogymnia physodes* at – 7°C (Godnev *et al.*, 1966).

The amount of water frozen in the thallus must be very small or a coherent ice crust will form that would decrease the CO₂ intake (Lange and Kappen, 1972). Perhaps that limitation is more applicable to species like *Peltigera subcanina* which holds much water. In addition, *Trebouxia* cells are active while extracellular freezing occurs (Lange and Bertsch, 1965).

Findings from the laboratory were confirmed in the field with *Hypogymnia physodes*. Using the conditioned gas-exchange chamber, Schulze and Lange (1968) showed that even at -6°C the thalli had a positive photosynthetic balance. Although the absolute assimilation rates below freezing temperatures are small, they may be of importance for lichens in polar environments (Lange and Kappen, 1972). The possible value of photosynthesis far below zero for the survival of lichens has been discussed by Levitt (1967) with respect to his SH-SS hypothesis of freezing injury as a means of maintaining a high reduction capacity.

In general, respiration survives lower temperatures better than photosynthesis (Lange, 1965a). Respiration of two arctic species (*Dactylina arctica* and *Thamnolia vermicularis*) was still measurable at -26°C (Scholander *et al.*, 1952, 1953). These authors did not find any significant differences in respiratory activity between arctic and tropical lichens (with the exception of *Sticta* and *Peltigera* species)—similar conclusions were made by Ahmadjian (1965) with isolated symbionts. The data of Bliss and Hadley (1964) and Bliss (1966) showed that net photosynthetic rates of *Cetraria nivalis*, *C. islandica*, and *Cladonia rangiferina* were optimal at low temperatures and thus fit well to the alpine environment of Mt. Washington. Gannutz (1969) mentioned that the temperature ranges of net photosynthesis of the antarctic *Xanthoria mawsonii* were optimal between -2° and $+14^{\circ}\text{C}$ and were conspicuously different from those of lichens from moderate and tropical regions (highest rates between $+10^{\circ}$ and $+32^{\circ}\text{C}$). With respect to gross photosynthesis, Lange and Kappen (1972) did not find any differences between antarctic, tropical, and hot desert species. The basic difference is that lichens from cold climates show higher respiration rates with increasing temperatures than those from moderate and warm climates, a phenomenon also known from phanerogams (Wager, 1941, James, 1953). This is illustrated by comparing the quotients (Stålfelt, 1939b) between maximum apparent CO_2 intake (at 10 klux) and the dark respiration of the same thallus at 20°C (see Table II) (Lange, 1965a; Lange and Kappen, 1972). Consequently, lichens from cold regions have optima of net photosynthesis near or below 0°C at low light intensities (*Neuropogon acromelanus* and *Lecanora melanophthalma*).

The high freezing tolerance of many lichens cannot be the reason for their restricted distribution over the world. As is evident from their photosynthetic activity, the phycobionts have a wide range of adaptation to cold environments. The metabolic nature of the mycobionts is probably a decisive reason for the distribution and adjustment of lichen species in extreme environments. Our knowledge about the response of lichens to cold stress is, however, based on a selected list of tested species. It may be that fast-growing species with a short life cycle (like some terricolous lichens or even

TABLE II
QUOTIENT BETWEEN MAXIMUM APPARENT CO₂ UPTAKE AT 10 klux AND DARK RESPIRATION AT 20°C WITH LICHENS FROM DIFFERENT REGIONS^a

Species	Region	Quotient
<i>Ramalina maciformis</i>	Hot desert	1.80
<i>Parmelia melanothrix</i>	Tropical region	1.21
<i>Parmelia tinctorum</i>	Tropical region	1.10
<i>Usnea pseudocyphellata</i>	Tropical region	0.87
<i>Cladonia alcicornis</i>	Temperate region	0.70
<i>Stereocaulon alpinum</i>	Alpine region	0.60
<i>Letharia vulpina</i>	Alpine region	0.58
<i>Xanthoria mawsonei</i>	Antarctic region	0.19
<i>Neuropogon acromelanus</i>	Antarctic region	0.17
<i>Lecanora melanophthalma</i>	Antarctic region	0.08

^aAfter Lange and Kappen (1972); and O. L. Lange (unpublished data).

folicolous lichens) represent a more sensitive type, especially those living in symbiosis with *Trentepohlia*.

5. OBSERVATIONS OF COLD RESISTANCE IN NATURAL HABITATS

Frost injury of lichens in their natural habitats has been rarely reported (Barkman, 1958) but this does not exclude its occurrence. After a severe winter (1962) in southeastern England, Laundon (1966) found thalli of *Parmelia caperata* totally bleached or discolored in the marginal zone. This must be exclusively due to winter killing and not to toxic damage because *Ramalina farinacea* and *Usnea* sp. growing in the same place were unharmed. Two years later the recovery of the lichen colony was observed.

The epiphytic lichens of the association *Parmeliopsidetum ambiguae* in subarctic regions are dependent on the shelter of snow. This was interpreted as psychrophobia (Barkman, 1954) because in moderate climates this association is not dependent on a long-lasting snow cover. The same reason probably applies to "snow-level" lichens (*Stereocauletum alpinae* and *Rhizocarpetum alpicola*) (Klement, 1955). Chionophilous species belonging to the lichen communities such as the *Ochrolechion tartareae* (Klement, 1955) and *Caloplacectum nivalis* are rarely free from snow for more than 2 months a year (Kalb, 1970).

D. Response to High Temperatures

In natural habitats high temperatures increase evaporation. Thus, it is difficult to decide whether a lichen responds to heat or to desiccation, etc. But the question can be answered by experimental investigation of the lichens or their symbionts under controlled conditions.

According to Thomas (1939), spores of *Xanthoria parietina* did not germinate at temperatures higher than 24°C. The symbionts of the fourteen species he investigated died at temperatures ranging between 21° and 30°C, with the average at 27°C. In most cases the phycobiont was somewhat more sensitive than the mycobiont. In some lichen species both symbionts were equally sensitive. The phycobiont of *Icmadophila ericetorum* tolerated a warmer temperature regime than the mycobiont. This also was found by Henriksson (1964) with the symbionts of *Collema* at 25°C. The mycobiont *Acarospora fuscata* under temperature conditions between 26° and 28°C produced a brown pigment instead of red as at lower temperatures. Under strong irradiation the pigment seems to become oxidized (Ahmadjian, 1961). Thalli of *Ramalina farinacea* and *Usnea dasypoga* showed irreversible depression of their CO₂ assimilation rate at a temperature warmer than 25°C (Stålfelt, 1939a).

1. HEAT RESISTANCE OF "SATURATED" THALLI

The degree of heat tolerance (determined by a standard method) does not directly reflect the injurious temperature in the natural habitat, because heat stress is greatly dependent on the period of high temperature in contrast to cold stress. Thus, heat resistance determined by experiments mainly illustrates the behavior of the species in relation to each other. A very detailed analysis of the heat tolerance of lichens was given by Lange (1953, 1965b). According to the viability of the phycobionts cultivated after a 30-minute heat treatment, the heat tolerance of the fully soaked thalli was found to vary between 35° and 43°C. Heat tolerance as indicated by respiratory activity, which is predominantly due to the mycobiont, was similar or a little higher (Table III). The highest tolerance (46.5°C for 30 minutes)

TABLE III
LIMITS OF HEAT RESISTANCE (°C) AFTER 30-MINUTES EXPOSURE OF THE THALLI
IN THE SOAKED AND IN THE DRY STATE, INDICATION BY TWO DIFFERENT
CRITERIA^a

Species	Heat resistance (°C) indicated by			
	Vitality of cultivated phycobionts (c.25% growth)		Rate of respiration of the thallus (reduced to 50%)	
	Soaked	Dry	Soaked	Dry
<i>Lobaria pulmonaria</i>	35.0	70.0	36.5	78.0
<i>Cladonia alpestris</i>	~44.0	80.0	43.0	80.0
<i>Umbilicaria vellea</i>	42.5	100.0	44.0	100.0

^a After Lange (1953).

was observed with *Cladonia rangiformis* var. *pungens* which was collected from a highly insolated place.

More recently, Lange (1965b) measured the photosynthetic reaction of lichens after a 60-minute heat treatment in the soaked state and confirmed his earlier observations (Fig. 10). It is remarkable that a lichen from the hot desert shows no greater tolerance to heat than a species from a moderate climatic region (cf. also Rogers, 1971). The heat tolerance of a marine *Verrucaria* species (Ried, 1969) is in the same order as the above-mentioned terrestrial species. Compared to fully turgid leaves of higher plants in the summer state, the heat tolerance of lichens is low.

2. HEAT RESISTANCE OF DESICCATED THALLI

In the air-dried state most lichens are extremely heat tolerant. This tendency was already suggested by the results with cultures of 20 mycobionts (Thomas, 1939). They continued growing on agar after being exposed for 60 hours to 45°C when desiccated. In contrast, the undried samples died under these temperature conditions. Desiccated thalli of *Teloschistes flavicans* from South Africa tolerated 3 days of continuous heating at 60°C but not at 98°C (Cuthbert, 1934).

The species specific differences of heat resistance (Table III) can be shown with dried as well as with soaked thalli, in contrast to the uniform behavior of dried lichens at very low temperatures. The limits of tolerance, as indicated by respiratory activity, were much higher with dry lichens and ranged

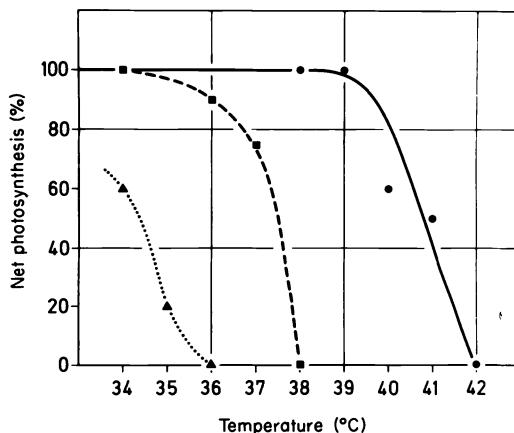


FIG. 10. Heat tolerance of soaked thalli of 3 lichen species, indicated by maximum restoration of rates of apparent CO₂ intake (% of normal) 2 weeks after a heat treatment for 60 minutes. ●—●, *Cladonia rangiferina*; ■---■, *Ramalina maciformis*; ▲···▲, *Roccella fucoides*. (Adapted from Lange, 1965b.)

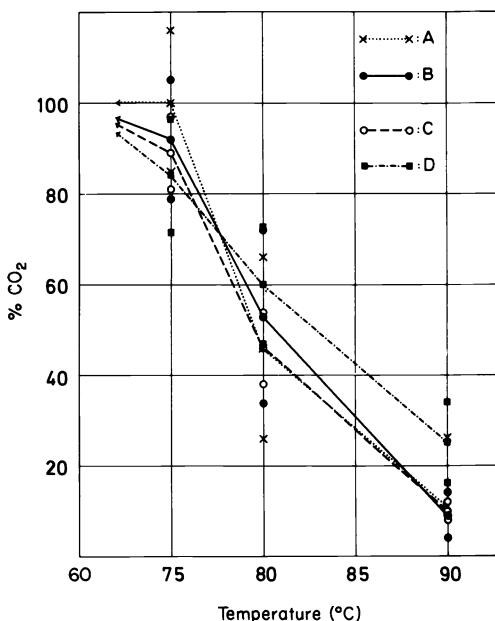


FIG. 11. Heat tolerance of *Cladonia alpestris* from various areas in Europe. The tolerance is indicated by the rate of CO₂ evolution (% of controls) 24 hours after a heat treatment for 30 minutes at different temperatures. (A) Bayerischer Wald (Germany), (B) Stockholmer Schärenhof (Sweden), (C) Rhön (Germany), (D) Jämtland (Sweden). (From Lange, 1953.)

from 70° to 101°C (Lange, 1953). The same specific heat tolerance was found (Lange, 1953) (Fig. 11) with different samples of the same species even from completely different areas. Consequently, it was evident that in Europe lichens are found in characteristically selected habitats. Different lichen communities could be distinguished by the difference in the heat tolerance of their species (Fig. 12). Those of the lichen community *Fulgensietum continentale*, characteristic of open, dry, and hot places in southwest Germany, tolerate up to 100°C in contrast to those of the mountaneous epiphytes (*Usneetum barbatae*) which were already severely damaged at around 75°C. On the other hand, the high heat tolerance (Lange, 1953), as measured with dry thalli, of hygrophilous *Umbilicariaceae* may appear surprising. But it must be realized that the thalli of these species can be severely overheated by the sun because of their dark color. The high heat tolerance of *Dermatocarpon fluviale* may be reasonable, since its habitat undergoes changes between cool-moist and dry-warm periods (cf. Lange, 1953).

Nifontova (1967) investigated the influence of heat on the metabolism of *Cladonia alpestris* and *C. amaurocraea* by measuring the ¹⁴CO₂ fixation rate 20 minutes after the treatment. After heating up to 200°C for 4.5 hours, the dry thalli did not completely lose their ability to fix ¹⁴CO₂ but their fixation

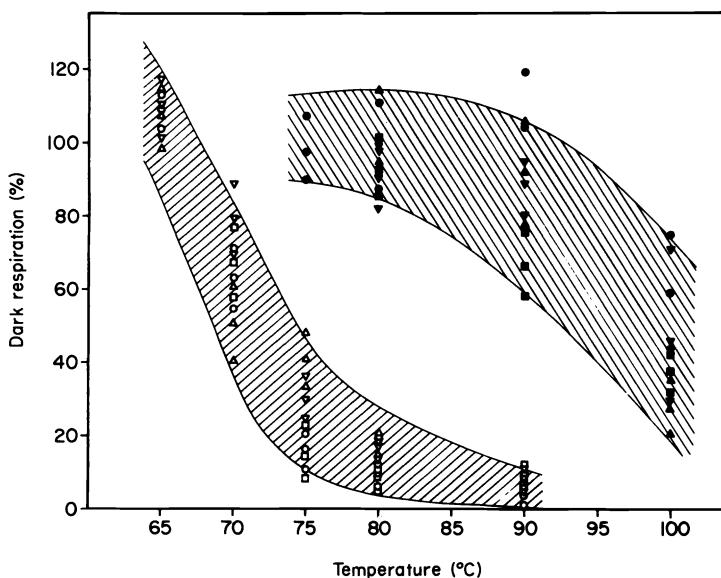


FIG. 12. Heat resistance of species of 2 lichen associations, indicated by rates of CO_2 release (% of the controls) after heat treatment at different temperatures for 30 minutes. *Usneetum barbatae*: Δ , *Alectoria sarmentosa*; ∇ , *Evernia prunastri*; \circ , *Usnea dasypoga*; \square , *Ramalina farinacea*. *Fulgensietum continentale*: \bullet , *Cladonia pyxidata* var. *pocillum*; \blacktriangle , *Cladonia rangiformis* var. *pungens* f. *foliosa*; \blacktriangledown , *Cladonia rangiformis* var. *pungens*; \blacksquare , *Cladonia convoluta*. (After Lange, 1953.)

rate was only 1% that of the controls. When exposed to 50°C, their fixation rate decreased to one-third of that of the controls. Among the partial processes of carbohydrate synthesis, mannitol synthesis proved to be most sensitive to heat stress, although it was not irreversibly damaged. According to Harley and Smith (1956), the ability to produce reducing sugars was not lost, but was raised even after 18 hours of heating at 75°C. The ability to take in sugar decreased strongly with predried thalli and ceased with moist-heated thalli.

A long period (9 weeks) of permanently moist culture induces loss of heat tolerance in isolated phycobionts. Phycobionts of *Cladonia convoluta* tolerated only up to 70°C after culture instead of 90°C when freshly isolated from the thallus. Lange (1953) discussed the possible ecological importance of this effect with respect to long rain periods that would make the algae more heat-sensitive.

3. HEAT STRESS IN NATURAL HABITATS

Many lichen habitats are open with only a scattered or dense but thin cover of cryptogams and in this respect represent extreme environments

even in moderate climates. Since Zopf (1890), a great number of measurements of temperature maxima in poikilohydric plants were carried out by many authors (cf. Lange, 1953). The temperatures in exposed places in temperate climates range between 50° and 60°C in crustose and foliose lichens. Von Kerner (1913) pointed out that temperatures above 58°C occurred for several hours in crustose thalli in the karst of Istria. In southwest Germany thalli of *Cladonia pyxidata* in their natural habitat were heated up to 66°C after 205 minutes. These conditions are injurious for dry thalli of some epiphytic species. Temperatures of around 53°C normally occur there not only for hours but days if one sums up the time over the hot vegetation period (Lange, 1953). In the deserts, thallus temperatures climb to over 60°C (Lange, 1953, 1965b) but not much higher. Even in Antarctica exposed lichen thalli were heated up to 32°C in summer (Rudolph, 1966).

Although there may have been methodological difficulties in the determination of temperature in earlier studies, it can be assumed that they all showed an intense heating of the lichens. With models Lange (1954) demonstrated (thermocouple measurements) that thalli fixed on cork warmed up more (56.9°C) than those fixed on sandstone (52.1°C) under the same intensive radiation conditions at an air temperature of 26.2°C. The measurement of the temperatures on the soil surface and in different heights of the podetia of *Cladonia furcata* var. *palamaea* are of special interest (Fig. 13) with regard to the temperature stress of the lichen. Some of the thalli in contact with the soil surface warmed up to 69.6°C. When a fruticose thallus becomes detached from the ground and remains fixed only in the lichen cushion the heat stress diminishes considerably.

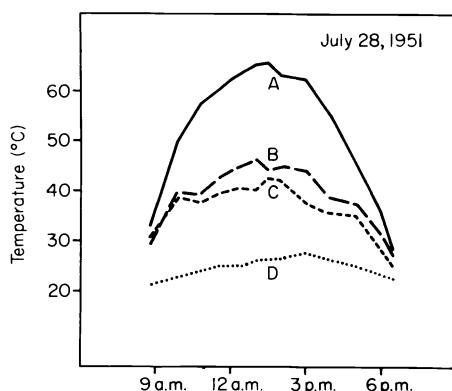


FIG. 13. Temperature course in the thalli of *Cladonia furcata* var. *palamaea* near the soil surface and in different heights of the podetia. (A) near soil surface, (B) 1 cm above, (C) 4 cm above, (D) air temperature. The measurements with thermocouples were carried out in the Kaiserstuhl (southwest Germany) on the plant association Xerobrometum. (From Lange, 1954.)

In a water-“saturated” condition, none of the tested lichens could resist these temperature stresses. Their existence is possible only because of their ability to desiccate quickly and intensively when exposed to the sun. However, Follmann (1965b) suggested that the abundance of protein and lichenin in lichens from the Atacama desert helps them to maintain their water content even under intense insolation. Such conclusions (Herre, 1911; Magnusson, 1940) seem to be paradoxical not only with respect to heat resistance but also considering that the net photosynthetic rate strongly decreases with increasing temperatures. Above approximately 30°C, respiration, even of a desert lichen, is so high that only CO₂ loss could be observed (Lange *et al.*, 1970a). This was demonstrated in the laboratory as well as in the field (Fig. 14). Thus, a prolongation of imbibition combined with an increase of insolation is unfavorable for lichens.

Heat damage in natural habitats may occur through a sudden change from a rain shower to bright sunlight. Heat injury may be indicated by damaged forms of *Cladonia* in wet places of a bog, where the water temperature rises considerably (cf. Lange, 1965b). Irrigated rocks or soil gutters, which are strongly insulated, are mostly bare of lichens or carry exclusively blue-green algae and a few cyanophilous lichens. The dark thalli of *Ephebe lanata* and others on these places are threatened by heat damage (Wirth, 1972). But it must be proved whether these cyanophilous lichens are more heat

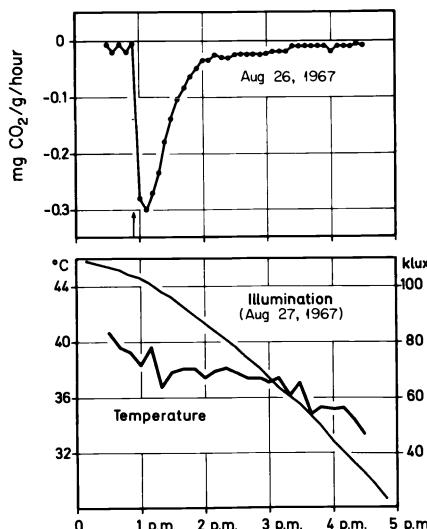


FIG. 14. CO₂ exchange of *Ramalina maciformis* after spraying the thalli with water (↑) under their natural environmental conditions in the Negev desert. In the curves below, the courses of temperatures and illumination are indicated. The illumination conditions on the 26th were just the same as on the 27th of August. (After Lange *et al.*, 1970a.)

tolerant or not. Lange (1965b) concluded that lichens from moist places in tropical rain forests must be especially resistant to high temperatures when they are in the hydrated state.

In the Hawaii Volcanoes National Park, *Cladonia cf. rangiferina*, *Cladonia cristatella*, and *Stereocaulon vulcani* were found occurring on hot volcanic soils near steaming vents. The thalli were soft and jellylike at the base and dry above (Fosberg and Lamoureux, 1966). While the thalli of the latter species were apparently damaged by heat, other samples were more recently reported to exist in this area (south of Puuhimau Crater) (Mueller-Dombois, personal communication). They occurred together with *Stereocaulon ramulosum* and *Cladonia oceanica* var. *descendens* in an open *Adropogon* community scattered on volcanic soils with temperatures ranging between 35° and 45°C in the upper 1 cm, temperatures which normally are lethal within 1 hour for lichens. These lichens must either be specifically adapted to hot conditions or the temperature gradient to the soil surface must be very steep. Possibly both are relevant. The temperatures in the thalli were not measured.

The explorations of Trass (1963) inform us about thermophilous lichen life within a radius of 2 meters around hot-water springs (96°C) in a geyser valley of Kamchatka. All seven listed lichens were *Cladonia* species. Three of them are regarded as "optional" thermophiles. *Cladonia corallifera* and its formae *gracilescens* and *foliolosa* and *C. grayi* f. *fasciculata* live preponderantly in thermal environments (optional-obligatory th.), and *C. vulcani* is an obligatory thermophilous lichen. The surface temperature of the soil on which *C. corallifera*, *C. scabriuscula*, and *C. pityrea* grew varied between 27° and 32°C. At a depth of 2 cm the temperature even rose to 32°–36°C. Other places with lichens had a surface temperature of 28°C.

E. Response to Visible Radiation

Radiation by the sun is an essential environmental factor for plant life. A great number of lichens live in extremely insolated habitats. Besides its direct illuminating influence, spectral energy affects the temperature and moisture conditions of a habitat. It is, therefore, often impossible to separate direct and indirect influences. Photophobic behavior is often a result of hygrophyly (Barkman, 1958; Rao and LeBlanc, 1965). The problem is whether illumination itself is a stress factor for lichens, how they respond to this influence, and whether low light intensities limit their existence.

1. INFLUENCE OF ILLUMINATION ON THE SEPARATE Symbionts

The intensity of illumination apparently has no influence on the growth of cultures of mycobionts (Thomas, 1939). However, mycobiont cultures of

Cladonia rangiferina in the light (10 klux) released about one-half the CO₂ that they evolved in the dark (Kappen and Lange, 1972).

Phycobionts, especially those of heavily pigmented lichen species, lose their color in cultivation when exposed to full summer sun (Warén, 1918–1919; Jaag, 1929) or as *Trebouxia decolorans* cease growing at 1.0 klux and die at 4.3 klux (Ahmadjian, 1959, 1962, 1967). Free-living green algae also were observed to avoid places of direct, strong insolation (Jaag, 1945; Vogel, 1955). But it is not clear to what extent illumination is a reason why algae live endolithically in rock fissures or under quartz particles.

2. PIGMENTATION AS A POSSIBLE RESPONSE TO STRONG ILLUMINATION

In the lichen thallus the phycobiont can generally be protected in two ways. Many lichens from open habitats such as rocks, especially in the less dusty atmosphere of the seashore and high mountains and less frequently in deserts, are strongly colored with yellow, red, brown, or black pigments. On heaths and bogs *Cladonia* species become black or brown colored apparently because of severe insolation. But thalli of scotophilous lichens are mostly green, gray-green, or at least greenish in the soaked state (Barkman, 1958).

Different coloring of the thalli was observed even with the same lichen species (*Parmelia saxatilis*, *Xanthoria parietina*) in relation to the illumination intensity of its habitat. Pigmentation has been interpreted as being a protection against irradiation (Bitter, 1901; Galløe, 1908; Laudi *et al.*, 1969). Ertl

TABLE IV
PERMEABILITY OF MORE AND LESS INTENSIVELY PIGMENTED LICHENS TO LIGHT, IN
RELATION TO THICKNESS OF CORTEX AND TO WATER IMBIBITION OF THE
THALLUS^a

	Relative illumination (%)	Thickness of cortex (soaked) (μm)	Permeability to light (%)	
			Thalli soaked	Air-dry thalli
Intensively pigmented				
<i>Nephroma laevigatum</i>	100	25	48	25
<i>Lasallia pustulata</i>	100	30	46	25
<i>Cetraria islandica</i>	100	35	56	28
Slightly or unpigmented				
<i>Lobaria pulmonaria</i>	—	30	73	50
<i>Evernia prunastri</i>	—	30	70	40
<i>Anaptychia ciliaris</i>	—	20	61	30

^a After Ertl (1951).

(1951) measured the light absorption of colored lichens in the fully soaked and in the dried states under strong illumination (Table IV) and showed that almost half of the light that reached the thallus was absorbed by the cortex. Tobler (1925a) stated from his observations with *Xanthoria parietina* that strong illumination induced a higher amount of parietin and a thicker layer of the phycobiont.

Rao and LeBlanc (1965) compared the fluorescence spectrum of lichen substances with the absorption spectrum of chlorophylls of *Parmelia caperata*, *Peltigera canina*, and *Xanthoria fallax*. They found that atranorin not only absorbed light of high intensity by quenching the incident radiation but also increased the use of light for photosynthesis at low light intensity by fluorescence. This can be of great importance in the early morning for desert and for polar lichens that live permanently under poor light conditions.

Pigmentation may protect the phycobionts from UV radiation, especially in lichens from high mountains (cf. Siple, 1938; Filson, 1966, cit. after Ahmadjian, 1970; Billings and Mooney, 1968). Siegel and Daly (1968) exposed thalli of *Cladonia rangiferina* to UV radiation (235 nm). After 12 hours of treatment, permeability to eosin Y did not change. Ultraviolet radiation in air caused a reversible threefold rise in CO₂ evolution. This single result may lead to an approximate estimation of the tolerance to radiation of the lichens at high altitudes or even under extraterrestrial conditions.

Some evidence about protection of algal chlorophylls is given by the following results: In contrast to Bruchet's (1959–1960) findings with *Trebouxia*, *Coccomyxa*, and *Hyalococcus*, De Nicola and Di Benedetto (1962) found different amounts of pigments between free-living algae and *Trebouxia albescens*. The nonlichenized *Trebouxia* contained more β-carotene but less chlorophyll and carotenoids than the phycobionts. Nevertheless, the chlorophyll–carotenoid ratio was better in the free-living *Trebouxia* than it was in the phycobionts, if one considers the fact that carotenoids play a role as protective pigments for algal chlorophylls.

Laudi *et al.* (1969) stated that *Trebouxia humicola*, presumably a free-living strain, would not be affected by strong illumination. However, isolated phycobionts of *Buellia punctata* and *Xanthoria parietina*, although not different in their chlorophyll content, did show changes in the lamellar system and in the pyrenoids (Fig. 15). The authors also tested the role of anthraquinones in protecting the chlorophyll of the phycobionts against photo-oxidation. Although they used filters with absorption maxima similar to the lichen pigments, they could not prevent bleaching of the phycobionts. It was suggested that the resistance of phycobionts to strong illumination was due to an exchange of compounds between mycobiont and phycobiont that resulted in structural changes of the pyrenoid.

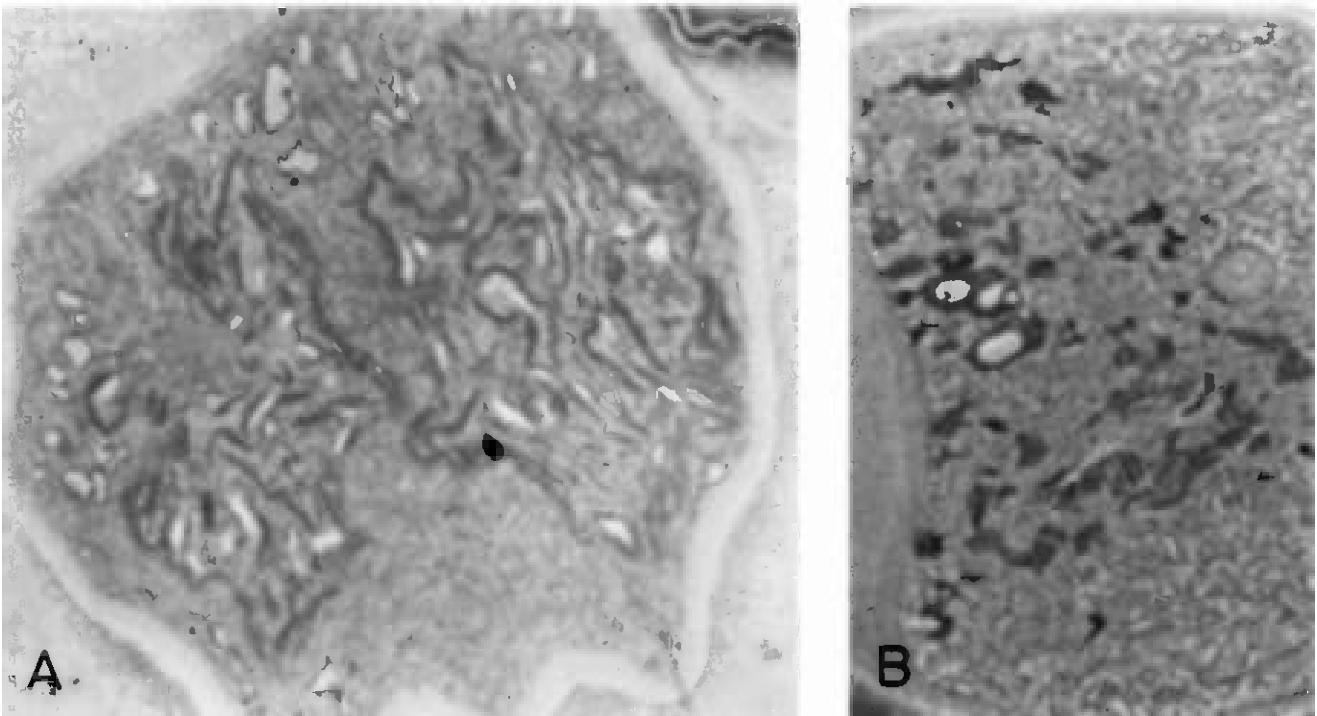


FIG. 15. Vegetative cells of *Trebouxia albulescens*; (A) unaffected vegetative cell, the arrangement of the lamellar system of the chloroplast and the grana formed by a limited number of thylakoids in the plastid stroma are visible ($\times 9200$). (B) vegetative cell from an achloric culture showing an advanced degeneration of the whole cytoplasm. The electron density of the lamellar system was strongly reduced by illumination with around 3000 lux. ($\times 10,000$). (From Laudi *et al.*, 1969.)

3. MORPHOLOGICAL AND OTHER ADAPTATIONS TO INTENSIVE ILLUMINATION

The cortex diameter has considerable influence on the light intensity reaching the alga. This has been stated by Zukal (1896) who observed that light intensity then is reduced almost to 10%. The same lichen species growing in full sunlight developed a cortex about twice as thick as that of a specimen in a shaded habitat (Bitter, 1901: *Hypogymnia physodes*; Tobler, 1925a: *Xanthoria parietina*; Galun, 1963: *Buellia canescens*; Looman, 1964: *Lecanora reptans*). Butin (1954) confirmed the effect of cortex thickening on the photosynthesis of lichens. Ellée (1938) reported a relationship between light permeability of the thallus and scotophily of the lichens *Lobaria pulmonaria* and *Peltigera rufescens*.

The extended investigations of Ertl (1951) demonstrated a clear relationship between cortex thickness and the environmental conditions of the thallus. Table V shows that when desiccation proceeds in the thalli, the protective effect against illumination rapidly increases (cf. Stocker, 1927; Ellée, 1938). This may be a very significant adaptation especially for lichens in bright desert and alpine habitats.

Morphological adaptations are very pronounced in lichens from desert stands (Fink, 1909; Fünfstück, 1926). According to Vogel (1955), two types of adaptations can be distinguished in the extreme districts of the little Karroo in South Africa. The first type is represented by species of *Endocarpon*, *Lecidea*, and *Toninia*. The small crustose thalli develop a cortex up to six times thicker than that of European species in sunny habitats. Besides this, their algal layer is very compact and two–three times thicker than that of European foliose species (Table VI).

TABLE V
COMPARISON OF THE RELATIONS BETWEEN THICKNESS OF THE CORTEX, WATER IMBIBITION, AND PERMEABILITY TO LIGHT IN DIFFERING THALLI OF THE SAME SPECIES FROM LIGHT AND FROM SHADY HABITATS^a

Species	Thickness of cortex (μm) imbibed with water		Soaked thalli (μm)		Air-dry thalli (μm)	
	From light habitat	From shady habitat	From light habitat	From shady habitat	From light habitat	From shady habitat
<i>Peltigera praetextata</i>	50	30	57	73	21	54
<i>Peltigera rufescens</i>	45	40	60	65	23	37
<i>Solorina saccata</i>	35	30	63	68	26	42

^aAfter Ertl (1951).

TABLE VI
THICKNESS OF CORTEX AND ALGAL LAYER OF SOAKED
LICHENS FROM XERIC HABITATS IN SOUTH AFRICA^a

Species	Thickness of cortex (μm)	Algal layer (μm)
<i>Lecidea decipiens</i>	200	90
<i>Toninia sp.</i>	175	120
<i>Lecidea sp.</i>	168	140
<i>Endocarpon sp.</i>	80–90	100

^a After Vogel (1955).

Lecidea crystallifera Tayl. (= *Eremastrella tobleri* after Vogel) is an extreme example of adaptation (Fig. 16a). The disk-shaped thallus has a long rhizinic string (up to 20 mm) at the base, and the cortex, which consists of several pyramidal cones (0.8–1.5 mm high), is formed by a dense, translucent colorless mass of parallel, vertical hyphae. Above the algal groups there is a brown pigment layer. Light is dispersed in the cortex cones and filtered by the pigment layer. The arrangement of the algae in vertical chains allows for a sufficient yield of the diminished illumination. Vogel parallels this with "sun leaves" of higher plants.

A second type of adaptation against strong illumination is seen with a *Buellia* species which lives on quartz blocks in Knersvlakte in South Africa (Fig. 16b). Unlike a normal lichen, the medulla forms the upper surface and consists of black, short-filamentous hyphae interspersed with grains of quartz, lime, etc. The phycobiont layer is situated underneath the light-impermeable medulla and is pressed close to the quartz surface. The rock itself appears to act as a cortex and light is transmitted through the rock and to the algal layer.

More recently, enormous thickenings of cortices or epinecral layers of placodial and crustose lichens were reported from northeast Afghanistan (Poelt and Wirth, 1968), from the Atacama (Follmann, 1965b), and from the Negev in Israel (Galun, 1963), where almost 60% of the heteromerous lichens have a more or less voluminous, amorphous upper layer. Until we can obtain experimental data from these desert lichens, the question of whether illumination is the primary cause of cortex thickening will remain unanswered. The thickening may also be a response to wind erosion.

The covering of the thalli and apothecia with a pruina, i.e., a layer of crystals or dust (Follmann, 1965a,b), has been interpreted as a protective device against insolation (Schulz, 1931; Galun, 1963; Poelt and Wirth, 1968). Weber (1962), however, disputes the protective role of such coverings since they might be only a response to the nature of the substrate.

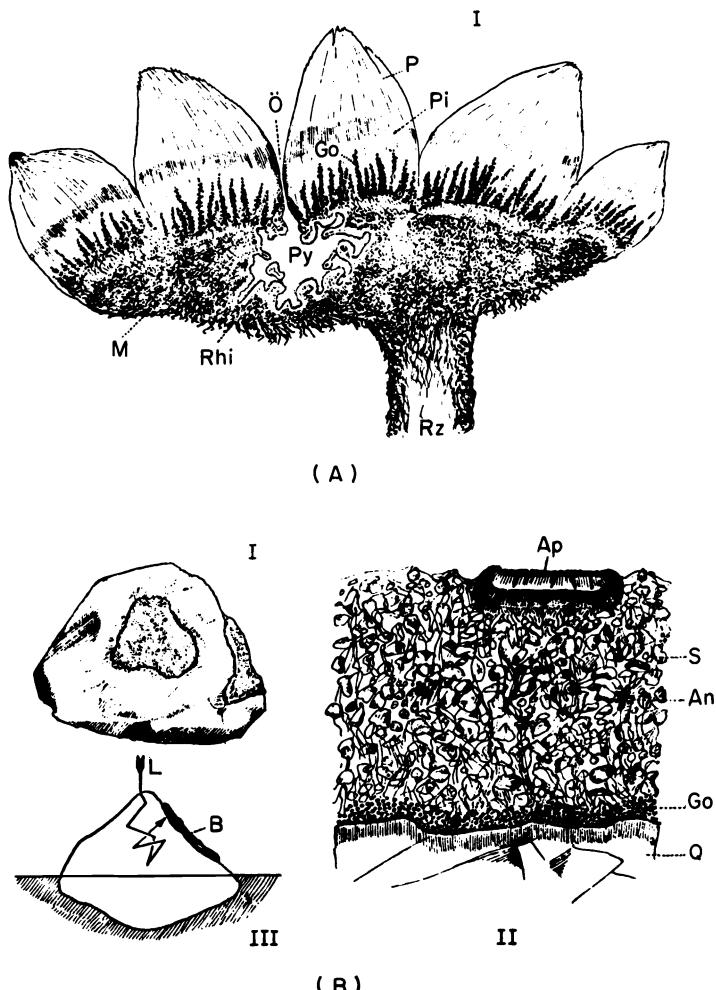


FIG. 16. Special kinds of adaptations to the desert environment in South Africa: (A) *Lecidea crystallifera* cross section of the thallus. P, cortex cone; Pi, pigment stratum; Go, chains of phycobionts; Py, pycnoconidia; Ö, aperture of the pycnoconidia; M, medulla; Rhi, rhizinic hyphae; Rz, rhizoidal string. (B) *Buellia* sp. on a quartz block. I, general view of the thallus on the quartz (natural size); II, cross section of the thallus; III, model of the course of light to the phycobionts of the inverse thallus. (Ap, apothecium; S, hyphae with soil particles; An, groups of phycobionts; Go, phycobiont layer; Q, quartz; L, incident light; B, thallus. (From Vogel, 1955.)

4. PHYSIOLOGICAL AND ECOLOGICAL RESPONSES TO EXTREMELY HIGH LIGHT INTENSITIES AND DARKNESS

In spite of many observations, there is a lack of experimental analyses on the resistance of phycobionts to strong illumination, in contrast with the studies on marine algae (Biebl, 1956). There is little evidence of the harmful effects of intensive illumination on photosynthetic activity. *Cetraria islandica* and *C. nivalis*, from alpine habitats on Mount Washington, underwent a decrease in their photosynthetic rate to 17–36% at 20°C, when they were exposed to 69.1 klux, a light intensity four times the optimum for photosynthesis of these species (Bliss and Hadley, 1964). *Ramalina maciformis* from the bright desert habitat, showed no drop in its photosynthetic rate at 40 klux (19°C) (Lange *et al.*, 1970a).

The question of how far poor illumination limits the existence of lichens also has not been answered. In contrast to bryophytes, the majority of lichens grow on open or well-lighted sites. There are several reports of degenerated thalli with apothecia which failed to mature (Zukal, 1896; Nienburg, 1919; Barkman, 1958) and of the dissolution of thalli into soredia (Schulz, 1931) in deep shady places in temperate as well as in tropical regions. Besides some aquatic species, a small percentage of lichens such as *Coenogonium*, *Graphis*, *Lecanactis*, *Lepraria*, and *Pertusaria* species are extremely sciotolerant. Many of them have a thin thallus and a thin algal layer or a loose structure. Lichens are rare in the deep shadow of tropical forests (Jaag, 1945) but some growth forms, such as Graphidaceae and lichens with blue-green algae, are adapted to these conditions.

Many authors failed to find lichens in caves, except for some soredial layers, while mosses are still abundant and grow luxuriantly (Dalby, 1966). However, Maheu (1906)—cf. also Gams (1922/1923)—found *Opegrapha endoleuca*, *O. hapalea*, *Verrucaria muralis*, and *V. rupestris* growing in semi-dark places in caves. In parts of an andine cave which were only periodically illuminated, Vareschi (1958) found two species of *Parmelia* and one of *Stereocaulon*. Only primitive fungus-alga associations and very tiny apothecia of Verrucariaceae were found scattered in the interior of southwest German caves (Wilmanns, 1960). In almost completely dark places Maheu observed a variety of *Placodium variable*, which was sterile, *Verrucaria rupestris*, which had greatly reduced and sterile thalli, and *Collema granuliferum*. Lichen existence in the dark is less surprising, since *Trebouxia* has been shown to grow quite well saprophytically in the complete absence of light (Ahmadjian, 1960).

F. Response to Gamma Irradiation and Radioactive Materials

Along with radiation from the sun, radiation by radioactivity must be taken into account as an environmental factor. Since 1960, great interest has

been directed towards the influence of radioactivity on vegetation. Besides the considerable fallout caused by atomic-weapons tests, natural sources of radioactivity also play a role, especially for the long-living organs of plants (cf. Hanson, 1967). Natural radionuclides such as ^{42}K , U, and Ra are present in various regions of the world. The concentrations of once naturally occurring ^{210}Pb , ^{210}Po (Beasly and Palmer, 1966), and ^{226}Ra (Holtzman, 1966) have been investigated in terms of their influence on plants and men.

Lichens, which are adapted to living on nutrients of the atmosphere, are the most efficient accumulators of fallout radionuclides (Table VII). Their function as a reservoir for fallout materials has been demonstrated repeatedly (Gorham, 1959; Grodzinsky, 1960; Poliakov *et al.*, 1962; Watson *et al.*, 1964; Hanson, 1967).

The fallout from atomic-weapons tests raised the radioactivity in lichens to high levels. This is especially due to the γ -emitting radioisotope ^{137}Cs (see Table VIII). The important role of lichens as a primary link in the arctic food chain is considered in Chapter 6.

With respect to artificial radiation, Brodo (1964) observed the after-effects of chronic γ irradiation on corticolous lichen communities in the Brookhaven National Forest. Cesium-137 was the radiation source. Growth measurements were made to determine vitality. After 9 months of irradiation, among the 145 thalli tested only 30 were slightly damaged or discolored. The greatest damage appeared with *Cladonia chlorophaea* and related species. Apparently, there was no clearcut relationship to distance from the source. At 1000 R/day most lichens remained almost undamaged after approximately 2 years' exposure. Even the average growth rate of *Parmelia sulcata* was above normal. Most of the trees on which the lichens lived had died within the first 6 months of radiation. Consequently, there were secondary effects due to changes in the microenvironment of the lichens.

Woodwell and Gannutz (1967) extensively studied the resistance of lichens in the same experimental area as Brodo and under almost iden-

TABLE VII
NATURAL RADIOACTIVITY IN UKRAINIAN PLANTS^a

	Number of species investigated	α -activity (imp/dm ²) _{av}
Angiosperms	16	345
Gymnosperms	15	418
<i>Polypodium vulgare</i>		1180
Masses	9	1892
Lichens	19	2837

^aAfter Grodzinsky (1960).

TABLE VIII
COMPARISON OF LICHENS WITH VASCULAR PLANTS WITH RESPECT TO THEIR
CONTENT OF ^{137}Cs IN THE FIELD

Lichens	Vascular plants	References
<i>Cladonia alpestris</i> 37.1 nCi/kg dry weight	Birch, willow, and others 3–7 nCi/kg dry weight	Häsanen and Miettinen (1966)
<i>Nephroma arcticum</i> 61.0 nCi/kg dry weight	Vascular plants 1–6 nCi/kg dry weight	Salo and Miettinen (1964)
<i>Cladonia silvatica</i> 46.4 nCi/kg dry weight	—	Lidén and Gustafsson (1966)

tical experimental conditions. Changes in species composition of the natural lichen communities were almost linearly related to the logarithm of daily radiation exposure. The diversity of the species* exposed along the irradiation gradient was observed for 3 years. The threshold for irradiation damage of the 43 species tested was lower than would be expected from the results of Brodo. At 300 R/day, the composition of lichens was affected. Some foliose species from the genus *Parmelia* showed great sensitivity. Fruticose species seemed to have a higher threshold of tolerance, while crustose lichens were very radioresistant (Fig. 17). Generally, the most abundant lichens of the forest were most radioresistant; some of them even survived under 2250 R/day. It was suggested that these lichens would be resistant to daily rates of 5000–10,000 R within a 3-year exposure. These findings generally agree with the observations of Jones (1965, cited by Hale 1967) who found that growth of *Parmelia conspersa* was still unaffected at 35,000 R for 11 days, but was reduced significantly at 64,000 R for 24 days.

According to Siegel and Daly (1968), in *Cladonia rangiferina* the succinate- and malonate-induced O₂ intake was only slightly affected, whereas malonate metabolism was completely inhibited by the comparatively high dose of 500 krad within 10 days. By comparing respiratory and photosynthetic rates of *Cladonia uncialis*, Gannutz (1969) found no effect on respiration even after exposure to 800 krad acute γ irradiation. Apparently, the fungus is indifferent to radioactivity. However, the photosynthetic rate remained on a low level for 28 days after irradiation with only 50 krad.

More extensive investigations and comparable data are required for the knowledge of the nature of resistance of lichens to irradiation. In general, lichens accumulate a higher level of radioactive nuclides than vascular plants and are far more radioresistant. This can be illustrated by comparing the

*The number of species per association or per square unit as a percentage of the control association.

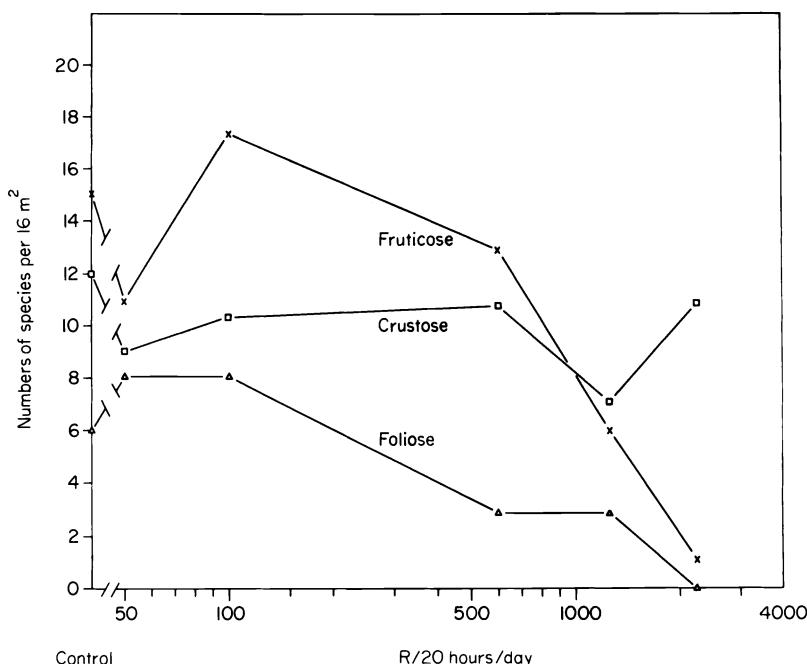


FIG. 17. Diversity per square unit of species of different growth forms of lichens along a radiation gradient ($R/20$ hours/day). (From Woodwell and Gannutz, 1967.)

results of Woodwell and Oosting (1965) with Woodwell and Gannutz (1967) in Table IX. A common feature of vascular plants and lichens is that the lower the growth and organization and the simpler the nuclear structure the greater the tolerance to irradiation. Lichens have symbionts with small nuclei and multinucleate cells, they form diffuse meristematic plectenchym, have relatively little differentiation or specialization of "tissues," and have a low ratio of nuclear volume to chromosome number (Brodo, 1964).

G. Response to Mechanical Influences

Erosion is not a negligible factor in harsh environments, especially for long-living organisms. Lichens that grow on exposed rocks or trees are subjected to the action of gales. If these winds bear clouds and rain then such habitats are favorable. In general, the lichens form very stable and elastic thalli that enable them to survive well along the seacoasts and in alpine environments. Several species even prefer wind-exposed habitats in open alpine sites where they are rarely covered with snow, as *Aleurotricha ochroleuca*, *Thamnolia vermicularis* (Gams, 1927; Klement, 1955; Churchill and

TABLE IX
THE RESPONSES OF LICHENS AND VASCULAR PLANTS TO
RADIATION (R/DAY)^a

Response after 3 years	Radiation exposure (R/day)	
	Vascular plants	Lichens
First effects of radiation on species composition	< 50	> 50
50% Reduction in diversity of species	160	~ 1000
Highest exposure tolerated by one or a few species	300	> 2250
No growth at all	> 3200	5000–10,000

^aAfter Woodwell and Oostings (1965); Woodwell and Gannutz (1967).

Hanson, 1958; Kalb, 1970), and *Usnea fasciata* and *Himantormia lugubris* on the South Shetland Islands (Lindsay, 1971). *Cladonia alpestris*, *C. impexa*, and other Cladinas prefer the opposite type of habitat (Ahti, 1961). Many of these relationships between lichens and the substrate are considered in detail by Brodo (Chapter 12).

Von Schrenk (1898) tested the amount of wind necessary to tear species of *Usnea*. He found that soaked thalli were torn at a wind velocity of 80 km/hour but a dry thallus still remained intact at 125 km/hour. Because these lichens desiccate very rapidly they are very wind resistant. In coastal areas many wind-exposed trees are bare of lichens apparently because of the intense drying effect of the winds (Klement, 1955). Deformed and fragmented thalli are the result of abrasion by sand in coastal areas (Bouly de Lesdain, 1953) and by ice blasts in polar and alpine regions (Gould, 1931; Rudolph, 1967; Poelt and Wirth, 1968). Dodge (1965) noted that parts of the cortex and sometimes even the algal layer are eroded by sandblast and described some adaptations to the rigorous conditions in Antarctica. For example, *Alectoria*, growing in small tufts, has a cortex that consists of thick-walled hyphae closely connected with each other. In members of the Umbilicariaceae there was a fastigiate cortex with an outermost amorphous layer of dead cells, sometimes up to 100 µm thick (epinecral layer). These modifications also can be observed in aquatic lichens in creeks and are very likely a response to mechanical stress. Modifications of the cortex as a result of intense insolation have been discussed in Section III, E.3.

Lichens affected by mechanical stress can undergo extreme morphological changes. *Ramalina fraxinea* in the Alps at 1200 m was so changed by prolonged sand-blasting that Bouly de Lesdain (1958) felt almost obliged to

describe a new variety "insignis." Forms that are a reflection of wind action include *Hypogymnia physodes* f. *papillosa*, and *Evernia prunastri* f. *ventosa*. In response to mechanical stress these lichens become knobby and verrucate. Even the well-adjusted crustose lichens often show traces of erosion in deserts (Weber, 1962).

The formation of long rhizinic strings that penetrate deeply into the soil is an adaptation of several placodial species in alpine and desert areas to winds and substrates that are subjected to loosening by freezing and thawing (Vogel, 1955; Poelt and Baumgärtner, 1964).

In marine and alpine environments the shaving of pack ice and glaciers and repeated freezing and thawing cause disruption and deformation of the lichens (cf. Lyngé, 1926; Gimingham and Smith, 1970; Ahmadjian, 1970). Finally, the ravaging influence of human action should be mentioned here, as scraping of trees or even complete destruction of natural materials and habitats.

IV. Extreme Habitats of Lichens

Up to this point it has been shown by experimental investigation of the physiological and morphological responses of lichens how well they can resist extreme environmental factors. The rest of the chapter will describe the effectiveness of these adaptations in the field and how far the abundance and diversity of lichens in specific habitats reflect general or special adjustments. The civilized areas, especially the role of cities which represent extreme environments for the lichens are discussed in Chapter 13.

A. Hot, Dry Environments

Hot deserts or desertlike habitats represent a complex of environmental factors. Besides the scarcity of water, there are extreme temperature changes within short periods of time, intense insolation, salinity, and strong, dry winds that erode the plants or cover them with sand or dust. Occasional heavy flooding by water also may occur.

Our knowledge about lichens in the deserts of the world is still incomplete. Recently, Galun (1970) made a survey of desert lichens, their floristic patterns, physiography, morphology, and physiology. From all the reports, it is evident that there exist only a few foliose and fruticose species in the desert. In the Middle East, for instance, they are represented by *Ramalina maciformis*, which is mostly sterile, *Teloschistes lacunosus*, and a few placiodoid species.

Several foliose desert species and sometimes even *Ramalina maciformis* (Galun, 1970) have developed a specialized life form. The thalli lie loosely on the ground and in the dry state they curl up their lobes and can be blown

easily by the wind. Additionally, they have often lost their foliose shape under the xeric conditions. For example, *Aspicilia affinis* and *Lecanora esculenta* form small balls that are very dissimilar to plant material. Since Eversmann (1831), erratic lichens have been found in nearly all deserts and steppes of the world [Asia: Elenkin (1901), Keller (1926), Tomin (1926), Reichert (1937), Klement (1965b), Schubert and Klement (1971); Africa: Faurel *et al.* (1953); Australia: Bibby (1956), Rogers (1971); South America: Follman (1966); North America: Tuckerman (1882); Antarctica: Lamb (1948a)]. There have even been characteristic lichen communities formed, e.g., the *Parmelietum vagantis* in the Irano-Turanian desert regions and the Gobi deserts (Klement, 1955).

Species with a crustose thallus are the most frequent in the desert. They are fixed closely on the substratum and show the smallest surface of all lichen types. Their shape can be altered so strongly by desert conditions that different specimens of one species look like different species, as Weber (1962) illustrated with *Acarospora*. Weber (1968) considers that up to 74 species of *Xanthothallia* (*Acarospora*), mostly from xeric regions of the world, that were described by Magnusson (1929), are environmental modifications of only two species. This view of Weber seems to be an overgeneralization (cf. Poelt and Steiner, 1971). The high plasticity of lichens is a striking phenomenon which was already pointed out by Herre (1942) (see Chapter 3). In the desert many places can be found with luxuriant lichen growth that covers stones completely or forms dense cushions on the soil. However, close to these areas we can frequently find the same substratum totally bare of lichens or only a few stones carrying endolithic species. Descriptions of frequent occurrence or even abundance of lichens in desert regions mostly refer to habitats that are influenced by fog (Follmann, 1960, 1963; Dawson, 1963; Thomson and Iltis, 1968; Walter, 1936) and by sufficient dewfall (Follmann, 1965a,b; Lange and Bertsch, 1965). Fink (1909) discussed a discontinuous lichen vegetation depending on the geographical exposition of the habitat. In the northern hemisphere favorable places face mostly in northerly directions (Herre, 1911; Roemer, in Poelt and Wirth, 1968). On northern exposures of the mountains of the Negev Desert it was observed that lichens are favored by shadow which provides more extended periods of high dew gain for photosynthesis in the morning than on other exposures (Kappen *et al.* 1973); consequently, the lichens form oases. With the help of dewfall lichens even occur on the inhospitable takyr plains in Turkmenistan (Rodin, 1963). In the desert highlands of the mongolian Altai the sparse lichen vegetation (*Amphoridium cariopsilum*, *Sphaerothallia desertorum*, *S. lacunosa*, *S. terrestris*) is concentrated on the undersides of translucent pebbles or on margins of stones facing the soil where dew water trickles (Schubert and Klement, 1971; cf. also Vogel, 1955).

According to Lange *et al.* (1970a), in a desert, besides the very few rainy days, the normal frequency of dewfall—allowing a period of carbohydrate production for around 2–3 hours a day—is sufficient for lichen growth despite considerable losses caused by respiration during the night (Fig. 18). The results of these calculations correspond to growth rates of *Caloplaca aurantia* (Lange and Evenari, 1971). Indeed, the increment of lichen thalli in a desert is not less than that of lichens in other climatic regions (see also the calculations of Rogers, 1971). It is striking that sometimes species occur in the driest sites of the desert as well as on exposed calcareous rocks in temperate and more moist areas, while species with distribution exclusively in arid regions occur in more sheltered places (Rogers, personal communication). In the Negev desert locally the standing biomass of epilithic lichens per square meter was even higher than that of higher plants (Kappen *et al.*, 1973).

There is evidence that several deserts are very poor in lichen species. According to Faurel *et al.* (1953), in the Algerian Sahara and Tibesti region only 114 lichen species were found, in contrast to 1000 species of phanerogams. In the Great Basin, in Arizona and New Mexico, there are altogether fewer genera and not all the same as in the Sahara (Flowers, 1952; cf. Rudolph, 1953). The Sinai peninsula also has a poor and scattered lichen vegetation. Large areas are totally free of lichens. Obviously, the lack of sufficient air moisture, infrequency of dewfall, and the powers of erosion cause the absence of lichens. These areas as well as those with loose sand (cf. Schubert and

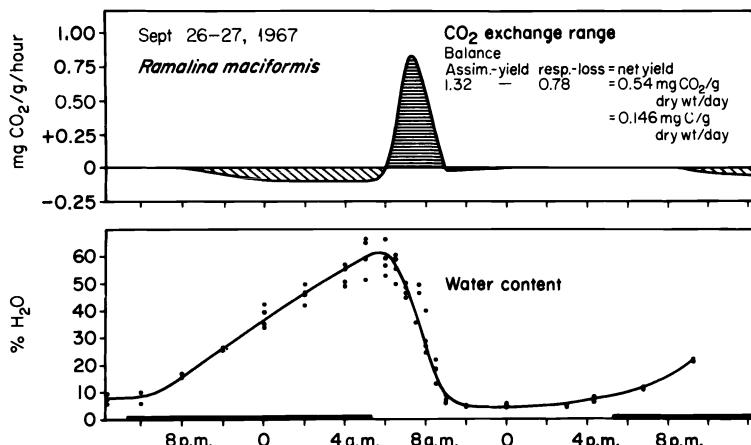


FIG. 18. CO_2 exchange of *Ramalina maciformis* in the Negev desert during a day with moderate dewfall. Water content in % of dry weight of the thalli is shown below. The calculated C-balance per day is about 0.146 mg C/gm dry weight. (From Lange *et al.*, 1970a.)

Klement, 1971) are lichen deserts. So, it frequently happens that in some places such as wadis, phanerogams can grow because they receive sufficient water from the ground while air moisture conditions are too meager to support lichens.

B. Polar and Alpine Habitats

In polar and high alpine regions, which are well-known extreme environments for plant life (cf. Billings and Mooney, 1968), the vegetation period is very short, temperatures are low, there are long periods of frost and snow cover, and abrasion and evaporation by winds are characteristic features. Lichens, because of their high resistance to freezing and their ability to endure long periods of inactivity in a frozen state, can survive in these environments. Moreover, they are adjusted by their poikilohydric nature. Many species are able to photosynthesize immediately when conditions become favorable and even maintain photosynthesis at temperatures below the freezing point. The scarcity of competitors allows even the slow-growing lichens to exist for long periods of time (Ahlmann, 1941; Beschel, 1958). There is no need to produce new biomass nor to reproduce each year (cf. Billings and Mooney, 1968).

Many species occur in mountains in moderate climates as well as in cold and dry regions. They are mostly saxicolous forms that are closely appressed to or grow within the substrate. Foliose and fruticose taxa appear compact and tiny in comparison to their normal shape (Rudolph, 1967). The dominance of crustose forms (e.g., *Buellia*, *Lecanora*, *Lecidea*, *Rhizocarpon*) in polar regions as well as in the desert shows their adjustment to extreme environments (Llano, 1959; Dodge, 1965).

1. POLAR REGIONS

With the "lichen coefficient" Mattick (1953) described the relative increase in the number of lichen species in polar regions. Antarctica ($Q = 175$) has a lichen flora of around 350 species but only two phanerogams (Rudolph, 1967). The abundance of lichens in the far north and the occurrence near the South Pole (Siple, 1938: seven species,* lat. $86^{\circ}3' S$; Wise and Gressitt, 1965: lichens at an elevation of 1980 m, lat. $86^{\circ}9' S$) have made them famous as pioneers of vegetation.

It was, therefore, not surprising that many lichenologists were attracted to investigating the polar regions. Just recently, research activity has focused on Antarctica (Llano, 1956, 1959; Dodge, 1964, 1965; Rudolph, 1967; Ahmad-

**Alectoria antarctica*, *Buellia Russelii*, *Lecanora fuscobrunnea*, *Lecidea Blackburni*, *L. cancriformis*, *L. Painei*, *Protoblastenia citrinigicans* (apparently exclusively antarctic species!)

jian, 1970). For the exploration of arctic lichen vegetation see the review by Dahl (1954) and the study by Thomson (1972). The lichen flora of far northern countries is rather rich, e.g., Jan Mayen (Sowter, 1958: 68 species), Ellesmere Island (Schuster *et al.*, 1959: 108 species) and Alaska (Krog, 1968: 375 species).

Several species are of bipolar distribution and may represent a kind of specialization for living in these environments (Lynge, 1941; Lamb, 1961). In Antarctica only one genus (*Himantormia*) is known to be endemic (Lamb, 1961). It occurs in the oceanic climate of Grahamland and the South Shetlands, but no further south than the Antarctic Circle. Ahmadjian (1970) feels that many other endemic species of Antarctica can be more easily interpreted as ecotypes of polymorphic species. Although antarctic lichens generally have well-developed apothecia and spores, vegetative propagation with thallus fragments is more efficient (Llano, 1962, 1965). Most of these lichens have neither soredia nor isidia (Dodge and Baker, 1938; Dodge, 1965). Also, in the high Arctic, most crustaceous species develop ascocarps while the foliose and fruticose species (which are more frequent than in Antarctica) are mostly sterile. The latter group may be disseminated by fragmentation because soredia are poorly developed or corticated (Lynge, 1926, 1932).

The Arctic, and especially Antarctica, show no continuous lichen vegetation. In Antarctica only the climatically favored Palmer peninsula and the areas near the Ross Sea are covered by a rich lichen vegetation. But the number of species on the antarctic continent comprise only 25% of the Antarctic lichen flora (Llano, 1959; Rudolph, 1967). Here the lichens occur only on the slopes of nunataks and on mountainsides in the coastal areas where air humidity is favorable (cf. Rudolph, 1963). Those lichens profit mainly from water-vapor intake. The continental climate of inner Antarctica produces severe drought on exposed or wind-blown habitats. In the large, inner dry valleys which have no snow and very little ice (Llano, 1967) and in well-drained places (Rudolph, 1963) lichens are completely absent.

In similar Arctic latitudes (up to lat. 83°N), the lichen vegetation is generally richer (cf. Longton and Holdgate, 1967). This is due to the relationship to the large continents surrounding the pole and to macroclimatic and ecological factors. However, locally strong impoverishment of lichen vegetation has been observed. Lynge (1939) remarked about some of the northernmost habitats on North-East-Land (near Spitzbergen) that "the reduced size and frost bitten habitus of many of them (the lichens) clearly show that they are not there for their pleasure." The lichens showed reduced vitality and no further outgrowth or multiplication. Lynge interpreted this feature as indicating a relic habitat which became more and more extreme with time. In the continental highlands of Greenland

(Hansen, 1962) and in the lower altitudes of the northeast Greenland coast (lat. 82°N) the occurrence of lichens is limited because these areas experience the drying action of frequent foehns (Lyngé and Scholander, 1932). In higher coastal altitudes (500–1000 m, and four species* even up to 1600 m), the lichens survive better. They must, however, be considered less as pioneers in extreme outposts than as colonists in an ecological niche, because the influence of the foehns is less effective there.

Ecologically favorable places for lichens in dry polar areas are found mostly under thin snow cover, ice sheets, or near snow fields. This shows the tendency of lichens to take advantage both of melting snow, the only source of liquid water (Bliss, 1956; Llano, 1959; Swan, 1961; Rudolph, 1963, Ahmadjian *et al.*, 1967; Lange and Kappen, 1972) and of thermal energy; i.e., as in a greenhouse. This tendency is enhanced by a dark-colored substratum (Llano, 1962; Ahmadjian, 1970). In Victoria Land, Antarctica, *Neuropogon acromelanus* was repeatedly found to colonize openings inside the scree slopes where air humidity remains very high (Lange and Kappen, 1972). In general, places with lichen growth in the high Arctic or the Antarctic continent must be considered analogous to oases in a desert. Therefore, lichens have a more limited distribution than several free-living algae (Dodge, 1965) and yeasts (Sinclair and Stokes, 1965) which were found in still more extreme regions of Antarctica.

2. REGIONS OF HIGH ALTITUDE

In mountains of northern regions, e.g., in Scandinavia, lichens are often the predominant vegetation. In most parts of the Alps, except the south, many species can live in the nival region and reach the highest peaks of the mountains (Frey, 1968/1969). There are lichens that live exclusively in high altitudes such as *Umbilicaria virginis* of the Eurasian Mountains. The lowest point at which this lichen has been detected was at 2550 m at Mount Pindus in northern Greece (Poelt, cited by Frey, 1968/1969).

The higher the elevation the fewer the species that occur (Table X). Records about lichen occurrence above 5000 m are scarce. In the Wakhan Mountains of East Afghanistan lichens were found between 5200 m and 5400 m by Roemer (Poelt and Wirth, 1968) but less frequently below 3200 m in the desert lowland. The upper border of lichen occurrence was not defined there. According to Gams (1960), the highest elevation in which a lichen was found was 7000 m (*Xanthoria parietina* on the K-2 in the Karakorum). Nevertheless, many of the lichens in the Himalayas, like *Stereocaulon myriocarpon*, show reduced vitality above 5200 m (Lamb, 1966). Swan (1961) reported that in the Makalu region (Himalaya), above 5486 m, lichens are very rare. How-

**Parmelia minuscula*, *Parmelia subobscura*, *Physcia muscigena*, *Umbilicaria cylindrica*.

TABLE X
RECORDS OF LICHENS FROM HIGH ALTITUDES

Area	Elevation (m)	Lichen species	References
Karakorum (K2)	7000	<i>Xanthoria elegans</i>	Gams (1960)
Makalu (Himalaya)	6200	"Few lichen thalli"	Swan (1961) cf. also Mattick (1953)
Mount Everest	5800–6000	15 species (<i>Cetraria</i> , <i>Letharia</i> , <i>Placodium</i> , <i>Rinodina</i> , <i>Lecanora</i> , <i>Aspicilia</i> , <i>Acarospora</i> , <i>Gyrophora</i> , <i>Lecidea</i>)	Paulson (1925)
Sikkim (Himalaya)	5660–5880	13 species	Hooker (1855, after Singh 1964)
Khumbu Himal (Himalaya)	5400	<i>Candelaria vitellina</i> var. <i>glacialis</i>	Poelt and Reddi (1969)
	5000	<i>Candelaria nepalensis</i> <i>Parmelia sinuosa</i> , <i>Candelaria himalayana</i> <i>Coccocarpia parmelioides</i> , <i>Lecanora rubina</i> , <i>L. chondroderma</i> , <i>L. terretiuscula</i> , and others	Poelt (1963, 1966)
Wakhan mountains (Afghanistan)	5200–5,400	<i>Xanthoria elegans</i> , <i>Lecidea tessellata</i> , <i>Lecanora melanophthalma</i> , <i>L. dispersa-areolata</i> , <i>Caloplaca paulii</i> , <i>Umbilicaria decussata</i> , <i>Staurothele elopima</i>	Roemer, Gilbert } cited by Poelt and Wirth (1968)
Kaukasus	5500	<i>Umbilicaria virginis</i>	Frey (1947)
Nevado de Toluca (Mexico)	4500	<i>Cora pavonia</i> , <i>Parmelia conspersa</i> , <i>Parmelia stenophylla</i>	Dix (1959)
Pico Espejo (Andes, Venezuela)	4750	15 species (reduced growth)	Hertel (1971)
Andes in North Chile	up to 4500	Species of the <i>Neuropogon acromelanus</i> association	Follmann (1965d)
Central West Alps	up to 4800	2 species	Gams (1960)
Mt. Rosa (Switzerland)	4638	<i>Parmelia encausta</i> , <i>Haematomma ventosum</i>	Maheu (1907)
Mt. Tacul (Savoie)	4200	<i>Solorina crocea</i> , <i>Squamaria concolor</i> , <i>Haematomma ventosum</i>	Frey (1968/1969)
Breithorn (Switzerland)	4171	<i>Stereocaulon condensatum</i> , <i>Solorina crocea</i> , <i>Squamaria concolor</i> , <i>Buellia discolor</i> , <i>Lecidea contigua</i>	Frey (1968/1969)
Grande Casse, Tarentaise (Savoie)	3861	16 species	Frey (1968/1969)
Großglockner (Austria)	3700	32 species of the genera <i>Umbilicaria</i> (4) <i>Lecanora</i> (4) <i>Parmelia</i> (2) <i>Cornicularia</i> (1) <i>Caloplaca</i> (1)	Frey (1968/1969)
Alaska	2140	18 species	Viereck (1967)

ever, according to Poelt (personal communication), in this elevation of the Himalayas numerous species, some with well-developed thalli, occur at sites which are not exposed to the avalanches of ice.

Above 6000 m sometimes even a vascular plant (e.g., *Stellaria decumbens*) seems to be better adapted to survive than the poikilohydric lichens because it can take up water from the sheltered subsurface of scree belts and rock taluses in which water from melting snow accumulates. Swan (1961) claims that "the lichens [unnamed] do not appear in their usual role as pioneers on rocks, they occur as secondary growth entangled in the spongy heads of prostrate rooted plants." *Candelaria vitellina* var. *glacialis* was found on rotted cushion plants in elevations of 5400 m in the Khumbu Himal (Poelt and Reddi, 1969). Mattick (1950) described how lichen life ceased almost immediately above the zone of passat clouds at the Pico Teyde, Teneriffa, a feature similar to several summits that are above the fog zone, like Maui in Hawaii or Mt. Kamerun (cf. Klement, 1965a, 1966).

In the high tropical mountains of Venezuela the occurrence of lichens is strongly limited along with phanerogams at around 4500 m (Vareschi, 1956). Hertel (1971) confirmed these observations, having found lichens up to 4750 m mostly with reduced vitality. The limitations, some hundreds of meters below the nival region, were interpreted by Vareschi as a consequence of abrupt diurnal climatic changes. Even in some parts of the South Alps (Pic Coolidge and Pic du Rabaons) the lichen flora is impoverished as a consequence of more extreme climates (Frey, 1968/1969).

Consequently, water deficiency in the mountains or low moisture in the air, as in the hot desert, and the mechanical influences of ice are limiting the existence of lichens. The nearer the mountains are to the equator, the more severe are the conditions in high altitudes. The few snowfalls that occur there are the only source of water and they normally sublime very quickly through intense insolation (Poelt, 1961; Swan, 1961; Follmann, 1965d).

C. Moist Habitats

1. AQUATIC ENVIRONMENTS

Depending on their sensitivity to moisture, lichens show very marked zonations. In marshes most taxa of *Cladina* can be found on tussocks or drier parts, but seldom in the wet depressions. In subarctic areas with very oceanic climates lichens are hardly able to compete with mosses. Foliose lichens that contain green algae cannot exist in any extremely humid area. The avoidance of temporarily inundated places by epiphytic and terricolous lichens in arctic regions has been reported repeatedly (Barkman, 1954; Dahl, 1954, Gjaerevoll, 1956). Snowy patches are extremely wet habitats, which are to a certain degree colonized by lichen communities character-

istic of places with long inundation (e.g., the *Stereocauletum alpini* or *Ephebetum lanatae*). In subarctic regions which normally show luxuriant lichen vegetation the decisive role of snow cover is remarkable (Nordhagen, 1928).

The very distinct zonations at lakes (Santesson, 1939), rivers (Beschel, 1954), and creeks (Ried, 1960a; Schubert and Klement, 1966; Wirth, 1972) can be clearly derived from the different resistances of lichens to submersion, drought, and water quality (Fig. 19). Foliose terrestrial and epiphytic lichens have their borderline above the highest annual high water level of lakes. Extreme flooding which lasts only 1 to 2 weeks kills them and recolonization progresses very slowly (Santesson, 1939). At places exposed to frequent oscillations of water level only lichens survive which are resistant to inundation as well as to strong insolation and drought. Additionally, erosion is involved, e.g., at the rapid waters of the alpine River Inn the zones are situated rather high above the water level (Beschel, 1954). The longer a habitat is submerged the more it becomes colonized by specialized lichens. In habitats with short inundation periods predominantly pyrenocarpous and almost exclusively crustose or verruculose lichens occur. Species of *Verrucaria* (Savicz, 1950), *Dermatocarpon*, *Jonaspis*, and *Staurothele* (Luther, 1954) are adapted to long submersion. Species of the lowest freshwater zone emerge from time to time, at least for a few days, every year or every second

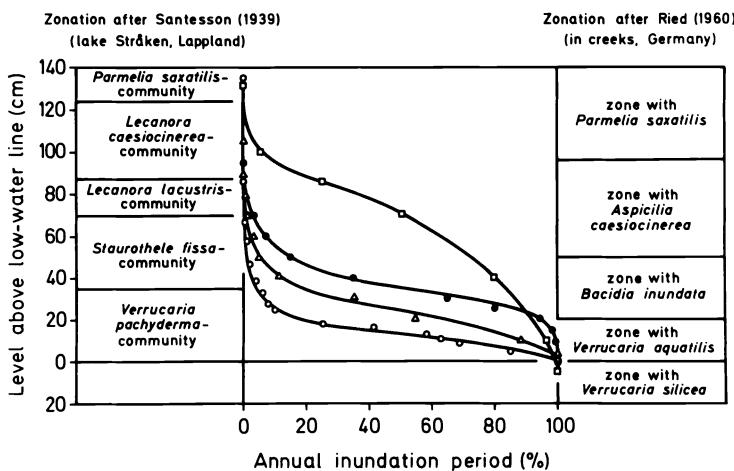


FIG. 19. Relation between the annual period of inundation (percents, abscissa) and elevation above the low waterline of different lakes and creeks (cm, ordinate), compared with zonations of different lichen communities as observed by Santesson (1939) at Lake Straken and by Ried (1960a) in Central European creeks. □, Lake Straken (average of 8 years); Δ, Seebach near Lunz; ○, Mauerbach (Wienerwald); ●, typical curve for European water levels.

year (Santesson, 1939; Klement, 1955, 1956). Permanently inundated habitats are usually free of lichens with the exception of *Verrucula* (= *Verrucaria*) *rheitrophila* (Luther, 1954), *Verrucaria silicea* (Wirth, 1972), and *Hydrothyria venosa* in North American streams (Smith, 1921).

Seashores, if they do not consist of loose or soft substrates, are colonized by lichens. On the unbroken substrates there are lichen belts with definite characteristics (Warming, 1906; Du Rietz, 1932; Southward, 1958; Lewis, 1964). Terrestrial lichens have their lowest border in the "supralitoral" zone. This zone is not washed by waves or tides, but salt spray always occurs ("spray belt"). Many lichens, especially nitrophilous species, seem to be very salt tolerant (e.g., *Acarospora*, *Caloplaca*, *Lecanora*, *Mastodia*, *Parmelia*, *Physcia*, *Ramalina*, *Roccella*, *Usnea*, and *Verrucaria*) (Gimingham and Smith, 1970). At the antarctic coast, members of the lichen alliance *Ramalineon terrebratae* colonize at about 2–73 m above sea level (Follman, 1965c). *Xanthoria parietina* can be found even on very salt-rich substrates such as the twigs of *Tamarix* or soil of salt marshes. At coasts in arid regions the lichen thalli become strongly incrusted by salt from seawater spray (*Chiodecton cerebriforme*, *Roccella portentosa*, *Roccellaria mollis*) (Follmann, 1967), while in humid climates salt is washed out very quickly (Almborn, 1955).

The ecology of marine lichens is rather unknown. Below the spray belt the more specialized lichens such as *Lichina* and *Verrucaria* occur (e.g., sixteen species in Europe; Zschacke, 1925). Here in the "litoral zone" (middle and lower hydrohalinic belt) the lichens still are not frequently inundated. The width of this belt depends on the exposure of the coastal sites (Fig. 20). At sheltered coasts the belt is very small and the lichens concentrate at the medium-high water level. This shows a dependence upon the direct influence of seawater, which may be due primarily to the competition with other, less water-resistant species. The species here which are normally considered as a uniform complex behave very differently (Grummann, 1937). *Lichina confinis* avoids action of waves but tolerates intense insolation (as observed on the Baltic seacoast), while the very thin thalli of *Verrucaria maura* are extremely scotophilous and, being closely fixed to the rocks, they tolerate stronger washing by the waves but not permanent inundation.

Thelidium (= *Arthopyrenia*) *crustense*, *Verrucaria microspora*, and *V. mucosa* occur locally within the belt of tidal oscillation (upper hydrohaline) provided they are not covered by sediments as happens in shallow bays (Degelius, 1939). *Verrucaria mucosa* was still found at the low-water level of exposed coasts of Wales (Moyse and Nelson-Smith, 1963). Yet the lowest occurrence of a lichen is below the lowest ebb-tide level (Lamb, 1948b). *Verrucaria serpuloides* lives permanently submerged on the rocks of the coast of Graham Land, Antarctica. The clearcut upper border of this lichen indicates that the submarine habitat is obligatory. More recently, Lamb found

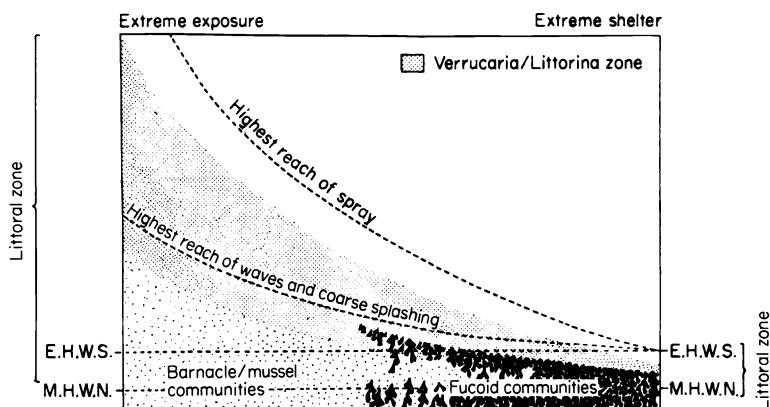


FIG. 20. Scheme showing the position of the *Verrucaria/Littorina* zone (densely spotted) in relation to the tidal levels and the reach of waves and sea spray. At exposed coasts is a broad littoral zone above the extreme high water surface (E.H.W.S.), including the upper border of the *Verrucaria/Littorina* zone. At sheltered coasts the littoral zone is small but descends below the medium high water level (M.H.W.N.). (From Lewis, 1964.)

this species growing abundantly in the "sublittoral" region down to a depth of 5 meters below the lowest ebb-tide level. During the period from July to October, these sites are covered by a thick sea-ice layer (Lamb, personal communication). Thus, its requirement for light must be very low.

Generally, for most marine lichens, the frequency of inundation, low light intensity, ice shaving in winter, and competition with seaweeds are the limiting factors. The very pronounced zonation of lichens in and around aquatic environments and the specialization of only a few taxa show that aquatic habitats represent extreme environments.

2. TROPICAL RAIN FORESTS

Most terrestrial lichens are sensitive to permanent humidity, especially when it is combined with high temperatures. Moreover, lack of sufficient light (Jaag, 1945; Singh, 1964) drastically limits lichen occurrence. Consequently, the dense tropical rain forest can be regarded as an extreme environment for lichens. This seems to be evident from the lichen coefficient that ranges from 0.006 for Java to 0.42 for Hawaii and shows that the lichens did not evolve in such abundance as higher plants (cf. Mattick, 1954). The absolute number of species, however, is greater in the tropics than elsewhere in the world. The humid tropics represent a vast complex of different habitats ranging from lowlands to high mountains. The mountainous region has an extremely rich lichen flora.

Our knowledge about lichens and their ecology in lowland rain forests is restricted. A great number of exclusively tropical taxa, which apparently

require high temperatures or other specifically tropical conditions, can be found in the lowland rain forest, while Umbilicariaceae, which are sensitive to permanent high humidity, and other epiphytic or epilithic species are absent (Tobler, 1925b; Mattick, 1954). Conversely, members of genera which mostly occur in humid tropical regions are not noticeable in other climatic regions because they require very humid environments (Saxen, 1963).

In places with sufficient light a particular type of lichen colonizes living plant leaves which have a life period of between 1 and 3 years (Jaag, 1943). These lichens have a short life cycle and become fertile within a few months. Tobler (1925b) believed that many of these lichens represent incomplete and changing symbioses (e.g., *Rhodothrix*, *Trichobacidia*) of algae and fungi, loose associations of both (e.g., *Coenogonium*) or fungi parasitic on algae (e.g., *Scytonema*). But the occurrence of parasymbiotic lichens and the transitions of lichens to parasitic fungi and slightly lichenized algae was overestimated. Santesson (1952) listed 236, mostly crustaceous, obligately foliicolous species which are distributed over several or all tropical parts of the world. They are stable, well-defined species. No doubt these lichens are generally primitively organized. Perhaps they were not compelled to evolve themselves higher under the conditions of the humid rain forest, in contrast to species of other climates (cf. Mattick, 1954). Whether this primitiveness is of a primary or secondary nature is not known. Up to 45 different lichen species were found on a large palm leaf in New Guinea. They formed typical and more or less constant lichen communities (Allan, 1928). Although they are not particularly dependent on the nature of the leaf surface, they all require or prefer habitats with permanently high humidity (Tavares, 1953).

D. Conjectures about Extraterrestrial Environments

Extraterrestrial regions are perhaps the most extreme environments we can imagine. Lichens often have been proposed as the most likely organisms for existence outside the earth (Becquerel, 1936, 1948; Kuiper, 1952; Firsoff, 1963).

The possibility of the existence of life in space was suggested by Becquerel (1950, 1951). Unicellular algae, bacteria, spores, tardigrades, and thalli of *Xanthoria parietina* withstood long-term desiccation, storage in high vacuum (some samples for 8 years) at temperatures near absolute zero. At -100°C the metabolic activity of lichens would be slowed down to 85,300 times the normal rate ($+20^{\circ}\text{C}$). An organism normally viable 1 year at $10\text{--}20^{\circ}\text{C}$ may then endure a period of 70.3×10^{12} years at -273°C (Becquerel, 1951; cf. also Scholander *et al.*, 1953). Becquerel's restriction that preservation of life would only be imaginable if radioactive and cosmic irradiation could be hindered may not be relevant for the radioresistant lichens. This may be a

concept for the existence of anabiotic states and dissemination of living material in the cosmos, probably by particles of lichens coming from the earth.

Special interest has focused on the planet Mars. Martian habitats with a thin atmosphere capable of supporting traces of moisture could most likely support some form of life in our solar system. Seasonal changes of color were also reasons for imagining that larger plantlike organisms similar to lichens existed on Mars. This idea seemed to be supported by spectrometric measurements of sunlight reflected from the dark area of Mars, which agreed with those of reflected light from lichen thalli (Kuiper, 1952).

Basa and Hawrylewicz (1962) interpreted the variation of color from green to black to be a change from active to anabiotic state of a kind of hygroscopic thalli. They used for an example *Umbilicaria dillenii*, which is green when wet, but reveals a black underside when the edges of the dry thallus curl inward. Some experiments were carried out under simulated Martian conditions (Basa and Hawrylewicz, 1962; Siegel, 1963). Microorganisms, eleven lichen species from Wisconsin, and three lichens from Antarctica were exposed to light of a wavelength of 4800 Å and more, circadian temperature change from 30°C in the light to -60°C in the dark, and an atmosphere composed of 95% N₂, 4% Ar, 0.3% CO₂, and less than 1% O₂ and moisture. Although *Trebouxia erici* separately survived these conditions for a period of 10 days of felsite and felsite-limonite, the results with the lichens were more or less negative.

The tested lichens were mostly foliose and fruticose forms, however (cf. also Siegel *et al.*, 1969), they do not represent the most resistant types. Crustose forms would be more suited for investigation of possible life under extraterrestrial conditions.

A weighty argument against the theory of lichens on Mars is that the lichen is a complex of alga and fungus. This implies a precedent evolution, especially of the fungus to a rather advanced life form until it became symbiotic (Salisbury, 1962).

It may be a matter of speculation whether such conditions have existed in the past on Mars or whether lichens came from the earth. In any case we should state that there is no evidence that the conditions on Mars allow even the poorest kind of metabolism or reproduction such as there is on earth. There are hypotheses about organisms with other metabolic pathways working only with traces of H₂O and not releasing O₂ but using other ways of energy transfer (Franck, 1949; Salisbury, 1962; Saunders, 1969).

V. Conclusions

The aim of this chapter was to determine the range of reactions and adaptations of lichens to different extreme environmental factors and to charac-

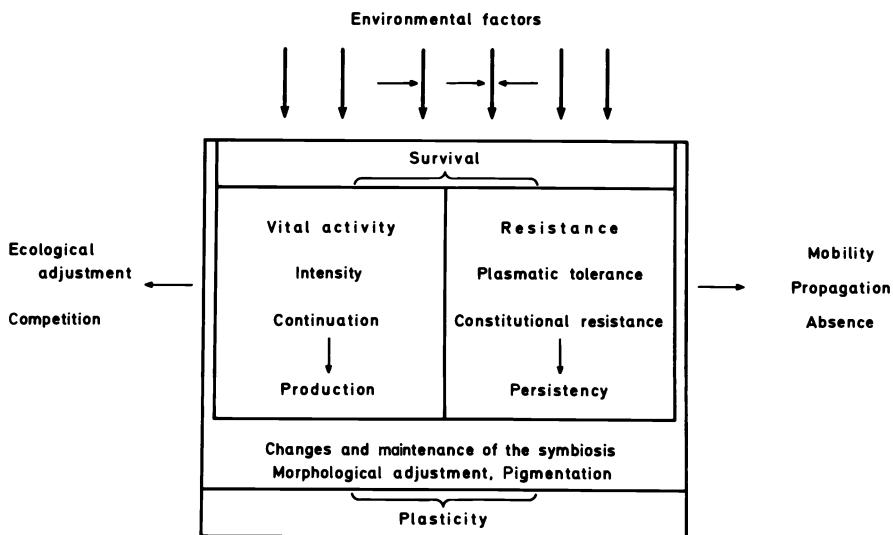


FIG. 21. Scheme showing how lichens can respond to extreme environmental conditions (see text, p. 366ff).

terize extreme environments with regard to lichens. We can summarize the given data about lichens by means of a model showing the dimensions of responding systems to different environmental influences (Fig. 21). The life of a lichen is characterized by rapid changes between active and inactive states. These changes can be optimally profitable if they allow for sufficient time (continuation) and intensity of metabolism and thus production of biomass, and minimally profitable if the lichen can persist only with reduced vitality. Their resistance allows lichens to persist under environmental stress either in the active state (plasmatic tolerance) or by diminishing the stress in the anabiotic, desiccated state, which is made possible by their poikilohydric nature (constitutional resistance). Vital activity and resistance are the elementary preconditions for survival guaranteeing production and persistency. If there are chances for survival, an individual lichen can "respond" to adverse conditions by morphological adjustments (strengthening of hyphae, cortical layers, ecotypes and various shapes), by pigmentation of the thallus, and by changes in the symbiotic state. The plasticity of lichens often appears as a result of the actions of a complex of environmental factors. This becomes evident in the extreme environments themselves. The different lichen species respond characteristically by ecological adjustments (colonization in special habitats, e.g., under shelter, under ice, on northern exposure), by which they can avoid the influences of extreme environmental factors. Apart from this, competition with other species is involved. Another kind of response to

extreme environmental conditions can be illustrated by their sexual and asexual reproductive vitality. Recently, this problem was strikingly illustrated by Poelt (1970) with so-called "primary" and "secondary" taxa. The first are always fertile and live in more favorable areas, the latter are obviously derivatives of the first and grow in larger and more extreme (cold and dry) habitats. The "secondary" species are usually sterile but produce many soredia and isidia and thus have the ability to be rapid colonizing pioneers. Erratic lichens in deserts and steppes indicate the ability to be translocated to more favorable environments. Finally, the recording of their absence helps to provide information concerning the limitation of lichens existing throughout the world.

Many properties enable the lichens to be extremists, but they are sensitive to other environmental conditions and in some respects even more so than other plants. It would be as unreasonable to consider all lichens as extremists, as it would be unreasonable to regard them all as cosmopolitan because even the widely distributed species are split into different races and chemical strains. When we know more about taxonomical differentiation with regard to ecotypes (Culberson, 1970) the ways of ecological and physiological adaptation will be more easily understood.

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Chapter 11

WATER RELATIONS

O. B. BLUM

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I. Introduction

Water relationships of lichens differ greatly from those of vascular plants. Lichen thalli do not have a stable water balance. They passively follow the fluctuations of atmospheric humidity. On the contrary, vascular plants because of their unique anatomical and morphological structure and physiologically active regulation of vital functions can maintain a relatively constant water content despite changes in environmental conditions, and can balance water content with the water content of the soil. These two types of water relations were designated by Walter (1931) as (1) poikilohydric, with fluctuating humidity, and (2) homoiohydric, with relatively constant humidity. These terms stress that the transition from the poikilohydric organization to the homiohydric one is as important for plant life as the transition from the poikilothermic state to homiothermic state is in the animal kingdom.

All lichen thalli have the poikilohydric type of water relations.* A few

*Some flowering plants whose vegetative organs have the ability to "revive" after absorbing sufficient moisture after drying out also belong to the poikilohydric type, i.e., representatives of the Gesneriaceae such as *Ramondia serbica*, *R. pyrenaica*, and *Haberlea rhodopensis* and some xerotic tropical ferns, such as *Ceterach cordatum*, *C. officinarum*, *Myrrothamnus flabellifolia*, and *Notholaena eckloniana*.

mosses can regulate their hydrature to a certain extent (Aleksiev and Gusiev, 1935; Aleksiev, 1937; Buch, 1945, 1947).

Lichens are one of the most characteristic groups of poikilohydric plants. Their anatomical and morphological structure differs greatly from that of vascular plants in that the lichen thallus is weakly differentiated.

The specific differences between lichens and vascular plants in regard to water relations are as follows:

1. Lichens do not have specific organs for the absorption or the transpiration of water. Both functions are carried out directly by the thallus surface. Thus, transpirational protection, similar to that found in vascular plants, is absent. Water is easily lost by evaporation from the thallus surface.

2. Unlike vascular plants that accumulate intercellular water, lichens are characterized by extracellular water accumulation, i.e., water is contained in swollen hyphal walls, in the gelatinous sheaths of blue-green phycobionts, and in intercellular spaces. There are no data on the water content in the cytoplasm of lichen components. However, as Hiltizer (1927) and Smith (1962) noted, the cellular water of lichens must be small compared with the general water content of the thallus. Both absorption and loss of water are caused by the colloidal-chemical properties of the lichens and are similar to analogous properties of agar gel. It should be noted, however, that one must not overestimate the importance of physicochemical processes of lichen water relations, as was done by many researchers (Bachmann, 1922, 1923; Goebel, 1926a; Stocker, 1927; Hiltizer, 1927; Degelius, 1935). Such an approach is not profound enough, since the state of water relations is determined by the metabolism of living matter that influences the state of the cytoplasm (Gusiev, 1959).

There is general acknowledgement that the area of water relations is an important ingredient of a plant's metabolism and is interconnected with the other aspects of metabolism, specifically with the processes of respiration, photosynthesis, synthesis of protein, synthesis of nucleic acids, and macroergic combinations (Gusiev, 1968; Gusiev *et al.*, 1969).

Although the basis of these notions stems from data obtained with homoiohydric plants, poikilophytes and lichens undoubtedly have analogous relationships. However, detailed physiobiochemical studies of lichen water relationships must remain the subject of future research.

II. Water Absorption

A. Absorption of Liquid Water

Simple field observations suggest that absorption of liquid water by dry thalli of most lichens is a rapid process. Experiments (undertaken by various

researchers under laboratory conditions) give us a more concrete idea of the rate of this process (Stocker, 1927; Hiltizer, 1927; Kolumbe, 1927; Degelius, 1935; Ellée, 1939; Ried, 1960b; Blum, 1964). These data of the rate of lichen saturation have significant discrepancies because of the differences in the measurement techniques. All the studies show an extremely high rate of the absorption of liquid water by lichens. Lichens are similar to a hydrophilic gel in the manner of liquid water absorption. More than half of the water contained in a saturated thallus is absorbed within one-quarter of the time it takes to achieve saturation. Such a quick rate of absorption is of great importance for lichens in nature (Stocker, 1927) because in order to absorb liquid water they have at their disposal, and for only relatively short periods of time, only the moisture of atmospheric precipitation (rain, fog, dew, or melting snow).

According to Stocker (1927) and Smith (1961), it takes foliose and fruticose lichens 1–2 minutes to achieve full saturation.

In our experiments (Blum, 1964), the time* required for saturation for most of the investigated fruticose and foliose lichens was 2–4 minutes, for gelatinous lichens (*Collema cristatum* and *C. flaccidum*) about 6 minutes, and for *Dermatocarpon vellereum* and *D. miniatum* about 9 minutes. The crustose, wandering, desert lichens *Aspicilia esculenta* and *A. fruticulosa* achieved saturation in 17 and 26 minutes, respectively.

Despite the very high rate of liquid water absorption of lichens in general, there are species of “nonwettable” lichens. Sievers (1908) found that a thallus of *Calicium chlorinum* encrusted with nonwettable “lichen substances” did not become saturated even after 6 hours immersion in water.

Once the initial rapid uptake of water by lichens is over, the thalli often can absorb considerably more water during a prolonged immersion of several hours. The free spaces take up water rapidly but then the water content in the thallus increases due to the increased ability of colloids to retain water. We call such a phenomenon a state of “oversaturation.” Our experiments showed that this state was most pronounced with *Ramalina farinacea* and *R. fraxinea*. During a 15-hour immersion, their thalli absorbed, respectively, 60 and 82% water in addition to the amount initially absorbed during the 30-second immersions. *Umbilicaria grisea* and *U. hirsuta* took up additionally only 8.2 and 7.7% water, respectively. Other investigated species were of intermediate values.

*The time required for saturation was determined by 30-second consecutive immersions. This time is somewhat less than the real time the thallus takes to become fully saturated, since we fail to take into account related activities, such as removing the thallus from the water, removing liquid water from its surface, weighing, and returning the thallus to the water.

B. Absorption of Water Vapor

The ability to use atmospheric moisture is a striking feature of the lichen's water relations. Except for some epiphytes, higher plants are inferior to lichens in this respect. But, according to new data (Breazeale *et al.*, 1951; Slatyer, 1956; Hübner, 1960, Samuilov, 1963), the ability to absorb atmospheric moisture, specifically during night hours with the increased atmospheric humidity may not be unimportant for the water relations of higher plants.

The ability of lichens to absorb water vapor from a saturated atmosphere was recorded long ago (Zukal, 1895; Sievers, 1908; Müller, 1909). Later, the lichen's ability to absorb water from a nonsaturated atmosphere was also proved (Bachmann, 1923; Hilitzer, 1927; Kolumbe, 1927; Pavillard, 1939; Butin, 1954; Heatwole, 1966).

Being similar in its character to the absorption of liquid water and also displaying a typical picture of swelling, the absorption of water vapor is several thousand times slower. In a humid atmosphere the water content of a thallus will slowly increase until it reaches a constant equilibrium value. The higher the relative atmospheric humidity, the higher is the equilibrium water content in the thallus and the longer it takes to reach equilibrium.

Studying how fast air-dried lichens absorbed water vapor from a non-saturated atmosphere, Stocker (1927), Smyth (1934), Ellée (1939), and Quispel (1943) found that the equilibrium water content was achieved in 6 to 9 days. According to Müller (1909) and Hilitzer (1927), even 14 days was not sufficient time for the lichens that they studied to reach equilibrium value with a saturated atmosphere. Kolumbe (1927), Cuthbert (1931, 1934), and Butin (1954) found that thalli required only 1 to 3 days to reach their equilibrium water content, but their results obviously cannot be considered as reliable. It seems likely that the authors used atmospheres of lower relative humidity than 100%.

In our experiments, some of the 27 investigated lichens (i.e., *Aspicilia esculenta* and *A. fruticulosa*) placed in a saturated atmosphere (relative air humidity 100%) still continued their absorption of water even after 22 days. However, the rate of saturation was infinitesimal.

The content of water in thalli that are in equilibrium with vapor pressure in a saturated atmosphere is lower than that attained after the absorption of liquid water because in a saturated atmosphere the lichen does not contain capillary-drawn liquid water in its thallus and water on its surface. According to the data obtained by a number of authors (Smyth, 1934; Ellée, 1939; Quispel, 1943; Butin, 1954) saturation by water vapor reaches a level of 50–75% of the maximum amount of water in a thallus saturated with liquid water. However, Ried (1960b) doubts the reality of this data and considers the findings to be overstated because of the possibility of

water condensation on the thallus surface in a completely saturated atmosphere.

The results of our experiments have shown that the relationship of water content in the thallus when the lichen is in a saturated atmosphere (in equilibrium with vapor pressure) to the water content in a thallus saturated by liquid water differs greatly in lichens. The lowest values were shown by *Collema flaccidum* (14%), *C. cristatum* (20%), *Aspicilia esculenta* (22%), and *A. fruticulosa* (23%). The highest values were held by *Cetraria islandica* (64%) and *Dermatocarpon miniatum* (68%). For most fruticose and foliose lichens the relation between the water content in the thallus in equilibrium with vapor pressure of the saturated atmosphere and the maximum saturation with liquid water was within 40–50%.

The study of the lichen's ability to use water vapor from a nonsaturated atmosphere is of particular interest. The main part of the water in a vapor state absorbed by lichens enters the thallus only at a relative humidity that is above 90%. For foliose and fruticose lichens placed in an atmosphere with a relative humidity of 95%, Butin (1954) and Ried (1960b) found that the maximum amounts of water absorbed by the thalli were 30–50% of the water content of thalli fully saturated with liquid water. These findings were confirmed by our experiments (Fig. 1) (Blum, 1965). The absorption of water vapor as well as liquid water to a certain extent depends on anatomical and morphological peculiarities of lichens. That is why various species have marked differences in the rate and amount of water vapor absorbed from equal relative atmospheric humidities. These differences become more pronounced with the increase of water vapor content in the atmosphere.

A noticeable divergence in the rate of water vapor absorption can be observed also in different specimens of the same species (Kolumbe, 1927). As a rule, highly sorediate or isidiate thalli absorb water vapor much more intensively than forms in which these structures are lacking or are poorly developed. This advantage of sorediate forms in water-vapor absorption is

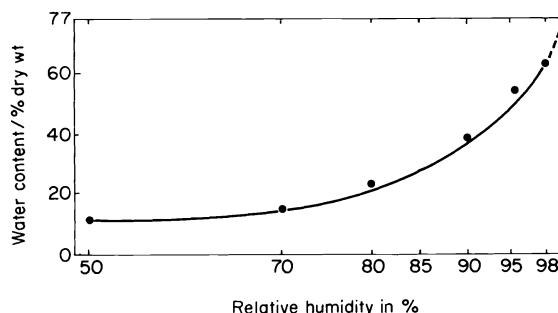


FIG. 1. Water-vapor absorption in relation to relative humidity ($t=30^{\circ}\text{C}$).

considered by Kolumbe as a compensation for the lower amount of liquid water in their much drier habitat. However the question of the ecological meaning of soredia and isidia remains open. The literature lacks reliable data on this subject. According to Du Rietz (1924) and A. N. Oxner (personal communication) the formation of highly sorediate and isidiate forms is caused mostly by atmospheric pollution.

The ecological importance of the lichen's ability to use atmospheric humidity was recognized long ago (Fries, 1831; Kolumbe, 1927). There are even indications (Smith, 1962) that for some unwettable lichens, i.e., *Calicium chlorinum*, atmospheric water vapor is the only source of moisture.

Some researchers (Stocker, 1927) feel that water vapor is of small importance for lichens and consider that they need liquid water for their assimilation activity. A conclusive answer to this question was given by precise and convincing laboratory and field experiments carried out by Lange and Bertsch (1965), Bertsch (1966), and Lange (1969). It was found that as a result of water-vapor absorption, air-dried thalli in a latent state could become reactivated and reach almost optimum photosynthesis. In Lange's (1969) experiments, dry thalli of *Ramalina maciformis* from the Negev desert placed in an atmosphere close to saturation were reactivated in 6–8 hours and reached photosynthetic levels that were 90% of the maximum possible value. Reactivation occurred not only in the saturated atmosphere but also in a nonsaturated atmosphere with a relative air humidity of about 80–85%. The net photosynthesis of *R. maciformis* in an atmosphere with a relative humidity of 90% was one-fourth of the maximum value. No other form of plant life has this ability.

The ecological importance of a lichen's ability to absorb water vapor from the atmosphere was displayed convincingly by Lange's (1969) field experiments. In the morning when the atmospheric humidity remained high and there was no dew, the water content of *R. maciformis*, exclusively due to water-vapor absorption, reached 31% of the dry weight. This water content was sufficient for 3 hours of net assimilation after sunrise, until the thallus temperature rose to 20°C. According to Lange *et al.* (1970), not only fruticose but also foliose and crustaceous lichens in natural habitats are able to manage without liquid water, absorbing moisture directly from an atmosphere that contains large amounts of water vapor at night. Thus, because of their ability to absorb water vapor from the atmosphere, lichens have the following advantages: First, they can achieve a positive metabolic balance during periods when liquid water is not available in their habitat. Second, during prolonged dry periods the lichen's photosynthetic apparatus is not inactive and its high stability is ensured. This remarkable ecological ability to use water vapor from the atmosphere makes it possible for lichens to exist in extremely arid regions where conditions are unfavorable for the growth of most kinds of plants.

According to Follmann (1967) some hydrotic lichens that are found on the seacoast of warm arid regions have an additional advantage for water-vapor absorption. As a result of sea-spray evaporation these lichens, i.e., *Chiodecton cerebriforme*, *Roccella portentosa*, *Roccellaria mollis*, develop a salt-crust film that significantly contributes to water-vapor absorption. Tiny inorganic crystals on the surface crust of some lichens, in particular on *Umbilicaria papulosa*, can act as condensation nuclei and thus facilitate the uptake of atmospheric water vapor (Showman and Rudolf, 1971).

III. Mechanism of Water Absorption and Water Conduction to the Thallus

Goebel (1926a,b) and Stocker (1927) were the first to find the reasons for rapid liquid-water absorption by lichens. Water first enters all the capillary and air spaces between the hyphal and algal cells. Thus, a large surface area of hyphal walls immediately and simultaneously swells. This phenomenon explains the extremely rapid water absorption by the thallus during the first moments of absorption.

Thallus hyphae have certain specializations, i.e., there are swollen and aerial hyphae. Goebel (1926a) claims that swollen hyphae may be of two types: thin-walled with large inner spaces and thick-walled. Between these two types, transitional forms are naturally observed. Swollen hyphae are easily moistened and thus they are very important for the absorption and preservation of water. Aerial hyphae are incrusted with lichen acids and are practically unwettable. Thus, the availability of a certain amount of air necessary for photosynthesis of the algal partner is always ensured.

At the initial stage of the dry-thallus saturation, water absorption by the cytoplasm appears to be an ordinary swelling process, for in a dehydrated state cytoplasm is not semipermeable. The latter ability is restored only after the cytoplasm swells. At this time osmotic forces causing cytoplasmic water conduction become effective (Stocker, 1956).

After the absorption of water from the adjoining walls, the cytoplasm comes into balance with the swelling cell membrane (Renner, 1933). As soon as the semipermeability of the cytoplasm is restored, osmotic forces begin to act until the absorption and swelling forces are in equilibrium with the atmospheric humidity (Stocker, 1956). A very slow rate of water-vapor absorption is explained by its slower diffusion compared with liquid water. Water vapor is condensed mostly by the cell walls (Müller, 1909; Mayer and Plantefol, 1924; Stocker, 1927; Goebel, 1930). Attaining equilibrium with a vapor-saturated atmosphere is very slow. Because of the thick hyphal walls and swollen cortex, the difference between vapor pressure and thallus water content becomes small. Showman and Rudolf (1971) state that the

algal layer may act as a sink, absorbing moisture until an equilibrium is reached with the adjacent tissues and, ultimately, with the water vapor in the atmosphere.

Very little is known about the osmotic pressure of the cells of lichens. Using Neubauer's (1938) measurements of the water content of lichen thalli in atmospheres with low relative vapor pressure humidity over a saturated solution of NaCl, Barkman (1958) calculated that the osmotic pressures of lichens were 300–1300 atmospheres. However, according to Smith (1962), much of the water in the thallus is held external to the cytoplasm in the cell walls, and thus these remarkably high values for osmotic pressure are not applicable only to the living cell contents. Barkman's estimates should be regarded as analogous to the estimates of the osmotic pressure of gels.

The most reasonable hypothesis for the mechanism of liquid water absorption is that it is mainly a process of imbibition by cell walls which act as a hydrophilic gel. The fine structure and composition of hyphal walls is of great importance for water retention during the process of swelling. However, one must not overstate the part of physicochemical processes of the lichen's water relationships. I do not agree with investigators, i.e., Smith (1962) in particular, who consider the water-relation process to be purely physical and who rule out the influence of metabolic processes. Showman and Rudolf (1971), who studied water relations of *Umbilicaria papulosa* in living and dead thalli and also in cellulose models, were more discreet in their statements. Although they spoke of water-absorption and water-loss processes as physical functions, this was not stated categorically: "Although it appears from these results that the living condition influences water-vapor uptake, this is apparently a consequence of the physical nature of the system rather than of the metabolic processes involved." Unfortunately, at present we do not have experimental data testifying to the active influence of metabolism on water absorption and loss by lichens. Such evidence was recently obtained for mosses (Karimova, 1971), a group of plants that ecologically have much in common with lichens, and this supports my contention that metabolic processes influence water relations. Studies of the importance of respiration in water relations of *Climacium dendroides* have shown that water uptake and loss are a combined process, involving both the physical process of diffusion when filling an empty space and the biochemical processes connected with expending energy at further water conducting into the cell. I feel that the absence of similar information for lichens may be explained by the lack of precise experiments.

The mechanism by which water passes from the cortex to the inner tissues is not known although the process is evidently a rapid one. It is only known that water is conducted into the thallus by capillaries among the cells, by swollen cell walls, and, according to Sievers (1908) and Stocker (1956), by

the cellular cytoplasm. It is a remarkable feature of most lichens that while the rate of centripetal conduction of water through a dry cortex is very rapid, the rate of longitudinal conduction along the cortex is very slow and limited—even in lichens with longitudinally oriented cortical hyphae, such as *Teloschistes flavicans* (Cuthbert 1931). Trying to explain this mysterious phenomenon, Smith (1962) supposed that the intermicellar spaces in the hyphal walls were specifically oriented in a centripetal direction. Further evidence for such a hypothesis must come from the studies of the fine structure of hyphal walls. The thick cell walls offer no significant barrier to water conduction into the cytoplasm. The proof of this is evident from the fact that the respiration of air-dried lichens increases greatly after moistening. However, until Smith's hypothesis of intermicellar spaces in cell walls is confirmed, it is difficult to speak of the degree of the barrier for water conduction into the cytoplasm. The process of water-molecule conduction through cytoplasmic membranes is complicated and is not understood even in higher plants cells. Slatyer (1967) claims that the diffusing molecule can cross the lipid layer of the cell membrane when it possesses the minimum kinetic energy needed to break the van der Waal's forces encountered between the adjacent $-\text{CH}_2-$ groups of lipid chains and can separate the lipid molecules. This, according to Slatyer, may be the manner in which a "pore" is created, a transient structure closing again when the kinetic energy of the hydrogen bonds between successive diffusing water molecules drops below the energy of the surrounding lipid molecules. At low temperatures (Slatyer, 1967), it is possible that only transient pore development occurs because most of the diffusing water molecules are single or transient files rather than continuous files. With increasing temperature, however, the extent and permanence of the pores may increase until, above the critical temperature, the activation energy would be such that water-filled pores could be effectively maintained. At this stage the low temperature coefficient value of water transport is similar to that for the viscosity of water, which implies a minimal resistance to water transport.

The dependence of water-transport rate upon temperature may help to explain why lichens are less able to take up liquid water and water vapor with decreased temperatures (Kolumbe, 1927; Smyth, 1934). However, I doubt whether the rough techniques used to measure saturation could detect the temperature dependence of the saturation process.

The data on water absorption by upper and lower thallus surfaces is contradictory. Nylander (see Elenkin, 1921) claimed that water is absorbed only by the upper surface, and the lower one along with rhizinae does not participate in the lichen's saturation. Berdau (1876), however, found that the lower surface of crustaceous and noncrustaceous lichens can conduct substratum solutions. But, he noted that the upper surface absorbed water at a

higher rate and more easily than the lower one. Zukal (1895) noted the advantage of the lower surface of some foliose lichens in water absorption. He paid much attention to the bundle of rhizinae and noted rapid conduction of colored solutions by rhizinae (Elenkin, 1921). However, the experiments by Poelt and Baumgärtner (1964) in which water along the rhizinae surface failed to rise showed that the long rhizinaelike strands of *Squamaria* type were unable to conduct water actively, while rhizinae of a more primitive type with a thick weft of hyphae could take up capillary-held liquid water and could conduct it farther into the thallus. The main function of rhizinae is supposed to be mechanical, i.e., as attaching organs. The absorption of water and nutrients by the dead basal part of the podetia of *Cladonia rangiferina* was shown by Barashkova (1963). According to Sievers (1908) and Bachmann (1922), the lower cortex is of great importance for liquid-water absorption by crustaceous lichens. In the central axis of *Usnea*, which consists of longitudinal hyphae joined by a chondroid substance, the rate of water conduction is as small as on the base of some fruticose lichens. In experiments with *Usnea* the rate was only 1 mm/hour (Goebel, 1926b). This testifies to the fact that such structures serve primarily mechanical functions.

Thus, the question of the mechanism of liquid-water and water-vapor absorption and the question of water conduction in the lichen thallus needs further detailed study.

IV. Water Contents of Thalli

Numerous authors give contradictory data regarding water content of the saturated thalli of the same lichen species. Such discrepancies are due mostly to subjective causes, i.e., methods of investigation and techniques of measurement. Objective differences based on a specimen's anatomical and morphological traits and other properties are also important. However, according to Hilitzer's (1927) statistic calculations and our data (Blum, 1964, 1965), these differences are not very significant, at least for nongelatinous lichens. Unfortunately, for many studies the results cannot be compared because of the different methods used to calculate the water content of the thallus; for example, grams per 1 gram of dry weight, percentage of the absolute dry weight, and percentage of the air-dry weight. While comparing the data of different authors about water-retention ability of lichens, it is important to consider the degree of liquid water removal from the thallus surface and also the time of saturation. The importance of the latter is proved by the results of our experiments, presented in Table I, where lichens are ranked according to the increase of absolute values of water content in an "oversaturated" state within the limits of different species.

TABLE I
WATER CONTENT OF DIFFERENT LICHENS IN RELATION
TO THE TIME OF SATURATION

Lichen	Water content (% of dry weight) reached in the state of saturation after consecutive 30- second immersions	Water content in an "over- saturated" state (% of dry weight) after immersion of the saturated thallus for		
		5 minutes	1 hour	15 hours
<i>Aspicilia fruticulosa</i>	116	117	121	131
<i>Aspicilia esculenta</i>	129	130	137	147
<i>Cladonia rangiformis</i>	118	119	127	133
<i>Cladonia crispata</i>	133	134	141	148
<i>Cladonia gracilis</i>	125	123	137	151
<i>Cladonia furcata</i>	126	128	243	155
<i>Cladonia uncialis</i>	129	134	144	158
<i>Cladonia mitis</i>	140	142	149	158
<i>Cladonia rangiferina</i>	145	149	160	167
<i>Anaptychia ciliaris</i>	120	144	153	157
<i>Cetraria islandica</i>	109	133	149	165
<i>Umbilicaria grisea</i>	152	157	161	165
<i>Umbilicaria hirsuta</i>	157	161	164	169
<i>Umbilicaria pustulata</i>	197	201	203	209
<i>Hypogymnia physodes</i>	128	152	166	171
<i>Hypogymnia tubulosa</i>	202	210	215	219
<i>Parmelia ryssoleae</i>	122	127	141	174
<i>Parmelia vagans</i>	124	131	148	173
<i>Parmelia prolixa</i>	160	167	185	205
<i>Parmelia sulcata</i>	151	158	173	213
<i>Ramalina fastigiata</i>	139	142	149	174
<i>Ramalina farinacea</i>	141	149	193	225
<i>Dermatocarpon miniatum</i>	142	150	164	185
<i>Dermatocarpon vellereum</i>	149	156	169	201
<i>Bryopogon implexus</i>	162	168	175	196
<i>Bryopogon jubatus</i>	145	149	158	219
<i>Lobaria pulmonaria</i>	150	180	193	199
<i>Usnea hirta</i>	154	163	183	214
<i>Pseudevernia furfuracea</i>	185	191	208	216
<i>Evernia prunastri</i>	149	173	212	248
<i>Xanthoria parietina</i>	174	178	188	227
<i>Peltigera polydactyla</i>	247	252	263	286
<i>Peltigera rufescens</i>	254	264	277	288
<i>Peltigera canina</i>	275	284	305	319
<i>Collema cristatum</i>	394	398	432	474
<i>Collema flaccidum</i>	566	588	609	738

Summarizing the existing data about water-retention ability of lichens, we can say that the saturated water content of most fruticose and foliose lichens lies between (130) 150–300% of the dry weight. Ried (1960b) indicated that crustaceous lichens are comparable in this respect to noncrustaceous forms. Much higher values—from 400–1300 and even 3900%*—have been obtained from gelatinous lichens (Jumelle, 1892; Sievers, 1908; Salomon, 1914). Such high water contents in saturated thalli of gelatinous lichens can presumably be explained by the ability of their phycobionts (blue-green algae) to hold large amounts of water in their thick gelatinous sheaths. However, studies by Moser-Rohrhofer (1965) with a polarizing microscope of the gelatine of *Collema* showed that this gelatine is formed mainly by the fungus and not by the alga as was previously thought. The cells of *Nostoc* have their own thin gelatinous walls and unlike the fungus gelatin, which contains chitin, the algal walls contain only cellulose.

Jacobs and Ahmadjian (1969), who examined the ultrastructure of ten foliose and fruticose nongelatinous lichens, found a thick (up to 1 μm) fibrillar material of a polysaccharide nature surrounding the fungal cell wall. A similar extracellular polysaccharide was also found between the cells of the *Trebouxia* phycobiont. This substance supposedly facilitates the water retention ability of the thallus.

Most lichens, except gelatinous forms, accumulate less water during saturation than other cryptogamic plants (fungi, algae, and mosses). Water accumulation in the thallus is very uneven. Different parts of the thallus and its layers contain different amounts of water. There are no data about the water content of mycobiont and phycobiont cytoplasm, but it is considered to be small.

There are contradictory data regarding water content in the medulla of a saturated thallus. Tobler (1925) reported that the medulla of saxicolous lichens absorbs much less water than the cortex and algal layer. Tobler referred to Beckmann's (1907) observation with *Haematomma*. But, according to Bachmann (1923), in the thick thallus of *Gyrophora vellea*, water accumulates mainly in the medulla. In experiments by Smith (1960), the medulla of *Peltigera polydactyla* contained 25% more water per unit of the dry weight than the upper cortex and algal layer. Goebel (1926a) found that the central axis of an *Usnea* species contained about one-third of all the water in the saturated thallus. Applying nonaqueous staining techniques Showman and Rudolf (1971) found that most of the water in a saturated thallus of *Umbilicaria papulosa* was held in the algal layer.

The well-developed hypothallus of some species is of great importance for the absorption and accumulation of water. Goebel (1926a), for example,

*This value is given by Jumelle (1892) for *Collema* sp.

noted that the lower part of the thallus of *Pannaria mariana* var. *radiata* has a cushion of thick black branching rhizoids that overhang the thallus edge and readily absorb liquid water. A similar function was noted by Degelius (1935) for the hypothallus of *Parmeliella plumbea* and by Bachmann (1923) for the hypothallus of *Pannaria pezizoides* and *Placynthium nigrum*.

Bachmann (1922, 1923) recognized another efficient device for water accumulation, namely the dead algal cells that make up necral layers in the thallus. A highly developed hyponecral layer was discovered by Bachmann in silicate lichens, i.e., *Aspicilia laevata* var. *albicans*, *Lecanora badia* and *Lecidea fuscocinerea*. This layer explains the high absorption ability of these lichens. It should be noted, however, that the great importance of the necral layers for the water relations of the lichens as described by Bachmann is questionable. He may sometimes have confused the medulla with the necral layer. Bachmann (1922) described lichens on silicate substrates whose medulla could be designated as a hyponecral layer if it contained dead, empty algal cells.

There are not any experimental data about the role of hypothallus and necral layers in the water relations of lichens.

The water content of a lichen thallus is closely correlated with the water content of the air. After a dry, sunny day the water content of *Umbilicaria dillenii* and *U. pustulata* was 6.4% of the dry weight (Scofield and Yarman, 1943). Neubauer (1938) obtained values for the water content of six epiphytic lichens on beechwood that ranged from 2% (*Parmelia caperata*) to 14.5% (*Usnea dasypoga*). Field measurements by Lange (1952) during periods of severe drought with thallus temperature often above 60°C showed that the minimum water contents of the investigated lichens ranged from 2% (*Collema multifidum*) to 8.9% (*Candellariella vitellina*) of the dry weight.

Thus, although in certain periods the water contents of lichens may be very low, the thalli do not dry out completely under natural conditions.

Water content of the thalli may rise considerably when there is dew. Stocker (1927) observed that epiphytic lichens such as *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina fraxinea*, *Usnea hirta*, and epigaeic lichens, such as *Cladonia rangiferina*, increased their water content as a result of dew absorption by about 50% of their dry weight. Butin (1954) found that the water content of *Pseudevernia furfuracea* rose from 10 to 60% of the dry weight during the 10 hours of night when there was dew and then dropped back to 10% during the 6 hours after sunrise. Scofield and Yarman (1943) showed that the water content of *Umbilicaria dillenii* (= *mammulata*) and *U. pustulata* rose after nightfall when there was dew by 85.4% and 112.3% of the dry weight, respectively. Lange (1952) gave the following values of water content in lichens after nightfall when there was dew: *Cladonia sylvatica*, 50%; *C. rangiformis* var. *pungens*, 73%; and *Peltigera*

canina, 116.6% of the dry weight. A lower water content in the thallus after dewfall was observed by Kershaw and Rouse (1971) for *Cladonia alpestris*. It was about 12% saturation. However, even at this water content the lichen had a net assimilation of 35% of the maximum. Thus, the ecological importance of dew as a source of sufficient moisture to reactive lichen metabolism is significant. Dewfall at night, according to Lange (1969) and Lange *et al.* (1970), who conducted field experiments in the Negev Desert, caused reactivation of all the investigated lichens and ensured considerable photosynthesis and a positive metabolic balance during several morning hours.

Mist also influences the water content of a thallus. Stocker (1927) considered misty days to be especially favorable for lichens because small drops of water carried by the wind assure the thalli of an even and prolonged moistening. Also, even on dense, foggy days the amount of diffused light (according to Stocker) is sufficient for assimilation. The ecological importance of mist is great because of its ability to retain water loss in the thallus (Butin, 1954).

Stocker (1927) claimed that the frequency of mists and their density were more important for the geographic location of lichens than the amount and frequency of rain. Degelius (1935), however, felt that the quantity and, especially, the yearly distribution of rain precipitation was more important for the distribution of ocean lichens than the frequency of mists. He observed that there are regions with a high frequency of mists where ocean lichens are completely absent and regions with rare fogs but with an abundant development of oceanic species.

The maximum saturated water content of lichens under natural conditions is achieved only during rain showers and for short periods thereafter. According to Stocker (1927) and other investigators, lichens do not achieve their maximum water saturation after a heavy, but short rain because water streams down from their thalli. Thus, even during humid periods, lichens are almost always in a state of insufficient saturation. The peculiarity of their gas-exchange processes is closely connected with this ecological property. Stocker (1927) showed that *Lobaria pulmonaria* and *Umbilicaria pustulata* reached their optimum assimilation at 70–75% of their maximum saturation. Stålfelt (1939) obtained similar data for *Ramalina farinacea* and *Usnea dasypoga*. At water contents above the optimum level the rate of photosynthesis decreases. Stocker (1927) supposed that the decrease in the rate of photosynthesis in saturated thalli (i.e., at a water content higher than optimum) was due to the reduced rates of gaseous diffusion through the thallus to the algal layer because of the swelling of the cortical hyphae and filling of the capillary spaces with water. According to other authors (Smyth, 1934; Ellée, 1939; Butin, 1954; Ensgraber, 1954), the investigated lichens in-

creased their assimilation until they were completely saturated. This discrepancy was analyzed by Ried (1960a). He found a clear relationship between thallus anatomy and the level of optimum water content for photosynthesis. The thicker and denser the thallus and the more that it is covered by a cortex, the lower the optimum water content for maximum photosynthesis. The smallest optimal level (65% of the maximum saturation) was that for *Umbilicaria cylindrica*. This thallus is covered with a thick upper and lower cortex. The highest optimum water content (90%) for photosynthesis, except for aquatic lichens, was that for *Peltigera canina*, which lacks a lower cortex and has a friable medulla. These results enabled Ried to interpret the earlier contradictory data.

The aquatic lichens, *Verrucaria elaeomelaena* and *Porina lectissima*, had maximum rates of photosynthesis even in a saturated state. Carbon dioxide could naturally penetrate the algal layer of these lichens only in solution. On the contrary, the optimum photosynthesis of the amphibian lichen *Dermatocarpon fluviatile* was at a water content less than 100% of saturation. The study of the gas interchange of different ecological groups of lichens, in relation to water content of the thallus, requires more detailed investigation. The peculiarities of gas interchange cannot be explained solely by differences in thallus morphology and structure. The lichens' physiological reaction to conditions of fluctuating saturation level is undoubtedly connected with their ecological type. This statement may be confirmed by the data of Rouse and Kershaw (1971) that the optimum rate of net assimilation for *Cladonia alpestris* was at 52% of thallus saturation. In this way the authors explain the absence of this mesoxerotic species in very wet habitats.

V. Water Loss

Water loss from the thalli is mostly a physical process, like water absorption, and it is a very rapid process. Field observations have shown that saturated thalli dry out within a few hours in dry weather.

Studying water loss by saturated thalli under laboratory conditions, Stocker (1927) and Hilitzer (1927) found a linear relationship between the loss of most of the water and time. They also found that loss of the remaining water was slower. The rates of water loss from lichen thalli under various conditions of temperature and atmospheric humidity, have also been measured by Sievers (1908), Bachmann (1922, 1923), Degelius (1935), Ellée (1939), Scofield and Yarman (1943), Mägdefrau and Wutz (1951), Ensgraber (1954), and Ried (1960b). However, since most of these experiments were not carried out under constant conditions the data cannot be used for the ecological evaluation of the rate of water loss by separate species. In this

respect, the experiments by Hilitzer (1927) and Ried (1960b) are more valuable since they were conducted at constant temperature and air humidity. Hilitzer, who found the mathematical expression of the dependence of the evaporating rate on the degree of thallus saturation and the deficiency of air humidity, concluded that this ratio (coefficient k) is unique for each species and can be characteristic of its ecological amplitude. The truth of this statement will be considered a bit further.

As was already mentioned, lichens cannot reserve water for relatively long periods of time regardless of environmental conditions. They do not appear to have special devices for transpiration protection. Zukal's (1895) idea that the cortex plays an essential role in retarding water evaporation was disputed by Goebel (1926b, 1927) and Stocker (1927) who stressed the significance of hyphal water reserve. Quispel (1943) considered the protection of the algae from drying out by the fungus, although effective only for several hours, to be a peculiar buffer in that hyphae prevent rapid changes in humidity. At the same time the ability of lichens to dry out quickly protects them from ruinous overheating in a saturated state.

The relation of the cortex thickness to the aridity of the habitat, stated in a number of investigations, does not appear to be natural. In some cases, lichens in dry habitats have a more developed cortex than lichens of shady, humid habitats (Bitter, 1901; Ertl, 1951). In other cases, there is a striking lack of correspondence in this respect (Galløe, 1908).

As noted earlier, Hilitzer (1927) considered the rate of water evaporation by thalli to be a very important ecological factor. He separated all the investigated species into the following ecological groups according to the rate of evaporation values: (1) xerotic species; (2) mesotic species; (3) aerotic and subaerotic species; (4) skyotic and psychrotic species. It is striking that there is not a single classification principle in this ecological diagram. That is why the ecological groups, singled out according to more or less close values of the coefficient k , are in most cases of an artificial character and do not reflect natural relationships. Our experiments (Blum, 1965) conform well to Hilitzer's conclusion about xerophytes having mostly an increased value of the coefficient k , which, nevertheless, is not sufficient to prevent the lichen from drying out during the day. However, we found that such mesotic species, such as *Cetraria glauca*, *C. hepatizon*, and *Cladonia squamosa*, and mesohygrotic species, such as *Leptogium saturninum* and *Nephroma resupinatum* (in Hilitzer's experiments), had rates of evaporation that were very close to those of xerophytes. Also, the studied xerotic forms, *Cladonia rangiformis*, *Peltigera rufescens*, *Umbilicaria grisea*, *U. hirsuta*, and *U. pustulata*, did not show considerable advantages in water retention compared with the lichens of other ecological groups. Mesohygrotic *Peltigera polydactyla*, mesotic *P. canina*, and xerotic *P. rufescens* did not show considerable differences in the rate of water loss (Blum, 1965).

Thus, we may assert, that although the evaporation rate of lichens is unique and relatively constant under uniform conditions for every species, it cannot serve as a satisfactory criterion for their ecological evaluation.

VI. Conclusions

Studying the features of the water relations of lichens we can better comprehend the peculiarities of this process for various groups of vegetation. It is the lichens' water relations that predetermine, in the main, their striking ability to exist under extreme environmental conditions both in arid deserts and in the frostbound Arctic and Antarctic. This vast geographic distribution of lichens is due to their evolutionary adaptation to unfavorable drought periods and is of a complex character. Under unfavorable conditions their metabolic processes are slowed, they attenuate, they become latent, while, on the other hand, they revive rapidly and make the most of short, favorable periods. Quick water loss by lichens and lack of special devices for transpiration protection is one of the ways of their physiological adaptation to xerotic conditions. The ability of lichens to dry out quickly protects them from insolation which is dangerous for a wet thallus. The process of gelification of cytoplasm during the lichen's dehydration prevents its submicroscopic structure from infringement and coagulation (Genkel, 1946; Genkel and Pronina, 1968; Genkel *et al.*, 1970).

Besides the lichen's universal physiological adaptation to drought due to quick dehydration, there are some anatomical and morphological peculiarities which allow them to prolong slightly their relatively short assimilation activity. Mostly it is the protection of the algal cells by fungal hyphae which serve as a peculiar buffer that prevents rapid changes in humidity (Quispel, 1959).

Blue-green phycobionts hold large amounts of water in their thick gelatinous sheaths. Some lichen forms have features of "xeromorphic" structures, i.e., a thick and solid cortex, cortical hairs, white pruina, etc. Many investigators see protective adaptation of the organism from water loss in such structures of lichens and mosses (Sapegin, 1910; Elenkin, 1921; Bachmann, 1922; Dombrovskaya, 1963, 1964, 1970). Besides their prime functions (mechanical, photoprotective), these structures sometimes give certain small advantages for water retention in some lichen forms. However, the statements about the "xeromorphic" structures of lichens and mosses in dry habitats as effective xerotic adaptation appear untenable and are often not confirmed by experiments (Blomquist, 1929; Ochi, 1957; Clausen, 1952; Melnichuk, 1961; Blum, 1965). It is appropriate to mention here that features of "xeromorphic" structure are not found in all lichens of arid habitats.

One can understand a lichen's adaptation to xerotic conditions only by

studying the inner molecular and biological mechanisms of adaptation to extreme environmental conditions. Since rapid dehydration involves all lichen-cell ultrastructure, we can speak of a certain latent period under these conditions when all vital processes are strongly inhibited. If during dehydration and saturation the cell structures that are responsible for biochemical functions, are not damaged irreversibly, then after the latent period all vital processes are reactivated. The species' ecological resistance depends on the relation of reactivation rates of these processes. Therefore, from the point of view of the present state of ecophysiology, the differences of various ecological types among lichens lie not in the quantitative criterion of water relations, but in reactivation rates of photosynthesis, respiration, and oxidative phosphorylation after the latent period and in preserving a normal correlation of these processes.

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Chapter 12

SUBSTRATE ECOLOGY

IRWIN M. BRODO

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I. Introduction

The most tangible element of a plant's environment is its substrate, the material on or in which the plant grows. In the mycological sense, the sub-

strate is often interpreted to mean the nutritive material used by the fungus. Lichenologists have avoided such a narrow definition since the role of the material supporting lichen thalli is a complex one. In fact, it is quite open to question whether lichens derive any nutriment from their support at all.

It is clear, however, that lichens have substrate "preferences." Few, if any, are found everywhere on anything. Terms such as "rock lichens," "bark lichens," and "ground lichens" are generally accepted and understood. Identification keys are often based on this substrate classification, studies are organized around it, and chapter headings reflect it. Technically, we speak of saxicolous, corticolous, lignicolous, muscicolous, terricolous, and foliicolous lichens, in referring to species found on rock or mineral, bark, wood (i.e., lignum), bryophytes, soil, and leaves, respectively. It is the purpose of this chapter to review what is known about these substrate preferences, and to discuss their causes and implications with regard to the ecology, distribution, and taxonomy of lichens.

II. Practical Aspects

The importance of having an understanding of the relationships between lichens and their substrates goes beyond our ordinary interest in lichen biology. Lichens are well-known accumulators of minerals from the soil and air (see Chapter 7 by Syers and Iskandar in this treatise; also Lange and Ziegler, 1963; Beschel, 1959), and their use as geobotanical indicators of mineral deposits has only recently been considered (Viktorov *et al.*, 1964; LeRoy and Koksoy, 1962; F. H. Erbisch, personal communication). Their accumulation of toxic materials from polluted air deposited on substrate surfaces is another area of concern (see Chapter 13 by Gilbert in this treatise). The harmful effects of lichens on substrates of economic importance will be discussed in Section V.

III. Substrate Factors

Of the many properties of a substrate one might examine to determine what causes the particular behavior of a lichen, the texture, the water relations, and the chemistry are most important. There are of course other factors to be considered which may affect lichen distributions, such as color, hardness, and physiology, but the first three are most often discussed and probably are most significant.

Barkman (1958), in his classic treatise on the ecology of cryptogamic epiphytes, has covered the subject of bark factors thoroughly, and so only some of the important papers which have appeared since 1958 will be covered here.

A. Texture

The smoothness, hardness, relative stability, and surface features of substrates have often been cited as factors causing the restriction of lichens to one substrate or another. Surface texture formed an important part of Hiltizer's (1925) ecological classification of epiphytic lichens. It has, in fact, been asserted that the physical properties are the only substrate factors determining the distribution of lichens (Richard, 1883). Some of these characteristics bear directly on other aspects of the substrate, such as the relationship of bark hardness to moisture capacity, but most seem to have their own influence.

Ease of colonization is certainly one of the more obvious effects of differences in substrate texture. Lichen diaspores can become trapped and begin to develop on rough surfaces more easily than on smooth surfaces. Trees with flaky bark, going through fairly frequent exfoliation cycles, will be uncolonizable by certain lichens. Des Abbayes (1934) and Kalgutkar and Bird (1969) found many species limited to the bases of pines due to the flaking of the bark near the treetops. This contrasts sharply with post oak (*Quercus stellata**) which becomes a less suitable substrate on the older parts of the trunk due to the increasing flakiness of the bark (Adams and Risser, 1971a).

The species on the smooth twigs of *Fraxinus excelsior* comprise a community quite different from that on rougher, older twigs from the same tree (Degelius, 1964). On rough and fissured bark, some lichens can be seen to colonize the crevices and some the plates (Brodo, 1968; Yarranton, 1967) although it is not clear whether in this case it is a matter of colonization ability on rough versus smooth surfaces, or survival in moist (crevice) versus dry (plate surface) microhabitats.

Rough rock surfaces normally bear a richer lichen flora than smooth surfaces, and undoubtedly the ability to compete successfully on very smooth stones evolved in some species surviving under extremely rigorous conditions of colonization. *Lecidea erratica*, for example, is largely restricted to smooth pebbles on Long Island, New York probably due to this ability to compete (Brodo, 1968). Lichens frequently first colonize the softer parts of rocks, such as mica, and the edges of layered rocks. On the other hand, some species of *Heppia* colonize the hard pebbles, not the soft matrix, of some types of conglomerate rocks, apparently due to the instability of the matrix (Wetmore, 1970).

In western North America, the leaves of *Thuja plicata* are regularly colonized by nearby twig-dwelling species, whereas the leaves of other ever-

*Scientific names of all vascular plants, bryophytes, and animals are used in the original author's sense. Lichen nomenclature follows Hale and Culberson (1970) or Grummann (1963) unless otherwise noted.

greens are generally free from lichens (Vitt *et al.*, 1973). The heavy lichen growth on the leaves of *Thuja* seems to be due entirely to the rough and scurfy nature of the leaf surface (Daubenmire, 1943).

Galun (1963) compared the lichen vegetation of various types of limestone rocks in the Negev of Israel and found the same community whether the rocks were crystal limestone, lithographic limestone, flinty limestone, or dolomite, all having different textures and hardness. A similar observation was made by Degelius (1955) with regard to crystalline and noncrystalline limestone except that the crystalline limestone had a richer flora. However, Braun-Blanquet (1951) reported that dolomite is species-poor compared to limestone.

Lichens, being slow-growing, normally cannot develop on moving or shifting sand and soil. In sandy areas, lichens normally gain a foothold on dead vegetation, especially on the dead stumps of grasses (Alvin, 1960; Robinson, 1959; Brodo, 1961) or on moss (Richards, 1929; Alvin, 1960; Brown and Brown, 1968). Species of *Peltigera* tolerate moderate sand coverage (Brown and Brown, 1968) and so are early colonizers of dune areas. McLean (1915) thought that the instability of the sand was the determining factor in the dune vegetation he studied. He recorded a succession of species along a gradient from bare sand to stone-stabilized "shingle," with *Cornicularia* (*sub Cetraria*) *aculeata* f. *acanthella* and *Cladonia furcata* invading the spots surrounded by stabilized sand.

B. Water Relations

The availability of moisture has long been recognized as a factor in the distribution of lichens. In a recent study by Kershaw and Harris (1971), for example, using the "systems model" approach based on physiological investigations, the authors concluded that the vertical distribution of *Parmelia caperata* on tree trunks was determined by water availability, and not by light. Barkman (1958) discusses the relationships between the moisture capacity of bark and the distributions of epiphytes in great detail. Both Smith (1962) and Harris (1971b) found that substrate moisture acts in the hydration of the thalli, thereby influencing the lichen's rate of photosynthesis and respiration.

1. BARK

In discussing the moisture relations of bark, there are two elements to consider: (a) the moisture of the bark originating from the tree's metabolic activities and (b) the moisture originating from the external environment through rain, snow, fog, dew, inundation, etc.

It has been suggested that the distribution of certain hypophloedal crustose lichens (those living under the outermost cork layers) is determined by

the moisture available in the living bark tissue of thin-barked trees (Johnson, 1940). It is interesting and somewhat surprising in this regard that smooth-barked trees such as *Betula* and *Fagus* have the lowest transpirational rates and those with fissured bark such as *Quercus* and *Pinus* have the highest transpirational rates (Geurten, 1950). Whether epiphytes benefit from this transpirational moisture is not known.

Most authors feel that externally derived moisture is the more important water source for lichens, and have examined the natural moisture content [the *humidité rémanente* of des Abbayes (1932)] and moisture capacities of many phorophyte barks in efforts to find correlations with epiphytic vegetation.

It is logical that barks with different densities, porosities, textures, and internal structures should differ in their capacity to absorb and hold water. In analyzing the epiphytic vegetation on oaks and pines, des Abbayes (1932) concluded that the oaks had considerably more *humidité rémanente* than did pines. He found that oaks not only retain more moisture, but liberate it more regularly and make it available to the lichens over a longer period of time and at higher levels. Differences in epiphytic vegetation between *Quercus velutina* and *Q. alba* (Hale, 1955) and *Pinus albicaulis* and *Larix lyallii* (Kal gutkar and Bird, 1969) were attributed largely to differences in bark water capacities between the phorophytes. Culberson (1955b) found that a "continuum" of epiphytic plants in northern Wisconsin agreed fairly well with a continuum of bark-moisture capacity, pointing to a possible relationship between them. Margot (1965) was able to relate differences in the epiphytic vegetation on the bark of poplars of different age to differences in moisture capacity over a tree-age gradient.

Various terms such as "substratohygrophilous" have been proposed for lichens requiring substrates with high moisture contents (des Abbayes, 1934; Barkman, 1958). Since the species given by des Abbayes as examples might well be found on substrates with low moisture capacities but in wet situations (e.g., rain tracks, seepage walls, close to bodies of water, etc.) I can see little value in adopting terms of that kind.

A list of trees in decreasing (or increasing) order of moisture capacity would be useful in the study of epiphyte ecology. Unfortunately, such a list will not be practical until methods for measuring and expressing bark-moisture relations are more or less standardized. Even then, variations within the trees themselves—whether within species, populations, or even individuals—will always be a serious problem, as will be seen below. First we will examine methods of moisture determination and expression.

Some authors have simply measured the field-water content of bark samples collected under "comparable" conditions, expressing it as a percent dry weight (Young, 1938; Billings and Drew, 1938). The difficulties of getting

reproducible results with differences in atmospheric humidity, and fluctuations in air temperature are obvious (see Barkman, 1958). It is known, for example, that natural bark moisture varies not only seasonally but diurnally as well. Moisture content is greatest in the rapid growing period, less in early spring and winter and least in autumn (Srivastava, 1964).

More frequently, authors have saturated bark samples in the laboratory in various ways and measured total moisture capacity either directly (as water absorbed per unit dry weight of bark, per unit volume, or per unit surface area of the sample), or indirectly, determining the rate of water absorption to saturation and/or the rate of water loss under given conditions. Barkman (1958) summarizes the subject admirably. It was he who first showed some dissatisfaction with the standard "dry weight" expression of water capacity, and suggested that a "per unit volume" expression was more realistic. LeBlanc (1962) and Margot (1965) determined the moisture capacities of their material and expressed their results both as ratios to dry weight and surface area of the sample. Indeed, ordinations of various species of trees, or age classes of the same tree, are entirely different depending on the method of expressing moisture capacity. Both LeBlanc (1962) and Brodo (1968) found that in comparing the moisture capacities of *Quercus rubra* and *Fagus grandifolia*, the former species is more mesic than the latter in a surface area expression, but quite the opposite is true with a dry-weight expression.

In measuring surface area it is probably better to use pieces of aluminum foil fitted as closely as possible to the contours of the bark sample and then weighed, deriving the surface area from a graph plotting foil weights against known surface areas (Brodo, 1968) rather than to use the external dimensions of the samples alone.

It is difficult to state unequivocally which method of expression is "better." The object, obviously, is to find a method which best reflects the factor which actually controls the distribution of the vegetation. Since the dry-weight expression is strongly affected by the density of the bark sample, it would seem that a surface area expression is more realistic. The surface area, of course, is much more difficult to measure accurately and has a narrower range than the dry-weight measurement (10–60 gm water/100 cm² versus [13–]29–178[–465]% water absorbed/dry weight).

The volumetric expression does not seem very valuable unless the sample volumes are carefully controlled, with the "nonabsorbing" portions of the sample kept to an absolute minimum. In addition, the problem of determining volume is compounded by the tendency of some bark types to trap air bubbles in standard water-displacement methods (Brodo, 1968).

All these methods of expressing moisture capacity still suffer from variations due to the characteristics of bark itself. For example, in some cases

the moisture capacities of bark at different exposures (Barkman, 1958) or vertical position (LeBlanc, 1962) of the same tree are different, and the same is true of trees of different age (Brodo, 1959; Margot, 1965). LeBlanc, however, could not detect any difference in the moisture capacities of bark samples from different exposures of the same tree. The moisture capacity of bark samples of a single tree species sampled in various vegetation types (wet versus dry) can be quite distinctive and can sometimes differ by a factor of three (Brodo, 1959). It therefore would seem highly unlikely that data derived from populations of a species in one part of its range would be directly comparable with data gleaned from trees in another part of its range. In fact, it is surprising that so close an agreement among the moisture capacities of some tree species has been found by various authors.

With all these things in mind, perhaps we might still hazard a few generalizations concerning the moisture capacities of various trees. Oaks, by and large, have low moisture capacities. This is even true of the "soft-barked" oaks such as *Quercus alba*, if surface-area expressions are considered. Elms have high moisture capacities, and pines are somewhere in between, as is beech.

The impact of these moisture capacities on the epiphytic vegetation apparently is determined, to a large measure, by the general moisture available to the epiphytes from the air. In moist woods, the effect is not nearly so marked as it is under xeric conditions (Billings and Drew, 1938; Barkman, 1958; Brodo, 1959; Margot, 1965).

2. ROCK AND SOIL

Much less has been published concerning the water relations of other types of substrates. Certainly various soil and even rock types absorb, bind, and release water at different rates and to different degrees. Sand holds less water than does a mixture of sand and humus, or even clay, and it holds this water for a much shorter period of time. The problem of relating lichen vegetation to moisture capacities of rock and soil types is seriously complicated by variations in available nutrients from one substrate type to another. The same is also true of bark, but presumably to a lesser degree, at least within each tree species. This will be discussed in the next section.

C. Chemistry

It is almost universally acknowledged that lichens are affected by their chemical milieu, and that many aspects of their microdistribution are determined by the chemistry of the substrate.

As a result of recent research on the physiology of the intact lichen thallus as well as of isolated lichen components, we now know that lichens are not

only able to absorb minerals but actually are efficient accumulators, and that lichens and their components can utilize a wide range of organic nitrogen and carbon sources as well (Smith, 1962).

1. MINERALS

The inorganic minerals and organic substances formed in, or washed from, the substrate surfaces seem to be of great significance in lichen distribution. Barkman (1958), in reviewing the influence of bark chemistry on lichens, concluded that it is more important to investigate the chemical composition of the water in contact with the bark and lichen than it is to investigate the chemical composition of the substrate itself. His conclusion can be applied equally well to other substrates, including soil and rock. Barkman pointed out that many minerals in the substrate are either insoluble, or in a state unavailable to the plant.

a. BARK. In their detailed study of the nutrient content of stem flow (the water flowing from the tree's crown down the trunk after a rain), Carlisle *et al.* (1967) have provided us with excellent data on the nutrients available to tree-trunk epiphytes. They found that although stem flow is only 1–2% of the annual throughfall (the rain water passing through the crown and falling to the ground), it is very rich in nutrients. Compared with throughfall water, the stem flow from *Quercus petraea* had high concentrations of K (up to 9.9 ppm), calcium (up to 15.4 ppm), magnesium (up to 4.9 ppm), sodium (up to 40.6 ppm), organic matter (up to 142.0 ppm), soluble carbohydrates (up to 14.1 ppm), and polyphenols (up to 9.0 ppm). Kaul and Billings (1965) showed, in their studies of *Pinus taeda*, *Acer rubrum*, *Cornus florida*, and *Liriodendron tulipifera*, that the concentrations of minerals in the stem flow will vary among different tree species. Carlisle and his co-workers analyzed the mineral content of the bark to determine the source of the stem flow nutrients, and concluded that whereas the leaching of the bark tissues could contribute to stem flow nutrients, some nutrients undoubtedly originate from the water passing over the leaves, twigs, etc. Although the importance of stem flow in the mineral supply of epiphytes has been acknowledged for a long time (see Hiltizer, 1925), it seems obvious that much more work of this kind is needed before we will really begin to comprehend the nutrient environment of epiphytes.

Bark chemistry has often been approached through studies of ash content. The principle involved was stated by Barkman (1958, p. 96): "... a close correlation exists between total electrolyte concentration and epiphytic vegetation. The former is expressed by the ash content of the bark." Even considering the ash content by itself, i.e., percent of dry weight, Barkman

found correlations with the epiphytic vegetation: The *Physcietalia ascendens*-*tis* is found on eutrophic bark (ash content [5–]8–12%); the *Arthonietalia radiatae* and *Parmelion caperatae* are associated with mesotrophic trees (ash content [2–]3–5%); the *Calicion hyperelli* and *Parmelietalia physodo-tubulosae* are, by and large, associated with oligotrophic tree bark (ash content 0.4–2.7%). Examples of eutrophic trees are *Acer pseudoplatanus*, *A. platanoides*, *Sambucus nigra*, and *Prunus avium*; some mesotrophic trees are *Quercus robur*, *Q. petraea*, *Fagus silvatica*, and *Fraxinus excelsior*; oligotrophic trees are species of *Betula*, *Picea*, and *Abies*. The bark of softwoods (i.e., conifers) only has an ash content of 0.6–2.5% whereas the ash content of hardwood bark is 1.5–10.7% (Chang and Mitchell, 1955). Margot (1965) investigated the electrolytic conductivity of poplar bark of various ages, and found that conductivity decreases regularly with bark age. Margot expressed conductivity as “micro-mhos/cm” rather than ash content, and therefore his data are not directly comparable with those of Barkman.

Barkman (1958) not only made comparative measurements of ash contents but provided detailed analyses of Ca, K, Na, Mg, Fe, P, S, Si, Cl, tannin, and resin concentrations (most as oxides) for 20 species of European trees.

More recently, Fabiszewski (1968) analyzed the chemical content of bark samples under different lichen communities. Besides finding clear pH correlations with the communities (see below), he found that bark associated with the *Lobarietum pulmonariae* had twice as much iron as did the bark associated with the *Calicietum viridis*, and also had by far the greater amount of magnesium and calcium.

b. ROCK AND SOIL. Attempts to analyze the distribution of saxicolous lichens according to lithochemistry are not very common. Generally, if rocks contain much calcium they will support very similar floras despite other constituents of the rock (see Galun, 1963). Lime-containing rocks have long been known to differ significantly from lime-free rocks in their vegetative cover. Werner (1956) determined the pH values and silica concentrations of various rock types and found that the granite of his area contained 65–74% silica, and the schists contained 57–61% silica. He concluded that the only difference between the two rock types which might explain their different lichen cover was the concentration of silica.

Serpentine presents a very interesting problem in substrate chemistry. Both Rune (1953) and Ritter-Studnička and Klement (1968) noted that serpentine rocks support lichens usually associated with siliceous rocks as well as lichens generally thought of as lime-loving. Ritter-Studnička and Klement, for example, found species of *Rhizocarpon* growing along with *Dermatocarpon miniatum* and *Physcia caesia*. Many observed that serpentine

rocks and soils are generally species-poor, and this led to investigations of serpentine chemistry. Rune (1953) found that serpentine soils were high in many heavy metals, MgO , SiO_2 and often Fe_2O_3 . The soluble potassium and phosphate were low, but no lower than in many relatively fertile soils. He found the Ca content varied widely. Rune concluded that the inhospitable character of serpentine, both for cryptogams and phanerogams, was due to toxic levels of nickel and chromium, low nutrition, low Ca , high Mg , and some mechanical characteristics (see also p. 441).

The possibility that the microdistribution of lichens on a rock surface might be due to a differential distribution of nutrients and minerals was raised by Scott (1967) in a study of lichen communities on granite "kopjes" (a special kind of outcrop) in Rhodesia. A complicated distribution pattern of lichens over the rock surface and around the small rock pools and channels seemed to the author to be due in part to the chromatographic effect of nutrient-laden water seepage over the rock surface, with the adsorption and chelation of certain minerals resulting in a differential zoning of nutrients. Even if no chromatographic effect were taking place, the author felt that considerable concentrations of precipitated salts would occur in certain areas of the drainage channels which could explain the lichen-free and lichen-rich zones which he observed. Each rain would bring about a repetition of the mineral precipitation cycle, bringing about "local concentrations of substances having a growth-promoting or retarding effect on different lichen species" (Scott, 1967, p. 376). It would seem to me, however, that his striking lichen-free zones on the lips of water channels and pools might better be explained by the net assimilation deficit which would occur in spots frequently subjected to flooding and drying (see Ried, 1960; Harris, 1971b). Scott himself mentions this possibility in a different context on p. 374 of his paper.

Although lichens absorb and accumulate minerals, their mineral composition does not always accurately reflect the composition of their substrate. Dormaar (1968) found that the infrared spectra of the rocks differed from those of the minerals in the lichens growing on them, although the residues and weathered rock zones were more similar. He found, for example, that a specimen of *Caloplaca* growing on dolomite showed no evidence of Mg or carbonate in the peroxidized material, not even $MgCO_3$. There was, however, a good deal of calcium in the thallus. It therefore seemed that *Caloplaca* picks up Ca in preference to Mg in dolomite. He concluded from his studies that "It is likely that lichens obtain at least part of their minerals from the substrate." A similar conclusion was expressed more negatively by Jenkins and Davies (1966). They found close correlations between the minerals in lichen ash and ash from material deposited from the air, and

poor correlations between lichen ash and the minerals of their substrate. They concluded that lichens are not nearly so dependent on their substrates for nutrients as was previously thought.

Hertel (1967) in a study of calcicolous *Lecideae* presented a most interesting diagram depicting the relative "calciphily" of 35 taxa of *Lecidea*. The diagram showed that each taxon has an optimum in a continuum of rock types from *reine Kalke und Dolomite* (pure lime and dolomite) to *harte Kieselkalke u.o. kalkarme Gestein* (hard siliceous limestone and/or lime-poor stone), and that some taxa had greater ranges of tolerance than others (e.g., *Lecidea rolleana* sens. str. versus *L. subrhaetica* Arn. ex Lett.). However, it might be argued whether or not these optima entirely, or in part, represent responses to other factors such as the rock hardness, Mg content, and Si content.

The response of certain lichens to extremely high heavy metal concentrations has already been mentioned with regard to serpentine rocks. *Acarospora sinopica*, growing on iron slag, accumulates incredibly high concentrations of some minerals (55,000 ppm of iron and 1100 ppm of copper, both per dry weight of lichen thallus), reflecting the concentrations in the slag, with apparently little ill effect (Lange and Ziegler, 1963). In New Brunswick (Canada) lichens were found surviving in copper seepage areas where the levels of Cu were 1% or 300 ppm at the surface (Beschel, 1959). Peat at the center of the swamps had up to several thousand ppm near the surface, and the lichens growing on the peat had the same high values. The question of how lichens are able to tolerate these concentrations is intriguing and possibly important.

2. HYDROGEN ION CONCENTRATION

Of all the aspects of substrate chemistry which might be considered, the hydrogen ion concentration or "pH" has certainly been the most studied and discussed. Many authors have cited pH as the main or entire cause of certain lichen distributions while others have found no pH-lichen correlations at all.

The acidity or alkalinity of the substrate can act on a lichen thallus in numerous ways. Various minerals and organic substances are in different chemical states under different pH regimes, some more "available" than others; diffusion rates may change at different pH's; some substances are toxic under acidic conditions and harmless when deacidified. Whatever the mechanism of influence, it is evident from numerous studies that the distribution of some lichens and lichen communities is strongly correlated with substrate acidity.

a. BARK. The bark pH of many species of trees was determined by a number of authors. Some investigated the pH of the stem flow (Carlisle *et al.*, 1967), but most took material from the surface layers of bark and tested mixtures of ground or chopped sample fragments and distilled water. They found that different species of trees have bark of different acidities, although the pH of bark samples of the same species taken from different localities or vegetation types in a region generally do not differ significantly (Brodo, 1961). Often, the range of pH for a given species is extremely wide, probably reflecting differences in sampling procedures (see p. 413). Hale (1955), for example, reported a range of 5.0–7.0 for *Ulmus rubra*. One must remember that pH is a logarithmic value and that a bark sample with a pH of 5.0 is 100 times more acidic than one with a pH of 7.0.*

As with the moisture-capacity values, certain trends do emerge. Oaks and pines are highly acidic, with the notable exception of white oak (*Quercus alba*) which is known to have an epiphytic flora unlike that of the more acidic oaks (Hale, 1955). *Acer rubrum* is much more acidic, and *A. negundo* closer to neutral than are other maples, and this is reflected in their epiphytic floras. Brodo (1959) noted lichens characteristic of elms on *Acer negundo*. Although Culberson (1955b) placed *Acer rubrum* midway in a pH series, he found that tree fairly similar in its epiphytic vegetation to *Pinus strobus* which had the most acidic bark. *Ulmus* and *Populus* are generally regarded as neutral-barked species (Barkman, 1958), but the bark of *Populus* apparently can be fairly acidic sometimes. Margot (1965) found pH values of 4.8–5.5 on his trees, Brodo (1968) had a value of 3.9 with a single tree on Long Island, and Culberson (1955b) found that "*Populus* spp." in Wisconsin had more acidic bark than *Quercus rubra* or *Fagus grandifolia* (although he presented no numerical values).

Du Rietz (1945) reported that the number of species on a tree is correlated with pH (acidic bark having fewer species than alkaline bark). He has been supported by evidence given by Fabiszewski (1968), but disputed by Almborn (1948) and Culberson (1955b) (see p. 424).

Fabiszewski (1968) presented an analysis of the chemical affinities of some lichen communities in Poland noting their pH optima. The precise nature of these "requirements" is somewhat obscured, however, by Fabiszewski's observation that significant differences in mineral content were found under the different communities (see p. 409), and that the lowest quantity of ash is from bark having the greatest acidity, and vice versa. [It should be noted

* In this connection, it should be noted that the practice of averaging pH values is not valid, although it is done almost universally by lichen ecologists. It is used here to the extent that "average pH" gives an indication, although an imperfect one, of relative hydrogen ion concentration of various bark types.

that Carlisle *et al.* (1967) observed precisely the reverse relationship between pH and mineral content.] Barkman (1958) has reviewed the work done on the effects of pH on epiphyte distribution, with particular emphasis on the European trees.

Variation in the pH of the bark of the same tree species or even the same tree makes pH-lichen distribution correlations difficult to interpret. For example, there are variations in the pH of stem flow of *Quercus petraea* in different seasons (Carlisle *et al.*, 1967) and significant differences with tree age in *Populus* (Margot, 1965). Young (1938) reported very small but consistently higher pH values on the north side of the trees she examined as compared with the south side, and Hale (1967) reported that bark acidity is greater at the base than at upper levels of a tree trunk.

Of course, the bark chemistry and pH can be severely altered by external as well as internal conditions. Barkman (1958) covered these conditions in detail. Briefly, however, one might mention: bark wounds are generally alkaline and salt spray raises pH as do most types of roadside dust, especially near farms where the dust is rich in ammonia. City air pollutants are generally acidic [SO_2 oxidizes to SO_3 which hydrates to H_2SO_4 ; nitrous oxides form nitric acid (Katz, 1961)] and can lower the bark pH of all city trees. That these external sources of acid or base have an effect on the lichen vegetation is widely recognized (see references in Barkman, 1958).

b. ROCK AND SOIL. The pH of rock substrates has not been studied as much as that of bark, but some data are available on certain rock types. Schatz (1963) found that granite is slightly alkaline, mica is slightly acid, and marl is very acid. Serpentine soils and, presumably, the serpentine rocks that weathered to form them, have pH's ranging from 4.5–8.3, but usually are between 6 and 7 (Rune, 1953). Perhaps this is why serpentine floras contain basicolous and acidicolous plants living together (see p. 409).

Perhaps the most comprehensive treatment of soil and rock pH with regard to lichen vegetation is that of Mattick (1932). Mattick classified and listed 83 species according to their substrate pH affinities. Basically, he recognized four groups: acidophilous: most frequently found on acidic substrates; basiphilous: most frequently associated with alkaline substrates; neutrophilous: usually on neutral substrates; and *bodenvage* or neutral species: having little association with any particular pH. Mattick found most lichens prefer decidedly acidic soil [quite the opposite from the situation in bark (Du Rietz, 1945)] and that most lichens have relatively narrow rather than broad pH "requirements." The pH correlations have to be considered with great care, however, because several other characters of the soil and rock are associated with pH, and one cannot be sure to what the lichen is responding. For example, as lime-rich rock, which is alkaline or neutral, starts

to weather and build up humus, it becomes more acidic (Mattick, 1932). The higher water-retaining qualities and organic nutrient content of acidic soils must then be taken into consideration. Mattick felt that these physical characters could not be the overriding influence, however, since he observed that rock or soil types having apparently identical physical properties, but which differed in chemical reaction, had a distinctive lichen vegetation. Conversely, rocks and soils greatly dissimilar in physical makeup but which were identical in reaction had the same sort of lichen vegetation.

Lichens growing on sand dunes show distinct correlations with soil pH. Alvin (1960) stated that analyses of the pH-lichen correlations in Dorset (England) agreed strikingly with those of Rypáček (1934), working in Czechoslovakia. Looman (1964) also observed close similarities between the pH optima of some lichen communities in Saskatchewan (Canada) and the pH values reported for equivalent European communities. On Long Island, New York, *Cladina* (*sub Cladonia*) *submitis* and its associated lichens were abundant on the south-shore and inland localities where the pH was 4.2–4.6, and absent from the north shore on identical sand types, but where the pH was 5.1–6.2 (Brodo, 1968).

3. ORGANIC NUTRITION

The role of organic nutrition on lichen distribution, or, more generally, the problem of the extent to which lichens make use of organic, and, in particular, living substrates is still unclear. Sugars and other carbohydrates of various kinds have long been known to be bark constituents (Srivastava, 1964). Many lichens produce an array of extracellular enzymes, especially close to their points of attachment, suggesting that the substrate is being used as a nutritional source (Moiseeva, 1961). For years, it was suspected that lichens could enter into a kind of semiparasitism with living substrates such as young trees and mosses, or at least live as saprophytes. Investigations of the physical attachments of lichens to their substrates revealed that many species, especially the crustose lichens, invade living tissue on occasion (see Section IV), and it was assumed that lichens could absorb and utilize organic substances encountered. Bouly de Lesdain (1912), Johnson (1940), and Brodo (1968) observed that some lichens associated with living trees die or at least fail to discharge spores after the tree dies. Bouly de Lesdain, however, attributed this to the possible formation of toxic substances in the tree after its death, and Johnson suggested that moisture, rather than nutrition, may be the cause. Johnson added, however, that he believed some hypophloedal species in the Pyrenulaceae and Trypetheliaceae "must acquire a portion of their nourishment from their substrate" (Johnson, 1940, p. 26).

The only direct evidence concerning a lichen's possible use of the sap

water and nutrients of living trees comes from the work of Trotet (1969). He studied the uptake of radioactive phosphorus (as monopotassium phosphate) by *Ramalina calicaris*, *Parmelia melanothrix* Wain., and *Usnea ceratina* from the branch of *Quercus suber*. Trotet found that while the woody tissue and leaves of the branch picked up the labeled phosphate, the lichens did not. Although concluding that lichens do not seem to absorb minerals (at least not phosphate) from the living host, he conceded that the story might be different with crustose species and recommended some further experimentation to investigate this. It would be particularly interesting to see what the uptake might be of the more substrate-specific hypophloedal lichens living within the primary periderm of certain trees and shrubs such as *Ilex*, *Fagus*, or *Alnus*, especially in view of the possibility that the mycobionts of the crusts may live within the bark tissue for considerable periods of time in a nonlichenized state (Fink, 1913). The nutritive contribution of the chloroplast-containing phellogen cells of these thin-barked phorophytes should be of particular interest in this regard. However, besides some studies of photosynthesis in aspen bark (Pearson and Lawrence, 1958), little is known about this aspect of bark physiology, and even less is known of the possible effect of such photosynthetic activity on epiphytic vegetation.

It has sometimes been suggested that foliicolous lichens parasitize the leaves upon which they grow, although it seems clear now that this is not the case (Fink, 1913; Santesson, 1952). Santesson observed that while most foliicolous lichens are obligate leaf-dwellers, they rarely show a specificity towards a host species or higher taxonomic group as do nonlichenized fungi on leaves. In addition, Santesson never noted any penetration of the leaf's epidermis.

There is some evidence, however, that a few lichens may derive nutriment directly from their bryophytic host. The physical invasion of moss tissue by lichen-fungus hyphae was described long ago (Zukal, 1879). That there may be such a thing as a double-symbiosis of lichen mycobionts with algae as well as bryophytic tissue was suggested by Buchloh (1952, cited in Poelt and Hertel, 1968) with regard to the lichen *Paryphaedria heimerlii* Zukal living on the liverwort *Tritomaria quinquedentata*, and by Poelt and Hertel (1968) with regard to *Pachyascus lapponicus* growing on the moss *Andreaea*.

D. Temperature

One other aspect of substrates, generally overlooked, but which can affect the distribution of lichens, is surface temperature. Under experimental conditions, it has been shown (Lange, 1953) that wet lichens are killed between 35°–46°C, although some seem to be able to survive temperatures over 70°C when they are dry. Smith (1962) also mentions that high tempera-

tures can have a harmful effect on certain physiological processes, although most lichens seem to tolerate heat very well. The indirect effect of substrate heating, by increasing the water loss and respiration rate, may be even more significant. Hoffman and Gates (1970) studied the effect of heating on lichens and found that while it is possible for the lichen to be heated to a lethal level under natural conditions, this seldom, if ever, happens due to convection and evaporational cooling. In addition, since lichens dry out so fast, it is unlikely that they would be subjected to very high temperatures in a moist condition (Haynes, 1964). On the other hand, Hoffman and Gates pointed out that the rock substrate provides an important mechanism in the regulation of thallus temperature. Rock, being an efficient heat sink, loses warmth accumulated during the day very slowly at night, and then helps to keep the lichen cool during the heat of the afternoon by its slow heat absorption. They note that small rocks are less effective in this respect than are large boulders, and this might help explain the restriction of certain lichens such as *Trapelia* (*sub Lecidea*) *coarctata* to small pebbles in exposed dry habitats (Brodo, 1968) where a special ability to compete under rigorous conditions might be a factor in their distribution. In very cold climates, the ability of a substrate to absorb and hold heat might be a factor in substrate "selection" by lichens. In Antarctica, for example, Rudolph (1963) recorded surface temperatures of 90°F (32°C) on insolated rocks.

The effect of bark color, heat-absorbing ability, and heat loss on the temperature of corticolous lichens was discussed in detail by Barkman (1958). Barkman suggested that possibly some lichens which are "anheliothetic" (against shade) might still be restricted to the north side of trees if they are also "thermophobous" (disliking heat). Studies by Brodo (1959) showed no relationship between bark-surface temperature and the distribution of lichens, but it is conceivable that where the rate of moisture loss is critical to a species, its increased heat due to substrate heating properties may be decisive.

IV The Lichen-Substrate Interface

A. Bark and Wood

Generalizations about the extent of penetration of lichens into tree bark cannot be made since the degree of penetration is dependent upon each lichen's ability and inability in this regard, as well as upon the bark type.

Certain tissues of fruticose lichens, such as *Ramalina*, *Usnea*, and *Evernia*, often extend deep into the corky bark tissue by growing between the periderm layers with "hapteral" wedgelike action. Studies of *Ramalina* species by Porter (1917) showed that these lichens gain entrance to the inner bark

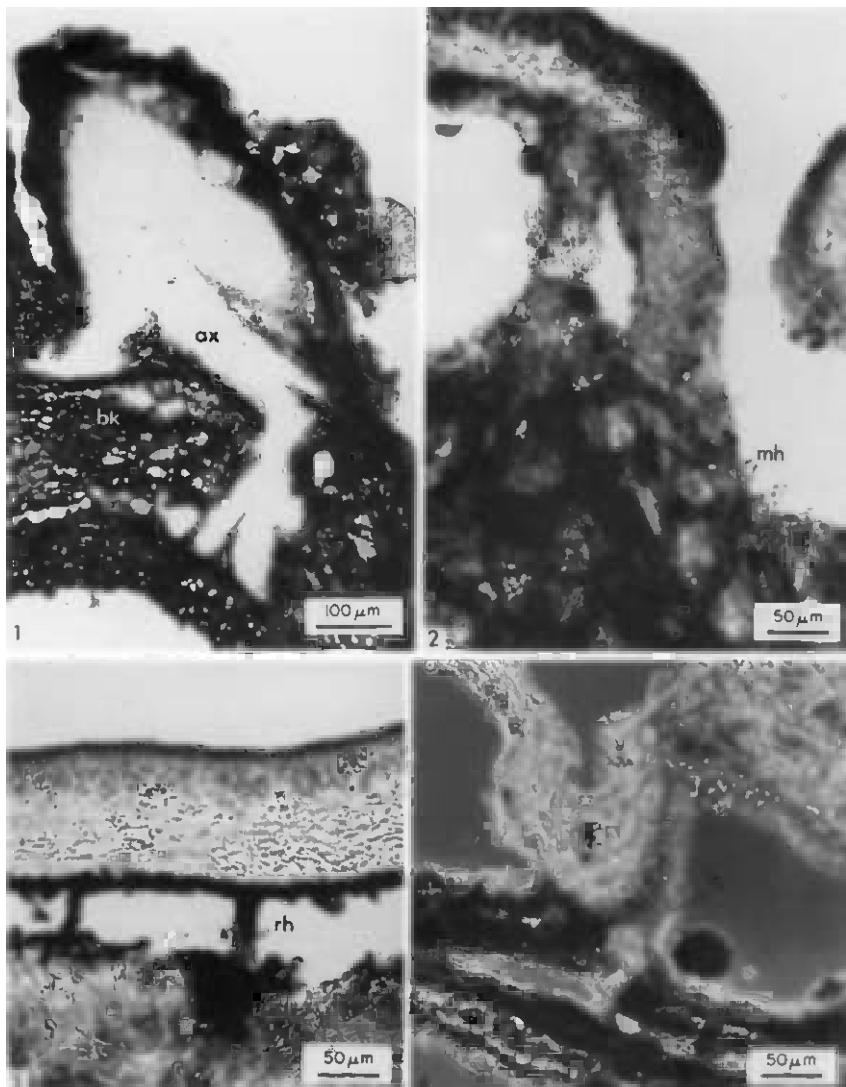
tissue through cracks and lenticels, extending even into the cambium and youngest cells of the wood. In *Ramalina*, the penetrating tissue is chiefly the cortex; in *Usnea* it appears to be mainly the axis (Fig. 1; see Porter, 1917, and references cited therein).

Foliose lichens penetrate less, but still are firmly attached by rhizines which grow at their tips among the loose cork cells. Porter (1919) said that a kind of hypothallus was formed on the surface of the bark by the tips of the rhizines. The hypothallus finally proliferates and grows between the cork cells. My sections of *Physcia* and *Dirinaria* species (Figs. 2 and 3) show traces of the development of a hypothallus of that kind, but only the most superficial invasion of the phellum. Even less penetration was seen with *Hypogymnia physodes* in which the lower cortex simply develops elongated hyphae which grow loosely among the surface bark cells thus providing firm thallus attachment (Fig. 4). Porter (1919), however, reported that this species had no lower cortex at the contact point and that the medullary hyphae grew into the bark layers much like an epiphloedal crust.

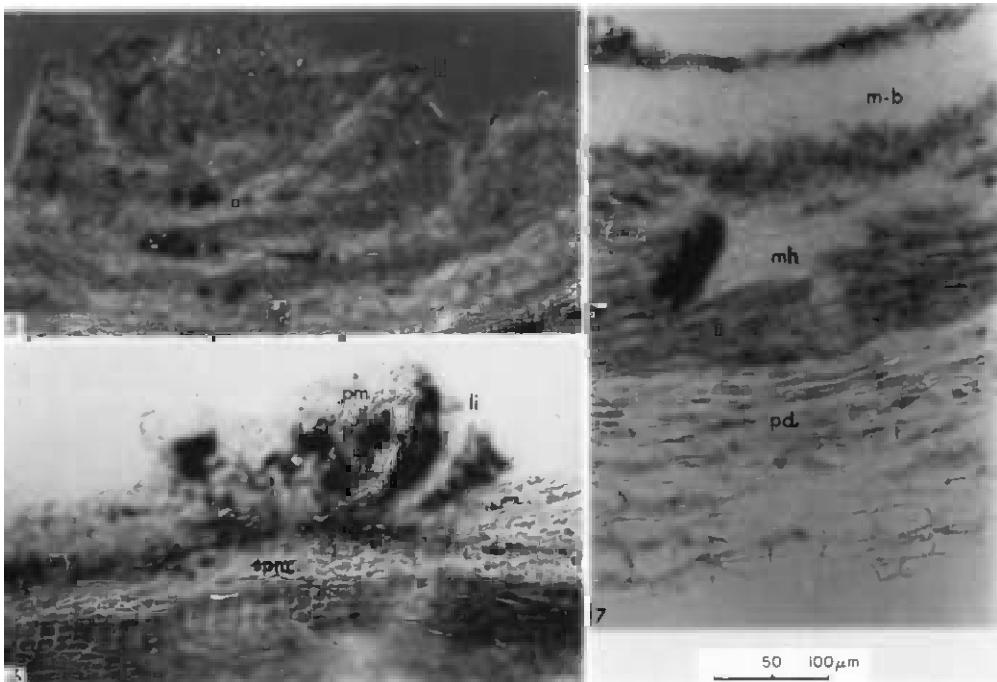
Corticulous crustose lichens are commonly divided into "epiphloedal" and "hypophloedal" species according to their position relative to the surface layers of bark. Epiphloedal lichens develop with most of the tissues (especially the algal layer) above the outermost corky layers, although some bark material is often incorporated into the lower portions of the thallus (Fig. 5). The thallus of hypophloedal crusts is entirely below the outermost periderm tissue. The fungal tissue does not actually pierce the cork cells, but rather grows around them, pushing them aside (Fry, 1926).

The fact that hypophloedal lichens generally are associated with trees having bark with a persistent, primary periderm which is still photosynthetically functional seems particularly significant with regard to the lichens' possible nutrition and specificity (see p. 414; Fink, 1913; Johnson, 1940; Dickinson and Thorp, 1968). However, sections of hypophloedal crustose species together with their bark substrate generally show the lichen thallus to be confined to the corky outer periderm layers and separated from the living and sometimes chlorophyllous phelloderm by one or several layers of suberized impermeable cork cells (Fig. 6). This can also be seen in some of Johnson's illustrations (especially his Plate 3, Figs. 1 and 4; and Plate 4, Figs. 1, 7, and 9). The close proximity of the mycobiont hyphae to the living phelloderm, however, still suggests the possibility of occasional contacts, especially at lenticels or natural fissures (Fig. 7).

In studies of members of the Trypetheliaceae growing on thin-barked trees, Johnson (1940) found that the entire thallus development occurred within the periderm, from initial lichenization with *Trentepohlia* filaments (which had already penetrated the bark 8–10 cell layers deep) to complete perithecial maturity.



Figs. 1–4. Fig. 1, the penetration of the dead bark tissue (bk) of *Picea* sp. by the axis tissue (ax) of *Usnea comosa* auct. (Wisconsin. Brodo 5707. CANL); Fig. 2, attachment of *Physcia stellaris* to *Pinus flexilis* showing the intricate winding of the mycobiont hyphae (mh) around the loose cork cells (Alberta. Bird and Kalguikar 19945. CANL); Fig. 3, the attachment of *Dirinaria picta*. Note the localized discolorations of the phellum where the cork cells and lichen rhizinae (rh) contact (Florida. Shchepanek 58. CANL); Fig. 4, *Hypogymnia physodes* attached by proliferations of the lower cortical hyphae (1c), (Ontario. Stewart 324. CANL.)



Figs. 5-7. Fig. 5, the epiphloedal crustose lichen *Lecanora caesiorubella* subsp. *caesiorubella* on *Acer saccharum* showing the infiltration of the looser outer phellum layers (pm) by the superficial lichen thallus (li) (Quebec. Lepage 15993. CANL); Fig. 6, the hypophloedal crustose lichen *Conotrema urceolatum* (li) growing within the outer phellum tissue (pm) of *Fagus grandifolia* and separated from the living phelloderm (pd) (containing chloroplasts) by a thick layer of suberized phellum (spm) (Quebec. Lepage 16776. CANL); Fig. 7, the hypophloedal crustose lichen *Trypethelium tropicum* on a young branch of *Liriodendron tulipifera* showing the penetration of some mycobiont hyphae (mh) almost to the living phelloderm (pd) through what appears to be a crack in the phellum tissue (pm). The mixture of mycobiont hyphae and decolorized cork cells (m-b) overlies a layer of lichen algae (al). (Louisiana. Tucker 7733. CANL.)

Ozenda (1963) presented a well-illustrated review of the substrate interfaces of corticolous crustose lichens.

B. Rock

Rock substrates prove to be a more formidable problem for the attachment of lichen fungal hyphae. However, many studies have shown how remarkably efficient lichens are in penetrating rocks of all kinds. Ozenda (1963) has provided an excellent review of this subject and Syers and Iskandar (see Chapter 7) give more specifics.

C. Leaves

Of the foliicolous lichens, all species except those of *Strigula* and *Raciborskia* are supracuticular; these two genera are subcuticular. Apparently, no obligately foliicolous lichens penetrate the leaf epidermis (Santesson, 1952). The same is true of facultative foliicolous crustose and foliose species (Daubenmire, 1943).

D. Soil

The attachment of terricolous lichens to the soil has not been studied in any detail. Except for a few species such as *Lecidea uliginosa* (Brodo, 1961) and some desert species (Shields *et al.*, 1957), few lichens have the ability to invade and consolidate sand. Most either are merely buried to a slight extent at the base (McLean, 1915), or, more frequently, become attached to dead vegetation (see p. 404).

V. The Effect of Lichens on their Substrate

Related to the mechanical attachment of lichens is the effect lichens have on their substrate. The issue is an important one because lichens have been said to be the cause of significant damage to various man-made materials serving as lichen substrates and to living plants bearing corticolous species. The problem is also of considerable significance with regard to the often cited role of lichens in soil formation.

A. Bark

The breakup of the surface layers of tree bark by the growth and development of lichens has been well documented, and the question as to whether lichens have the ability to actually damage or even kill a tree is often raised. Certainly, lichens sometimes do form extensive growths over large areas of

bark. The lichen cover thereby traps moisture, which can lead to increased fungal activity (Kaufert, 1937) and decay rates, and can harbor insect life which may infest and damage the tree (Fry, 1926). Thick growth of *Ramalina* and *Physcia* has been reported to interfere in the development of adventive shoots in tea plants, mostly through competition for available sunlight (Asahina and Kurokawa, 1952). Apparently, some lichens can invade the bark so completely that lenticels are blocked, causing, as with *Ramalina pollinaria*, a "hypertrophy of the periderm and an erosion of the wood" (Porter, 1917, p. 25). The penetrating "hapteral system" of the *Ramalina* branches beneath the surface crushing and discoloring the cells (Porter, 1917). Bouly de Lesdain (1912) claimed that the bark of willow trees was completely removed by the action of some lichens, especially *Xanthoria parietina* and *Physcia adscendens*, through penetration and alternate wetting and drying of their rhizines.

Hypophloedal crusts such as *Trypethelium* and *Melanotrichia* apparently can discolor and, to some extent, decompose the bark tissue they contact (Johnson, 1940). Aware of some of the effects of lichens on tree bark, Plitt (1929), in a study of lichens growing on the bark of "official drugs," wondered if the bark beneath the lichen had the same drug properties as bark having no lichen cover.

B. Rock Degradation and Soil Formation

The varied role of lichens in rock degradation and soil formation is the subject of a separate chapter in this volume and the reader is referred there (Chapter 7).

There seems no reason to doubt that the decomposition of crustose and foliose lichens invading bare rock surfaces contributes small amounts of humus to the surface, and that the lichens themselves trap more, permitting the establishment of other humus-requiring species of plants. It is equally clear, however, that except in certain areas and on certain rock types, chemical breakdown of minerals by lichens is extremely slow and, relative to the humus-forming role, almost insignificant. There is also abundant evidence that, except on the smoothest of rock surfaces, lichens are often not pioneers, but actually follow the establishment of other pioneer plant types, especially bryophytes.

The effect of terricolous lichens on the chemistry of the soil can be either detrimental or beneficial to soil fertility. Shields *et al.* (1957) showed that lichens growing on alkaline desert soil may help to hold particles together and sometimes contribute to the nitrogen content of the surface layer (especially in the case of lichens such as *Collema* with blue-green algal phycobionts).

It is possible that at least lichens producing usnic acid can adversely affect soil fertility. Malicki (1965) found that some usnic acid is leached out of usnic-producing *Cladinae* and can be found in the soil beneath them. He also found that ammonification bacteria and those decomposing cellulose are affected by the antibiotic properties of usnic acid (although there is no effect on *Azotobacter*) (Malicki, 1967).

Another, unrelated, effect of lichen products on soil chemistry was reported by Pyatt (1967). He established that an exudate of *Peltigera canina* can inhibit the growth and germination of certain vascular plants, thus influencing competition and succession in certain habitats. This was borne out by field observations.

VI. Substrate Specificity

The extent to which a lichen is restricted to a narrowly defined substrate type can be called its "substrate specificity." (The term "host specificity" implies a nutritional relationship which may or may not exist.) Some lichens are narrowly substrate specific, e.g., confined to one or two tree species or a particular rock type, and others are found not only on trees of different kinds but sometimes also on wood, soil, or rock.

A. Species Which Have High Specificity

No attempt will be made here to review extensively the subject of corticolous lichen specificity since Barkman (1958) devotes a large portion (44 pages) of his monograph to this subject.

Among the most specific of tree lichens are crustose, hypophloedal species, especially those found on thin-barked shrubs and trees such as *Ilex*, *Fagus*, and to some extent, *Acer*. Members of the Trypetheliaceae are largely confined to *Ilex*, *Fagus*, (young?) *Liriodendron*, and species of *Magnolia* (Johnson, 1959; Brodo, 1968); *Pyrenula nitidella* to *Fagus* (Barkman, 1958); and *Conotrema urceolatum* to *Acer saccharum* (Gilenstam, 1969). Other lichens, which are extremely specific to a particular tree species in one area, may be found on many others in different regions. An excellent example is *Parmeliopsis placorodia*, which is found almost exclusively on *Pinus rigida* on the east coast of North America, only on *P. banksiana* in the Great Lakes region, and on *P. ponderosa* in the west (Culberson, 1955c; Brodo, 1968). Of the three species said to be restricted to a single tree species in the Netherlands (Barkman, 1958), all are found on numerous other trees elsewhere.

Other very substrate-specific species such as *Pachyascus lapponicus* (living on the moss *Andraea*) (Poelt and Hertel, 1968), *Calicium curtisii* (on *Rhus typhina*), *Stenocybe major* (on *Abies balsamea*), and *Leptorhaphis epidermidis*

(on species of *Betula*) are suspected of being only partially lichenized or living as double-symbionts, semi-parasites, or saprophytes.

Foliicolous lichens show little specificity and, apart from being restricted to leaves, are rarely associated with specific taxonomic groups of plants. *Strigula elegans*, for example, is found on 99 different genera in 51 families, and *Porina epiphylla* (Fée) Fée is on 55 genera in 30 families (Santesson, 1952). Santesson noted, however, that *Strigula* is found only on one monocot and no ferns.

Among the saxicolous lichens restricted to one rock type, the serpentine species *Rhizocarpon sphaericum* (Schaer.) Mig., *Aspicilia serpentinicola* (Suza) Räs., *A. polychroma* var. *ochracea* Anzi, and *A. crusii* Klem. should be mentioned (Ritter-Studnička and Klement, 1968). In northern Sweden, however, Rune (1953) found no serpentine-specific lichen. Many obligately calciphilous rock species are known, such as the "Gloeolichens": *Phylloscum* (*sub Omphalaria*), *Peccania*, *Anema*, *Psorotrichia*, and *Forssellia* (*sub Enchylium*) (Forssell, 1885, cited in Smith, 1921); *Caloplaca heppiana* (Müll. Arg.) Zahlbr., *Lecanora calcarea*, and *Rhizocarpon umbilicatum* (Syers et al., 1967); some species of the Heppiaceae (Wetmore, 1970); certain *Lecideae* (Hertel, 1967); certain *Collemae* (Degelius, 1954); and others (see Degelius, 1955; and references in Smith, 1921). Among the lichens confined to acidic granitic or siliceous rock, one might mention members of the *Rhizocarpon geographicum* group, species of *Umbilicaria*, and *Parmelia conspersa*.

B. Lichens with Exceptionally Low Specificities

At the other end of the spectrum, one can list those species found on a variety of substrates, including trees, rocks, soil, and wood. Perhaps the most eurysubstratic lichen is *Parmelia sulcata*, found almost everywhere in temperate and boreal regions of the world. It is apparently equally tolerant of bark, stone, and wood, and is known not infrequently from soil as well. Perhaps more important, it apparently has no strong preference with regard to neutral versus acidic substrates, although I have never seen it on limestone. Other lichens found on bark and rock often prefer acidic bark and siliceous rock or neutral bark and calcareous rock (Barkman, 1958, and references therein). A well-known example is *Xanthoria parietina* and its associated species (the "Xanthorion" community). It is not surprising that the lichens with the broadest substrate ranges have the broadest geographic (Barkman, 1958) and environmental (Hale, 1955) ranges as well.

C. Similarities of Phorophytes

In an effort to discover what characteristic of the substrate causes the specificities we see, it is interesting to determine which trees are most similar in their epiphytic flora. The most similar substrates are then compared in

order to discover the characteristics they have in common. The literature before 1958 has been thoroughly reviewed and summarized by Barkman (1958) with particular reference to the work of Koskinen (1955) in Finland, and Hale (1955) and Culberson (1955b) in Wisconsin.

Attempts to correlate certain tree species with large numbers or small numbers of epiphytes ("rich-bark species" and "poor-bark species", cf. Du Rietz, 1945) generally prove unsuccessful. Trees that are "rich" in one area or under certain conditions may be "poor" when the area or conditions are different (Barkman, 1958; Almborn, 1948). Barkman concluded (1958, p. 134) "... epiphyte abundance is not directly correlated with either relief, water capacity, or pH of the bark in most regions except with regard to the poorness of conifers as distinct from other trees." He also stated that trees with centrifugal crowns such as *Picea* and those with rapidly flaking bark tend to be poor in species. Exceptions to this do occur on a local level, however, as was shown by Fabiszewski (1968) who found that the less acid the reaction of the bark the richer the association in species.

The similarity of various tree species with regard to their epiphytic vegetation has most fruitfully been studied by means of unbiased sampling methods and statistical analyses (Hale, 1955; Culberson, 1955b; Brodo, 1961, 1968; Adams and Risser, 1971a). These analyses have generally been based on mathematical comparisons of shared versus unshared epiphytic species between two trees (e.g., using Kulczinski's "coefficient of community"). The comparisons are only valid within small regions, of course, regions having the same general epiphytic flora. However, finding relationships between trees in their flora is easier than finding correlating similarities in their bark characters. Culberson (1955b) found that the vegetational "order" of a series of trees did not agree with the order obtained with pH, water capacity, or bark hardness, although it did resemble, to some extent, a kind of "average" of all three factors.

It should not be surprising that ordinations of trees according to their lichen flora are difficult to reconcile with orders of bark parameters. If the correlations were perfect one might conclude that all lichens respond to the same parameter, a situation which seems highly unlikely. Since some lichens may find moisture limiting in one vegetation type or geographic area and not another (Brodo, 1959; Barkman, 1958; Adams and Risser, 1971a), bark-moisture capacity may be important to them in one place and not another, where pH or nutrition might be limiting. [The changes in substrate specificity in different vegetation types have been discussed at length by Brodo (1961, 1968) and mentioned by Margot (1965).] Even if one compares trees within the same vegetation type, one still has to contend with the individual requirements and limiting factors of the individual species making up the communities on each tree. Lichens respond to bark characters, not to tree species, a point strongly made by Fabiszewski (1968).

D. Quantitative Studies of Lichen-Phorophyte Association

The computation of the frequency with which a particular lichen is found on a particular tree was the basis for expressions of substrate specificity in several North American studies (Hale, 1955; Culberson, 1955b; Brodo, 1961; Kalgutkar and Bird, 1969; Adams and Risser, 1971a,b). Hale and Culberson were the first investigators to express substrate specificity quantitatively and provide us with an objective way of comparing specificities as they vary from place to place. The use of Cole's "index of interspecific association" (Cole, 1949) as the expression of specificity permits the immediate recognition of both positive and negative lichen-phorophyte associations, as well as their measurement. Cole's index was used by Hale (1955) and Brodo (1961).

A study in New York (Brodo, 1968) added one facet to the consideration of specificity. Under the assumption that the highest degree of specificity would be evidenced by close substrate-lichen relationships over several vegetation types, I set up five categories of substrate specificity and classified eight species on three phorophyte species in four segments of a pine-oak forest continuum. The various relationships seen were: "(a) significant positive association of the lichen with the tree species over the entire continuum, (b) significant positive association in some segments, but not in all, (c) no significant positive or negative association with the tree in any segment, (d) significant negative association in some segments but not in others, and (e) significant negative association in all the continuum segments. I interpreted the above relationships, respectively, as follows: (a) the lichen shows constant substrate specificity indicating possible substrate requirements, (b) the lichen shows some specificity for the tree but exhibits no clearcut requirement for it, (c) the lichen shows considerable flexibility in substrate requirements, varying in degree of association with any particular tree species as the bark characteristics such as texture, chemistry, and moisture relations change in the different stands, (d) the lichen shows some tolerance for the normally unfavorable substrate, but will occur more abundantly on other more favorable trees if they are available, and (e) the lichen has some sort of physical or physiological inability to inhabit the substrate" (Brodo, 1968, p. 21). Margot's (1965) five categories of specificity *obligatoires, préférentielles, facultatives, eurysubstratiques, indifférentes*, are somewhat comparable.

This method of looking at substrate specificity perhaps could also be used to analyze tree-lichen relationships in various parts of the lichen's normal geographic range.

In studies of the lichens growing on only a few of the available tree species of an area, as with the work of Kalgutkar and Bird (1969) regarding the relationships of lichens to *Larix lyallii* and *Pinus albicaulis*, it would be better to speak of substrate preferences (among the trees studied) than substrate specificity since the entire substrate potential has not been sampled.

E. *Ornithocoprophilous or Neutrophilous Species*

One of the more striking and intriguing examples of a kind of substrate specificity is displayed by a certain community of lichens, generally species of *Xanthoria*, *Physcia*, and *Caloplaca*, tending to be found on substrates subjected to bird excrement. Complicating the issue somewhat is the fact that these same species are very often associated with limestone, bone, and neutral-barked trees. The confusion over the possible influencing factor (or factors) is evidenced by the host of terms which refer to this type of specificity: neutrophily, nitrophily (Hilitzer, 1925; Barkman, 1958), ornithocoprophyly (Sernander, 1912), coniophily (Almborn, 1948), and ammoniophily (Räsänen, 1927) to which might be added "calciphily" (see Ochsner, 1928, cited in Barkman, 1958).

The only thing that everyone seems to agree on is that some kind of chemical or nutritional factor is involved. Many authors have discussed this subject. The best summaries are probably those by Almborn (1948) and Barkman (1958). The fact that related, but not necessarily identical, species are involved in some of these discussions does not make analysis of the results any easier. In general, however, the following observations have been made: (a) almost all the substrates are alkaline, or at least neutral; (b) bird excrement is high in N, P, and Ca; (c) limestone is high in Ca but low in N and P; (d) neutral-barked trees have higher ash contents than acid-barked trees and are often rich in Ca and P; (e) rain tracks of trees or bark wounds are rich in nitrogen but can be either alkaline or slightly acid; (f) road dust is usually nitrogenous, especially near farms.

This community, or rather, the lichens in this community are therefore not at all substrate specific in the usual sense, but apparently are very specific for a certain chemical environment. While acknowledging Barkman's (1958) objections to the use of "neutrophilous" in describing these lichens [mainly because of point (e) above], I feel that term is the safest to use in the majority of cases, at least until careful studies are done of the nutrient requirements and tolerances of the species. Massé's (1966) study of the correlation of nitrogen content of bird excrement with the distribution of "ornithocoprophilous" lichens merely confused the issue, since no account was taken of the myriad of other factors which might correlate equally well (calcium and phosphorus, for example). On the other hand, it seems entirely proper to use the terms "ornithocoprophilous" and "coniophilous" to refer to communities or even species which seem to be responding positively to the presence of bird excrement and dust, respectively, particularly in areas or on substrates where these lichens do not normally occur. I think the terms "nitrophilous" or "calciphilous" imply a knowledge of the requirements of lichens that we do not yet have.

F. Substrate "Switches" and Unusual Substrates

In the absence of carefully controlled experiments, some of the best clues we have as to the bases of some substrate preferences can be gleaned from a consideration of the occurrences of lichens on substrates normally foreign to them. An evaluation of the substrate characters involved can sometimes lead one to discover certain "common denominators" which reveal substrate requirements.

Ohlert (1871) has one of the oldest, extensive treatments of lichens with regard to their substrate range. In considering the potential "normal" substrates, i.e., bark, wood, stone, and soil, he listed first those species found on all four, then combinations of three, and finally two. All these are the *bodenvagen* or, loosely, "substrate-indeterminate" species. Among the *bodenvagen* species, Ohlert distinguished those which reach their best development, or fertile condition, only on one of these substrates, and those that are equally well-developed on all. The latter he termed "substrate-indifferent."

Ohlert's lists are interesting but largely out of date, with many of his "species" now divided into smaller units, each much more substrate specific. Ohlert himself pointed out that of his twelve *bodenvagen* species, only three are more or less substrate indifferent, and that even among these, some substrate preferences occur within each substrate category. For example, *Bacidia (sub Lecidea) sabuletorum* is found only on calcareous rocks and at the bases of deciduous trees.

Lambert and Maycock (1968) found that the substrate preferences of *bodenvagen* species change along a forest continuum, apparently due to secondary ecological factors such as availability of moisture and light.

But most interesting are not the eurysubstratic species in which substrate perambulations are relatively meaningless, but rather the narrowly substrate-specific types which are occasionally found on a foreign substrate (at least foreign to them). Koskinen (1955) provided us with a wealth of such data in a table of the substrate occurrences of about 570 taxa.

It is best not to consider too seriously more or less accidental occurrences of lichens on abnormal substrates (as when a corticolous species falls to the ground and continues to grow over the soil). Even some of the remainder apparently are able to change substrate purely by virtue of the number of propagules in an area, flooding the area, as it were, with "experiments" in substrate tolerance. If enough genetic potential is represented, some of the "experiments" are bound to be successful, and a substrate "switch" will occur. Lambinon (1968) presented a thorough and well-developed discussion of this phenomenon using as his framework the phytogeographic concept of *accessibilité* introduced and developed by Heimans (1954).

A number of lichens normally confined to siliceous rocks are sometimes found on very old, hard lignum (i.e., decorticate wood), generally coniferous and therefore acid. Darrow (1950) cites *Parmelia conspersa* on *Pinus arizonica* wood; Alvin (1961) noted *Rinodina "demissa"* on old wooden posts. In Ontario, I have seen both *Parmelia taractica* and *Umbilicaria papulosa* on conifer lignum close to populations of these species on rock. I have also seen good growths of *Lecanora cenisia*, a silicicolous species, on *Juniperus communis* lignum from dry exposed sites in Wisconsin and on fence rails in Oregon. Lambinon (1968) mentions an occurrence of the siliceous rock lichen *Haematomma coccineum* on the bark of a beech tree near a rock bearing this species, and Richard (1883) noted *Umbilicaria deusta* (*sub flocculosa*) and a species of *Roccella* on old wood and the stems of *Rhododendron ferrugineum*. Corticolous occurrences of such siliceous rock lichens as *Lecidea macrocarpa* (*sub L. steriza*), *Aspicilia* species, *Rhizocarpon*, and *Verrucaria* were found to be restricted to the bark of *Betula* by Koskinen (1955).

Lichens often shift between neutral-barked trees and calcareous rock (Barkman, 1958), or the shells of molluscs (Peake and James, 1967), or bone (Richard, 1883), substrates sharing the characters of high Ca content and high pH, among others.

Among the lichens adopting "abnormal" substrates, glass-dwelling species and those on roof tiles are, not unexpectedly, siliceous rock lichens, but so are those on iron and lead; those found on leather generally are associated with lignum (Richard, 1883). Species growing on odd bits of debris such as felt, silk, paper, linoleum, and wool are generally substrate-indifferent, fast-growing, ubiquitous species (Richard, 1883; Smith, 1921).

Lichens which colonize animal material have been known for many years; some were reported on chrysalid cocoons and bird feathers by Bouly de Lesdain (1912). Recently there have been reports of lichens on living animals. Gressitt (1966a,b) described the occurrence of lichens in New Guinea on various species of specially adapted weevils of the genera *Gymnopholus*, *Poropterus*, and *Pantorhytes*, as well as on one colydiidaean beetle, *Dryptops phytophorus*. These insects have evolved special secretions and surface features on their elytrae which provide for the establishment of several species of foliose lichens common in the area on bark: *Parmelia reticulata*, *P. crenata*, *P. sp.*, *Anaptychia* sp., and *Physcia* sp.

Hendrickson and Weber (1964) recorded the occurrence of colonies of *Dirinaria* (*sub Physcia*) *picta* on the giant land tortoise, *Geochelone elephantopus*, in the Galapagos Islands. This *Dirinaria* is found on all types of substrates in the area including lava rock, bark, wood, and evergreen leaves.

G. Causes of Specificity

The response of a lichen to a potential substrate will depend on the plant's requirements and tolerances. These requirements and tolerances may

change to some extent under different conditions, but basically each population of an organism will have its own physiological needs and limits. When we speak of a "calciphilous" species, we imply that under most circumstances, this lichen will find calcium limiting and so will respond to (i.e., colonize) any substrate in which the calcium concentration is high enough to supply its needs. The more crucial the requirement for that substance, the more "specific" the lichen will be to substrates containing it. (Lichens found only on trees with a living periderm may require special metabolites produced only in the living tissue of those trees [see Section III, C, 3].)

If the lichen requires large quantities of a mineral, the specificity will seem obvious, since few substrates contain large quantities of any particular essential element. (Plants found only on limestone seem to be obvious calciphiles.) If a lichen has an absolute requirement for moderate levels of boron, the substrates to which it can adapt will still be highly limited, but the cause of its specificity will be much more obscure.

Tolerances for high concentrations of some substances and needs for lower concentrations of other substances may combine within a species to give a confusing picture of its substrate requirements. An example might be a high tolerance of certain coastal bird-rock lichens for Cl^- ions together with a requirement for Ca or N in high concentrations, or, conversely, a tolerance of Ca or N and a requirement for Cl^- . The fact that obligate calcicoles apparently accumulate calcium oxalate whereas nonobligate calcicoles do not (Syers *et al.*, 1967) indicates a possible mechanism to deal with excess Ca ions which has evolved in these species (see p. 432). Here is an instance in which substrate specificity has been tied directly to a physiological process in the organism itself.

Other substrate factors discussed in the preceding sections (substrate moisture, hardness, or stability, various minerals, and organic products such as sugars, glycosides, amino acids, vitamins, etc.) can be limiting factors depending upon the organism's needs and tolerances. Hydrogen ion concentration can also be limiting, in a way, but its effects are almost always secondary. That is, the solubility, availability, or toxic state of various substances changes at different pH levels. Direct influence of pH might operate through effects on the activity of enzyme systems and the like.

It should be remembered that lichens are dual organisms, and what may be limiting to the algal component may not be limiting to the fungal component, and vice versa. In addition, what is limiting for growth may not be limiting for establishment, and, conversely, what is needed for germination may not be needed for development. These points were well made by Hale (1967) and Harris (1971a).

In the framework of the principle of limiting factors, the substrate preferences of many lichens are easier to understand. In a very xeric woodlot, certain moisture-requiring lichens will tend to be found on trees having bark with high moisture capacities. In mesic woods, the same lichens may

show no such preference since moisture is not limiting (see p. 407). Siliceous rock lichens apparently have a strict requirement for (or tolerance of) extremely stable, hard surfaces for colonization, a requirement which can be met, under certain circumstances, by old, very hard lignum as well as rock. If such a surface is encountered and is therefore no longer "limiting," other requirements of the species, such as chemical requirements, come into play, and the lignum of certain tree types becomes acceptable where that of others becomes unacceptable. (See the discussion by Koskinen, 1955, pp. 31–32.) Coniophily and ornithocoprophyly (see Section VI, E) must involve a great complex of limiting factors, tolerances, and perhaps even synergistic effects. This is why nitrogen appears limiting in one instance and calcium or phosphorus in another. It would be rather naive to speak of the cause of the substrate tendencies observed since, for any given species, the limiting factors will differ under different circumstances.

By this point, it may have become clear that experimental data are needed. Thanks to the work of Barkman, Koskinen, Hale, Culberson, and others, the substrate tendencies for at least the corticolous lichens of the temperate zone are well known. Less work has been done with saxicolous and terricolous species, but correlation data are available for certain communities. Some essential information on the utilization of organic and inorganic substances is beginning to accumulate and methods are now available for routine laboratory investigations of lichens (see the reviews in Smith, 1962; Ahmadjian, 1967). I believe the time is now ripe for a major new effort in experimental ecology, both in the field and in the laboratory, since only with well-controlled experimentation will the causative factors in lichen ecology be unravelled.

H. The Influence of Specificity on Community Composition

A factor of considerable importance in the probability of two or more species of lichens occurring together is obviously their specificity to certain substrates. The more specific they are to the same trees or rock types, the more likely they are to be found in the same community. Indicator or "faithful" species will often be those with narrow substrate requirements. Of course, the substrate is not the only factor involved in community relationships and "fidelity," but it is clearly important in quantifying community structure. This point was well documented by Adams and Risser (1971b) who showed that lichen–lichen association values can vary from positive to negative depending on the phorophyte investigated. When considering a region containing many phorophyte species, the substrate then plays an important part in determining the overall interspecific relationships.

I. The Influence of Specificity on Geographic Distributions

If lichens do indeed have substrate specificity, one would expect their geographic distribution to be determined by the distribution of a substrate to the extent to which they are specific to it. In general, that appears to be the case. Exceptions occur, however.

Not all corticolous lichens are found below the tree line, for example. Many species shift to soil habitats in arctic or alpine situations (Barkman, 1958). These substrate changes are not due to *accessibilité* (see p. 427) but rather appear to be genetically controlled.

The distribution of lichens with broad substrate tolerances is largely determined by climatic and historical factors (Almborn, 1948; Brodo, 1968; Imshaug and Brodo, 1966) although substrate specificity may play an important role, especially in the relative abundance of the species (Culberson, 1955a; Hale, 1955; Adams and Risser, 1971a). As the specificity of a corticolous lichen becomes more pronounced, its distribution is determined to a greater and greater degree by the distribution of the phorophyte. This was graphically shown in the distribution of many lichens on Long Island (Brodo, 1968). *Conotrema urceolatum* is another example, being only found in the Appalachian-Great Lakes region where *Acer saccharum* is found.

Lichens with disjunct distributions often show interesting substrate "switches." *Acroschyphus* grows on rocks in Patagonia, Himalaya, Yunnan, and Japan, but on soil and trees in Mexico and Peru (Sato, 1967). *Sphaerophorus melanocarpus* grows exclusively on siliceous rocks in Norway, but is often or always on trees and logs elsewhere in its range (Lye, 1969). *Coccotrema (sub Perforaria) cucurbitula* (Mont. in Gay) Müll. Arg. is a species of tree bark and rarely rock in the moist forests of Australasia and east Asia (Oshio, 1968), but is exclusively on maritime rocks on the Queen Charlotte Islands (British Columbia, Canada).* *Lecanora rugosella* is generally on *Fraxinus* and *Sorbus* and similar trees in Europe, but almost exclusively on *Thuja occidentalis* in North America. In the extreme northern and southern portions of its range, *Lecanora farinacea* Fée is found only on bark; the collections in between are saxicolous (Imshaug and Brodo, 1966).

Substrate "switches" such as these are probably due to heavy selection on isolated populations with respect to available niches. For example, the ancestral North American population of *Coccotrema*, as it existed in western North America, probably in the Tertiary, was likely corticolous, but as the available habitats were lost in the Pleistocene, remnants became adapted, and then restricted, to shoreline rocks in coastal refugia which the ice did not reach.

*I have recently recognized the Queen Charlotte Island population as a distinct species: *Coccotrema maritimum* (Brodo, 1973).

VII. Substrates and Speciation

Whether substrate selection can influence speciation in lichens is an interesting but almost unanswerable question. In other organisms, substrate selection is frequently an important isolating mechanism and may thus lead to speciation. The genetic mechanisms of lichen fungi are very inadequately known. Since most lichens appear to be functionally "apomicts" the appearance of a genetically determined substrate tolerance may become fixed in a population if it is locally advantageous.

A. Substrate-Induced versus Genetically Induced Morphological Changes

In certain cases, we can be reasonably sure that a special morphotype is substrate controlled. If there is a continuum of morphologic change along a substrate-character gradient such as hardness or mineral content, or if numerous unrelated taxa react to certain substrates in the same way, we can usually assume that the morphotype is not genetically determined.

For example, various types of rock and rock crystals influence the thickness, form, and sometimes color of tissues of endolithic crusts (Doppelbauer, 1959). Similarly, Hertel (1967) noted that the harder the rock, and/or the lower the calcium content, the greater the tendency for a normally endolithic species to form an epilithic thallus.

That the chemistry of the substrate can affect lichen morphology has been established by several authors. Massé (1964) stated that *Lecanora muralis* only attains its juvenile forms (f. *squamata* Nyl., f. *areolata* Harm., or f. *ecrustacea* Nyl.) when growing on rock walls not subjected to the excrement of birds. Certain varieties of *Peltula obscurans* (Nyl.) Gyeln. show a decreased development of medullary and lower cortical tissue when growing on alkaline substrates (Wetmore, 1970). These examples of arrested development are quite probably due to certain biochemical precursors which are missing or limiting on one substrate and not another, and, as such, would not be genetically controlled. Under certain circumstances, however, these characters could become fixed by selection.

Examples of morphological changes across taxonomic lines in response to substrate changes are common in the literature. Both Weber (1962) and Schade (1970) concluded that calcium oxalate production in many taxa, especially as pruina, is correlated with calcium-rich substrates, although it appears that species growing on limestone differ widely in their oxalate content (Syers *et al.*, 1967). Schade regarded calcium oxalate production as a kind of excretory mechanism in the presence of excesses of calcium ions together with oxalic acid, and he pointed out that calcium is much more common in "noncalcareous" substrates than might be supposed. Weber

(1968) reduced to synonymy dozens of species of the yellow *Acarosporae* after concluding that they were merely substrate-induced morphological variants.

Several species of crustose lichens, especially in the genus *Lecidea* (e.g., *L. macrocarpa* and *L. lapicida*), have what is commonly called "oxidated" forms on rocks containing iron. In these forms, iron is absorbed by the thallus and laid down in the cortex as a red-orange pigment shown to be iron oxide by Weber (1962). The fact that the ability to deal with iron in this way is shared by many lichens indicates that it is not a very fundamental biochemical maneuver, even though the ability seems to be genetically restricted to certain species. The fact that there are species which are found only in the "oxidated" state (e.g., *Rhizocarpon oederi* and *Lecidea dicksonii*) is a possible indication that the forms on iron-rich substrates may develop into genetically distinct populations (even species in the strict sense), given enough time and the right selective forces.

A clear example of substrate-induced morphological change is the effect of wood or bark grain patterns on the growth patterns of the lichens. The variety *recta* of *Graphis scripta* results from apothecial development following the wood grain with the apothecia becoming greatly elongated (Ohlert, 1871). The same pattern of development can be seen with other graphidlike species such as *Xylographa* and *Arthonia* growing on heavily grained wood.

The tendency of pendent lichens such as *Evernia* and *Alectoria* to take up a horizontal and often flattened growth pattern when growing on the ground is widely recognized. *Alectoria sarmentosa* subsp. *vexillifera* (Nyl. in Kihlm.) D. Hawksw. appears to be a genetically fixed population of *Alectoria sarmentosa* which has evolved the prostrate habit under vigorous selection in arctic and alpine conditions. It is, in fact, regarded as a full species (*A. vexillifera* [Nyl. in Kihlm.] Stizenb.) by most lichenologists.

The real problems arise with regard to clearly distinct populations on different substrates which show no substantial integradations correlating with substrate gradients, and which seem to be more or less unique in kind. There are far too many examples of this situation.

Is *Cladonia calycantha* a genetically determined taxon with the ability to live on acid substrates, perhaps having recently evolved from a race of *C. verticillata* which can only reach full development in more neutral sites (see Brodo, 1968, p. 86), or is it merely an ecological modification?

Cladonia subrangiformis is regarded in some recent works to be distinct from *C. furcata* on morphological, chemical, and ecological grounds (e.g., Ozenda and Clauzade, 1970), but asserted to be merely a substrate modification by Schade (1966). Schade claimed that the calcium content of the soil is responsible for the morphological changes, and the differences in thallus

chemistry are not constant. There is a question as to whether the variety *nitidella* of *Pyrenula nitida* (syn. *P. nitidella*) is restricted to thin-barked trees, or whether *P. nitida* only produces full-sized perithecia on bark substrates thick enough to accomodate them (e.g., *Fagus*, 1871).

B. Substrate-Induced versus Genetically Induced Chemical Changes

Variations in metabolic abilities and chemical production are no less important than morphological variations in this discussion. In fact, the selection of biochemical characters may lead to speciation faster than selection based on morphological characters, especially if nutrition is involved. For this reason, the observations of Shields *et al.*, 1957) concerning the amino N content of thalli growing on different substrates are of particular significance. Shields and her associates found that *Collema nigricans* had less than one-third the concentration of amino nitrogen when on lava rock than had thalli on lava soil. (The phycobiont in the various thalli was checked and found to be the same.) *Dermatocarpon squamellum* had just slightly more than one-half the concentration of amino nitrogen when growing over moss than it had growing alone.

Cherkasskaya (1965) discovered significantly different concentrations of "resinoids" (concentrated alcoholic extracts) in samples of *Evernia prunastri* collected from different species of deciduous trees, although the variation in concentrations of evernic acid and atranorin + usnic acid was not significant. Unfortunately, without knowing how many (if any) replicates of each substrate sample were tested and what the ecological setting of each tree was (e.g., with respect to light and moisture), it is impossible to distinguish between substrate effects and general habitat effects on the production of these substances. In fact, Cherkasskaya mentioned the noticeable effect of temperature and seasons on chemical activity in this lichen.

It is known that, in some cases, chemically distinct races of a species or population show certain substrate preferences [e.g., in the *Cladonia chlorophaea* group (Wetherbee, 1969), and the *Cladonia cariosa* group (Culberson, 1969)], although it is equally clear that others do not [e.g., *Cetraria ciliaris* (Hale, 1963; Graham, 1969)].

There is certainly no unequivocal evidence to show that a change in substrate will influence the qualitative production of lichen substances (e.g., depsides and depsidones), although quantitative differences may indeed result from the indirect effect of decreased or increased assimilatory activity due to substrate-derived nutrition.

Careful laboratory and field experiments in conjunction with considerations of phytogeography, morphology, development, and related disciplines may make it possible to sort out the mechanisms, genetic or ecological,

controlling substrate-correlated phenotypic variation. I know of no study where this has been done, but see no reason why it is impossible, especially if the study material is carefully selected for ease of experimentation. It is a challenge which awaits an imaginative experimental ecologist or taxonomically interested physiologist, or, better still, both.

VIII. Summary and Conclusions

1. Lichens are found on a great variety of substrates which provide relative permanence and surface stability. Known substrates include virtually every form of rock, tree, wood, and soil; all kinds of man-made materials such as cloth, paper, glass, iron, and leather; and even certain long-lived animals with parts persistent enough to be colonized, such as the exoskeleton of weevils and the carapace of land tortoises.

2. Some lichens are connected to their substrate only tenuously, while others, such as certain crustose species, are entirely immersed in the substrate, be it rock or bark.

3. Epiphytic lichens may conceivably harm their phorophyte by blocking lenticels or harboring harmful insects, but this has never been proven to be a widespread or serious effect. Some lichens can etch glass and marble and have been a serious problem in the preservation of ancient buildings and artifacts.

4. Some lichens can dissolve certain rock types by slow weathering action due to metabolic acidity and by chelation due to the action of some lichen substances. Their main pedogenic role, however, is in their early colonization of rock surfaces with subsequent humus production by decay and dust entrapment.

5. Some lichens are so substrate-indifferent that they are found almost as frequently on any of numerous substrate types. An example would be *Parmelia sulcata*. Others are so substrate-specific, they are found only on one or two species of trees and shrubs. Examples are *Calicium curtisii* restricted to *Rhus typhina* and *Conotrema urceolatum* largely restricted to *Acer saccharum*.

6. Substrate-indifferent species tend to be widespread, and substrate-specific species tend to be restricted in distribution.

7. The most substrate-specific species are those in closest contact with the substrate, and they may, in fact, derive certain essential and specific nutrients from it.

8. Relative substrate specificity is likely determined by the principle of limiting factors in some cases coupled with certain tolerances. The substrate's moisture capacity, mineral content, pH, metabolite production,

physical texture, and stability are all important as potential limiting factors.

9. A substrate can influence the geographic distribution of a lichen in direct relation to the specificity of the lichen to that substrate. The ability of a lichen to make a substrate "switch" at the edge of its normal host range would, of course, increase its own potential range.

10. Certain very ancient species characteristically found on one substrate have disjunct populations living on entirely different substrates. This type of vicariism apparently arose due to a gradual elimination of suitable habitats in the disjunct area. Many of these species have not changed morphologically despite their disjunctions and substrate differences.

11. Whether substrate-distinct populations are developing into new species as a result of their new habitats is a question which is difficult to answer without a great deal of experimental and phytogeographic evidence.

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Note Added in Proof

Wirth has recently published a thorough and important discussion of his observations of silicate rock lichens (V. Wirth, Die Silikatflechten-Gemeinschaften in ausseralpinen Zentraleuropa. *Dissert. Bot.* **17**, 1–306, 1972). He includes some very interesting observations on substrate specificity, and properties of silicate rocks affecting lichens (such as mineral content, rock hardness, surface texture, porosity and water-holding capacity, color, and temperature). His comments on weathering with and without lichen cover, the role of lime and silicate concentrations, and especially the influence of heavy metals on lichen vegetation are particularly noteworthy. Under the last-mentioned heading, Wirth discusses serpentine (finding that in his area, basi- or neutrophytic lichens are *not* found mixed with acidophytic species) (see p. 409), and lichens characteristic of ore rocks very high in heavy metals (concluding that these lichens occupy this niche not due to high heavy metal requirements, but rather that they compete successfully in a habitat toxic to most other species). He also discusses bird-rock communities coming to conclusions similar to those presented here (see p. 426).

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Chapter 13

LICHENS AND AIR POLLUTION

O. L. GILBERT

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I. Historical

It is over 100 years since Nylander (1866) suggested that the absence of lichens in the outskirts of the Jardins de Luxembourg in Paris was due to air pollution originating from the surrounding buildings. Since then similar and increasingly extensive examples of lichen deterioration have been observed around towns and industrial complexes all over the world.

The early work was done in Europe, especially Scandinavia, but since the last war detailed accounts have come from cities as far apart as Montreal, New York, Christchurch, Caracas, London, and Zagreb, indeed, wherever damage has been looked for. The recent concern with all forms of pollution has stimulated research into the use of lichens as indicators of air pollution and there is now a large literature on the subject.

The nineteenth-century observers simply noted a decline in the abundance and luxuriance of epiphytic lichens as towns were approached. Early floras and the journals of many local natural history societies in Britain carried comments of which the following are typical. Grindon, writing about lichens in his "Manchester Flora" (1859), stated, "the quantity has been much lessened of late years through the cutting down of old woods and the influx of factory smoke, which appears to be singularly prejudicial to these lovers of pure atmosphere." In 1879 the Rev. W. Johnson visited Gibside woods 7.5 km from Newcastle on Tyne and on his return wrote, "The lichens which flourished here in the fine condition spoken of by Winch (1831) have perished and this obviously from the pollution of the atmosphere by the smoke and fumes of Tyneside and the collieries of the surrounding district."

Sernander (1912, 1926) appears to have been the first worker to make a critical study of the lichens around a town. Working in the Stockholm region, chiefly on epiphytes, he recognized a central lichen desert (*lavöken*), a struggle zone (*kampzon*) where lichens covered up to half the surface of the trunks, and an outer normal zone (*normalzon*). Haugsjå (1930) mapped Sernander's zones in Oslo, but Vaarna (1934) working in Helsinki found it necessary to divide the struggle zone into an inner stunted foliose lichen zone (with *Hypogymnia physodes* marking its inner limit) and an outer stunted fruticose lichen zone (with *Evernia prunastri* forming the boundary between the two). So, by 1934, detailed maps had been prepared for the three Scandinavian capitals Stockholm, Oslo, and Helsinki, which must be regarded as forming the classic ground; this reflects the advanced state of lichen ecology in those countries at that time.

Soon, similar maps and town studies were being produced all over Europe. Vareschi in Zürich (1936) and Caracas (1953), Felföldy in Debrecen (1942), Sauberer in Vienna (1951), Barbalić in Zagreb (1953), Mattick in Danzig and Dresden (1937), Almborn in Lund (1943), and many others all reported a zoned pattern of deterioration similar to that in the type area.

Jones (1952), faced with the vast and poorly demarcated conurbation of Birmingham, made a careful study of lichens along a 64-km transect cutting through the center of the city. He found that the drop in diversity as the town was approached started a long way out and was continuous and that extinction depended partly on features such as growth form and species of tree examined. Fenton (1960) later used the same technique around Belfast in Ireland.

By now workers were mostly confirming the same pattern and from a research point of view the subject was not progressing. In 1958 Barkman reviewed the subject and increased the scale of operations by producing a map of epiphyte deserts throughout Holland which showed how much they had increased in size this century. He used Vaarna's definition of a desert—

an area from which foliose and fruticose lichens are absent—and was surprised at their size. The limits of the Scandinavian ones often lay in the outer suburbs of the city, but in Holland they extended over large areas of rural countryside. In 1958 Skye extended our knowledge by publishing the first account from around an isolated factory, in this case an oil-shale works at Kvarntorp, Sweden.

Throughout this period it was gradually becoming clear that sulfur dioxide (SO_2) was the pollutant involved in the formation of lichen deserts, and an important advance was made when Tallis (1964), Fenton (1964), Skye (1964), and Gilbert (1965) felt able to attach tentative SO_2 levels to some of the boundaries they were mapping. Rydzak (1958, 1968) and Rydzak and Krysiak (1970), however, maintained that drought, not toxic gases, was responsible for the deserts. Surprisingly, most studies were still concerned only with epiphytes where the pollution effect is seen at its most spectacular, but Gilbert (1965) working in Newcastle and Laundon (1967) working in London studied the entire lichen flora of their regions in equal detail.

By now important papers were coming thick and fast as authors started to specialize in certain aspects of lichen/pollution relationships. LeBlanc (1969) studied damage around the huge isolated point sources of SO_2 represented by iron- and nickel-sintering plants at Wawa and Sudbury in Canada. Skye (1968), Laundon (1970), and Gilbert (1968a, 1970a) became interested in the way the environment could apparently modify the effects of SO_2 ; Martin and Jacquard (1968) studied the effects of fluorine and calcium oxide dust on lichens in the Romanche Valley; while on the other side of the Atlantic Nash (1971) was examining the effects of HF in rural Pennsylvania. The first accounts of the reinvasion of lichen deserts following declining SO_2 levels were prepared by Skye and Hallberg (1969) and Pyatt (1970), while Brodo (1961), Schönbeck (1969), and LeBlanc *et al.* (1971) developed several types of transplant experiment. At last detail was being filled in and questions being answered by well-designed experiments instead of well-meaning speculation. This prepared the way for the first biological scales for the estimation of air pollution (Gilbert, 1970b; Hawksworth and Rose, 1970).

In the last few years papers reporting on the physiological and biochemical effects of SO_2 have appeared. A subject which has till recently been the preserve mainly of the field botanist and ecologist is now moving into the laboratory.

II. Lichen Deserts

The lichen deserts so far studied are broadly similar; therefore general principles will be elucidated and followed by a comparison on a world scale to highlight floristic variation.

A. General Features

A rapid method of investigating lichen deterioration around a town or city is to examine a series of strictly standardized habitats along a belt transect stretching upwind from the center of pollution. This enables species to be placed in an approximate order of tolerance and at the same time the effect of substratum on survival can be studied. The results of such a transect (Fig. 1) shows the number of species of lichen gradually falling off in each habitat with increasing pollution until in the city center only a few remain. Though diversity in all habitats is strongly affected, sensitive species are seen to disappear first from trees and then sandstone walls, while asbestos roofs retain their flora the longest. Thus the behavior of lichens in polluted areas is to some extent governed by the nature of the substratum. That the enhanced survival on basic substrata such as asbestos is a product of the habitat rather than innate in the species which colonize them is indicated by *Physcia tenella*, *Xanthoria parietina*, and *Lecanora dispersa* coming in 10, 11, and 13 km further on asbestos than on the boles of the ash trees.

Despite the variety of growth form displayed by lichens the process of extinction is strikingly similar. Diminishing luxuriance is followed sooner

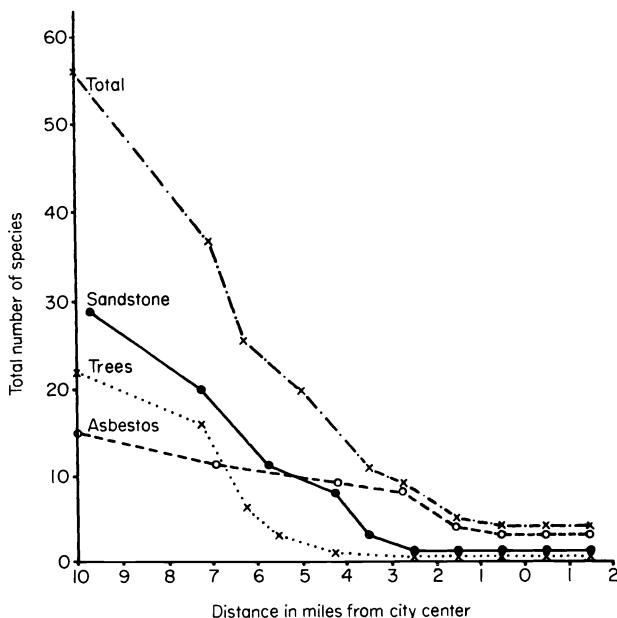


FIG. 1. Transect showing how the number of lichens growing on the top of sandstone walls, on asbestos roofs and on "standard" ash trees decline as Newcastle is approached from the West. (From Gilbert, 1965.)

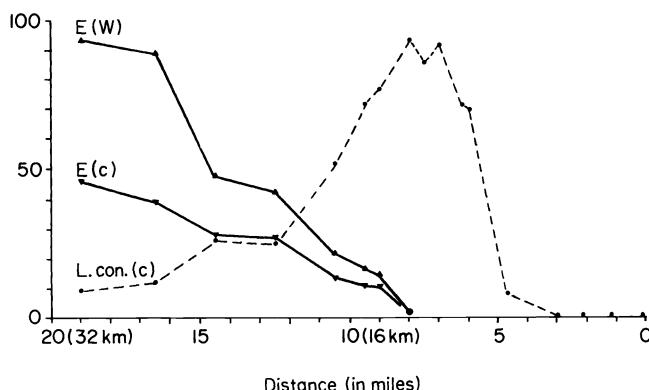


FIG. 2. Some changes in the lichen cover of ash trees moving away from Newcastle to the West. E(W), biomass (oven dry weight) of *Evernia prunastri*; E(c), cover of *Evernia prunastri*; L. con.(c), cover of *Lecanora conizaeoides*. (From Gilbert, 1969.)

(*Parmelia sulcata*, *Evernia prunastri*) or later (*Xanthoria parietina*, *Physcia millegrana* (LeBlanc and DeSloover, 1970) by a depression of fruiting. At their inner limit all species are sterile, compact, and individuals tend to be small with a low cover. The gradual suppression of *Evernia prunastri* is illustrated in Fig. 2, which shows how biomass and percentage cover start to decrease long before the species is eliminated.

Centers of old industrial towns support very few species and in the areas of highest pollution acid stone and trees remain completely uncolonized. In Britain the only common town lichens are *Lecanora dispersa*, *Candelariella aurella*, *Lecania erysibe*, and a few others, all on asbestos or similar calcareous substrata; *Lecanora conizaeoides* (*L. pityrea*) on acid stone and tree bases; and occasional *Cladonia* spp. in sheltered localities. These species which show an enhanced resistance to air pollution are of great interest to lichenologists but remain little studied. Figure 2 shows how *L. conizaeoides* increases in abundance as a town is approached. Where the last sensitive lichen becomes eliminated it reaches its maximum cover and its thallus is thicker and more luxuriant than at any other point on the transect.

Around the edge of most habitat deserts is a zone in which certain species show a mild form of this distribution; thus, in England, *Parmeliopsis ambigua*, *Cetraria chlorophylla* and *Buellia punctata* appear to be enjoying an enhanced abundance and ecological amplitude around the edge of towns compared to unpolluted areas and compared with their distribution last century.

London and Stockholm probably possess the most exhaustively studied lichen floras to date. Laundon (1970) included detailed habitat surveys in his study and reports the following preferences for London's 71 lichens (give in the tabulation on p. 448).

Substrata	Total	Per-cent-age	Habitats	Total	Per-cent-age
Calcareous stone	48	68	Woods	8	11
Acid stone	21	30	Parks	29	41
Acid soil	15	21	Heaths and commons	17	24
Bark	9	13	Churchyards and cemeteries	46	65
Wood	8	11	Old brickwalls	32	45
			Private gardens	24	34
			Sewerage farms	21	30
			Reservoirs	11	15
			Bombed sites	1	2
			Asbestos roofs	11	15

His substrate study supports the well-known fact that air pollution has a much greater adverse effect on corticolous communities than those on other types of substratum and confirms the relative tolerance of acid-soil species. His list ascribing the present lichen flora to different habitats is so far unique among city studies and has highlighted the importance of churchyards and cemeteries as refugia for lichens in the built environment.

Stockholm has lower levels of air pollution than London, and this enabled Skye to carry out detailed work on the extinction of epiphytes. He found that while most species decline steadily as the town is approached, there is a ring around the heart of the city where *Bacidia chlorococca*, *Hypogymnia physodes*, *Lecanora conizaeoides*, *Lecidea scalaris*, and *Lepraria incana* have an enhanced abundance and then decline in frequency towards the outlying districts. A little further out was another zone in which certain of the species which penetrate only moderately deeply into the city also show an increased frequency compared to rural areas. Among these are *Cladonia coniocraea*, *Cetraria glauca*, *C. pinastri*, *Physcia dubia*, and *Parmeliopsis ambigua*. This type of distribution becomes apparent only after careful recording on standard trees and is not shown by every species on all types of tree, e.g., it is strongly displayed by *Hypogymnia physodes* on *Fraxinus* but not on *Pinus sylvestris*. This is a classic example of how plant communities adapt to adverse conditions by simplification and the selection of new dominants.

The size and shape of an area affected by pollution can be discovered rapidly (and cheaply) by mapping indicator species. Ideally these should be widespread, easy to recognize, have shown a sharp extinction point in any transect studies. To obtain meaningful maps it is important to carefully standardize the habitats examined as field observations suggest that sheltered sites, sites subject to nutrient flushing, species of tree, and even position on tree can have a pronounced effect on survival. By using different species and varying the substratum it is possible to determine a boundary at almost any

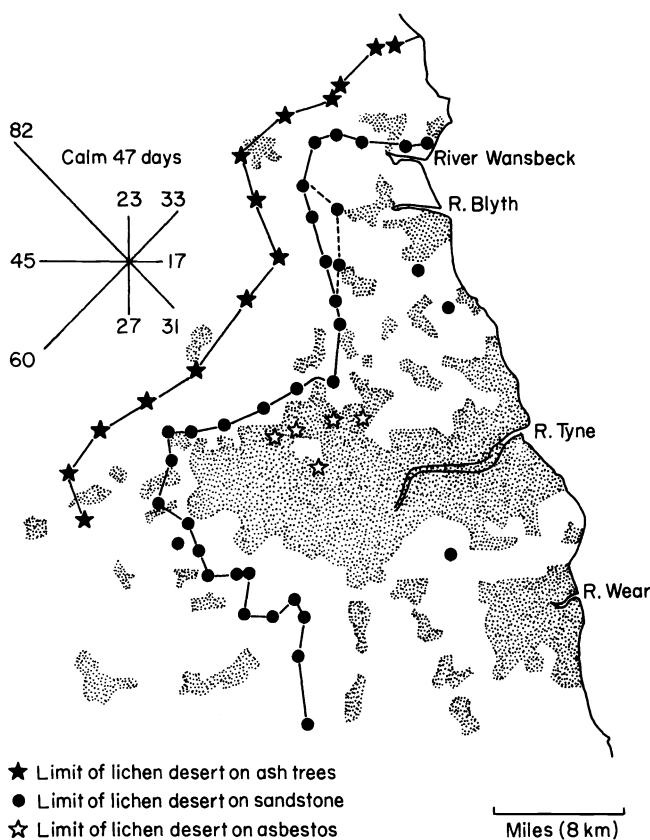


FIG. 3. Map of Lower Tyne Valley and adjacent areas showing the continuously built-up area (gray) surrounded by concentric deserts. Wind star for Newcastle 1960–1964. (From Gilbert, 1969.)

distance from a conurbation. The pioneers usually mapped the inner limit of foliose lichens on trees, but other parameters can be used as Fig. 3 shows. Here the shape of the zones is of interest as they keep more or less parallel, providing information on the steepness of the pollution gradient.

B. Urban Areas: A World Picture

A lack of data makes it impossible to compare lichen deserts on a truly worldwide scale. There are, however, enough studies from Europe and North America to make it clear that in these areas the zoning of epiphytes (the only widely studied group) is outstandingly similar.

The biggest disparity in temperate latitudes is between cities with and without *Lecanora conizaeoides*. Where it is well established this lichen is

conspicuously more resistant than any other epiphyte and forms dense pure stands. This species, which appears to have been first collected about the middle of the nineteenth century in England (Laundon, 1967), is now expanding vigorously to fill up the lichen deserts of Europe. Brightman (1964) mentions it from Norway, Sweden, Germany, Holland, and Belgium and it has recently been reported from New Zealand (P. W. James, private communication), Newfoundland (Ahti, 1965), and Iceland (Bailey, 1968). In areas where it is not yet known to occur, such as Montreal (LeBlanc and De Sloover, 1970) and New York (Brodo, 1966), the small leprose lichen *Bacidia chlorococca* often replaces it as the most resistant corticolous species, and in the more continental parts of Europe *Lecanora hageni* has been reported as filling this role, as in Oslo (Haugås, 1930) and Austria (Beschel, 1958). There is, however, some uncertainty about the exact identity of the latter species (Skye, 1968).

Workers are agreed that over much of Europe the most resistant epiphytic foliose lichen is *Hypogymnia physodes*, closely followed by *Parmelia sulcata* and *Physcia tenella* (including *P. adscendens*). The upper end of a generalized list of increasing sensitivity runs *L. conizaeoides/Buellia punctata*, *Cladonia coniocraea* (tree bases), *Lecanora expallens*, *Leparia incana*, *Bacidia chlorococca/Hypogymnia physodes*, *Lecidea scalaris*, *Parmelia saxatilis*, *P. sulcata*, *Physcia tenella*, *Xanthoria parietina*. There are local differences; for instance, *L. scalaris*, *Physcia dubia*, and *P. orbicularis* have variously been reported as highly resistant and in England *B. chlorococca* is not as resistant as elsewhere. The orders of sensitivity described from Canada and New York are, despite the presence of some different species, strikingly similar to the lists from Europe. In Christchurch, New Zealand, *Buellia punctata*, *Lecanora varia*, and *Rinodina exigua* have been reported as forming the inner limit of epiphytic lichen vegetation (Daly, 1970).

It is generally agreed that on basic substrata *Lecanora dispersa* is the most resistant lichen. It appears to be almost unaffected by urban levels of air pollution and in this habitat it (and several other species) are conspicuously more resistant than *L. conizaeoides* is on trees and walls. Laundon (1967), who has worked on the phytosociology of urban lichen communities, described the new federation *Lecanorion dispersae* from artificial calcareous substrata in the center of London and observed that it was replaced by two unions of the *Xanthorion* (*Caloplacetum heppianae*; *Physcietum caesiae*) in the suburbs where levels of air pollution are lower. Acid saxicolous habitats have also only been studied in Britain. Here pure stands of *L. conizaeoides* are gradually replaced by communities of sterile white crusts and nitrophilous species such as *Acarospora fuscata*, *Lecanora intricata*, *L. polytropa*, and *L. muralis* as pollution levels fall. *Parmelia saxatilis* is the most resistant foliose lichen in this habitat. An interesting recent development has been the

spread of saxicolous *Stereocaulon* spp. into the lichen deserts of lowland England.

At the other end of the sensitivity scale there is even closer agreement. All lists highlight the sensitivity of *Usnea*, *Ramalina*, and *Evernia* spp., in spite of different species frequently being involved. Other taxa widely accepted as being highly sensitive are *Anaptychia ciliaris*, *Physcia aipolia*, *Arthopyrenia biformis*, most *Graphis* and *Opegrapha* spp., and the majority of the *Caliciaeaceae*. Sharp variation is shown in the position accorded to *Cetraria glauca*, *C. chlorophylla*, and *Parmeliopsis ambigua*, and as one approaches the Atlantic seaboard of Europe it appears that *Evernia prunastri* and *Ramalina farinacea* become less sensitive. Close agreement is, however, the general rule which is remarkable as only rarely have tree species or sampling procedures been standardized.

At present there is not much precise information on the sensitivity of *Peltigera* spp. or the *Collemataceae*.

The size of lichen deserts differs greatly throughout the world but until there are pollution-recording gauges in more cities the significance of this can not be determined. It appears that those in the wetter, Atlantic parts of Europe are larger for a given population and have more widely spaced



FIG. 4. Epiphytic vegetation in the Netherlands. Black area, epiphyte deserts; white area, epiphytic vegetation poor to locally subnormal (transitional zone); gray area, epiphytic vegetation normal to rich and luxuriant. (Adapted from Barkman, 1969.)

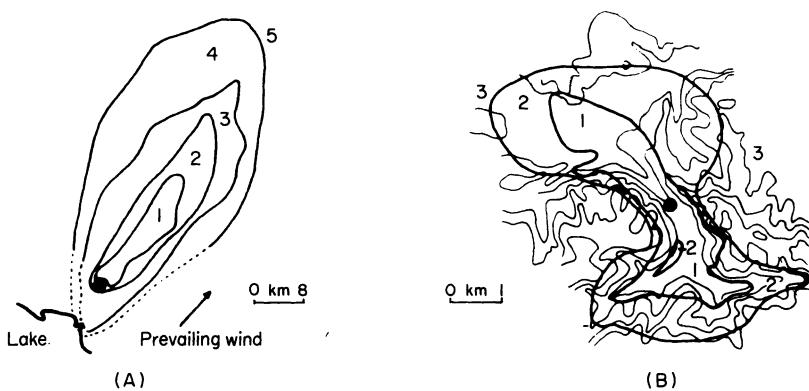


FIG. 5. Zonation patterns around isolated industrial plants influenced (A) by the wind (Wawa, Ontario, adapted from Rao and LeBlanc, 1967); (B) by local topography. (From Rudnany, Czechoslovakia, adapted from Pišút, 1962.)

zones than ones in more continental climates. It has been estimated that in the last 100 years Holland—the most densely populated country in the world—has lost 27% of its epiphytic lichens (up to 1954) due mainly to the spread of air pollution (Fig. 4) (Barkman, 1969). Lichen deserts are often elliptical and elongated in shape downwind but local topography and the pattern of town development can destroy this (Fig. 5).

C. Industrial Areas

The effect industrial point sources of SO_2 have on lichens is similar to that of cities despite the different patterns of fumigation involved. Around an oil-shale works at Kvarntorp in Sweden, Skye (1958) found certain epiphytic lichens absent up to 8 km downwind and interpreted a zone of relative lichen abundance at 2 km as lying between the active spheres of pollution originating from slag heaps at ground level and from lofty chimneys. Rao and LeBlanc (1967), investigating the effect of an iron smelter at Wawa, Ontario, found that air pollution had killed all trees for 13 km downwind, the first epiphytic lichens (*Bacidia chlorococca*, *Cladonia coniocraea*) coming in on tree bases at 16 km and tree boles at 30 km (Fig. 5). Nickel smelters at Sudbury, Ontario were having an equally devastating effect.

Richardson and Puckett (1971), who have also examined the Sudbury site, report that the first macrolichens (*Stereocaulon saxatile*, *Cladonia deformis*) are found at 6 km and receive 5–10 damaging fumigations a year. At 50 km from the smelter, 25 macrolichens were present which are fumigated on the average only once per year.

The only accounts we have of recovery following falling pollution levels come from industrial areas. The oil-shale works at Kvarntorp closed in 1966

and in the following year Hallberg visited the area and reported recovery in the form of strong lobe growth leading to a definite difference in appearance between the central and peripheral parts of single lichens (Skye and Hallberg, 1969). Pyatt (1970) has interpreted the numerous young (very small) lichen thalli in parts of the steel town of Port Talbot as indicating that the area is recovering from a previous period of more intense atmospheric pollution.

Observations around point sources in Britain suggest that they produce lichen deserts, the margins of which are frequently characterized by damaged thalli, e.g., chalky white *L. conizaeoides*, or *Parmelia saxatilis* and *P. sulcata*, which show abnormal pink, white, or brown coloration in the thallus. These colors which may to some extent be reversible are caused by sublethal fumigations associated with abnormal weather conditions or an unusually heavy exposition of pollutants. The edge of deserts associated with powerful point sources are thus not in as complete a state of equilibrium as urban deserts where pollution levels are more stable.

III. Causes of Lichen Deserts

A. Background

Strong views have been expressed over the cause of lichen deserts and when Barkman reviewed the subject (1958) there was genuine controversy between the drought and toxic-gas hypotheses. Since that date, however, nearly all published work has upheld the air-pollution explanation, two phytotoxicants (SO_2 and fluorine) have been identified, and the controversy is now whether lichen distributions reflect their mean, seasonal, or peak levels. A brief review of the growing weight of field, analytical, and experimental evidence which supports SO_2 as the major "city influence" will be given.

The size and shape of lichen deserts—elongated downwind and often extending for many miles beyond the built-up area—clearly point to some airborne influence and in all studies where measurements of SO_2 have been made the severity of damage correlates well with its distribution. Due to a lack of recording apparatus not all workers have been able to make this correlation, but in Britain results from the National Survey of Air Pollution, which employs more than a thousand 24-hour sampling gauges for smoke and SO_2 , are available for all to consult. Having this detailed information coupled with a generally wet climate is, perhaps, why British workers more than any others are severely critical of the drought hypothesis.

Over many cities and industrial complexes an atmospheric soup of pollutants exists which makes it difficult to separate the effects of any one,

and here SO₂ can be implemented only by analogy with areas where the situation is simplified. One of the best areas for collecting primary data has been the coal-burning cities of northern England where most of the industry is constructional or textile. Here the problem is smoke or SO₂, and when their relative toxicity is compared by means of transplants to parts of the conurbation which have different ratios of smoke: SO₂, it is seen that deterioration is rapid at sites with high levels of SO₂ but damage correlates poorly with smoke. In Newcastle upon Tyne this has been confirmed by observing the natural colonization of asbestos roofs close to pollution gauges where it was found that floristic diversity is strongly linked to SO₂ levels (correlation coefficient $r = -0.84$) but only slightly ($r = -0.5$) with smoke (Gilbert, 1970a).

Good correlations between the richness of the lichen flora and measured levels of SO₂ were shown by Skye at Kvarntorp and by LeBlanc at Sudbury, but the presence of a complex range of pollutants, including metals, around most industrial sources and levels of SO₂ often sufficient to kill trees makes them less suitable for obtaining primary data.

Field evidence for the toxicity of SO₂, then, is strong and analysis of lichen thalli before and after exposure to urban pollution has confirmed that sulfur is rapidly accumulated. In addition, analysis of specimens from the edge of polluted areas shows that they contain much greater concentrations of S both on and in their thalli than matched samples from further afield (Table I). More confirmatory evidence for the toxicity of SO₂ is coming from laboratory experiments in which lichens (and bryophytes) exposed to low concentrations of sulphite or SO₂ in solution are showing differing sensitivities which correlate well with their known sensitivity to air pollution in the field (Baddeley *et al.*, 1971; Hill, 1971).

TABLE I
THE SULFUR CONTENT OF *Parmelia saxatilis* FROM DIFFERENT PARTS OF THE
TYNE VALLEY^{a,b}

	Distance west of town center				
	4 miles	4.5 miles	6.25 miles	8 miles	21 miles
S content(ppm)	3290(670) ^c	2870	1420(560) ^c	659	225

^aFrom Gilbert, 1968b.

^bReplicated, washed 500-mg air-dry samples were used.

^cFigures in parenthesis refer to the amount of sulfur in ppm removed by shaking a matched sample for 1 hour in deionized water.

B. Effects of SO_2 on Growth and Metabolism

1. GROWTH EFFECTS

The functioning of lichen propagules may be the first mechanism to be affected by low levels of SO_2 . This is suggested by both Laundon's work and that of Margot (1971) who has discovered that the algal component of soredia from *Hypogymnia physodes* are easily damaged by traces of SO_2 . Saunders (1966) working with the leaf fungus *Diplocarpon rosae* found ascospore germination was the stage in the life cycle most sensitive to SO_2 . So there is a certain amount of evidence that establishment is the first process affected.

Under greater pollution stress an accelerated rate of senescence sets in. To date, this has been demonstrated only for saxicolous foliose lichens, e.g., *Parmelia saxatilis*, *P. glabratula*, *Xanthoria parietina*, the colonies of which take on a highly characteristic crescent-shaped appearance due to the older central parts becoming detached and flaking off (Gilbert, 1971a). This may be accompanied by the decomposition of lichen acids but there is no slowing down in the extension rate of young margins.

At still higher concentrations of SO_2 a whitening, browning, or violet tinging of marginal lobes takes place; this eventually spreads through the thallus which shrivels and dies. This syndrome has been widely reported in transplant experiments (Schönbeck, 1969; Brodo, 1961; Gilbert, 1968b) and has been observed in the field where new pollution sources have started up in rural areas.

2. PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS

It is widely assumed that the highly efficient, nonselective mechanism which lichens possess for accumulating substances from very dilute solutions is at least partly responsible for their acute sensitivity to atmospheric SO_2 which is concentrated until present in toxic amounts (Smith, 1962). Some confirmation for this was obtained when the total sulphur content of *Parmelia saxatilis* growing at different distances upwind of a pollution source was measured. The results (Table I) showed that thalli from the edge of a heavily polluted area contain much larger amounts of S than specimens collected at a distance. From a further experiment in which live and dead *Usnea* material (enclosed in net bags) was exposed to town air for 8 weeks and then analyzed for S, it was shown that the living material accumulated S over six times faster (Gilbert, 1969). This suggests that S uptake is metabolically dependent and that it can be concentrated beyond expectable needs.

Early work on the harmful effects of SO_2 on lichen metabolism was carried out by Pearson and Skye (1965) and Rao and LeBlanc (1966). The

latter found that the algal component (*Trebouxia*) of lichens which have been fumigated with 5 ppm SO₂ for 24 hours develop certain abnormalities. Chlorophyll is bleached, a permanent plasmolysis sets in, and brown spots develop on the chloroplasts. They produced evidence that chlorophyll a was decomposed to phaeophytin a and also reported that less damage occurred to samples maintained at 45% relative humidity during fumigation than those exposed at 92% relative humidity. Pearson and Skye, taking a different approach, carried out Warburg experiments on *Parmelia sulcata* which had been exposed to various concentrations of gaseous SO₂. They decided that the low apparent rate of photosynthesis shown by the fumigated samples was due to atmospheric SO₂ destroying chlorophyll.

Several criticisms can be made of the work reported above, the main one being that the levels of SO₂ used were unrealistically high. Undoubtedly these pioneer experiments suggest a possible mechanism for SO₂ toxicity but it is not necessarily the important one under field conditions. More recently Hill (1971) and Baddeley *et al.* (1971), appreciating the difficulty of working with realistically low concentrations of gaseous SO₂, have been studying the effect of buffered solutions. Hill examined the effect that sulphite solutions (Na₂S₂O₅) have on the incorporation of H¹⁴CO₃⁻ (in the light) into a small number of lichens. The concentration which prevented incorporation was least in *Usnea* and greatest in *Lecanora conizaeoides* (Fig. 6), differences which correlate with the known sensitivity of these

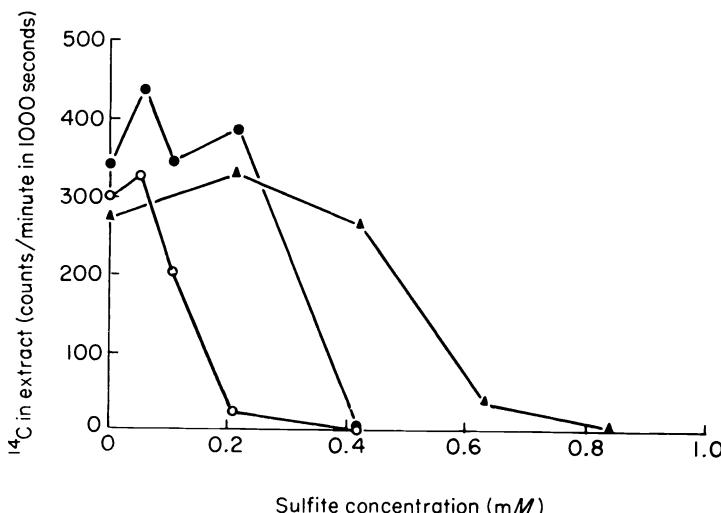


FIG. 6. The effect of sulfite on ¹⁴C incorporation in *Usnea subfloridana* (○), *Parmelia physodes* (●), and *Lecanora conizaeoides* (▲). (From Hill, 1971.)

lichens to air pollution. He also showed that sulfate was less toxic than sulfite and that the latter was toxic at pH 4 and below but not at pH 5 and above.

Baddeley *et al* (1971) have examined the effect on respiration of short duration exposures to increasing concentrations of SO₂ in solution (buffered at pH 4.2) using an oxygen electrode. Results from several different types of experiment show a marked reduction in the respiration rates of all lichens so far tested when immersed in solutions containing 10–33 ppm SO₂. Respiratory response seems to be an immediate one and also bears some relation to the sensitivity of lichens as observed in the field. This lack of an absolute correlation with field data is only to be expected as the experimental work is still at an early stage.

The relevance of solution studies to field observations cannot be established at present. However it is tentatively suggested by Saunders (1966) that 100 µg SO₂/m³ will maintain an SO₂ concentration in surface water approximately equivalent to 35 ppm so it seems probable that realistic results are being obtained, though more evidence for this is urgently needed.

It should be stressed that both SO₂ toxicity and lichen metabolism are little understood at present, which makes the interpretation of results difficult. To date every process examined appears sensitive so there may be some basic metabolic control which is disrupted and what we are recording are the results of this disruption.

C. Other Pollutants

Research has concentrated on the devastating effects of SO₂, and there is surprisingly little information available about other forms of air pollution. The only other pollutants so far identified as having a major effect on lichens are fluorine and fertilizer dust.

Not many parts of the world are at risk from high concentrations of air-borne fluorides, but lichens have been recorded as absent or deteriorating around aluminium smelters in France (Mazel, 1958; Martin and Jacquard, 1968), Scotland (Gilbert, 1971b), and Canada (LeBlanc *et al.*, 1971), and also near a chemical factory in Pennsylvania (Nash, 1971). Lichens transplanted into the vicinity of these F sources (LeBlanc *et al.*, 1971; Nash, 1971) have accumulated F and gradually died after becoming first chlorotic and then necrotic, finishing up a brownish, pinkish, or yellowish color before disintegrating. Nash has suggested that the critical level of F within the lichen thallus may lie between 30–80 ppm. Gilbert found that at their inner limit washed thalli of *Usnea subfloridana* and *Parmelia saxatilis* contained 20 and 47 ppm soluble F, respectively, which largely bears this out.

Ecological data are available only from the Scottish site where it was noted that lichens survive conspicuously better in sheltered sites and on the

ground, and that nitrophilous conditions enhance survival. We do not know about behavior on calcareous substrata nor is much known about the relative sensitivity of acid saxicolous and epiphytic communities. The fluorine desert at Fort William in Scotland is strongly elongated down the prevailing wind and has *Stereocaulon pileatum* as the most resistant species.

The considerable effect that blowing fertilizer dust can have was apparently noticed first by Dr. Francis Rose who realized that pockets of rich epiphytic vegetation in southern England were invariably associated with parkland and other sites where intensive agriculture was not practiced. It now seems clear that fertilizer dust brings about a marked and widespread eutrophication of bark, causing acidophilous communities to be replaced by common, aggressive, and vigorous species belonging to the Xanthorion federation.

A few authors have reported that car exhaust is responsible for eliminating lichens along roadsides (Barkman, 1958, p. 117; Domrös, 1966). These are mainly casual observations and no attempt was made to separate the effect of toxic exhaust gases from de-icing compounds and dust effects. Lichen deserts became widespread before the internal combustion engine was invented. Photochemical smog associated with car exhaust fumes is known to be phytotoxic and may be having an effect in the United States but damage to lichens has not been reported. A. V. Fletcher has suggested that the recent expansion of *Stereocaulon* spp. into urban areas of Britain could be associated with increasing lead in the environment. As these species are especially characteristic of the spoil heaps of old lead mines, the idea might repay study.

D. Resistance to Pollution

Many problems center around resistant species of which *Lecanora conizaeoides* is the outstanding example. Its remarkable resistance to SO₂ under acid conditions is still unexplained, though it seems likely that a fast growth rate—as this feature is common to all resistant lichens and bryophytes—confers a certain advantage. At present there is insufficient evidence to decide whether *L. conizaeoides* is a recently evolved acidophytic toxotolerant lichen in the process of extending its distribution along well-defined urban invasion paths—hence an apparent connection with pollution—or if it has some nutritional requirement which is satisfied by urban pollution.

Experimental work with bryophytes (Gilbert, 1968a) indicates that the difference between sensitive and resistant species does not lie in the resistant ones possessing a less efficient mechanism for sulfur accumulation. From this point the possibilities for speculation are endless. Sulfur could be removed by chelation in resistant species and quickly oxidized to sulfate; alkaline cell sap could render it harmless, and it has been suggested that low wettability of the thallus might be protective.

As *L. conizaeoides* is conspicuously more resistant than other acidophytic species, it may have a method of protection not developed by other lichens, so when investigating general sensitivity/resistance mechanisms it might be advisable to work on other species.

IV. Effect of Environment

In asserting that SO₂ is responsible for the lichen deserts which have developed around urban and industrial areas one could be accused of oversimplification as such an unrefined statement hides a wealth of fascinating interactions between lichens, pollution, and the rest of the environment. These interactions can accentuate the effects of pollution so they are still operating 50 or 100 miles from the source or minimize them so that certain attenuated lichen communities survive almost into the city center.

Where SO₂ levels are high, as around the Sudbury and Wawa smelters, pollution does tend to be a master factor incapable of modification, but once lichens start to return it is noticeable that certain niches are colonized first and the marked influence habitat has on survival starts to become apparent. Many workers have noted how lichens and bryophytes tend to return first to terricolous habitats, followed by saxicolous calcareous, saxicolous acidic, corticolous rich bark, and last to corticolous poor bark habitats such as the acid boles of conifers and birch. The effect of individual habitat factors which help to explain this will be dealt with.

A. Shelter

Though there is nothing in the air-pollution literature to suggest that shelter can play more than a small part in modifying pollution levels, most lichenologists know that sites sheltered by dense woodland, tall herbage, deep valleys, and stonework at ground level regularly carry sensitive species to well inside their normal limit. The more sheltered the site the further they come in, the combination of trees in a deep ravine being especially favorable. This suggests that shelter has a relatively large effect in reducing SO₂ levels.

One such site has been investigated with a series of portable pollution gauges which were operated for 10 weeks in and around a sheltered valley near Newcastle upon Tyne. Typical of the results obtained (Gilbert, 1968a) was a 76% reduction of SO₂ in short grassland at an exposed windy site (compared to readings at 1.3, 0.6, and 0.15 m above ground level). The valley showed SO₂ reductions of 60% at 2 m and 92% at ground level compared to the surrounding area sampled at 2 m. Reductions of this order easily explain the persistence of sensitive species in sheltered niches.

Reports of *Cladonias* from heavily polluted areas (Gilbert, 1965; Seaward, 1966; Brodo, 1966; Laundon, 1967) has led to the belief that many of them possess a certain resistance to air pollution. However, this resistance may be more apparent than real, merely reflecting their ability to grow vigorously in sheltered sites and on the ground.

An extension of these observations is being made by F. Rose and B. Coppins, who are examining the lichen flora of southern England on a regional scale. Their results confirm that the richest communities now survive only in the valleys and they have evidence that the converse is true, pollution acting most powerfully on high ground. So, at some distance from a source it is lichen communities on exposed hills and ridges which deteriorate the fastest. The observation that in the New Forest it is the twig epiphytes which disappear first is probably another example of this phenomenon.

The modifying effect of shelter may be a result of "scrubbing," as air readily deposits its strongly polar SO₂ molecules onto surfaces of all kinds. An experiment in which nylon hairnets containing glass wool, cottonwool, and dead *Usnea* were hung for 8 weeks in a polluted atmosphere (average SO₂ = 0.05 ppm) and on analysis were found to have gained 342, 248, and 155 ppm S, respectively, confirms that SO₂ impacts readily onto surfaces (Gilbert, 1969).

B. pH

Most lichenologists have observed that a high pH can reduce the effects of pollution. For example, Laundon (1967) found 66% of the lichen flora of

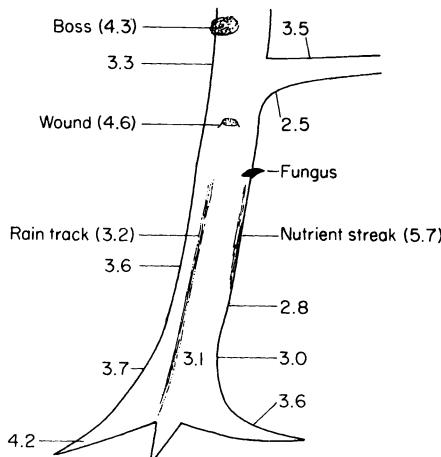


FIG. 7. Variations in pH on the bole of an ash tree, 11 miles (18 km) west of Newcastle. The boss is 10 feet above ground level. This tree stands on the edge of the lichen desert and much of the trunk has started to become acidified. (From Gilbert, 1970a.)

London growing on basic substrata; around Newcastle upon Tyne concrete and asbestos remain well colonized at pollution levels which have cleared all corticolous and acid saxicolous habitats, and certain normally wide-ranging species like *Lecanora muralis* behave as strict calcicoles under pollution stress. Epiphytes show the same phenomenon, persisting longest on the alkaline bark of ash and elm and on those parts of the tree which have the highest pH (Fig. 7).

Two explanations have been put forward to account for this pH effect. Skye (1968) believes that acidification of the substratum finally makes it impossible for certain epiphytic communities (e.g., *Xanthorion*) to exist, so that pH per se operates to exclude sensitive lichens from all habitats except those strongly buffered at a high pH. The alternative idea (first put forward by food chemists trying to inhibit bacteria and mold in fruit juices) is that pH acts indirectly by affecting the ionization and rate of oxidation of sulfurous acid.

1. DIRECT EFFECT

Acidifying effects of SO₂ on the environment have been looked for in soil, water, and tree bark. The magnitude of soil pH changes has been reviewed by Webster (1967) who concluded from the limited information available that they are small, even in the neighborhood of long-established smelters.

Detailed surveys of bark acidification have been carried out at Krakow in Poland (Grodzinska, 1971) and in Stockholm (Skye, 1968). In both areas the wide range of tree barks sampled showed a progressive acidification as the center of pollution was approached though the magnitude of the change varied greatly. In Stockholm the drop was usually (1 *Pinus*) 2–3 (4 *Ulmus*) units with the final pH in the range 2.4–2.9, while in Krakow it is less than half a unit to a final pH about 4. Around Newcastle upon Tyne an intermediate situation exists, the shift being about 1.5 units to a pH just above 3. This geographical variation is puzzling. It may be partly related to sampling procedure as pH varies widely over a tree, but is more likely to be connected with soot contamination on the Newcastle and especially on the Krakow barks. Soot has a pH of about 6.

The trees in central Stockholm have some of the lowest bark pH's ever recorded, but certain observations suggest that some other factor eliminates sensitive lichens before pH levels have dropped low enough to become toxic. An examination of Table II clearly shows how the percentage cover of sensitive lichens starts falling before the pH of the bark on which they are growing begins to decline. It appears, therefore, that the marked acidification of tree bark which occurs in polluted areas is not sufficiently low in the transition zone to cause widespread elimination of the lichen flora,

TABLE II
THE pH OF SURFACE FLAKES OF BARK COLLECTED FROM STANDARD ASH TREES AT
VARIOUS DISTANCES WEST OF NEWCASTLE (THE PERCENTAGE COVER
OF SENSITIVE LICHENS IN THE SAMPLING ZONE IS ALSO GIVEN)^a

Distance (miles)	19	16½	14½	12½	10½	9½	9	8	7	6½	6	4½	3	1½	1	0
west of central Newcastle																
pH value	4.4	4.2	4.3	4.3	4.4	4.4	3.9	3.6	3.4	3.6	3.4	3.9	3.4	3.6	3.0	4.0
% cover sensitive Lichens	66	66	57	54	25	23	12	1	0	0	0	0	0	0	0	0

^aFrom Gilbert, 1970a.

though it can result in the development of more acidophilous communities as noted by Skye.

2. INDIRECT EFFECT

Food chemists who use SO_2 as a preservative to inhibit microorganisms have known for a long time that its preservative value is greatly influenced by pH as this determines the form in which the sulfurous acid is present (Vass and Ingram, 1949; Cruess *et al.*, 1931; Rahn and Conn, 1944). These workers discovered that at high pH's sulfurous acid was quite ineffective against yeast and molds, while at low pH's only a trace was needed to inhibit their growth.

Experiments designed to investigate the effects of presenting very dilute sulfurous acid, buffered at several pH's, to a variety of bryophytes (Gilbert, 1968a) and germinating spores of the sensitive leaf fungus *Diplocarpon rosae* (Saunders, 1966) clearly showed that neither SO_4^{2-} nor SO_3^{2-} (H_2SO_3 at pH 6.6) were toxic to the material tested. In contrast, bisulfite ions (H_2SO_3 at pH 4.2) were moderately toxic while at pH 3.2 when about 5% undissociated H_2SO_3 is present the solutions were highly toxic. Control experiments showed that it was not pH per se which was damaging. Recent physiological work (Section III,B) has extended these observations to a wide range of lichens.

3. BUFFER CAPACITY

The above experiments showed that below pH about 3.5 only traces of SO_2 in solution can have devastating toxic effects. It is buffering against acid substances which is critical and when a variety of barks are tested (Gilbert, 1970a; Skye, 1968) it becomes clear that the order of increasing buffer capacity above pH 3.5 (*Betula* and *Pinus*, *Quercus robur*, *Fraxinus excelsior*, *Salix alba* and *Ulmus* bark, asbestos) mirrors the order of habitat

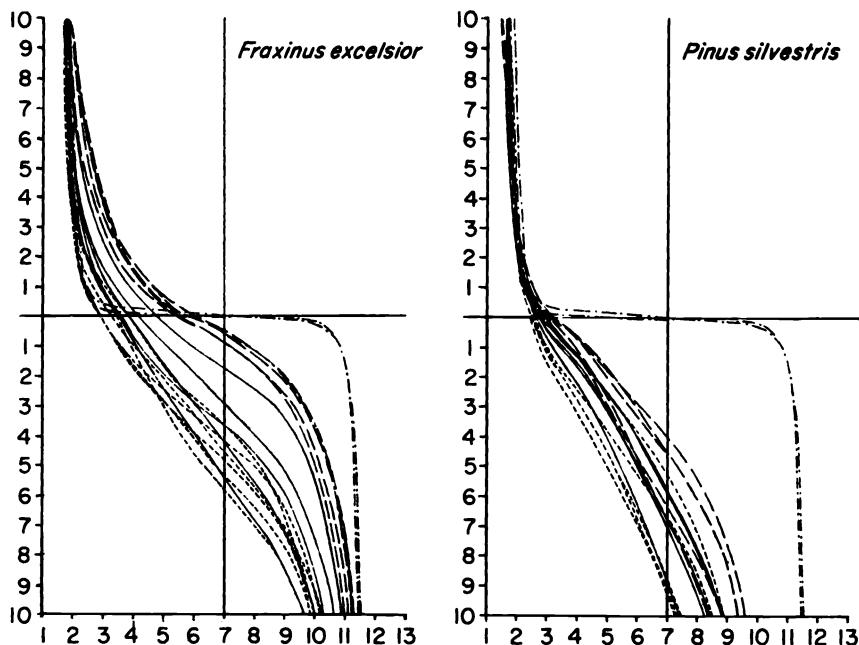


FIG. 8. Buffer capacity in bark samples from the normal zone (—), the transitional zone (---), and the lichen-free zone (-----) of Stockholm. Curves for the two grades of distilled water used are also shown (—·—). (From Skye, 1968.)

sensitivity (Fig. 8). Skye also determined the pH and buffering capacity of lichens around Stockholm and reports that with a few exceptions it is the species with the lowest buffer capacity for acid substances which disappear first.

The above experiments provide a satisfactory explanation for most of the pH phenomena observed in the field.

C. Nutrients

It has been observed repeatedly that survival in polluted areas can be enhanced by nutrient flushing. In Britain the spectrum of nutrients provided by roadside dust encourages the persistence of *Candelariella vitellina* and *Acarospora fuscata* on walls, and even more effective is the strong flushing provided by bird droppings. Before lichens disappear from roofs they become restricted to the edges, ridge, and gable ends where birds perch, such niches carrying lichens far into the suburbs of many large cities. Other spectacular examples can be found associated with bird "song posts" in cemeteries, around sewage farms (Laundon, 1967), and on the trunks of old deciduous trees where nutrient-rich streaks frequently develop below

wounds and old rotting limbs. Such sites invariably support nitrophilous vegetation belonging to the Xanthorion federation.

Bark analysis (Gilbert, 1970a) has confirmed that nutrient streaks on ash trees (*Fraxinus excelsior*) contain more nutrients than adjacent parts of the trunk, but it has not been possible to link enhanced survival with any one or any particular combination of nutrients. As the best correlation was with pH, the role of nutrients could be chiefly their effect on this, but it may be significant that it has been reported from a number of sources that the resistance of many higher plants to SO₂ injury can be increased by an application of nitrogen (Zahn, 1963).

D. Water Relations

Several authors (Beschel, 1952, 1958; Klement, 1956, 1958; Rydzak, 1958, 1968; Rydzak and Krysiak, 1970) have considered dryness of the air to be chiefly responsible for the absence of lichens around cities, which they regard as warm, dry stone deserts. This idea had maximum support in the fifties but fell out of favor once actual levels of air pollution became known and it was possible to appreciate the good correlation between SO₂ levels and lichen distribution. Recently, only J. Rydzak in Poland has given support to the drought hypothesis, and in a paper published just before his death (Rydzak and Piorecki, 1971) he admitted that air pollution was to some extent responsible for lichen deserts in Poland. It has never been disputed that town centers experience a slightly dryer climate than neighboring rural areas; Kratzer (1937) quotes the difference in relative humidity as about 2% in winter and about 8% in summer, but this is at least partly compensated for by increased rainfall (Barrat, 1964; Weigel, 1938).

It was field evidence such as the extensions of lichen deserts over areas of open country downwind of towns and their existence around isolated factories where urban development could have no desiccating effect which always formed the biggest objection to the drought hypothesis. It is now of historical interest only.

It would be naive to assume the slightly dryer town climate and the slightly different drying/wetting régime experienced there to have no effect on lichen survival but it must be very small as it has not been picked up in the field. When the pattern of water uptake and loss from a variety of bark disks was compared, no correlation between the water relations of these substrata and their ability to alleviate pollution could be found (Gilbert, 1970a). As most lichen studies have been carried out in temperate climates, the possibility remains that city-induced drought is of some consequence in continental areas.

E. Age of Substratum

While preparing distribution maps around pollution sources, workers in Britain sometimes observed that near to their inner limit lichens became restricted to the older trees and walls in a way that could not satisfactorily be explained in terms of the modifying effects of shelter, pH, or nutrient enrichment. This apparent anomaly was eventually explained by Laundon (1967). Working on dated limestone memorials in London he recognized the community *Caloplacetum heppiana* as having persisted for over 70 years in areas where rising levels of air pollution made the colonization of new surfaces impossible. During this period a resistant species *Lecanora dispersa* continued to colonize newly erected memorials (Fig. 9). Once recognized, this phenomenon of relic lichen communities has been of immense help in interpreting field data. It has been observed on old asbestos roofs, brick-walls, sandstone walls, and trees, but is at its most spectacular on calcareous substrata.

The implications of this phenomenon are considerable. Even if no further increase in ground-level concentrations of SO_2 occur at a moderately polluted site, the lichen flora will continue to deteriorate as nonviable communities slowly die out.

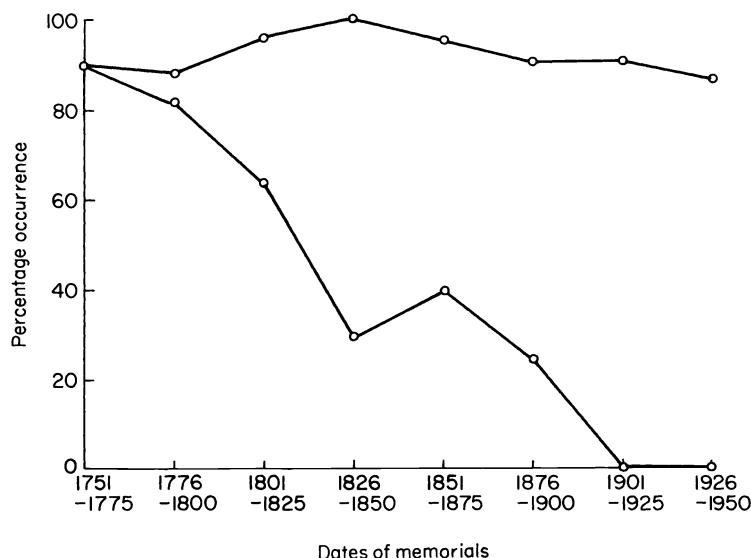


FIG. 9. The percentage occurrence of *Caloplaca heppiana* (lower graph) and *Lecanora dispersa* (upper graph) on limestone memorials erected from 1750 to 1950 at St. Peter and St. Pauls churchyard, Mitcham, London. (From Laundon, 1967.)

F. Other Factors

1. SERAL STAGE

Some evidence is on hand that late seral stages of both lichen and bryophyte communities are more sensitive to SO₂ than pioneer ones. This may be associated with parallel trends of decreasing pH and shelter as isolation from the substratum proceeds and more luxuriant growth forms are attained. Thus, pollution often gives the appearance of putting the community back to an earlier seral stage.

2. GROWTH FORM

In the early days it was thought that growth form was a reliable guide to sensitivity (Fenton, 1960) but it is now known that this is not the case, though it may be a pointer. For instance, fruticose lichens tend to be rather sensitive and most highly resistant ones are crustaceous. A general trend of increasing resistance can be represented by fruticose → foliose → crustaceous → leprose. In practice, however, there are so many exceptions that these trends are of little use in assessing pollution levels except to experienced observers who can also appraise luxuriance.

3. SYSTEMATIC POSITION

The loose connection between growth form and susceptibility allows links with taxonomy. Thus whole families such as the Usneaceae and Ramalinaceae are prone to be susceptible due to the predominance of profuse growth forms. Resistant species are rare, being scattered apparently at random through a wide range of unrelated genera.

V. Biological Estimation of Air Pollution

Any map showing the distribution of lichens also contains information on regional levels of air pollution. To enhance this faculty certain authors have mapped selected species (Skye, 1958; Gilbert, 1970a) or delimited broad zones (Fig. 4) in such a way that the distribution of air pollution becomes immediately apparent.

LeBlanc and De Sloover have developed a formula from which the richness of the epiphyte flora at a site can be represented by a single number called the "index of atmospheric purity" (IAP). They have applied this technique to an industrial valley in northwest Belgium (De Sloover and LeBlanc, 1968) and to the city of Montreal (LeBlanc and De Sloover, 1970).

TABLE III
QUALITATIVE SCALE FOR THE ESTIMATION OF SULFUR DIOXIDE AIR POLLUTION
IN ENGLAND AND WALES USING EPIPHYTIC LICHENS^a

Zone	Noneutrophiated bark	Eutrophiated bark	SO_2 ($\mu\text{g}/\text{m}^3$)
0	Epiphytes absent	Epiphytes absent	?
1	<i>Pleurococcus viridis</i> s.l. present but confined to the base	<i>Pleurococcus viridis</i> s.l. extends up the trunk	> 170
2	<i>Pleurococcus viridis</i> s.l. extends up the trunk; <i>Lecanora conizaeoides</i> present but confined to the bases	<i>Lecanora conizaeoides</i> abundant; <i>L. expallens</i> occurs occasionally on the bases	~ 150
3	<i>Lecanora conizaeoides</i> extends up the trunk; <i>Lepraria incana</i> becomes frequent on the bases	<i>Lecanora expallens</i> and <i>Buellia punctata</i> abundant; <i>B. canescens</i> appears	~ 125
4	<i>Hypogymnia physodes</i> and/or <i>Parmelia saxatilis</i> , or <i>P. sulcata</i> appear on the bases but do not extend up the trunks. <i>Lecidea scalaris</i> , <i>Lecanora expallens</i> and <i>Chaenotheca ferruginea</i> , often present	<i>Buellia canescens</i> common; <i>Physcia adscendens</i> and <i>Xanthoria parietina</i> appears on the bases; <i>Physcia tribacia</i> appears in S	~ 70
5	<i>Hypogymnia physodes</i> or <i>P. saxatilis</i> extends up the trunk to 2.5 m or more; <i>P. glabratula</i> , <i>P. subrudecta</i> , <i>Parmeliopsis ambigua</i> and <i>Lecanora chlorotera</i> appear; <i>Calicium viride</i> , <i>Lepraria canicularis</i> , <i>Pertusaria amara</i> may occur; <i>Ramalina farinacea</i> and <i>Evernia prunastri</i> if present largely confined to the bases; <i>Platismatia glauca</i> may be present on horizontal branches	<i>Physconia grisea</i> , <i>P. farrea</i> , <i>Buellia alboatra</i> , <i>Physcia orbicularis</i> , <i>P. tenella</i> , <i>Ramalina farinacea</i> , <i>Haematomma coccineum</i> var. <i>porphyrium</i> , <i>Schismatomma decolorans</i> , <i>Xanthoria candelaria</i> , <i>Opegrapha varia</i> and <i>O. vulgata</i> appear; <i>Buellia canescens</i> and <i>X. parietina</i> common; <i>Parmelia acetabulum</i> appears in E	~ 60
6	<i>P. caperata</i> present at least on the base; rich in species of <i>Pertusaria</i> (e.g., <i>P. albescens</i> , <i>P. hymenea</i>) and <i>Parmelia</i> (e.g., <i>P. revoluta</i> (except in NE), <i>P. tiliacea</i> , <i>P. exasperatula</i> (in N); <i>Graphis elegans</i> appearing; <i>Pseudevernia furfuracea</i> and <i>Alectoria fuscescens</i> present in upland areas	<i>Pertusaria albescens</i> , <i>Physconia pulverulenta</i> , <i>Physciopsis adglutinata</i> , <i>Arthopyrenia alba</i> , <i>Caloplaca luteoalba</i> , <i>Xanthoria polycarpa</i> , and <i>Lecania cyrtella</i> appear; <i>Physconia grisea</i> , <i>Physcia orbicularis</i> , <i>Opegrapha varia</i> and <i>O. vulgata</i> become abundant	~ 50

TABLE III (continued)

Zone	Noneutrophiated bark	Eutrophiated bark	SO_2 ($\mu\text{g}/\text{m}^3$)
7	<i>Parmelia caperata</i> , <i>P. revoluta</i> (except in NE), <i>P. tiliacea</i> , <i>P. exasperatula</i> (in N) extend up the trunk; <i>Usnea subfloridana</i> , <i>Pertusaria hemisphaerica</i> , <i>Rinodina roboris</i> (in S) and <i>Arthonia impolita</i> (in E) appear	<i>Physcia aipolia</i> , <i>Anaptychia ciliaris</i> , <i>Bacidia rubella</i> , <i>Ramalina fastigiata</i> , <i>Candelaria concolor</i> and <i>Arthopyrenia biformis</i> appear	~ 40
8	<i>Usnea ceratina</i> , <i>Parmelia perlata</i> or <i>P. reticulata</i> (S and W) appear; <i>Rinodina roboris</i> extends up the trunk (in S); <i>Normandina pulchella</i> and <i>U. rubiginea</i> (in S) usually present	<i>Physcia aipolia</i> abundant; <i>Anaptychia ciliaris</i> occurs in fruit; <i>Parmelia perlata</i> , <i>P. reticulata</i> (in S and W), <i>Gyalecta flotowii</i> , <i>Ramalina obtusata</i> , <i>R. pollinaria</i> , and <i>Desmaziera evernioides</i> appear	~ 35
9	<i>Lobaria pulmonaria</i> , <i>L. amplissima</i> , <i>Pachyphiale cornea</i> , <i>Dimerella lutea</i> , or <i>Usnea florida</i> present; if these absent crustose flora well developed with often more than 25 species on larger well lit trees	<i>Ramalina calicaris</i> , <i>R. fraxinea</i> , <i>R. subfarinacea</i> , <i>Physcia leptalea</i> , <i>Caloplaca aurantiaca</i> , and <i>C. cerina</i> appear	> 30
10	<i>L. amplissima</i> , <i>L. scrobiculata</i> , <i>Sticta limbata</i> , <i>Pannaria</i> spp., <i>Usnea articulata</i> , <i>U. filipendula</i> or <i>Teloschistes flavicans</i> present to locally abundant	As 9	"Pure"

^aFrom Hawksworth and Rose, 1970.

for which a five-zone map with IAP values ranging from 1 to 122 was prepared. The zones distinguished correlate well with suspected levels of SO_2 .

By working in areas where the distribution of SO_2 is known, British lichenologists have been able to produce scales from which levels of this pollutant can be estimated. After studying lichen/bryophyte communities on sandstone walls, deciduous trees, and old asbestos roofs, Gilbert (1970b) constructed a six-point scale (for use in lowland Britain) from which mean annual SO_2 levels below ~170 $\mu\text{g}/\text{m}^3$ can be assessed. At about the same time Hawksworth and Rose (1970) published their ten-point epiphyte scale (Table III) which provides separate lists for eutrophiated and noneutrophiated bark. They went on to show its potential value by using it to construct a preliminary air-pollution map of England and Wales, and a highly detailed one of southeast England (Fig. 10).

A number of adjustments will need to be made to these scales as it is still a matter of opinion whether distributional data reflect mean or peak con-

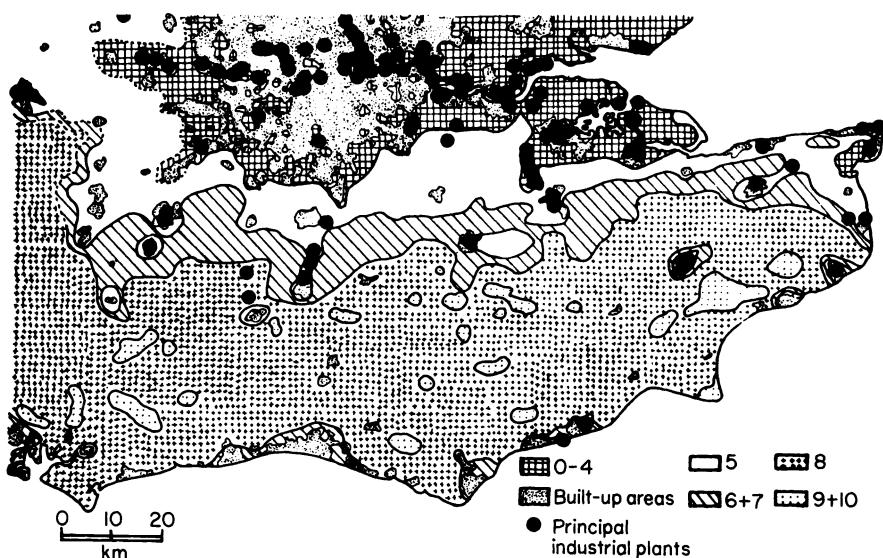


FIG. (10). Preliminary map showing the extent of air pollution in southeast England based on the scale given in Table III. (From Hawksworth and Rose, 1970.)

centrations experienced at a site. Further uncertainty surrounds the possibility of resistant ecotypes (Gilbert, 1971a), the accuracy of the scales outside the area in which they were constructed, and the speed with which lichens respond to changes in air pollution. The question of synergistic effects between pollutants also needs investigating.

Built on the foundation of knowledge which has accumulated since the days of Sernander, Haugsjå, and Vaarna, these scales provide a semiquantitative method of monitoring the spread of air pollution and as such are a positive contribution the study of lichens is making to our industrial society. Eventually, such methods are likely to prove more acceptable than costly and energy-consuming instrumentation.

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Chapter 14

GROWTH

MASON E. HALE

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I. Introduction

Growth of lichens has long held a peculiar fascination for lichenologists. In other groups of cryptogams such as fungi and mosses growth is rapid and obvious, and these organisms often complete their life cycles in a matter of weeks or months. The slow growth of lichens, however, is legendary, and it is safe to say that no one has followed the entire life span of a lichen thallus. Indeed, if we believe some reports of longevity, many lichens that existed when Meyer reported the first serious measurements in 1825 are still alive and growing quite well today!

The purpose of this chapter is to summarize in broad detail the background on growth of lichen thalli, methods of measurement, and factors that seem to influence growth. There are a number of reviews that touch on the subject at least briefly (for example, Schneider, 1897; A. L. Smith, 1921; Tobler, 1925; Beschel, 1958; Barkman 1958; D. C. Smith, 1962; Ahmadjian, 1965, 1966; Hale, 1967), and these may also be consulted to gain perspective on growth studies in relation to other work being done in lichenology.

A. Growth Phenomena and Mechanisms

Growth is first of all an increase in mass that can be determined by weighing plant colonies at various intervals. This method is commonly used in physiological studies of isolated mycobionts but would be difficult to apply to a symbiont thallus because the continuity of colonies being measured would have to be destroyed. Growth is also any increase in surface area or a linear extension of the margins of colonies irrespective of mass. Elongation of thallus part, while not as accurate a measure as mass, does provide a convenient tool for comparative studies and virtually all workers have adopted this method.

Increase in size or weight of a thallus is largely attributable to the fungal component which comprises 90–95% of the plant body. The algae are apparently carried along passively by the growing hyphae and remain (in heteromerous lichens) as a thin scattered layer only 20–40 μm thick. Assimilates synthesized by the algae are utilized by the fungus at the growth site. Evidently lateral transport of nutrients from the central parts to the apical growing region is of very minor importance, and in fact no experiments have proved that such transport occurs.

The growth pattern in lichens is centripetal and apical. Intercalary growth that might contribute to lobe elongation is minimal, ceasing in portions 1 or 2 years old (Hale, 1970). Further growth of the central more or less stationary parts of a thallus is manifested as a thickening of various histological layers, often doubling thallus thickness, and as production of fruiting bodies or vegetative propagules (Porter, 1927).

Each lobe of a foliose species (and presumably each branch of a fruticose species) grows independently of other lobes, even adjacent ones (Hale, 1970). This phenomenon was first observed by Linkola (1918), who was also the first worker to measure individual lobes. The possible magnitude of this variation was shown by Phillips (1969), who measured three lobes on the same specimen of *Lobaria pulmonaria* in Tennessee. Growth averaged 0.4, 10.4, and 15.0 mm over a 3-year period.

B. Factors Affecting Growth

Growth occurs when there is positive net assimilation, a physiological process that responds to proper conditions of moisture, temperature, light, etc. Significant deviations in growth rate in the natural habitat will occur only when one or more of these factors falls far outside of the normal range, as in the temperature contrast between summer and winter in temperate zones. Most observations and correlations have been made from uncontrolled field studies. The difficulties of whole-lichen culture in the laboratory have prevented more accurate assessments of the factors affecting growth.

1. MOISTURE

When a thallus is wetted, as by rain, it attains saturation quickly and begins to metabolize almost at once. It is quite easy to show that growth occurs as a result of this. A colony is photographed before a rain, during a rain, and after a rain when it is again air-dry. Data taken by Hale (1973) for the foliose *Parmelia baltimorensis* in Maryland show increments of up to 0.10 mm without subsequent shrinkage as the result of a single one-day long rainstorm.

Total rainfall over a whole season has significant effect on growth. Nienburg (1919), for example, points to heavy rains in May, 1889 as a reason for greater than average growth observed by Lotsy for lichens being measured in Germany. Similarly, Hausman (1948) felt that the high rates for *Parmelia centrifuga* during one summer in New England could be correlated with the unusually high cloud cover that year.

Kärenlampi (1971), using another more rigid approach to the problem, tabulated growth of various *Cladonia* species during a summer in Finland where total rainfall was 150–300 mm (May to November). He investigated several climatic factors that would be expected to influence growth but concluded that amount of rain per day was highly correlated with the relative growth, more so than temperature or light (Fig. 1).

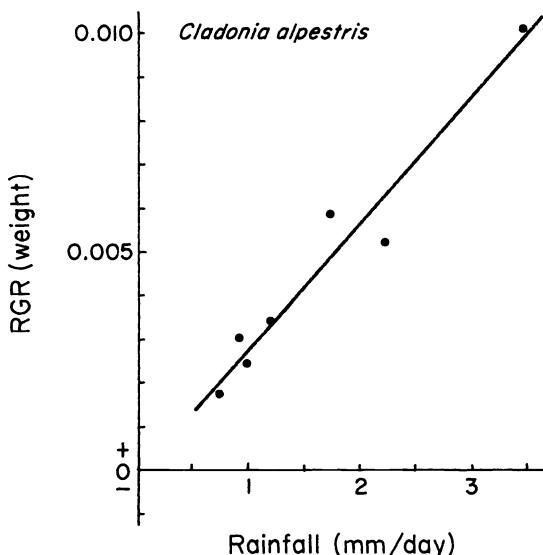


FIG. 1. Relative growth rate (RGR) of young *Cladonia alpestris* specimens in Finland plotted against mean daily rainfall and the linear regression line. (From Kärenlampi, 1971.)

TABLE I
GROWTH OF LICHENS AT VARIOUS HEIGHTS ABOVE SEA LEVEL IN
FINLAND^{a,b}

	Dry zone	2 m above shore	Surf zone
<i>Parmelia centrifuga</i>	1.50	2.08 (2.59)	2.36
<i>P. conspersa</i>	0.83	1.83 (2.48)	3.43
<i>P. saxatilis</i>	1.67	2.08 (2.28)	3.18

^aAverage annual radial lobe growth over 3 years; values in parentheses stand for nutrient-enriched colony growth.

^bAfter Hakulinen (1966).

Further effects of moisture on growth may be observed with lichens growing near lakes or other bodies of water subject to wetting, splashing, or spraying in addition to any moisture received as rain. Hakulinen (1966), in a significant series of observations, examined colonies of four foliose species in three subjectively delimited habitats along a seashore in Finland: normally dry rocks well above water level, shoreline rocks about 2 m above water level, and rocks in the surf zone. His data are summarized in Table I for three of the species. While we cannot tell precisely how dry or how wet the various habitats were, the relative differences in moisture regimen are rather strongly correlated with growth rates, and it would be hard to argue that the added moisture nearer the waterline did not augment growth. Lichens must have the capacity to grow much more than average if they are wetted more than normally.

Moisture available as dew is an important source for many lichens, particularly in desert regions (Lange and Bertsch, 1954). It appears to be less crucial in temperate and boreal regions, for Miller (1966), using an accurate dew balance, showed that while foliose species in southern Canada absorb 30–50% of oven dried weight, they rapidly dry out as the sun comes up. At other times, there may be no dew absorption. Dew formation does retard water loss from rain-wetted thalli, helping to prolong an active metabolic state for a day or more after rains. The same holds true for high humidity (for example, daytime relative humidity of 70–80%), which is encountered in subtropical areas. As a rule, air-dry thalli take up water vapor very slowly and probably reach equilibrium much below the level where assimilation can occur.

2. LIGHT

Light affects growth by limiting the rate of photosynthesis and ultimately the amount of assimilates available to the fungus. Most lichens are a

matter of fact photophils, and any light reduction would probably come about by gradual closing of the forest canopy over many years. I know of no data on growth rates in this kind of situation. Hakulinen (1966) reported an annual rate of 2.6 mm for *Hypogymnia physodes* growing on the north side of trees in Finland, whereas the same species on the south side, exposed to sunlight, grew 2.75 mm. Other workers give conflicting data, and in no case are the differences great enough statistically to allow a categorical statement that increased light promotes growth. Any reduction caused by less light might conceivably be offset by an increase in moisture in a shaded habitat.

3. TEMPERATURE

Effects of temperature have been little studied because of the problems of maintaining appropriate metering equipment in the field. Ideally one should use recording instruments in order to identify maximum and minimum temperatures and duration of the extremes. All data so far indicate that most rapid growth is in the spring in temperate zones when daytime temperature is about 19°C, the most favorable range determined for laboratory culture of the mycobionts.

While lichen colonies obviously grow where the average temperature is tolerable, extreme temperatures unfavorable to growth may still occur. Very low temperatures in winter (0°C or less) without snow cover not only stop growth but can kill thalli by freezing (Laundon, 1966), apparently destroying the algae. Brodo (1965) ascribes poor growth of lichens on Long Island, New York, in 1959–1960 to an exceptionally cold January.

4. NUTRIENT ENRICHMENT

It is axiomatic that lichens thrive in nutrient-deficient habitats, not necessarily because they prefer them but because they face less competition there. This fact is cited as one reason for their slow growth. What if the nutrient supply were artificially increased? Just such an experiment was run by Hakulinen (1966) on four common foliose species on rocks 2 m above the shoreline in Finland. Data for three of these are given in Table I. *Physcia caesia* showed the same trend. A solution of bird excrement (2 gm per 100 ml) was poured over the thalli at weekly intervals during the summer. Growth was consistently greater than in control plants that received only natural rainfall over the 2-year period.

Jones and Platt (1969) independently demonstrated the beneficial effect of nutrient enrichment and incidentally showed that the added moisture in experiments such as Hakulinen's was not entirely responsible for the accelerated growth. They moistened specimens of *Parmelia conspersa* under controlled laboratory conditions twice a month with (1) distilled water or

(2) standard nutrient solution at the rate of 1 ml per 3 cm². The mean monthly rate for lobes receiving only distilled water was 0.20 mm radial growth, while that for nutrient-supplied lobes was 0.28 mm, a statistically significant difference.

C. Seasonal Differences

The combined effects of moisture, light, and temperature lead to seasonal growth in lichens, especially in boreal and temperate zones, less so in the low land tropics. All studies where several measurements are made during the year show best growth in summer and least growth in winter. The amount of growth in winter depends on the severity of the climate. Rydzak (1961) found little or no growth of common species in Poland where winters are rather severe with a long snow cover. Phillips (1963), on the other hand, made some careful measurements over a 2-year period in Tennessee where snowfall is infrequent and average winter temperatures are not much below freezing. For two colonies of *Parmelia conspersa* he found 1.2 and 0.6 mm in the winter (November to April) and 6.75 and 5.6 mm for the same plants in summer. Data taken by Hale (1970) at various times during a whole year show maximum growth of *P. baltimorensis* in early summer but with measurable growth in every month, except when the thalli are actually covered with snow (Fig. 2).

More exact determinations of seasonal growth, as spring versus fall, remain to be done. What is also needed is the kind of sophisticated metering equipment used by Miller (1966) to measure microclimate in the vicinity of the plants. Data taken from broad synoptic weather summaries are not

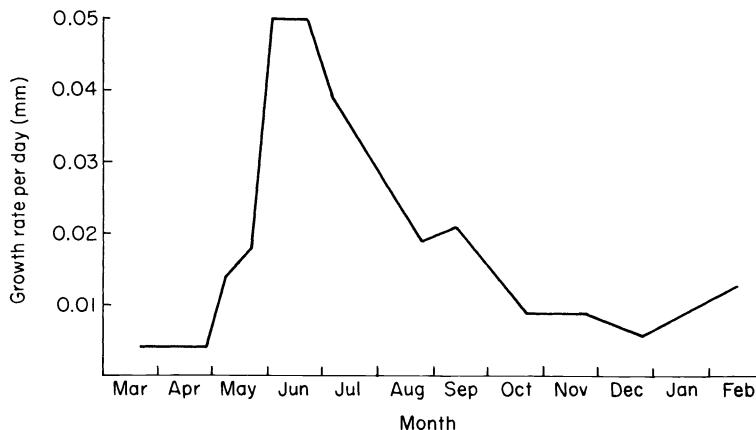


FIG. 2. Average daily growth rate of *Parmelia caperata* near Washington, D. C. for 1 year (14 measurements). (From Hale, 1970.)

sufficiently exact. Finally, we need further refinements in handling data, such as proposed by Kärenlampi (1971), subsystem modeling, and regression techniques to measure effects of climate on growth.

D. Annual Variation

When data are taken for more than 1 year, it is easy to show that year-to-year variation may be quite large. Hakulinen (1966), for example, averaged annual radial increase for four lobes of *Parmelia centrifuga* and found in a consecutive 3-year period (1961–1964) rates of 1.5, 1.3, and 2.3 mm/year. Phillips (1963) averaged data from 25 plants in Tennessee from 1959–1961 and obtained annual increments of 4.7, 4.5, and 5.7 mm/year for *P. conspera*. Brodo (1965) measured rather large differences from two series (1959–1961 and 1961–1962) for four corticolous lichens: *P. caperata* (1.45 mm/year versus 2.05 mm/year), *P. saxatilis* (1.46 mm/year versus 2.08 mm/year), *P. sulcata* (0.91 mm/year versus 1.91 mm/year), and *Lecanora chlarotera* (0.56 mm/year versus 0.98).

II. Techniques of Measuring Growth

Thallus growth has been measured by various techniques, depending largely on what the researcher wants to find out. One can measure thallus diameter, radius, surface area, or individual lobes. Unfortunately, some workers have failed to specify how they took measurements and their data are virtually useless. Even when methodology is clearly stated, data from different studies are not always comparable. Various methods will be discussed below with particular emphasis on general application and limitations.

A. Direct Measurement

Direct measurement means that some growth dimension is measured at two different points in time. Since lichens grow very slowly, this discourages rapid accumulation of data and affords a chance for natural forces to damage a thallus before measurements are completed.

Linkola (1918), one of the earliest workers, employed the simplest technique. He set up a base point, either a nail driven into wood near the thallus or a chisel mark for rock quadrats, and measured from the base point to the lobe tip, expressing his data as radial increase of individual lobes in millimeters per year. This exact technique was followed by Hakulinen (1966). A modification has been proposed by Hale (1970) where a point or mark on the lobe surface becomes a base point, thus reducing the slight error caused by expansion of the mature central parts of the thallus.

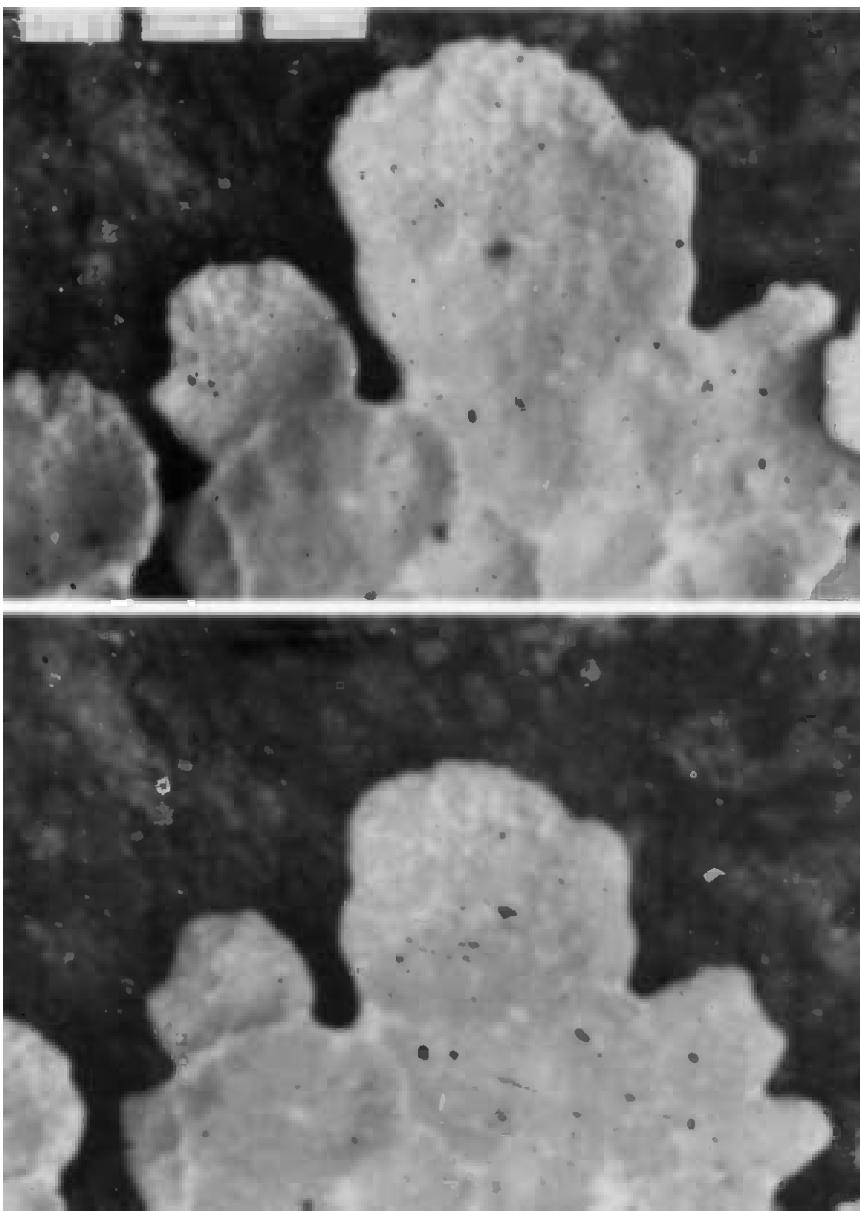


FIG. 3. Comparison photographs of *Parmelia baltimorensis* taken on October 6, 1968 (lower print) and November 24, 1968 near Washington, D.C. (Scale in mm.)

Most workers have used far less precise techniques. A roughly orbicular thallus is selected, the diameter measured, and the same plant remeasured a year or more later. Dividing by two gives average radial growth (cf. Vallot, 1896; Fink, 1917; Frey, 1959; Hale, 1954). This method does not give extremes of growth, and average values have only very general significance.

Another method of direct measurement can be used for certain fruticose lichens that are not attached to the substratum. Kärenlampi (1971), for example, placed loose colonies of various reindeer mosses (*Cladonia*) in boxes on the forest floor. Every 1–4 weeks these were removed from the boxes, air-dried, weighed, and returned to the boxes without damage. A rather similar technique was tried by Miller (1966) for foliose lichens but with less success because of breaking up of the thalli.

Actual recording of data is best done with direct photography. Photographic prints provide permanent records for immediate and future study and with suitable enlargement permit measurements with an error of as little as 0.01 mm (Fig. 3) (Hale, 1970). Hakulinen's (1966) excellent series of photographs shows how useful this technique is for a detailed study of lobe growth.

The plastic method (Fig. 4) enjoyed brief popularity (Hale, 1954; Rydzak, 1956; Brodo, 1964), but when compared with photographs it suffers from several defects. The marking of thallus outlines with a pen or wax pencil is tedious and not especially accurate. Identification of different species on

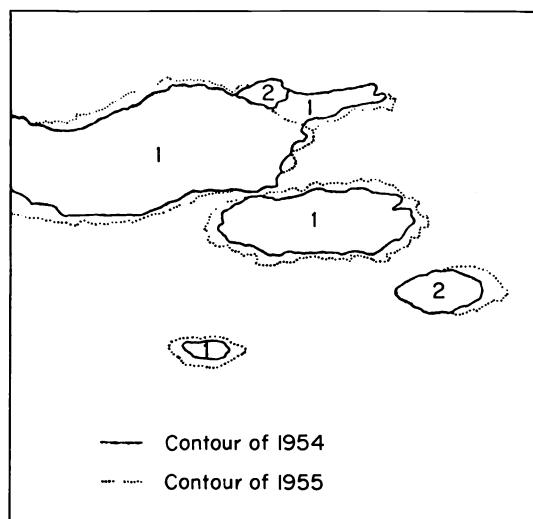


FIG. 4. Outlines of two crustose lichens in Poland. (From Rydzak, 1956.)

the sheets is always open to question. And finally, accurate placement of the plastic sheets over the quadrat creates additional errors.

Rydzak (1961) relied entirely on surface-area increments in his studies of lichen growth in Poland. He accurately measured surface area of individual thalli at different times and expressed growth as spatial increment

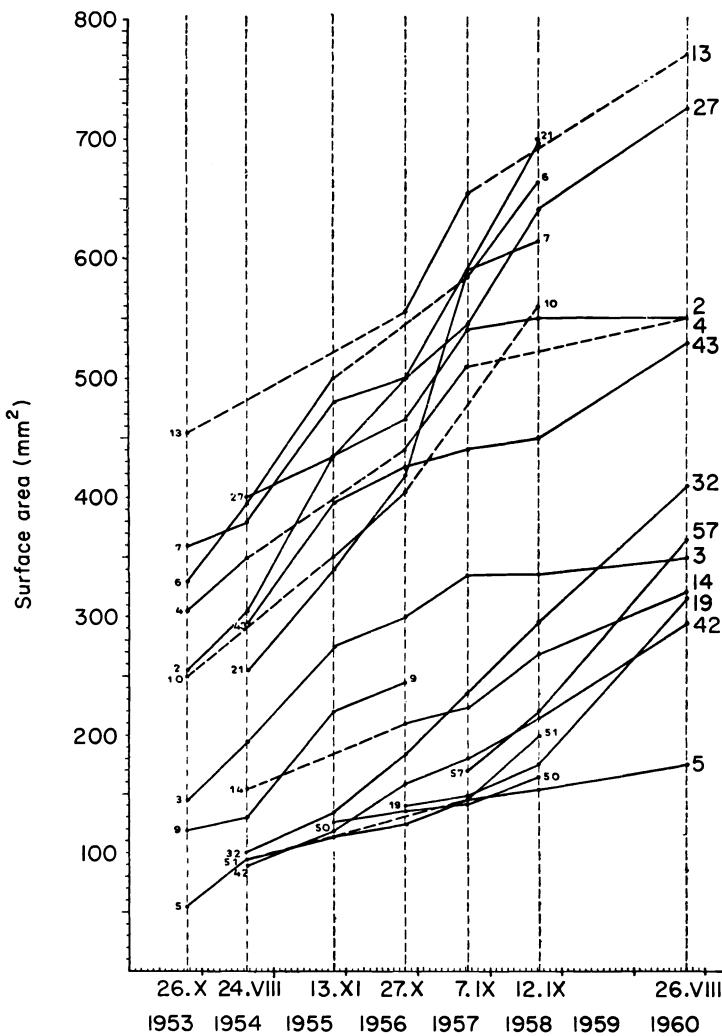


FIG. 5. Growth of various lichens in Poland from 1953–1960 calculated as increase in surface area. (From Rydzak, 1961.)

in square millimeters per time unit (Fig. 5). While this is an interesting way to measure growth, Brodo (1965) objects on the grounds that growth here would largely be a function of the original size of the thalli, that is, small thalli would seem to grow faster than large thalli. Surface area can also be used to determine average radial growth by using the standard formula $r = (A/\pi)^{1/2}$ (Hale, 1959). Woolhouse (1968) proposed a more complex formula in order to equalize differences in thallus size when thalli of different diameter are used; it defines the growth that occurs in a given time interval in relation to the already existing area of the thallus.

Most workers have taken measurements when the thalli are air-dried and often indicate this in publication. The thalli absorb water readily and expand, a typical foliose thallus several centimeters in diameter increasing in size about 1 mm when saturated. Phillips (1962) measured expansion experimentally by sealing specimens of *Parmelia conspersa* in chambers of controlled relative humidity. Significant radial increase occurred only in wetted thalli and this amounted to 0.25–0.70 mm for this species, at most an increase of about 3%. No expansion occurred in nonwetted thalli even with 100% relative humidity.

Direct measurement of single intact lobes cannot usually be carried on indefinitely. Published studies thus far run at most 4–6 years. Barring a loss of patience or interest by the investigator, lobes when followed this long will have branched considerably and often lose their identity. Natural atrophy and destruction by animals or insects (Hale, 1972) are other elements that interfere with continuity of the thalli. Even when photographs have lost usefulness for growth measurements, they can still provide a graphic and instructive record of succession and ecesis of lichens. By far the best example of this is the work by Frey (1959) for numerous lichen quadrats photographed in Switzerland for over 23 years.

B. Indirect Methods

Indirect measurement involves selecting colonies of lichens on substrates of known age (or estimated age), measuring their size or biomass, and extrapolating growth rates by dividing the lichen dimension by the age of the substrate. Thus a lichen thallus 20 mm in diameter growing on a substrate exposed for 10 years must have grown 1 mm/year in radius at the minimum. This method circumvents the long waiting period required to accumulate data through the direct method. There is obviously some loss in precision, since we arrive at a composite picture of growth drawn from many unrelated thalli at different stages of growth and must express the results as relative or minimal rather than absolute growth rates.

1. TERMINAL TWIGS

Many trees in boreal and temperate zones produce terminal bud scars. The internodes between these may be accurately dated and the age of lichens growing on them estimated. The first application of this method would have to be attributed to Nienburg (1919), who chose twigs of *Abies alba* and concentrated on *Hypogymnia physodes* and other conifer lichens. Degelius (1964) did a much more exhaustive study on *Fraxinus* twigs in Europe, which was repeated on a small scale by Hale (1967) for *Fraxinus* twigs in northern Minnesota. By taking into account a 2- or 3-year lag in colonization, one can easily determine growth rates that are very close to those calculated with direct methods.

Platt and Amsler (1955) proposed an interesting variation of this method for lichens growing on twigs of *Juniperus virginiana* and *Ulmus alata* in Georgia. Lichens were cleanly removed from areas on twigs which were dated by ring counts of the woody tissue. Dry weight of the samples plotted against age of the substratum showed a high degree of correlation.

Other datable substrates have been used. Beschel's (1958) thorough study of lichens on dated grave markers in Switzerland must stand as a classic. He followed growth patterns for up to 40 years, very nearly the life span of some of the species studied. One could also examine a series of bridge abutments, road banks, abandoned fields, etc., where the date of exposure is known.

2. LICHENOMETRY

Lichenometry is the converse of the twig method described above. One uses the estimated age of a lichen colony to date the substratum on which it is growing. The pioneer in this technique was Beschel, who refined the method during the 1950's to date moraines of glaciers. Since arctic lichens grow extremely slowly and persist for hundreds of years intact, Beschel (1957, 1961) estimated some colonies to be as much as 4000 years old. Extrapolation curves of thallus diameter are consulted to date the moraines. Several glaciologists (Andrews and Webber, 1964; Burrows, 1971) have employed this method to date moraines back to the 1600's. *Rhizocarpon* species are most commonly used.

Follmann (1961) used the same principle to assign a date to stone images on Easter Island. He had found old photographs that showed lichen colonies (*Dirinaria picta*, *Lecidea paschalis*, etc.) in 1914 and was able to rephotograph the same quadrats in 1961. By extrapolation from thallus diameter he calculated values of 380–850 years depending on the locality. This technique would be more difficult to use in moist temperate zones for dating archeo-

logical artifacts since the life span of lichens there is known to be less than 100 years.

3. LENGTH OF INTERNODES IN *Cladonia*

The podetia of the common reindeer mosses grow upward as a loose mat on soil. The internode between branches is believed to represent 1 year's growth so that the age of a podetium is estimated by dividing the number of joints into the total height (Scotter, 1963; Kärenlampi, 1970, with references to the original Russian studies). The average annual growth rates determined by this method are comparable to those obtained by direct methods (Tengwall, 1928).

4. MARGINAL ZONATION

Certain species of *Pertusaria*, *Lecanora*, *Ochrolechia*, etc., have concentrically zoned margins. The zones arise because rapid summer growth is whitish and the narrow dormant zone in winter is darker. Each zone is therefore 1 year's growth and the width is an accurate measure of radial growth

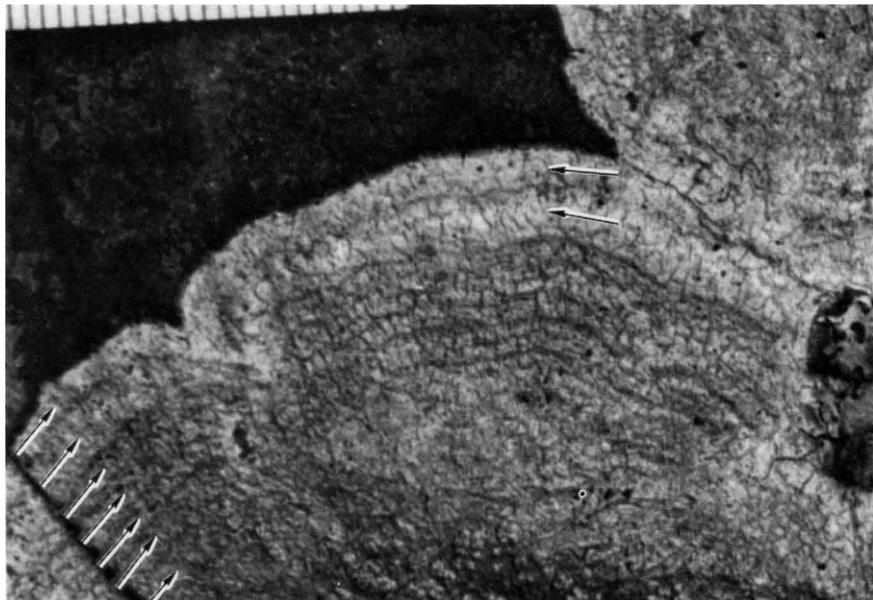


FIG. 6. Marginal zonation in *Pertusaria* sp. in Virginia. (Scale in mm; arrows point to center of annual zones.)

which may be traced back 3–7 years (Fig. 6). Unfortunately, the vast majority of lichens do not manifest any annual zonation.

III. Growth Rates and Life History

A. Growth Rates

Anyone who has searched the literature will find no lack of data on how fast lichens grow. Many authors have taken measurements in the past 50 years, and the aggregate data give us a fairly good idea of the amount of growth that can be expected for various kinds of lichens in different regions. Some representative values of annual radial increment are listed below.

Foliose species

- Cetraria pinastri*: 1.15 mm (Hakulinen, 1966)
Hypogymnia encausta: 1.00 mm (Frey, 1959)
Lobaria pulmonaria: 4.82 mm (Phillips, 1969)
Lobaria quercizans: 5.62 mm (Phillips, 1969)
Menegazzia terebrata: 2.54 mm (Phillips, 1969)
Parmelia centrifuga: 2.50 mm (Linkola, 1918), 0.85 mm (Hausman, 1948)
Parmelia conspersa: 1.60 mm (Hale, 1959), 5.30 mm (Phillips, 1963)
Parmelia pulla: 1.0–1.2 mm (Størmer, 1934)
Parmelia sulcata: 1.60 mm (Linkola, 1918), 2.22 mm (Degelius, 1964)
Parmeliopsis ambigua: 0.70 mm (Linkola, 1918), 0.90 (Hakulinen, 1966)
Physcia aipolia: 1.30 mm (Hakulinen, 1966)
Umbilicaria deusta: 2.30 mm (Hakulinen, 1966)
Xanthoria parietina: 2.15 mm (Hakulinen, 1966), 2.50 mm (Degelius, 1964)

Fruticose species

- Cladonia rangiferina*: 2.7–6.0 mm (Scotter, 1963)
Evernia prunastri: 2.00 mm (Degelius, 1964)
Ramalina reticulata: about 30 mm (Herre, 1904)

Crustose species

- Diploschistes scruposus*: 0.44 mm (Hale, 1959)

Foliicolous species

- Strigula*: 1.5–1.8 mm (de Wilde-Deyfjes, 1967)
Lecanora alphoplaca: 0.95–1.40 mm (Frey, 1959)
Lecanora muralis: 1.30 (Hakulinen, 1966)
Pertusaria shenandoahensis: 1.6–3.6 mm (Hale, 1973)
Rinodina oreina: 0.57 mm (Hale, 1959)
-

All of the values above are expressed as millimeters per year since most workers have taken measurements at intervals of 1 or more years. For

smaller intervals of weeks or months a better way of expressing growth is probably millimeters per day (Hale, 1970) (see Fig. 2).

The maximum amount of radial increment of which a thallus is capable will depend partly on the size of the species measured. Small lichens, those less than 20–30 mm in diameter at maturity, do not grow as much as larger thalli. Hakulinen (1966), for example, measured a growth rate of 0.98 mm/year for a colony of *Physcia caesia* (20.7 mm in diameter), whereas a larger lichen, *Parmelia centrifuga* (60.8 mm in diameter), grew 1.50 mm/year at the same time. In this connection (and as will be discussed more fully below), we should remember that growth rate may vary over the life span of a lichen, being as a rule significantly slower in early juvenile and senile stages and most rapid in the middle (maturing) stages. This could mean that the rate determined with a large specimen might actually be lower than for a smaller one of the same species.

B. Life History

The normal life cycle of a plant includes a juvenile stage, a period of rapid growth to maturity, and a final stage of senescence, following along a sigmoid curve ideally. Lichens are no exceptions, as so explicitly stated by Vallot (1896) over 75 years ago. We have, however, virtually no direct measurements of life cycles, only guesses and extrapolations from indirect or very short-term studies. Beschel (1958) has covered the subject rather thoroughly and defines several stages in the life cycle of lichens as deduced from his grave-marker investigation.

1. JUVENILE PERIOD

This is a stage of variable duration following establishment of a propagule (or symbiotic recombination of alga and fungus) on a substratum. No lichenologist has defined it accurately in terms of thallus size. Brodo (1965) considered as juvenile thalli those less than 4.1 cm in diameter, but he was unable to show that even thalli 2.8 cm in diameter grew at a slower rate. Miller (1966) used seven size classes beginning with plants 0.0–4.9 cm and was still not able to show convincingly that small plants grew at a different rate from "mature" plants. Phillips (1963) examined very small thalli of *Parmelia conspersa* (0.6–0.7 cm) in Tennessee and found them to be growing at 1.7–2.7 mm/year. Another series of thalli 1.4–14.3 cm in diameter in the same area grew much more, 4.5–8.2 mm/year. Clearly the juvenile stage would cover plants no more than 1 cm in diameter and probably much less than this for small foliose species.

Indirect measurements compiled by Nienburg (1919) and Beschel (1958) show fairly conclusively that the first 2–3 year period after establishment,

when the propagule is only 0.1–0.5 mm across, contributes little to thallus diameter. Twig studies suggest that a juvenile stage might last 3–4 years at the most, but considerably more work needs to be done in this area.

2. THE "GREAT" PERIOD

It was Beschel (1958) who coined this term for the period of maturation. His data show that it may be a relatively short time in the life span of a lichen, lasting from 2–10 years directly after the juvenile stage. He suggested that this is a critical stage when a lichen must occupy its habitat as quickly as possible and would have the highest relative growth rate. Once again, we have no direct measurements of this stage in the lichen life history, only indirect ones.

3. MATURITY AND SENESCENCE

Some lichens lack a "great" period and simply have a linear growth rate until maturity is reached (Beschel, 1958) (Fig. 7). In any event a point is soon reached in the life history of many species where growth slows down signif-

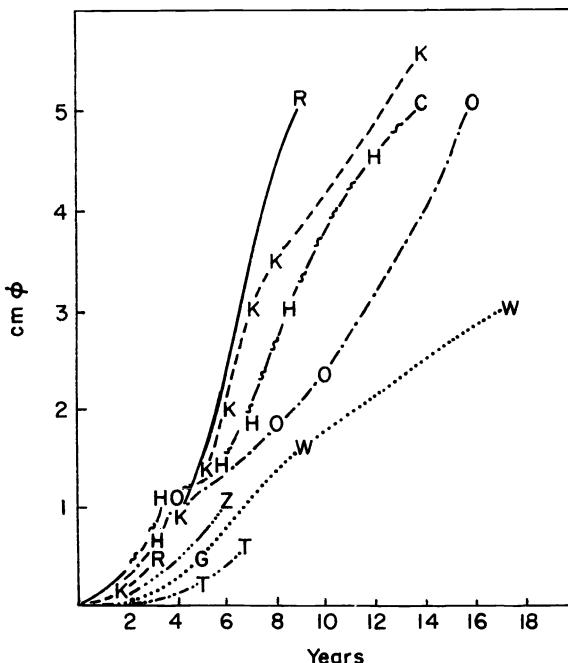


FIG. 7. Growth curves of 7 colonies of *Hypogymnia physodes* in Switzerland calculated from dated grave markers over 16 years. (From Beschel, 1958.)

icantly and remains so for an undetermined number of years. These would be considered "mature" lichen thalli, the kind that is most often collected for herbarium specimens. The decrease in growth rate has been well documented by Frey (1959) for a colony of *Placodium alphoplacum* in Switzerland. By taking measurements of diameter from photographs, he calculated a rate of 1.4 mm/year radial growth for one colony from 1923–1927, but the same colony grew only 0.07 mm/year from 1927–1934. Another set of measurements for the same species gave 0.95 mm/year from 1923–1927 and 0.20 mm/year from 1927–1934.

Indirect measurements give a very distinctive flattening of the growth curve for a number of species (Beschel, 1958), reflecting a slower rate of growth (sometimes even a cessation) when the thallus approaches maximum size. Vallot (1896) was probably the first worker to hypothesize that lichens have a certain maximum size, beyond which the plant does not enlarge but instead begins to disintegrate, first at the center and finally toward the periphery. Some marginal areas may survive and go on to initiate new colonies but the integrity of the mother plant is lost. Vallot estimated that the maximum size for *Parmelia saxatilis* is 20–24 cm. Beschel (1958) estimated 6 cm for *P. exasperatula* and 9 cm for *P. sulcata* on the basis of grave-marker studies. The maximum size for *Parmelia conspersa* appears to be about 12 cm and for *P. caperata* about 20 cm (Hale, 1973).

4. LONGEVITY

The age of a lichen growing on an undated substrate cannot be determined, and even indirect methods tell us merely that a particular thallus cannot be older than a dated substratum. There is still a great temptation to extrapolate the age of a thallus from the diameter and an approximation of the rate of growth. This can be a foolhardy operation since rate of growth varies throughout the life history of a thallus and a senescent period with little or no growth can last an indeterminate length of time. Linkola (1918) nevertheless calculated the age of "larger individuals" of the following species on the basis of 6-year growth averages: *Parmelia centriguga*, 50–80 years; *P. olivacea*, 50–60 years; *P. physodes*, 30–40 years; *P. sulcata*, 30–40 years; *Parmeliopsis aleurites*, 20–25 years; *P. ambigua*, 15–20 years.

On a similar basis Hausman (1948) estimated a colony of *Rhizocarpon geographicum* 86 mm in diameter to be 120 years old, and Tengwall (1928), studying podetia of several Cladonias (reindeer mosses), guessed that mature plants at a maximum of 60 mm high with a rate of 1–5 mm/year would be 15–45 years old. Those working in lichenometry of arctic lichens, of course, claim longevity of hundreds or even thousands of years.

IV. Conclusions

Past work in lichen growth has shown that most species in temperate areas grow 0.5–8 mm a year and that both seasonal and annual variation is large. Availability of moisture and temperature have the greatest effect on growth. Little is known about life cycles except that a brief juvenile period following establishment of a propagule precedes a longer period of rapid growth leading to a mature thallus. Senescence is reached as the thallus approaches a maximum diameter or length that is species specific. Growth rate slows and the central or older portions of the thallus finally die away. Details of the life cycle are still known only from indirect measurements. More definitive answers to the many questions about lichen growth will have to come from well-planned long-term studies.

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Part IV

SECONDARY METABOLIC PRODUCTS

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Chapter 15

NATURE OF LICHEN SUBSTANCES

S. HUNECK

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I. Introduction

Lichen substances are organic compounds synthesized by lichens. They have long attracted the attention of organic chemists, and their chemistry, structure, properties, and distribution have been treated in detail recently in major works by Asahina and Shibata (1954) and Culberson (1969, 1970). This field is also reviewed by Huneck (1968, 1971). Further details on the biosynthesis of lichen substances may be found in Chapter 16 by Mosbach.

Although their chemical nature is extremely varied, lichen substances can be classified on the basis of their biogenesis and brought together into larger groups. Amino acids, amines, peptides and proteins, polyols, and mono-, oligo-, and polysaccharides are examples of the products of the primary metabolites. By far the majority of typical lichen substances belong to the group of acetogenines, i.e., the aliphatic, cycloaliphatic, and aromatic derivatives as well as terpenoids such as mevalonate derivatives, and lastly are the polyporic acid, thelephoric, and pulvinic acid derivatives derived from phenylalanine. The approximately 220 lichen substances of known structure will be discussed in this order. Structural determinations of this group of natural products are accomplished today primarily with physical methods: infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Optical rotatory dispersion (ORD) as well as circular dichroism (CD) provide valuable information. Concentra-

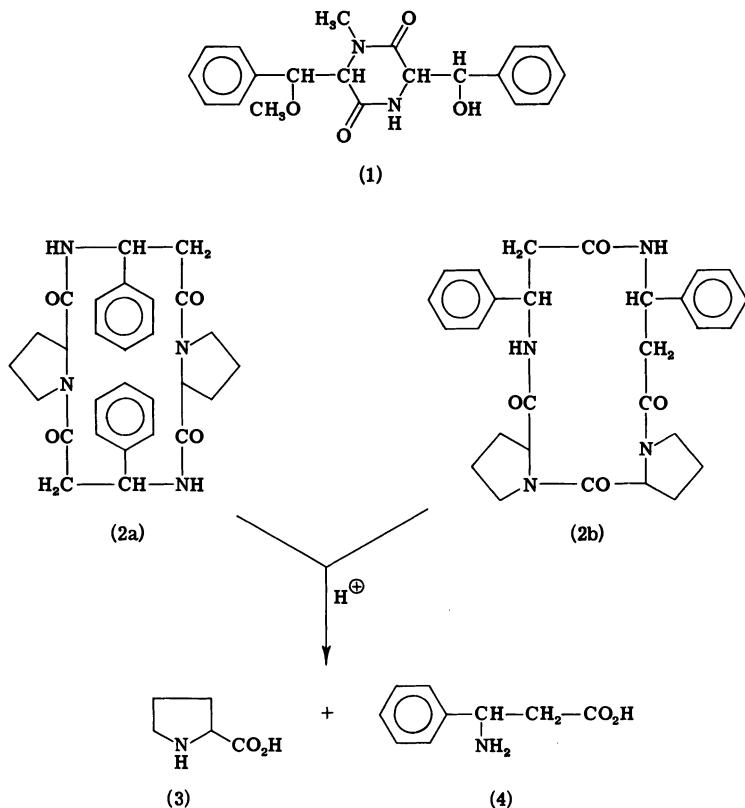
tion of lichen substances varies within relatively wide limits and can moreover reach high values such as in *Pentagenella fragillima* Darb., which has 30% of its weight in psoromic acid. Methods of identifying lichen substances are discussed by Santesson in Appendix B of this book.

II. Structure of Lichen Substances

A. Products of Primary Metabolism

1. AMINO ACIDS, AMINES, PEPTIDES AND PROTEINS

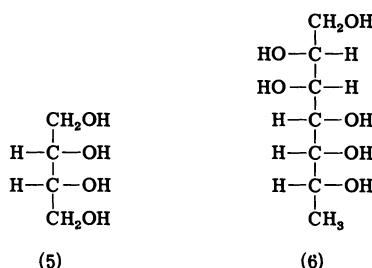
The lichens contain important amino acids similar to those in higher plants. Picroroccellin (1) and the cyclic tetrapeptide (2a or 2b), which yields L-prolin (3) and D- β -amino- β -phenylpropionic acid (4) on hydrolysis, have been isolated from *Roccella fuciformis* (L) Dc., *R. canariensis* Darb., and *R. vicentina* Vain.



Protein content of lichens varies between 1.6 and 11.4% of the dry weight but can go as high as 20%. Methyl- and trimethylamine, amines that have been detected in various species of the Stictaceae, are apparently degradation products of amino acids.

2. POLYOLS AND MONO-, OLIGO-, AND POLYSACCHARIDES

Polyols and carbohydrates are derived by photosynthetic activity of the phycobiont, and these can be converted into related compounds by the mycobiont. Polyols occurring in lichens include glycerin, ribitol, D-arabitol, meso-erythritol (5) isolated from many species in the Roccellaceae, D-mannitol, myo-inositol, D-volemitol, and D-siphulitol (6). All of these are water soluble.

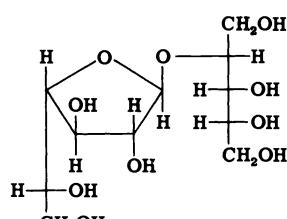
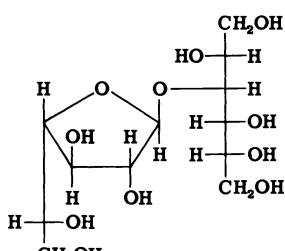
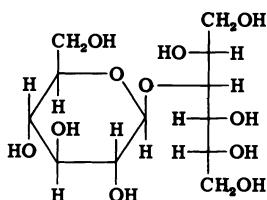


Lichens may contain the following mono- and disaccharides that also occur widely in higher plants: D-glucose, D-fructose, D-galactose, D-xylose, D-tagatose, arabinose, saccharose, and trehalose. The following sugar alcohol glycosides, however, are restricted to lichens: 4-O- β -D-glucopyranosyl-D-mannitol (7), peltigeroside (8), and umbilicin (9).

Lichenin is poly- β -D-glucopyranose with β -(1→3)- and β -(1→4)-glycosidic linkages in the ratio 27:73. Isolichenin, which gives a positive (blue) iodine test, is a linear polyglucoside with α -(1→3) and α -(1→4) linkages in the ratio 45:55. Pustulan, known from *Lasallia pustulata* (L.) Mér., is a linear glucan with β -(1→6) linkages. *Umbilicaria esculenta* Miyoshi and *Lasallia papulosa* (Ach.) Llano both contain a poly- β -(1→6)-glucan O-acetylated to about 2% and with a molecular weight of 20,000. This particular compound has an anticancer effect on sarcoma-180 in mice. Finally, a linear α -(1→3)- and α -(1→4)-glucan has been found in *Parmelia caperata* (L.) Ach.

3. SULFUR-CONTAINING COMPOUNDS

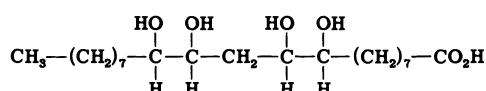
The only sulfur-containing compounds known from lichens are dimethylsulfone, the internal salt of choline sulfuric acid, and thioethanol amine.



B. Acetogenines

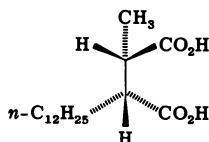
1. ACETATE AND MALONATE DERIVATIVES

a. MONO-, DI-, AND TRIBASIC ALIPHATIC ACIDS. Oleic and oleinic acids have so far been reported only in *Alectoria ochroleuca* (Hoffm.) Mass. In contrast, 9,10,12,13-tetrahydroxyheneicosanoic (10) and 9,10,12,13-tetrahydroxydocosanoic acid appear to have wide occurrence in lichens.



(10)

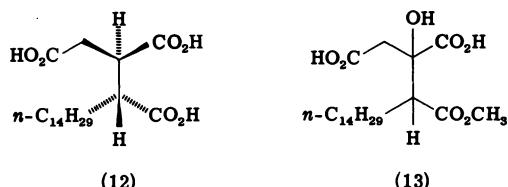
The dibasic fatty acid (+)-roccellic acid is (+)-2S-methyl-3R-dodecylsuccinic acid (11) and can be synthesized as a racemate through anodic coupling of lauric acid with erythro-3,4-dimethoxycarbonylpentanoic acid.



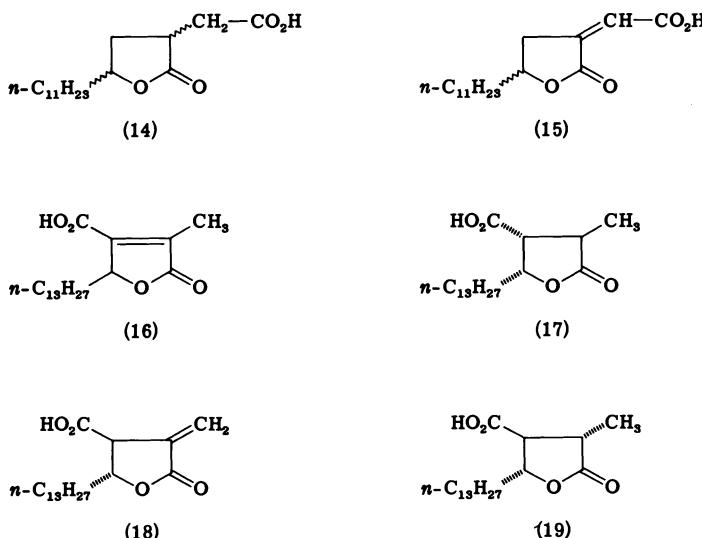
(11)

Norrangiformic acid (12) can be approached through total synthesis from threo-3, 4-dimethoxycarbonyl-6-oxoheptanoic acid by means of electrolytic coupling with myristic acid, following hydrolysis and oxydation with hypobromite.

Rangiformic acid is the monomethylester of (12). Caperatic acid (13) is derived from rangiformic acid by hydroxylation.

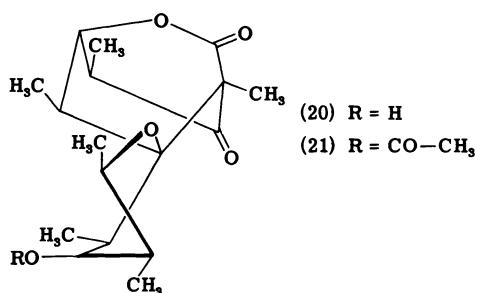


b. γ -LACTONIC ACID DERIVATIVES. Acaranoic acid (14), acarenoic acid (15), lichesterinic acid (16), nephromopsic acid (17), nephrosteranic acid, nephrosterinic acid, (+)-protolichesterinic acid (18), alloprotolichesterinic acid, and (+)-roccellaric acid (19) are long chain γ -lactonic acids.



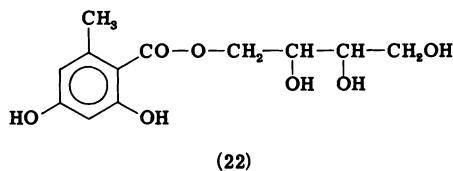
The stereochemistry of these acids has been elucidated in part by past workers. The absolute configuration of some of these lactones has been determined from ORD and CD data. Compound (16), for example, has a negative Cotton-effect at 260 nm. Protolichesterinic acid with an UV maximum at 217 nm ($\log_{10} \epsilon = 5.5$) can be isomerized easily to lichesterinic acid by heating with acetic anhydride.

c. CYCLOALIPHATIC COMPOUNDS. The only compounds known in this group are the bitter-tasting lactones, portentol (20) and acetylportentol (21), both described from various *Roccella* species. Their structure can be verified by chemical degradation to acetylmesitol methylether or by X-ray analysis of the *p*-bromobenzoate of acetyldihydroportentol.

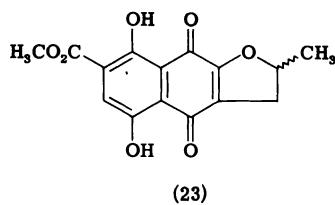


2. AROMATIC COMPOUNDS

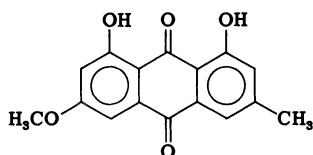
a. ORCINOL DERIVATIVES. Although many aromatic lichen substances, especially the depsides and depsidones, are derived from phenolic carboxylic acids, up to this time only orcinol, ethylorsellinate, methyl β -orcinolcarboxylate, and (+)-montagnetol (22) have been found.



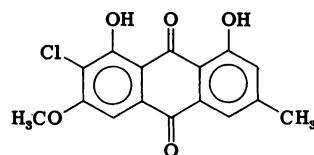
b. NAPHTHOQUINONE DERIVATIVES. The pigment in the apothecia of *Haematomma ventosum* (L.) Mass., identified as haemoventosin (23), is a dihydrofuranonaphthoquinone.



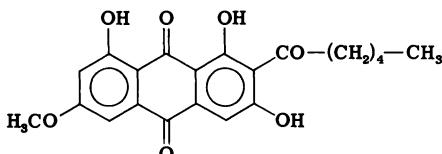
c. ANTHRAQUINONES AND ANTHRONES. A number of lichens, in particular in the genera *Xanthoria* and *Caloplaca*, are pigmented orange or red by hydroxyanthraquinones which are distinguished by a violet coloration with potassium hydroxide. We will discuss here parietin (24), fragilin (25), solorinic acid (26), and everythrin-6-monomethylether (27), four of the approximately 22 known lichen anthraquinones. Parietin, for example, absorbs in UV at 220 ($\log_{10} \epsilon = 4.31$), 254 ($\log_{10} \epsilon = 4.03$), 264 ($\log_{10} \epsilon = 4.05$), 286 ($\log_{10} \epsilon = 4.03$), 374 ($\log_{10} \epsilon = 3.44$), and 430 nm ($\log_{10} \epsilon = 3.86$).



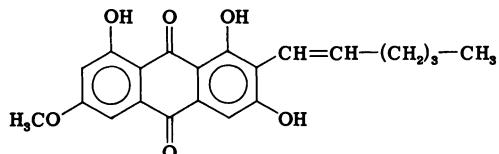
(24)



(25)



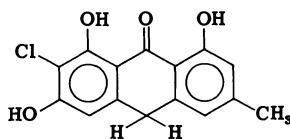
(26)



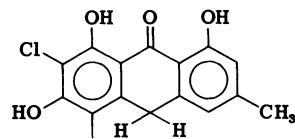
(27)

Especially noteworthy is the joint occurrence of anthrones (28) and (29) in *Anaptychia obscurata* (Nyl.) Vainio. Perhaps they are precursory biogenetic stages of the corresponding anthraquinones.

d. CHROMONES. Few of the colorless or pale yellow chromones are presently known in lichens. These include siphulin (30) from *Siphulaceratites* (Fr.) Th. Fr., 8-chloro-5,7-dihydroxy-2,6-dimethyl chromone (31) from *Lecanora rupicola* (L.) Zahlbr. and *L. carpinea* (L.) Ach., lepraric acid (32) from *Lepraria latebrarum* Ach. and a few Roccellaceae, as well as 6-ethoxymethyleneugenitin (33). Chromones are easily recognized from

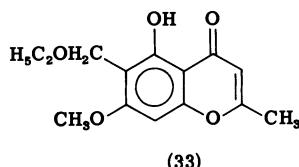
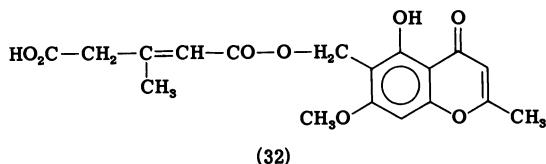
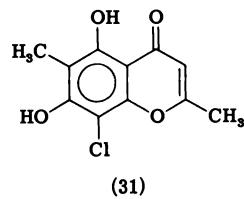
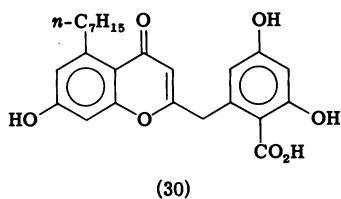


(28)

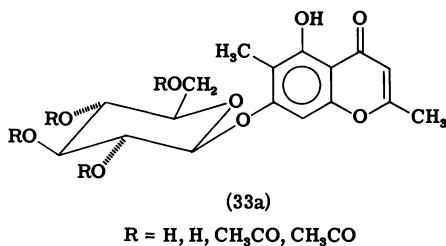


(29)

their characteristic UV spectra; thus, compound 31 has the following: $\lambda_{\text{max}}(\log_{10}\epsilon)$ 225 (4.5), 260 (4.5), 295 (4.1), and 330 nm (3.8).

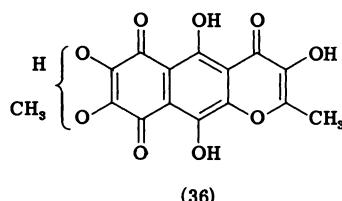
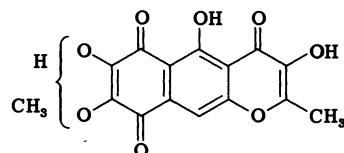
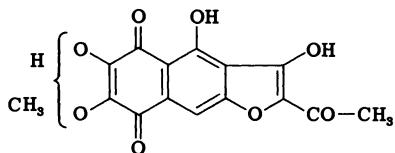


Roccellin (33a), mollin, and galapagin are the first phenolic glycosides known from lichens; they have been isolated from *Roccellaria mollis* (Hampe) Zahlbr., *Schismatomma accedens* (Nyl.) Zahlbr., and *Roccella galapagoensis* Follm.

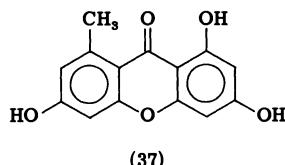


The furane structure (34) was originally proposed for rhodocladonic acid, the red apothecial pigment in various *Cladonia* species. More recently, however, the chromone structure (35) has been considered. Chiodectonic acid,

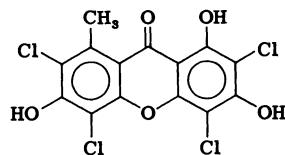
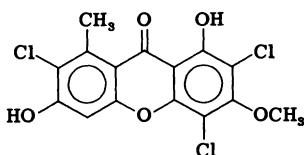
which is present in the tropical corticolous lichen *Chiodection sanguineum* (Sw.) Vain., apparently has the structure (36).



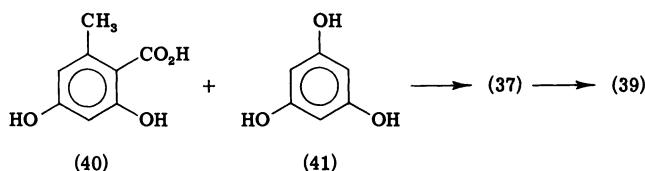
e. XANTHONES. All lichen xanthones are derived from norlichexanthone (37), which has been found in *Lecanora straminea* (Wahlbg.) Ach. and *L. reuteri* Schaefer. A number of chlorine-containing xanthones occur in the crustose lichen genera *Pertusaria*, *Lecanora*, *Buellia*, and *Leclidea*.



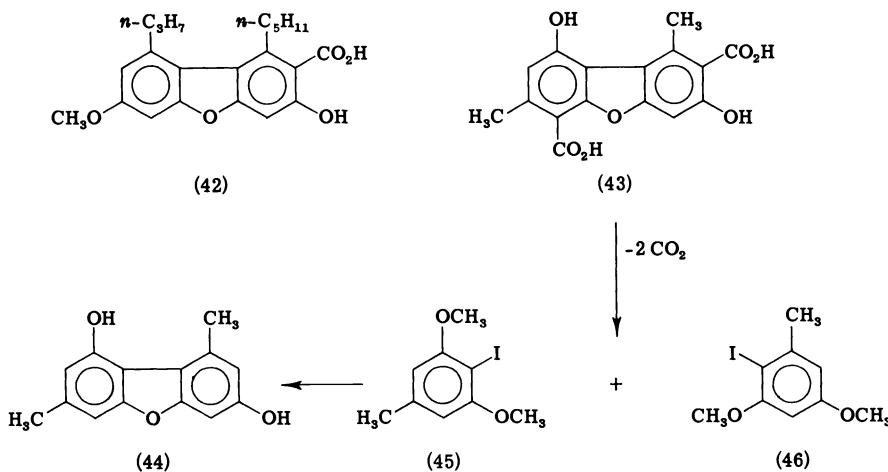
Examples are thuringion (38) and thiophanic acid (39), which absorbs in UV at 248 ($\log_{10} \epsilon = 4.65$), 320 ($\log_{10} \epsilon = 4.12$), and 360 nm ($\log_{10} \epsilon = 4.22$).



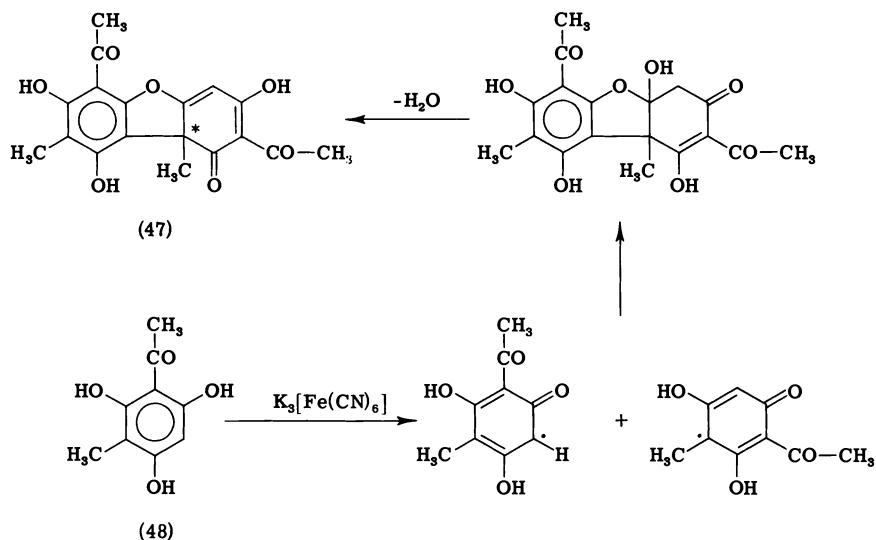
Condensation of orsellinic acid (40) with phloroglucinol (41) leads to norlichexanthone which can be chlorinated to thiophanic acid (39) with chlorine in acetic acid.



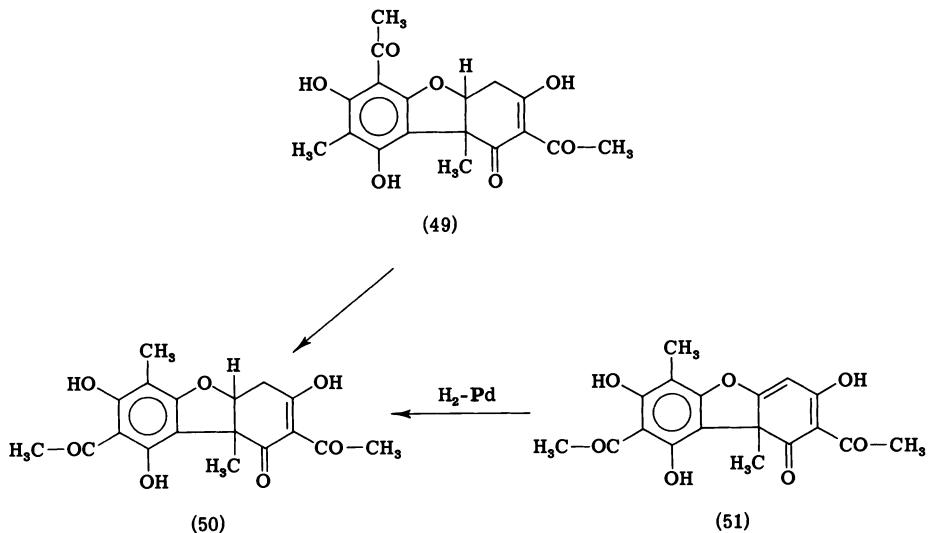
f. DIBENZOFURANES. Five dibenzofuranes are known from lichens: didymic acid (42), pannaric acid (43), porphyrilic acid, schizopeltic acid, and strepsilin. They also have characteristic UV spectra, for example, schizopeltic acid with a maximum at 241 ($\log_{10} \epsilon = 4.49$), 274 ($\log_{10} \epsilon = 4.17$), 290 ($\log_{10} \epsilon = 4.13$), 301 ($\log_{10} \epsilon = 4.00$), and 314 nm ($\log_{10} \epsilon = 4.02$). The structure of pannaric acid follows from decarboxylation to pannarol (44) which is available synthetically from 3,5-dimethoxy-4-iodotoluol (45), and 3,5-dimethoxy-2-iodotoluol (46).



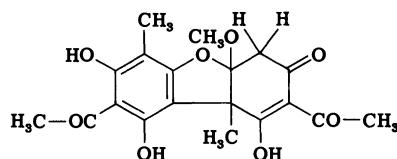
g. USNIC ACID, ISOUSNIC ACID, AND PLACODIOLIC ACID. Usnic acid (47) [UV: λ_{max} ($\log_{10} \epsilon$) 232 (4.41) and 282 nm (4.31)], one of the most widespread lichen acids, is optically active (chiralic center indicated with an asterisk) and occurs in both genus-specific (+) and (-) forms. The racemate can be synthesized in a biogenetic type pathway by oxidation of methylphloracetophenone (48) with potassium ferricyanide and separated into the optical antipodes with brucine.



(+)-Usnic acid may be hydrogenated to (-)-dihydrousnic acid (49) with hydrogen in the presence of palladium; 49 is isomerized to isodihydrousnic acid (50) by heating in hydrogen. The structure of isousnic acid (51), isolated so far only from a few species of *Cladonia*, *Lecanora*, and *Sphaerophorus*, follows from catalytic hydrogenation with palladium in tetrahydrofuran to compound (50).



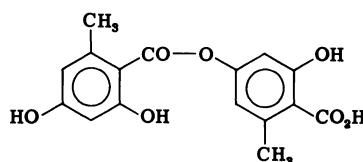
The structure of (–)-placodiolic acid from *Lecanora rubina* (Vill.) Ach. has been established as (–)-isousnic acid isomethoxide (51a).



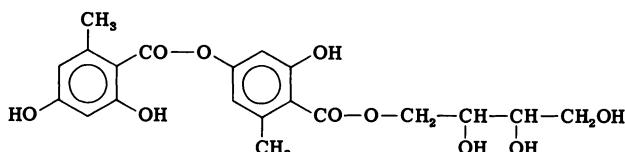
(51a)

h. DEPSIDES. Lichen depsides may be categorized as shown in Fig. 1. Of the 43 structurally determined depsides, 37 are didepsides and five are tridepsides; only one tetradepside has been discovered. Depsides have typical UV spectra with two bands, such as lecanoric acid with λ_{max} ($\log_{10} \epsilon$) at 270 (4.3) and 307 nm (4.2).

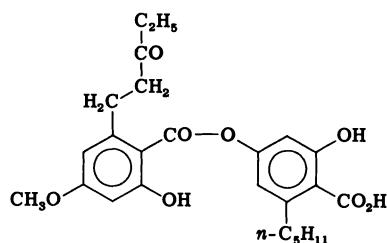
p-Depsides of the orcinol series include among others lecanoric acid (52), erythrin (53), especially widely distributed in the *Roccellaceae*, and miriquidic



(52)



(53)



(54)

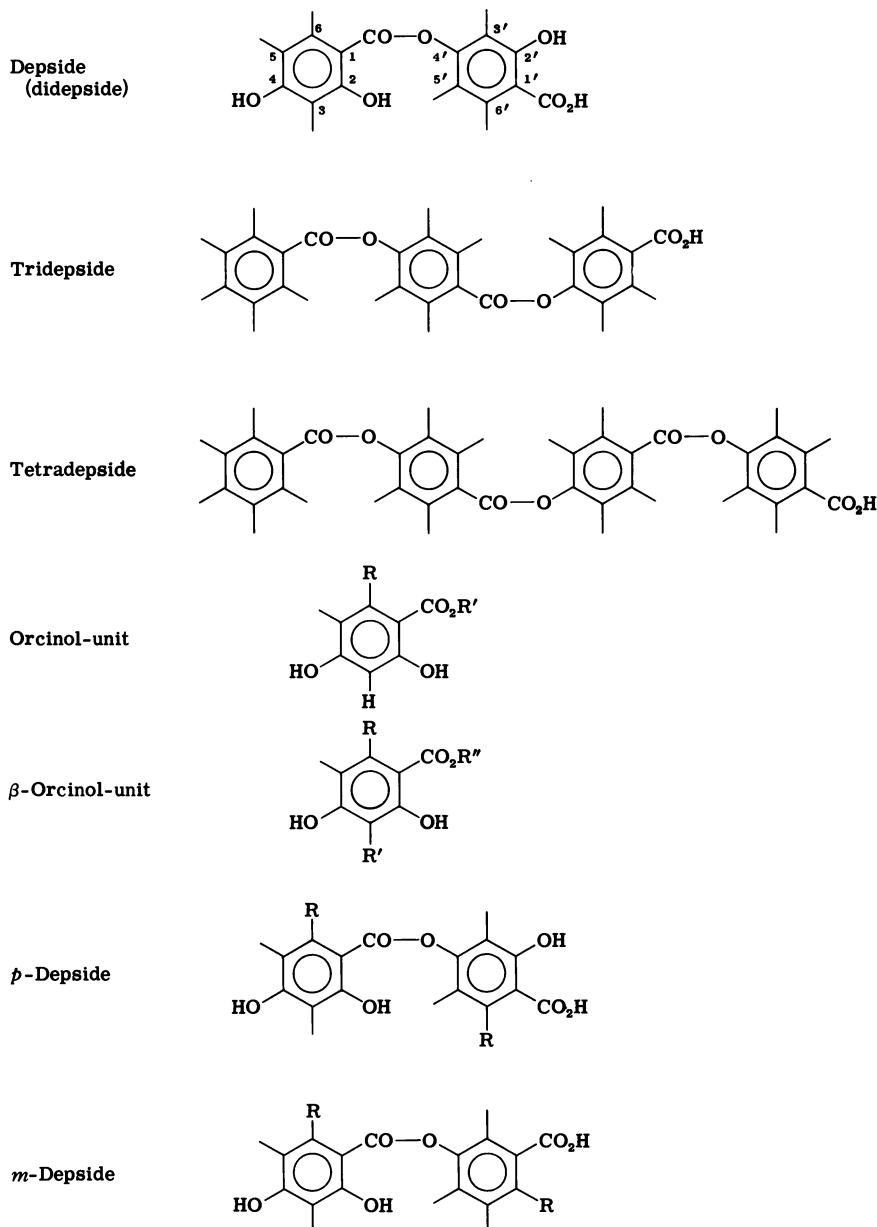
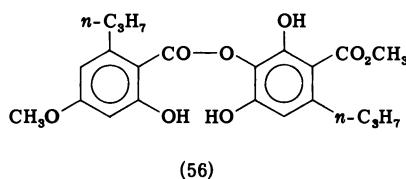
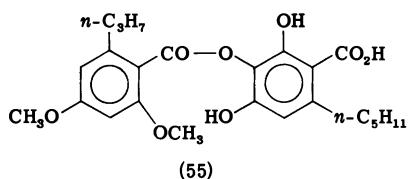


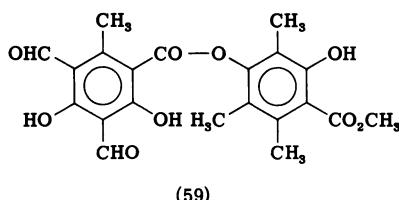
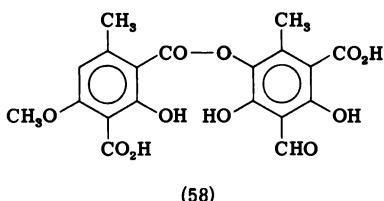
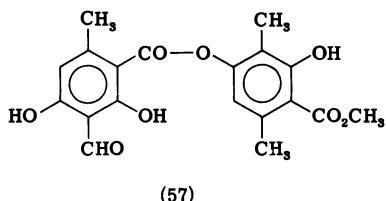
FIG. 1. Basic structural formulae of the main classes of lichen depsides.

acid (54), which deviates from the acetate rule with reference to the position of the carbonyl group in the side chain.

Merochlorophaeic acid (55) and scrobiculin (56) are examples of *m*-depsides of the orcinol series.

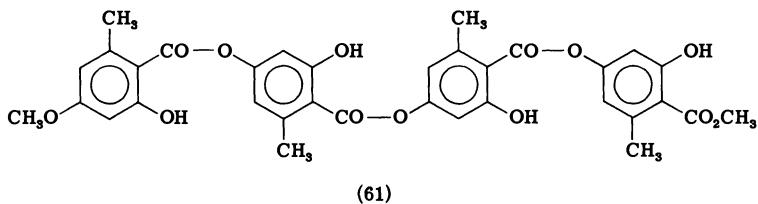
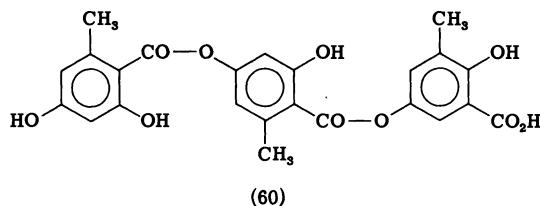


The most frequently encountered *p*-depside in the β -orcinol series is atranorin (57) and the most widely occurring *m*-depside, thamnolic acid (58). Phenarctin (59) is characterized by complete substitution of the depside skeleton.

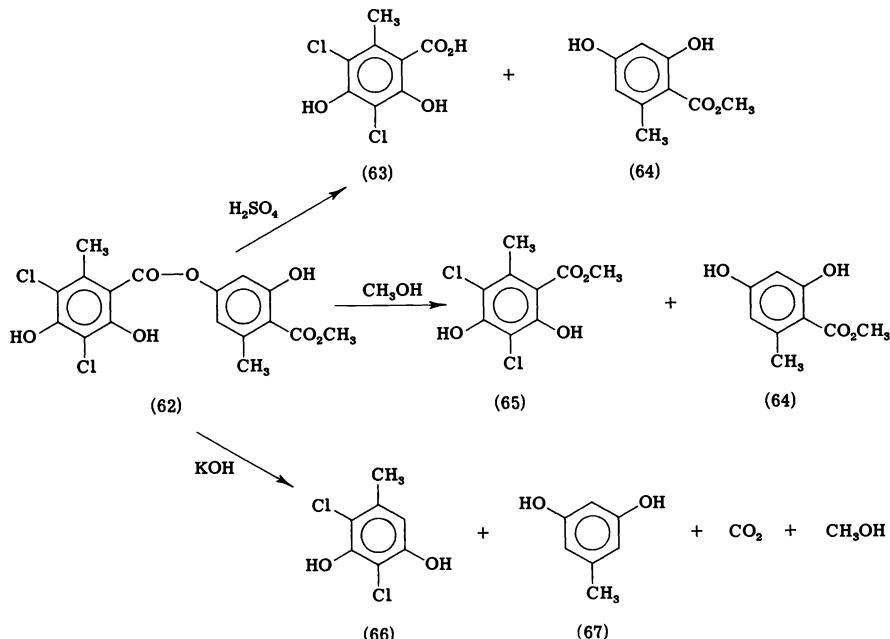


The tridepside gyrophoric acid (60) is the chief component in the Umbiliariaceae, while a tetradepside, aphthosin (61), has been isolated from *Peltigera aphthosa* (L.) Willd.

The ester linkage in depsides can be hydrolyzed with concentrated H_2SO_4 at $0^\circ C$ or with aqueous or methanolic KOH. The corresponding phenolic carboxylic acids, their methylesters, or their decarboxylation products are obtained. Hydrolysis is especially easy with depsides having a β -positioned carbonyl group in the 6 and 6' side chains. One therefore obtains from

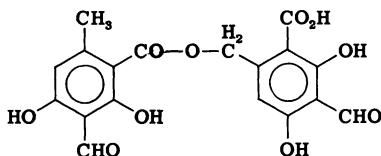


methyl 3,5-dichlorolecanorate (62) the following compounds: 3,5-dichloro-
orsellinic acid (63), methyl orsellinate (64), methyl 3,5-dichloroorsellinate
(65), dichloroorcinol (66), and orcinol (67).

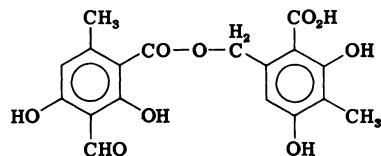


Depsides can be synthesized by condensation of the S and A units in the presence of trifluoroacetic anhydride or *N,N*-dicyclohexylcarbodiimide.

i. BENZYL ESTER DERIVATIVES. Barbatolic acid (68) and alectorialic acid (69) are two closely related benzyl esters from lichens.

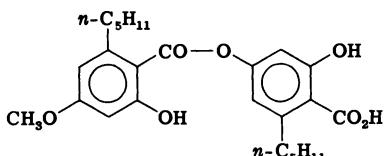


(68)

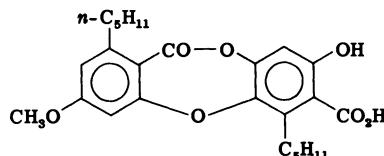


(69)

j. DEPSIDONES. The approximately 23 lichen depsidones are derived biogenetically from depsides by phenoloxidation, giving among others the following depside-depsidone pairs: 4-O-desmethylbarbatic acid-hypoprotocetraric acid, microphyllinic acid- α -collatolic acid, and perlatic acid (70)-colensoic acid (71). As with depsides the UV spectra of depsidones show two peaks, hypoprotocetraric acid, for example, having λ_{max} ($\log_{10}\epsilon$) at 256 (4.14) and 314 nm (3.55).

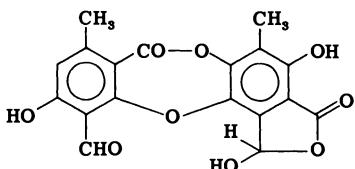


(70)

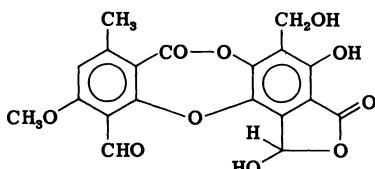


(71)

A number of depsidones in the meta series are aldehydes: fumarprotoce tric acid, stictic acid, norstictic acid (72), constictic acid, (73), pannarin, physodalic acid, protocetraric acid, psoromic acid, salazinic acid, and virensic acid. They react with *p*-phenylenediamine to give yellow to red condensation products (P+ substances).



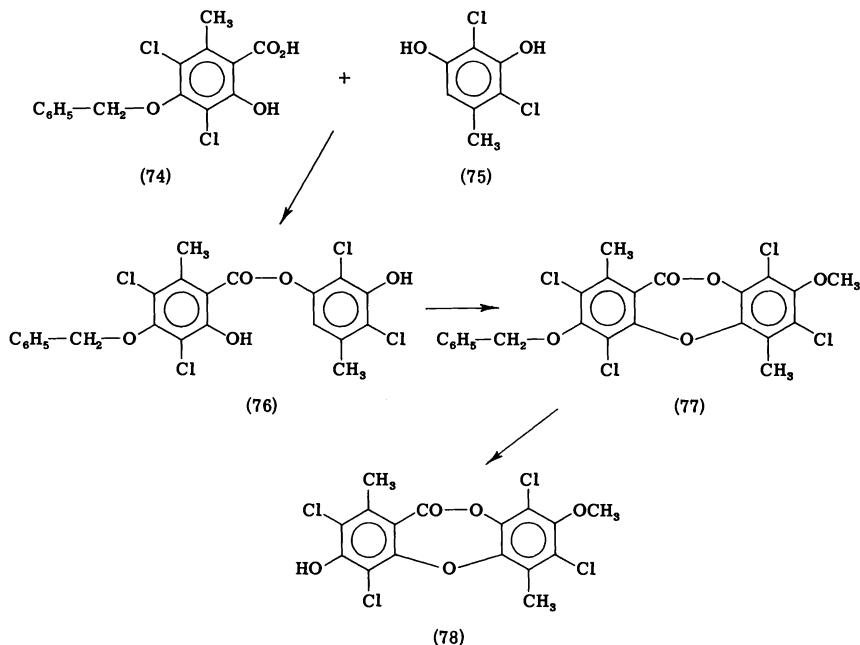
(72)



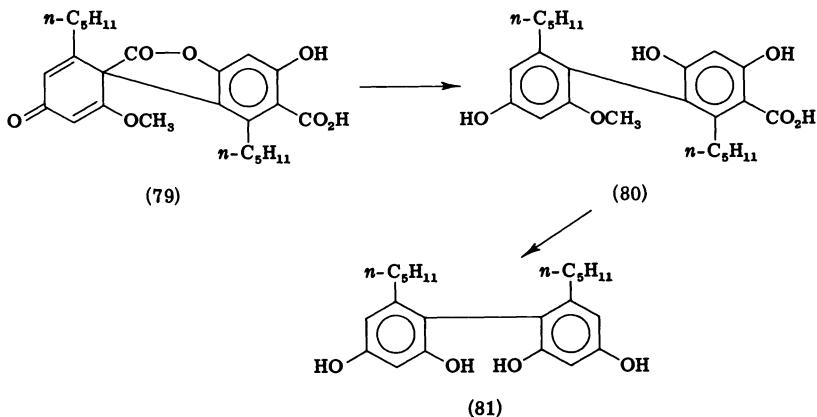
(73)

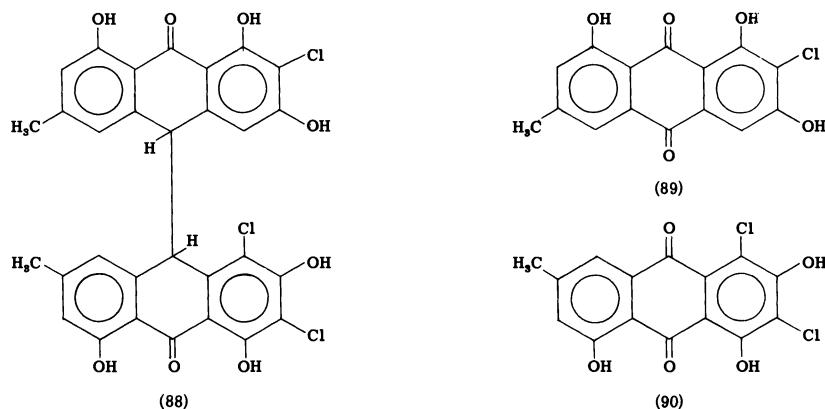
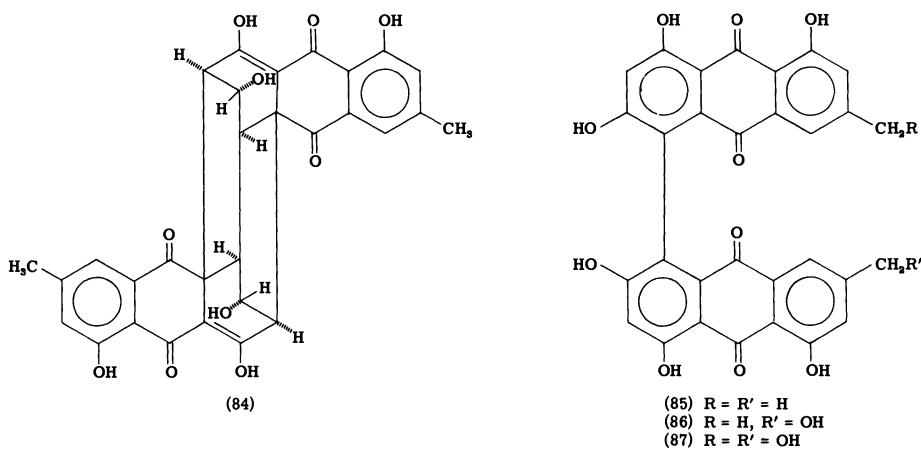
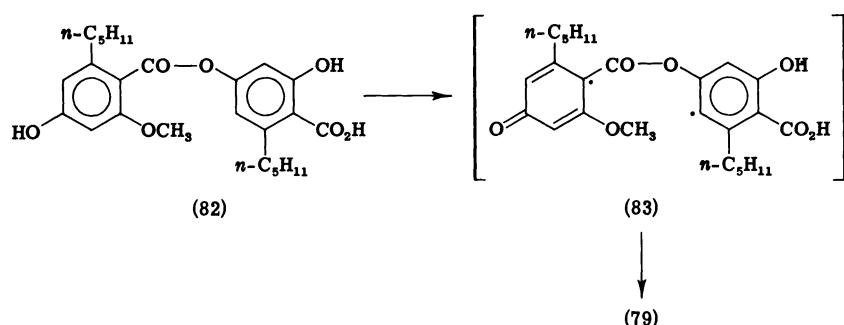
The ester linkage of depsidones is easily hydrolyzed, as is the case with depsides, but cleavage of the ether linkage calls for more drastic means such as fusion with KOH or oxidation with nitric acid.

In the synthesis of diploicin, 3,4-dichloro-4-*O*-benzylorsellinic acid (74) is esterified with 2,4-dichloroorcinol (75) in the presence of trifluoroacetic anhydride to (76), this product is oxidized to (77) with manganese dioxide, and this methylated and catalytically hydrolyzed to diploicin (78).



k. DEPSONES. The only hitherto known depsone from lichens is picrolichenic acid (79), the bitter principle from *Pertusaria amara* (Ach.) Nyl.; struc-



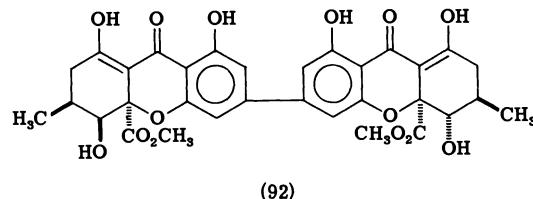
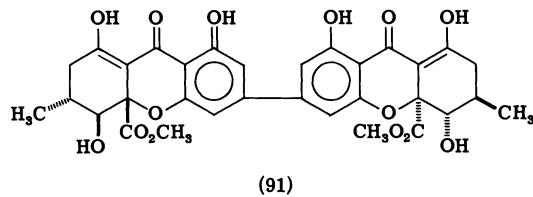


ture (79) is hydrolyzed to 80 which yields by decarboxylation and demethylation 2,2'-di-*n*-pentyl-4,6,4',6'-tetrahydroxydiphenyl (81).

The structure of picrolichenic acid can be further verified by a biogenetic type synthesis. Oxidation of anziaic acid 2-methylether (82) with active manganese dioxide goes directly to (79) through the diradical (83).

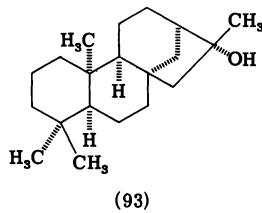
1. BISANTHRAQUINONES AND BISANTHRONYLES. The structure of rugulosin (84) was proved by X-ray analysis of (+)-dibromodehydrotetrahydrorugulosin. Skyrin (85) is chiral because of the limited rotation around the C-5—C-5'-axis and shows a positive Cotton effect. Other bisanthraquinones are oxyskyrin (86) and skyrinol (87). The bisanthonyl flavoobskurin A (88) yields two anthraquinones, (89) and (90), on oxidation with chromium trioxide.

m. BISXANTHONES. *Parmelia entotheiochroa* Hue and several other *Parmelia* species contain both ergot pigments ergochrome AA (91) and ergochrome AB (92).



3. MEVALONATE DERIVATIVES

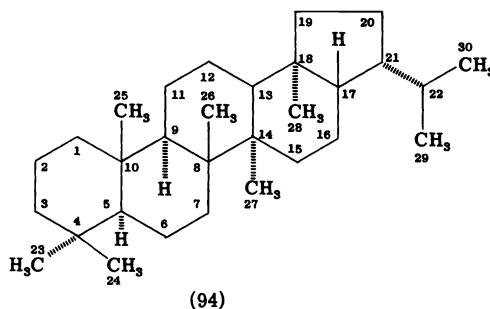
Compounds derived biogenetically from mevalonic acid are considered as mevalonate derivatives. In the lichens these comprise diterpenes, triterpenes, steroids, and carotenoids.



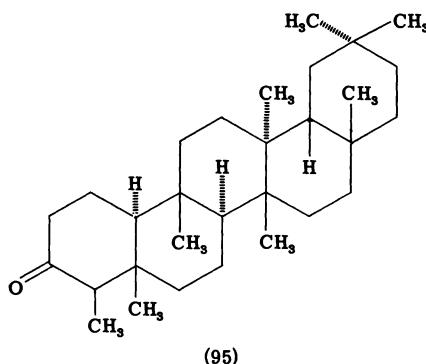
a. DITERPENES. The single structurally determined lichen diterpene is (\rightarrow)-16 α -hydroxykauran (93) known from different *Ramalina* species.

b. TRITERPENES. Lichen triterpenes are easily crystallized, colorless compounds with a relatively high melting point ($> 200^\circ\text{C}$). Most are neutral compounds readily separated and purified by chromatography on aluminum oxide or silica gel. Leucotylic acid, phlebic acid A and B, pyxinic acid, and ursolic acid are very weak acids.

Most of the 20 odd triterpenes that have been structurally determined are derived from pentacyclic hopane (94). The exceptions are friedelin (95), epifriedelanol, taraxerene, ursolic acid, and diacetyl pyxinol.

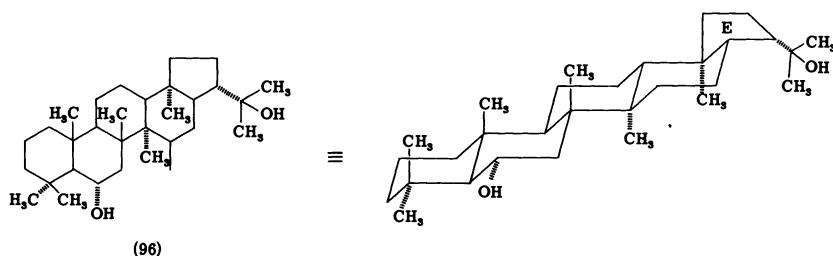


(94)

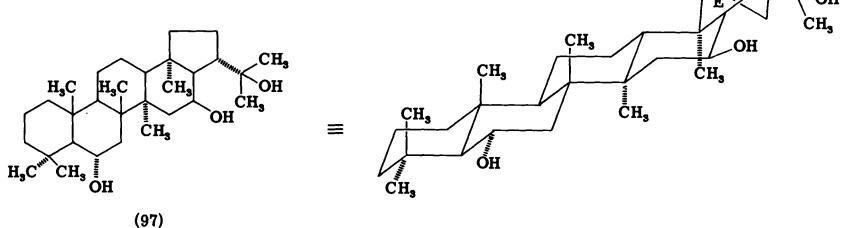


(95)

Zeorin (96), the most common lichen triterpene, has long been known. Its structure and stereochemistry were ultimately confirmed by X-ray analysis of 6 α -bromobenzoyl zeorin. Leucotylin has structure (97) according to X-ray analysis of the 6-keto-16 β -*p*-bromobenzoyl derivative. While the side chain has also α -configuration, the E ring exists in a half chair form in contrast to zeorin with an envelop conformation.

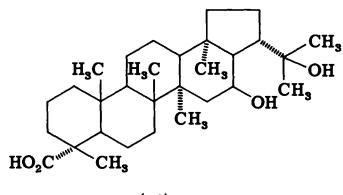


(96)

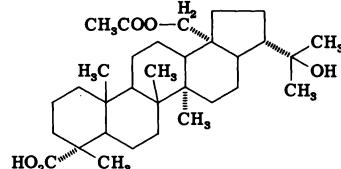


(97)

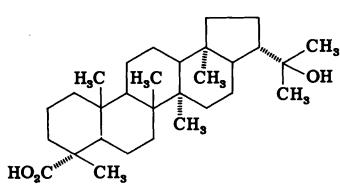
Leucotylic acid (98), phlebic acid A (99), and phlebic acid B (100) are hopane derivatives with a 23-carboxylic acid from *Parmelia leucotyliza* Nyl., *P. entotheiochroa* Hue, and *Peltigera aphthosa* (L.) Willd. Diacetyl pyxinol (101), known from *Pyxine endochrysina* Nyl., possesses a dammaran skeleton from X-ray analysis of 3β , 12β -di-*p*-bromobenzoyl pyxinol.



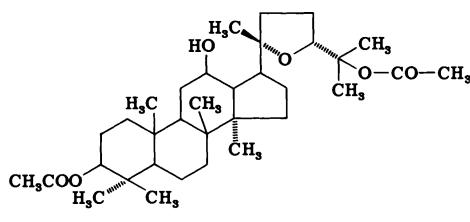
(98)



(99)



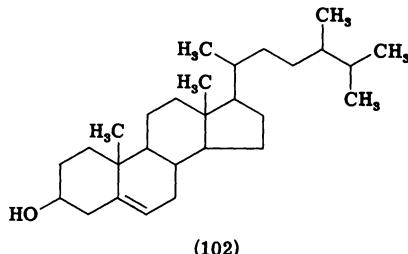
(100)



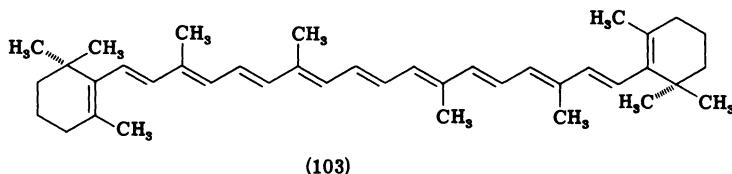
(101)

$6\alpha,7\alpha,22$ -Trihydroxyhopane, 6α -acetoxy- $7\alpha,22$ -dihydroxyhopane, 7α -acetoxy- $6\beta,22$ -dihydroxyhopane, and $11\beta,22$ -dihydroxyhopane have been isolated from *Pseudocycphellaria mougeotiana* (Del.). Wain. var. *dissecta* Del.

c. STEROIDS. Ergosterol, fungisterol, and β -sitosterol (102) occur in very low concentrations in some lichens. Recently ergosterol peroxide has been found in *Peltigera aphthosa* (L.) Willd. and *P. dolichorrhiza* (Nyl.) Nyl.



d. CAROTINOIDS. β -Carotin (103) has been demonstrated in several species of *Roccella*.

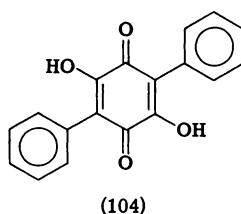


C. Phenylalanine Derivatives

The biosynthesis of phenylalanine derivatives goes through shikimic acid and phenylalanine in contrast to the aromatic units of the anthraquinones, depsides, depsidones, depsones, dibenzofuranes, and xanthones, which are formed on a polyacetate pathway. This justifies a separate classification for the phenylalanine derivatives, which include polyporic acid, thelephoric acid, and pulvinic acid derivatives, all deeply pigmented (yellow, orange, red, or violet).

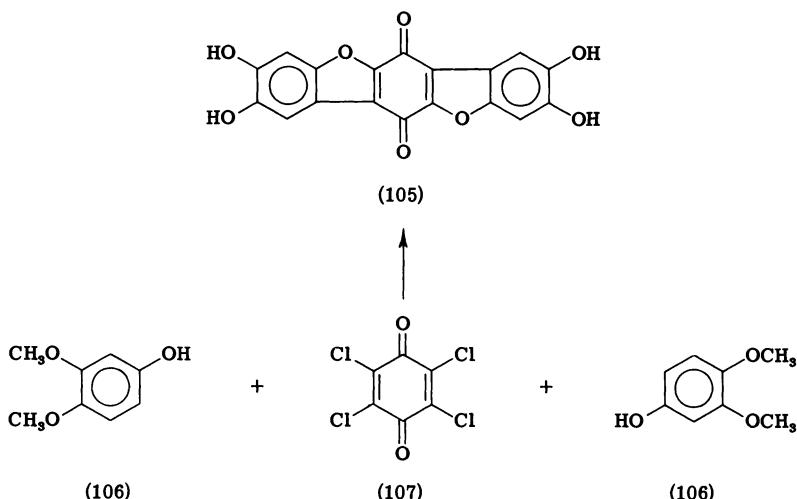
1. POLYPORIC ACID

The red-violet pigment in the lower cortex of some *Sticta* and *Pseudocycphellaria* species is polyporic acid (104), the structure of which has been proven by synthesis.



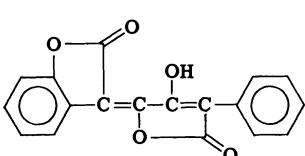
2. THELEPHORIC ACID

Thelephoric acid (105) from *Lobaria* species can be synthesized by condensation of 2 moles 3,4-dimethoxyphenol (106) with chloranil (107) and subsequent demethylation.

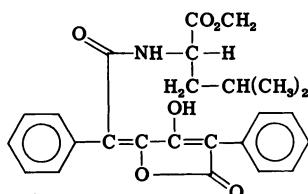


3. PULVINIC ACID DERIVATIVES

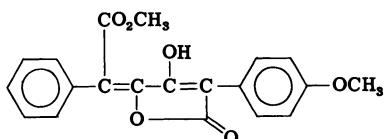
Pulvinic acid derivatives now include calycin (108), epanorin (109), leprarinic acid, leprarinic acid methylester, pinastriic acid (110), pulvinic acid (111), pulvinic acid amide, pulvinic acid lactone (112), and vulpinic acid. Taking calycin as an example, the UV maxima are at 241 ($\log_{10} \epsilon = 4.20$), 253 ($\log_{10} \epsilon = 4.21$), and 430 nm ($\log_{10} \epsilon = 4.40$) (in ethanol). Pulvinic acid lactone may be synthesized simply by condensation of 2 moles phenylacetyl chloride (113) with oxalyl chloride (114).



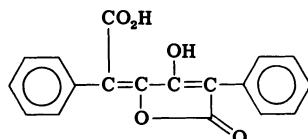
(108)



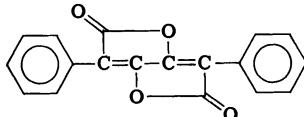
(109)



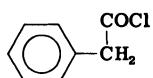
(110)



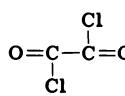
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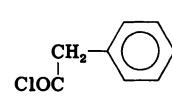
(112)



(113)



(114)



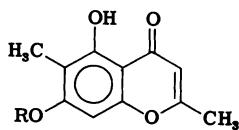
(115)

D. Vitamins

The following vitamins have already been demonstrated in lichens: ascorbic acid, biotin, folic acid, folinic acid, nicotinic acid, panthotenic acid, riboflavin, and vitamin B.

E. Substances Isolated from Mycobionts

Considering the large number of lichens, very few mycobionts have been investigated for their ability to produce lichen substances. The following compounds have been isolated from lichen mycobionts: parietin from *Xanthoria parietina* (L.) Fr., pulvinic acid, pulvinic-acid lactone, calycin, and vulpinic acid from *Candelariella vitellina* (Ehrh.) Müll. Arg., roccellic acid, eugenitol (115), eugenitin (116), and 8-chloro-5,7-dihydroxy-2,6-dimethyl



(115) R = H

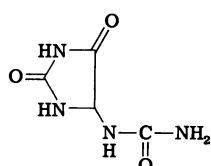
(116) R = CH₃

chromone from *Lecanora rupicola* (L.) Zahlbr., (+)-usnic acid and salazinic acid from *Ramalina crassa* (Del.) Mot., and (+)-usnic acid from *R. yasudae* Räs.

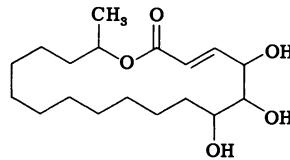
III. Recent Results

Solberg (1971) has found in *Xanthoria parietina* (L.) Th.Fr. allantoin (117). The structure of roccanin, the cyclic tetrapeptide from *Roccella canariensis* Darb. has been established by synthesis as 2 a [cyclo-(R- β -phenyl- β -alanyl-L-prolyl-)₂] (Bohman-Lindgren, 1972; Bohman-Lindgren and Ragnarsson, 1972).

Aspicilin, the first macrocyclic lactone from a lichen (*Aspicilia gibbosa* Körb.) is 4,5,6-trihydroxyoctadec-2-en-1,17-olide (118) (Huneck *et al.*, 1973).

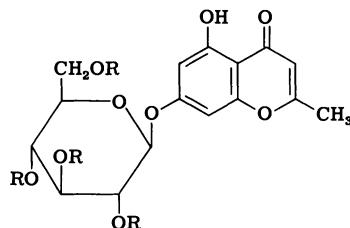


(117)



(118)

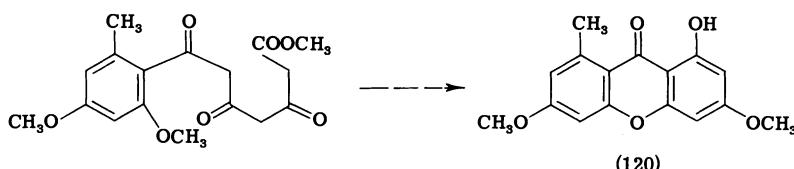
A further chromone glucoside, lobodirin (119) was isolated from *Lobodirina cerebriformis* (Mont.) Follm. (Huneck, 1973).



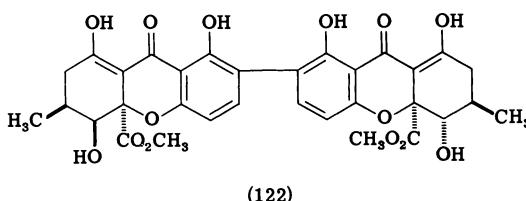
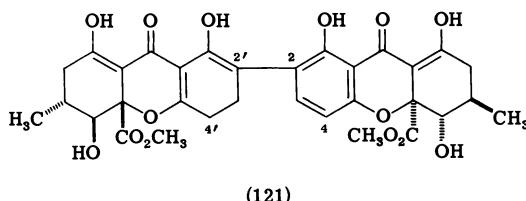
(119)

(R = H, CH₃CO, CH₃CO, CH₃CO)

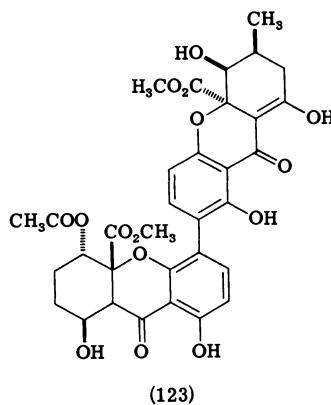
Lichexanthone (120) was synthesized in a biogenetic-type reaction by intramolecular condensation of methyl-7-(4-orcinyl)3,5,7-trioxoheptanoate (Hay and Harris, 1972).



By NMR spectroscopy and by chemical degradation it has been established that the linkage between the two halves of ergochrome AA [= secalonic acid A(121)] and ergochrome AB [= secalonic acid C(122)] is 2,2'- and not 4,4'-as previously suggested (Hooper *et al.*, 1971).

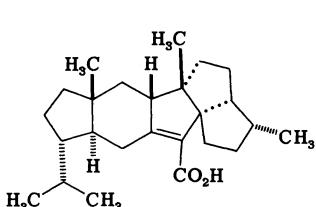


Usnea bayleyi (Stirt.) Zahlbr. contains the bisxanthones eumitrin A₁ (123), A₂, and B (Yang *et al.*, 1973).

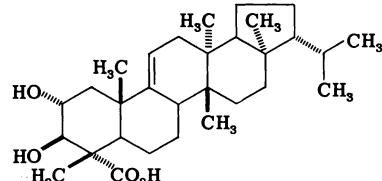


Retigeranic acid (124) is a novel sesterterpene from *Lobaria isidiosa* var. *subisidiosa* Asah. and *Lobaria subretigera* Inum.; its structure was established by X-ray analysis of the corresponding *p*-bromoanilide (Kaneda *et al.*, 1972).

Also from *Lobaria* species were isolated the new farnene derivatives retigeric acid A (125) and retigeric acid B (Takahashi *et al.*, 1972).



(124)



(125)

The Shibata group reports the occurrence of a number of anthraquinones in the cultivated mycobionts of *Xanthoria mandschurica* (Zahlbr.) Asah., *X. fallax* (Hepp) Arn., and an unidentified *Caloplaca* species (Nakano *et al.*, 1972). The mycobionts of *Ramalina crassa* (Del.) Mot. and *R. subbreviuscula* Asah. convert ribitol into arabitol and mannitol (Komiya and Shibata, 1971).

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Chapter 16

BIOSYNTHESIS OF LICHEN SUBSTANCES

KLAUS MOSBACH

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I. Introduction

The structures of about 220 secondary metabolites found in lichens have been established so far. A classification of these compounds based on the biogenetic progenitors, carbohydrate, acetate-poly malonate, mevalonate, and shikimate, is a convenient starting point for a discussion of their biosynthesis. The carbon metabolism involved is summarized in Fig. 1.

A study of the biosynthesis of lichen metabolites is warranted for several reasons:

1. Many of the structural classes, e.g., depsides, depsidones, depsones, dibenzofurans, and pulvic acid derivatives, are almost exclusively confined to lichens.
2. Where analogous metabolites occur in lichens and fungi, it is of interest to compare their biosynthetic pathways.
3. Biosynthetic studies may give valuable insight into the biochemistry of symbiosis, answering questions concerning the roles of the separate symbionts in synthesis and breakdown of lichen metabolites as well as information on general lichen metabolism.

Prior to 1964, the slow-growing lichens had been considered unsuitable for direct experimental biosynthetic investigations. About that time, however,

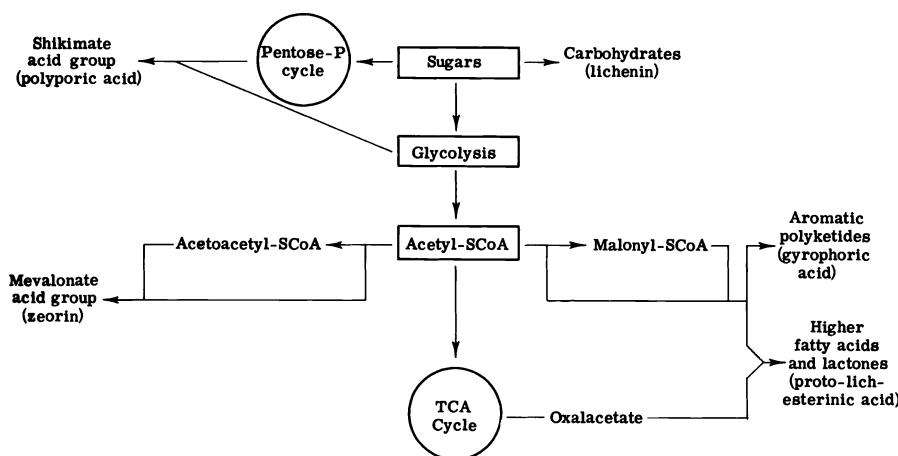


FIG. 1. Carbon metabolism leading to the formation of the major groups of lichen substances with a typical representative given in parentheses.

a limited number of experimental reports began to appear in the literature dealing with aspects of the biosynthesis of lichen metabolites (Mosbach, 1964a,b,c; Maass *et al.*, 1964; Yamazaki *et al.*, 1965). There is still surprisingly little data, but these can be supplemented by appropriate analogies drawn from parallel studies, in particular those on closely related free-living fungi. In the studies to be discussed below, the basic methodology involves almost exclusively administration of radioactively labeled precursors to intact lichen thalli. The first reports recently appeared on the isolation and properties of enzyme systems directly involved in the metabolism of typical lichen substances (Mosbach and Schultz, 1971; Schultz and Mosbach, 1971).

II. Biosynthesis

A. Carbohydrates

Three main types of carbohydrate have been recognized in lichens.

1. POLYOLS

Lichens are notable for their high polyol content. Eight different compounds have so far been found: glycerol, *meso*-erythritol, D-arabitol, D-ribitol, D-mannitol, *myo*-inositol, D-volemitol ($C_7H_{16}O_7$), and siphulitol ($C_7H_{16}O_6$), the latter being the first deoxy sugar alcohol found in nature. Erythritol also occurs in some lichens as orsellinate (montagnetol) and lecanorate (erythrin)

esters. From investigations of the sugar alcohol content of 60 types of lichens it appears that the occurrence of these compounds varies from genus to genus. D-Mannitol, however, occurs in all the species investigated (Lindberg *et al.*, 1953).

2. POLYOLGLYCOSIDES

Three lichen polyol glycosides have been described: 3-*O*- β -D-glucopyranosyl-D-mannitol, peltigeroside, and umbilicin (Lindberg and Wickberg, 1962).

3. POLYSACCHARIDES

The cell walls of the hyphae consist mainly of the polysaccharides, lichenin and isolichenin, and, to a lesser extent, pustulan and other glucans, some of which occur solely in lichens. The widespread lichenin is a linear β -D-glucopyranosyl polymer of molecular weight 20,000–40,000 lying between those of starch and cellulose (Peat *et al.*, 1961).

The biosynthetic pathways leading to lichen carbohydrates seem unexceptional, relevant parts of this topic being covered in the preceding chapters. The role of sugar alcohols, which are generally present as intermediates during the carbon flow from the alga to the fungal partner, appears to be of special significance.

B. Acetate-Polymalonate Group

By far the greatest number of lichen substances are derived by condensation of a "starter" unit, usually acetyl-SCoA(*),† and *n*-malonyl-SCoA units with concomitant decarboxylation. The putative poly- β -ketothioester thus formed undergoes modification to give the observed metabolites, also known as "polyketides" (Collie, 1907), a name best restricted to non-fatty-acid compounds.

1. HIGHER FATTY ACIDS AND LACTONES

The higher fatty acids and their lactones occurring in lichens can be divided into three groups: γ -lactone acid derivatives [nine members, e.g., protolichesterinic acid (1)]; dibasic fatty acids [roccellic acid (2)]; and tri-basic fatty acids [caperatic, nor- and rangiformic acids (3)]. In addition, two tetrahydroxy fatty acids, e.g., ventosic acid [$\text{CH}_3(\text{CH}_2)_8(\text{CHOH})_2-\text{CH}_2-(\text{CHOH})_2(\text{CH}_2)_7\text{COOH}$] have been reported (Solberg, 1957). All aliphatic lichen acids are optically active.

†(*) = coenzyme A.

The only reports of biosynthetic studies of this group of aliphatic lichen acids deal with the biosynthesis of (+)-protolichesterinic acid in *Cetraria islandica* (Bloomer *et al.*, 1968, 1970; Bloomer and Hoffman, 1969). Feeding experiments using ^{14}C -labeled acetate and succinate strongly indicate formation of this γ -lactone by condensation of palmitic acid with a C₄ unit (Fig. 2) thus justifying earlier biogenetic speculation (Mosbach, 1969). In close analogy are the enzymic syntheses of the related fungal metabolites decylcitic (4) and spiculisporic acids which proceed by condensation of lauryl-SCoA with oxalacetate and α -ketoglutarate, respectively (Gatenbeck and Måhlén, 1966). Formation of the lichen acids is believed to proceed similarly by standard elaboration of a long-chain alkanoyl-SCoA unit, the α -methylene group of which undergoes aldol type condensation with a ketoacid. The condensing units of the lichen substances shown in Fig. 2 are therefore most likely the fatty acids myristoyl- or palmitoyl-SCoA, on

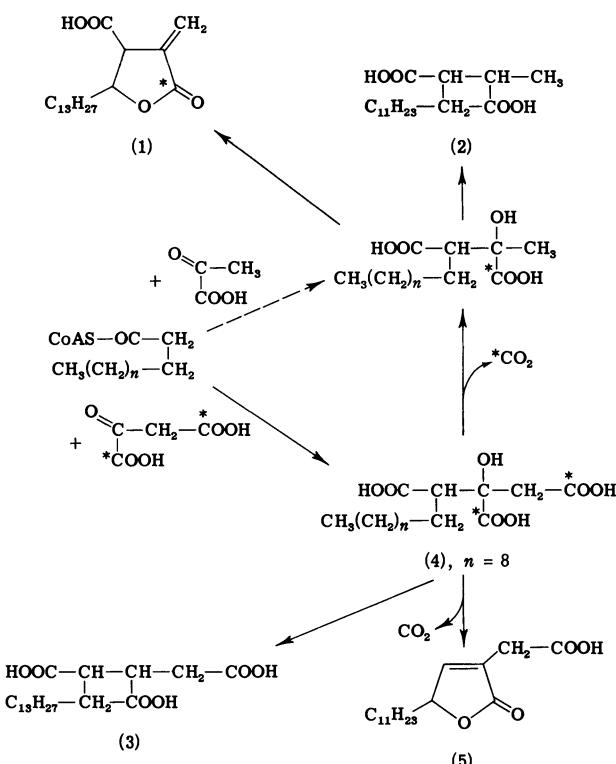


FIG. 2. Probable biosynthesis of protolichesterinic acid (1), roccellic acid (2), rangiformic acid (3), and acarenoinic acid (5). The labeling given for (1) is the one expected after administration of the likely precursor oxalacetate by analogy with the results reported for succinate.

the one hand, and the keto acids oxalacetic acid and perhaps pyruvic acid, on the other. In the γ -lactone acids the corresponding β -hydroxy fatty acids are possible condensation units. Recent enzymic studies using a highly purified and kinetically well-characterized decylcitrate synthase from a *Penicillium* mold are in full accord with the scheme given (Måhlén, 1971).

2. AROMATIC POLYKETIDES

The structures of about 130 aromatic lichen substances have so far been elucidated; about 120 of these are polyketides. In most cases orsellinic acid (6) and its homologues are the principal structural units. Contrary to molds where orsellinic acid occurs generally in the free form (Reio, 1958; Mosbach, 1959), although occasionally as a part of a metabolite (e.g., Merlini *et al.*, 1970), in lichens it is present almost exclusively condensed as di-, tri-, and tetracyclic compound. Isotopic studies with ^{14}C and ^{18}O on this mold metabolite (Mosbach, 1960, 1961; Bentley and Keil, 1961; Gatenbeck and Mosbach, 1959) established that its formation involves condensation of one acetyl-SCoA unit with three malonyl-SCoA units, probably via an enzyme-bound thioester of 3,5,7-triketooctanoic acid, which undergoes internal aldol condensation, dehydration, and hydrolysis. An alternative aldol condensation could lead to 3,5-dihydroxyphenylacetic acid (8), which, however, has never been shown to occur. A Claisen-type condensation leads to the acetylphloroglucinol skeleton (7) (Fig. 3). The formation of these compounds most probably takes place on a multienzyme complex in analogy with the biosynthesis of fatty acids, as has been indicated by enzymic studies with the related polyketides, 6-methylsalicylic acid (Lynen and Tada, 1961) and alternariol (Gatenbeck and Hermodsson, 1965). In a recent study, the enzyme system responsible for the formation of the former compound has

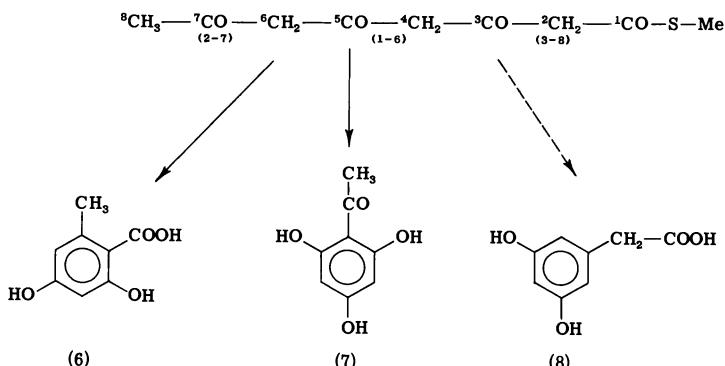


FIG. 3. Three possible alternative cyclization of 3,5,7-triketooctanoic acid leading to the compounds (6)–(8). During cyclization the intermediate compound is likely to be bound to its multienzyme complex (= ME) as thioester or, less likely, to coenzyme A.

been isolated from *Penicillium patulum* and shown to comprise a single multienzyme particle with a molecular weight of about 1.1×10^6 to 1.5×10^6 (Dimroth *et al.*, 1970).

Several groups of characteristic polyketides occur in lichens. Biosynthetically they are closely related, being derived mainly from 6-alkyl- or 6-(β -keto) alkyl-2,4-dihydroxybenzoic acids which further vary in their details of "extra" ring substituents (Table I). β -Orsellinic acid units are not so widespread as fungal metabolites. The only report of such a recognized depside component in a free-living fungus concerns the occurrence, biosynthesis, and metabolism of 3-methylorsellinic acid ($R^3 = CH_3$) in *Penicillium stipitatum* (Scott *et al.*, 1971).

This large number of variations on the orsellinate template is augmented by the diversity of modes by which the monomers are connected (Fig. 4) and according to which the metabolites are usually classified. The two principal mechanisms involved are esterification, as with the depsides (9), and oxidative coupling, which leads to the C-C bond joining the two rings of the dibenzofurans (12) and dibenzoquinones (13). The biosynthesis of the depsidones (10) and depsones (11) involves both linkages. Apart from the dibenzoquinones, these types constitute, together with the pulvic acid derivatives, the typical aromatic lichen substances and are, with very few exception such as the nidulins (mold depsidones) and the *m*-digallic acid occurring in tannins (depsides in algae and higher plants), restricted to the lichens.

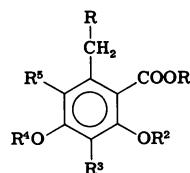


TABLE I.
LIST OF SUBSTITUENTS OF OSRELLINIC ACID^a FOUND SO FAR IN LICHEN SUBSTANCES

R	R ¹	R ² , R ⁴	R ³	R ⁵
C ₂ H ₃	CH ₃	CH ₃	CH ₃	
n-C ₄ H ₉	Erythrityl		CH ₂ OH	
CO-n-C ₃ H ₇			CH ₂ O-COCH ₃	
n-C ₆ H ₁₃			trans-CH ₂ O-CO-CH=CH-COOH	
CO-n-C ₅ H ₁₁			CHO	CHO
			COOH	
OH			OH	OH
			Cl	Cl

^aR = R¹ = R² = R³ = R⁴ = R⁵ = H.

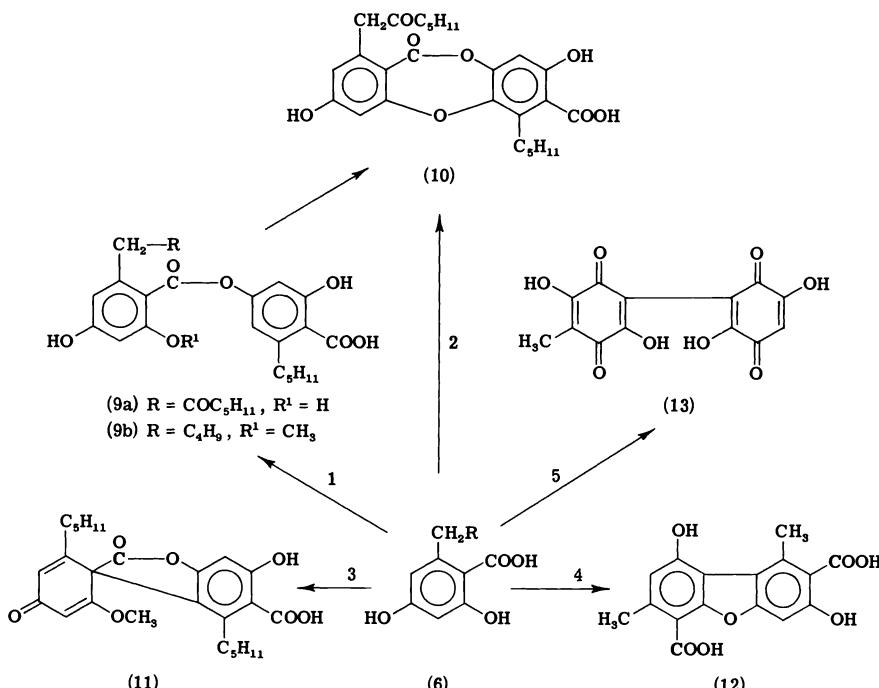


FIG. 4. Structural relationships and possible formation of the five types of condensation products of orsellinic acid (6) or its homologues taking place in lichens. (9a) = olivetoric acid, (9b) = dihydropicrolichenic acid, (10) = physodic acid, (11) = picrolichenic acid, (12) = pannaric acid, (13) = pyxiferin.

a. ORCINOL DERIVATIVES. Metabolites based on orsellinate monomers (or monomeric homologues) are rare in lichens; known are ethyl and erythrityl orsellinate and methyl β -orsellinate. To my knowledge, no studies have been carried out on the formation of these compounds. What will be said below under depside biosynthesis and in the conclusion is likely to apply to these substances as well.

b. DEPSIDES. The 40 depsides presently known constitute by far the largest group of aromatic lichen substances. The first biosynthetic investigation on these metabolites was carried out with gyrophoric acid (14) from *Umbilicaria papulosa*. [1,3-¹⁴C]diethylmalonate was administered to the lichen thallus (Mosbach, 1964a) in Erlenmeyer flasks on a rotary shaker and partly submerged in the medium. After an incubation period of 3 days, radioactivity was found to have been incorporated into gyrophoric acid at the surprisingly high level of about 4%. Later studies with the same organism using ¹⁴CO₂ further emphasized the rapid metabolism of the organism since

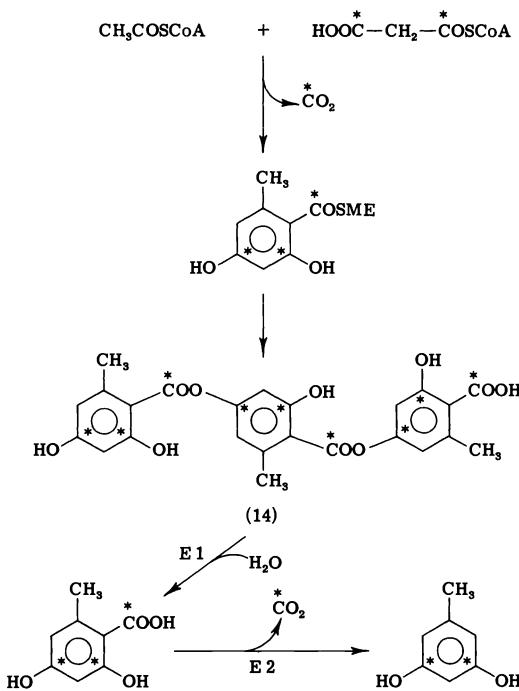


FIG. 5. Biosynthesis and enzymic degradation of the tridepside gyrophoric acid (14). Labeling pattern (*) obtained after incubation with $\text{CH}_2\text{-(}^{14}\text{COOC}_2\text{H}_5\text{)}_2$. Enzymic degradation involves hydrolysis, catalyzed by orsellinate depside hydrolase (E1) followed by decarboxylation with orsellinate decarboxylase (E2).

radioactivity was detected in the depside after incubation for only 1 minute (Fox and Mosbach, 1967). Subsequent degradation of labeled gyrophoric acid obtained after incubation with diethylmalonate gave the same specific activity for all three orsellinic acid molecules in the positions emanating from the malonyl-SCoA, while much less activity appeared in the positions derived from the acetyl-SCoA "starters" (Fig. 5).

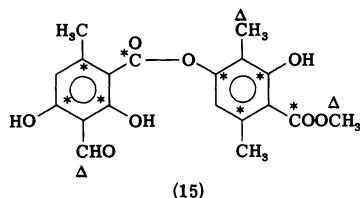


FIG. 6. Labeling pattern in the depside atranorin (15) after incubation with $\text{CH}_3\text{-(}^{14}\text{COOH)}$ and $\text{H-(}^{14}\text{COOH})$.

In similar studies [1^{-14}C] acetate and [^{14}C] formate were incorporated into the depside atranorin (15), belonging to the β -orsellinic acid group, with specific incorporation of formate into the "extra" CHO and CH₃ groups (Fig. 6) (Yamazaki *et al.*, 1965), the immediate precursor of which is likely to be S-adenosylmethionine. Subsequently it was shown by Yamazaki and Shibata (1966), that tritium-labeled β -orsellinic acid was incorporated into atranorin whereas tritiated orsellinic acid was not, thus implying that insertion of the "C_i" unit occurs before aromatization.

It would appear that the many higher orsellinic acid homologues such as olivetoric acid (9a) are derived from a single acetate-polymalonate chain in analogy with the fungal "C₁₀ acid" (6-acetyl-2,4,-dihydroxybenzoic acid) (Manchanda and Stickings, 1963). A condensation of a separately formed alkanoyl-SCoA unit with orsellinic acid appears less likely.

c. DEPSIDONES. Approximately 23 lichen depsidones have been characterized. Their formation probably proceeds via one of the following pathways: (1) intramolecular oxidative coupling of the depside (Barton and Cohen, 1957; Erdtman and Wachtmeister, 1957) by a separate "depside dehydrogenase", (2) oxidative coupling, without the participation of a free depside intermediate, on a "depsidone multienzyme complex." The first alternative has support in the coexistence of the depside-depsidone pair olivetoric acid (9a)-physodic acid (10) (Fig. 4) in species of *Cetraria ciliaris* (Culberson, 1964). Other such pairs, which have not so far been reported to occur jointly, are atranorin (15)-virensic acid, microphyllinic acid- α -colatolic acid, and sphaerophorin-grayanic acid. A biogenetic-type synthesis of the depsidone diploicin, involving oxidation of the corresponding depside with manganese dioxide has been reported (Brown *et al.*, 1960).

d. DEPSONES. Only one depsone, picrolichenic acid (11), has so far been discovered (Wachtmeister, 1958). It is probably formed from the corresponding depside, dihydropicrolichenic acid (9b) (not itself a recognized metabolite), by an intramolecular oxidation leading to C-C coupling. Here the methoxy group prevents the presumably favored C-O coupling of depsidone formation. The reported synthesis of the above depsone by oxidation of the corresponding synthetic depside (9b) with manganese dioxide is in agreement with this postulate (Davidson and Scott, 1961).

e. DIBENZOQUINONES. Only a tentative structure for the only known lichen dibenzoquinone pyxiferin (Neelakantan, 1965) has been given; speculation as to its biogenetic origin should therefore be treated with greatest care. Pyxiferin is reported to be closely related structurally to the fungal dibenzoquinones oosporein (16b) and phoenicin (16c). The biosynthesis of the monocyclic quinones probably proceeds via orsellinic acid, as was shown for the fungal toluquinone fumigatin (17) (Pettersson, 1966). The two

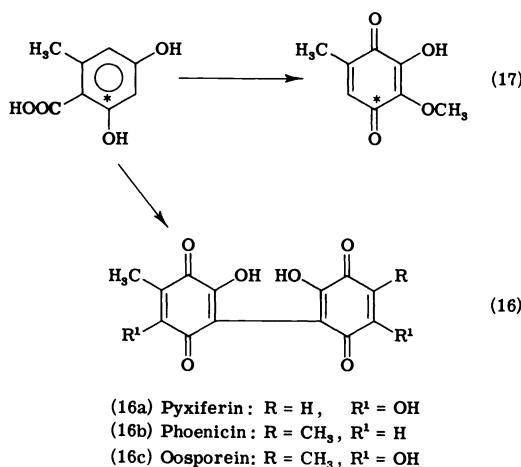


FIG. 7. Possible biosynthesis of pyxiferin (16a) and structures of the fungal dibenzoquinones phoenicin (16b) and oosporein (16c). Labeling pattern in fungal toluquinone fumigatin (17) after incubation with [2-¹⁴C] orsellinic acid.

monocyclic portions of dibenzoquinones are thought to combine by oxidative phenolic coupling (Westerfield, 1942). Furthermore, it has been shown that orsellinic acid and orcinol are incorporated specifically into phoenicin (Charollais *et al.*, 1963).

f. DIBENZOFURANS. Dibenzofurans are essentially unique to lichens. Only one representative, rhodomyrt toxin, has tentatively been identified outside the lichen group (Trippet, 1957). Of the seven dibenzofurans whose structures have been elucidated, five are condensation products of orsellinic acid and homologues, e.g., porphyrilic acid (18) and strepsilin (19), and represent the usual lichen dibenzofurans; usnic (21) and isousnic acid, however, are based on acetylphloroglucinol(7) units and are the sole aromatic lichen substances to be derived by Claisen condensation of the polyketide chain (Fig. 8). Barton *et al.* (1956) were the first to succeed in synthesizing usnic acid in their classical work involving oxidation of acetyl-methylphloroglucinol with potassium hexacyanoferrate (III). The initially formed hydroxydihydrousnic acid (20) was subsequently dehydrated with sulfuric acid to give usnic acid. Pentilla and Fales (1966) and Taguchi *et al.* (1969a) succeeded in effecting the same coupling enzymically with a peroxidase system to give (20). In a series of incorporation studies using in particular ³H- and ¹⁴C-labeled acetyl-methylphloroglucinol, the biosynthetic pathways leading to the formation of usnic acid were established as depicted in Figure 8 (Taguchi *et al.*, 1966, 1969a,b). Administered acetyl-methylphloroglucinol was incorporated intact into usnic acid whereas

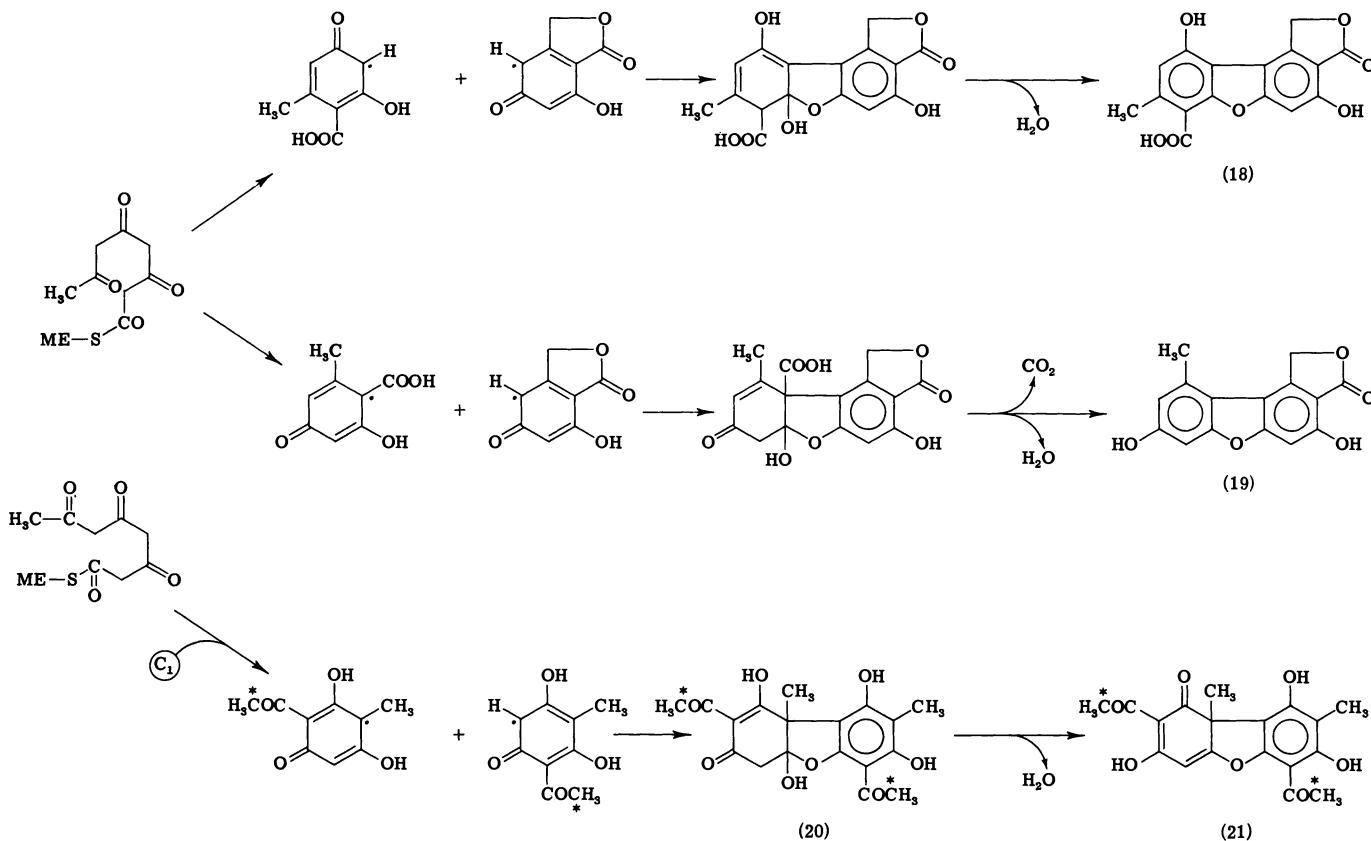
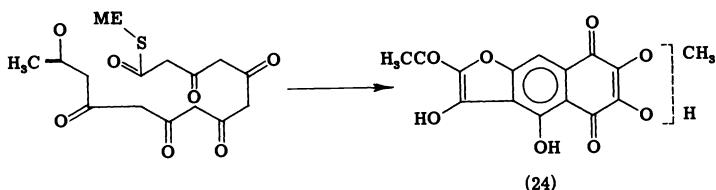
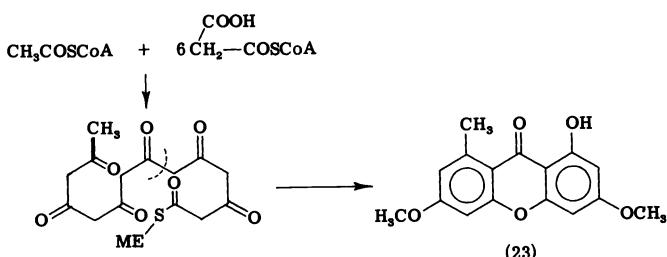
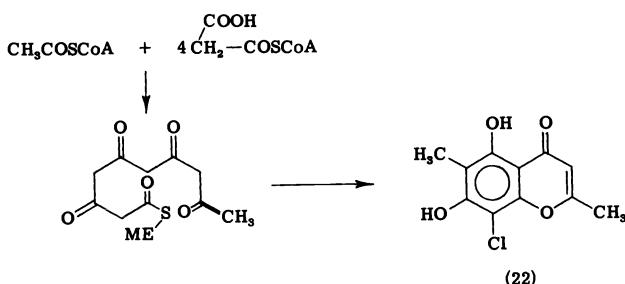


FIG. 8. Proposed biosynthesis of dibenzofurans; porphyrilic acid (18), strepsilin (19), and usnic acid (21). Labelling pattern in (21) was obtained after incubation with (¹⁴CH₃ — CO)-acetyl methyl-phloroglucinol.



acetylphloroglucinol was not. According to these findings, the biosynthesis of usnic acid and probably that of other dibenzofurans proceeds by oxidative phenol coupling and subsequent dehydration. The question of whether separate dehydrogenases and dehydratases are involved or whether the complete synthesis takes place *in vivo* on a multienzyme complex has yet to be answered. In these above studies the observation has also been made (Taguchi *et al.*, 1969b) that the efficiency of synthesis of usnic acid in lichens is subject to seasonal variation. This may be a result of the variable supply of the early precursors in the total biosynthetic sequence.

g. CHROMONES, XANTHONES, NAPHTOQUINONES, ANTHRONES, AND ANTHRAQUINONES. Chromones, xanthones, naphtoquinones, anthrones, and anthraquinones cannot be regarded as unique lichen metabolites since they are

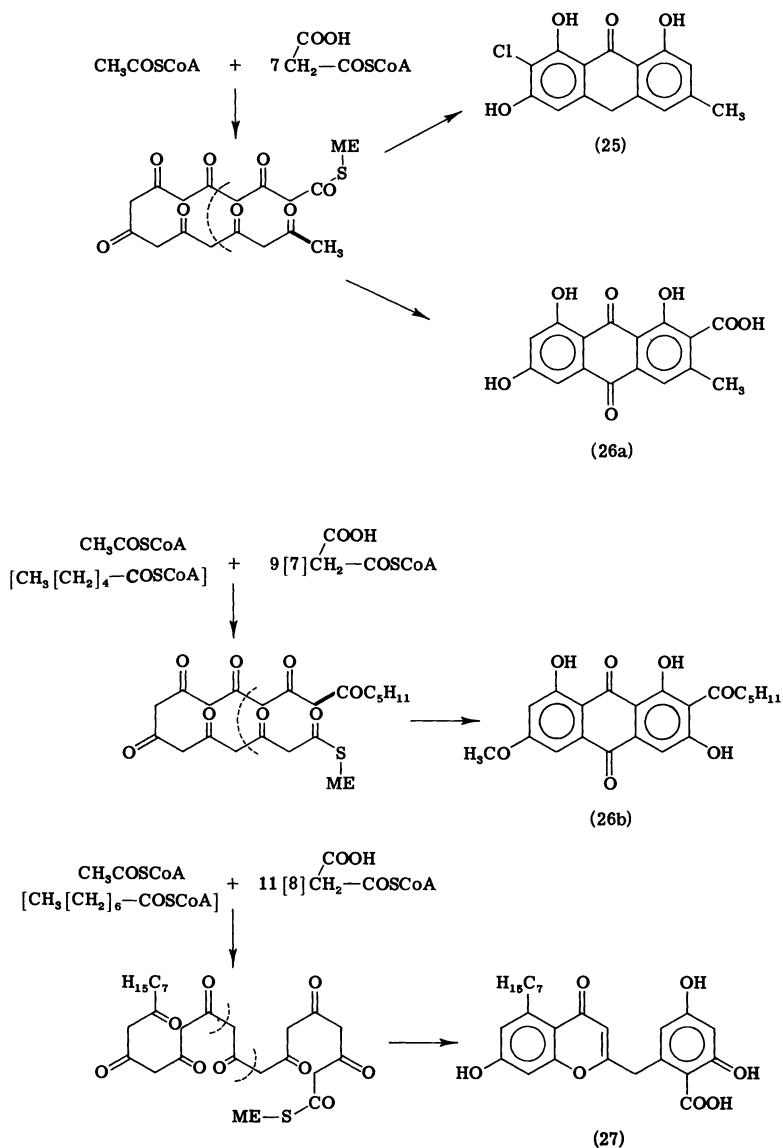


FIG. 9. Possible biosynthetic pathways of noncondensed aromatic lichen polyketides from a β -polyoxomethylene chain. The "starting" positions are indicated by heavy lines, while the dotted lines indicate the theoretically still possible formation of such compounds from two separate units.

represented in other organisms. They are, however, unusually common in lichens, at least 40 different compounds having been described. No biosynthetic studies on the lichen compounds have yet been reported but the biosynthesis of analogous fungal metabolites is well documented and biogenetic parallels can usefully be drawn. It is most likely that all originate from poly- β -oxomethylene precursors as outlined in Fig. 9. The much less likely formation by condensation of two separately formed polyketide chains or their cyclized products, as demonstrated for the biosynthesis of citromyctecin (Gatenbeck and Mosbach, 1963) and mollisin (Bentley and Gatenbeck, 1965; Tanabe and Seto, 1970), cannot, however be entirely ruled out.

The four known chromones found in lichens, lepraric acid, 6-ethoxy-methyleugenitin, rupicolin (22), and siphulin (27), probably result from the condensation of an acetyl-SCoA "starter" unit with either four malonyl-SCoA units or, in the latter case, eleven. The six xanthones, e.g., lichexanthone (23), are probably formed analogously from one acetyl-SCoA molecule and six malonyl-SCoA molecules. Noteworthy is the structure of thiophanic acid (2,4,5,7-tetrachloro-1,3,6-trihydroxy-8-methylxanthone), a tetrachloroxanthone (Huneck, 1966), one of a large number of characteristic chlorinated aromatic lichen metabolites. Three naphthoquinones have been isolated so far, haemoventosin, chiodectic acid, and rhodocladonic acid (24). Although it is not established whether the ring fused to the naphthoquinone moiety of (24) is 2-acetyl-3-hydroxyfuranyl (as shown) or 2-methylchromonyl in nature, the biosynthesis of either probably involves a polyketide chain of the same length as for the xanthone precursor but folded differently.

The structures of about 26 lichen anthraquinones have so far been elucidated. Their biosynthesis is discussed together with that of the anthrones, biosynthetically obviously closely related compounds of which two representatives have been recently isolated (Yoshioka *et al.*, 1968). Taking the information gained from a series of investigations by Gatenbeck (1962) and Shibata and Ikekawa (1963) on fungal anthraquinones, the formation of the corresponding lichen metabolites will involve one acetyl-SCoA and *n* malonyl-SCoA units. The β -polyoxomethylene chain formed, leading to endocrocin (26a), corresponds to the ring closure leading to orsellinic acid, while that leading to solorinic acid (26b) parallels the formation of the acetylphloroglucinols. The anthrones (25) can be envisaged as being formed more or less directly through cyclization of the chain and are probable precursors of the anthraquinones which require a further oxidation step. Of interest is the occurrence of dimers of both lichen anthraquinones, anthrones, and xanthones.

h. PORTENOLS. Recently, occurrence and structure of two interesting lactones, portenol (28a) and acetylportenol (28b), from *Roccella* species have

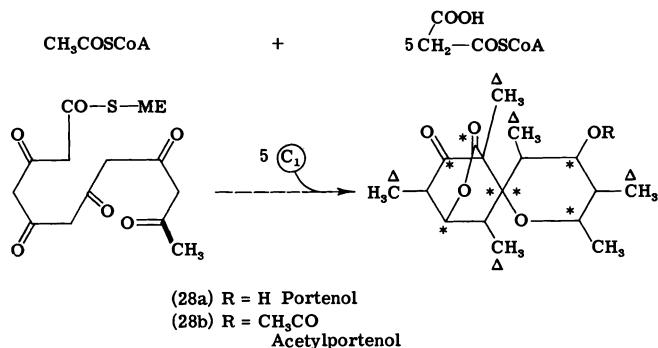
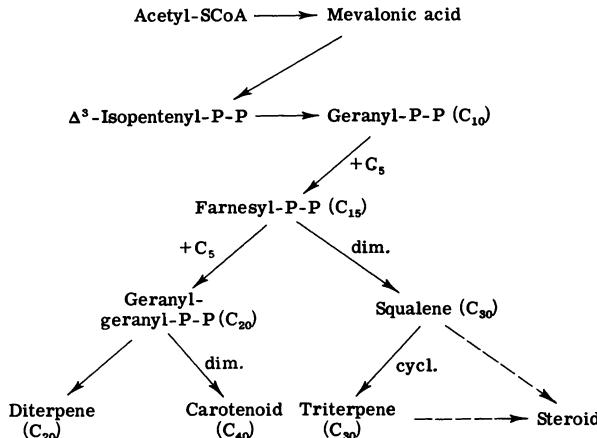


FIG. 10. Biosynthesis of portenol. Labeling pattern after incubation with $\text{CH}_3^{14}\text{COOH}$ - $(^*)$ and (Me^{14}C) methionine (Δ).

been reported (Aberhart *et al.*, 1969). Their biosynthetic formation would appear to involve condensation of one acetyl-SCoA unit and five methylmalonate units. However, a recent report (Aberhart *et al.*, 1970) on biosynthetic investigations carried out on these lichen metabolites of apparent polypropionate origin indicates, surprisingly, their formation from one acetyl-SCoA and five malonyl-SCoA units with not fewer than five “ C_1 ” units originating from methionine (Fig. 10). A fungal polyketide with a somewhat similar high degree of C_1 substitution is citrinin (Birch *et al.*, 1958).

C. The Mevalonate Group

Known lichen metabolites of probable mevalonate origin include one diterpene, $(-)$ - 16α -hydroxykaurane, a series of about 20 triterpenes, several



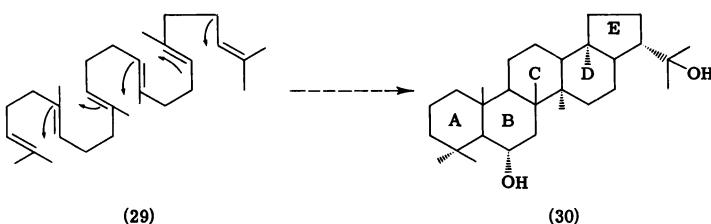


FIG. 11. Cyclization of squalene to form zeorin.

steroids (ergosterol, β -sitosterol, fungisterol, vitamin D₂), and carotenoids (β - and γ -carotene as well as several xanthophylls). Studies of their formation in lichens have not been reported, but it may be assumed that this follows the established pattern of "poly-isoprene" biosynthesis summarized on p. 537 (Richards and Henrickson, 1964).

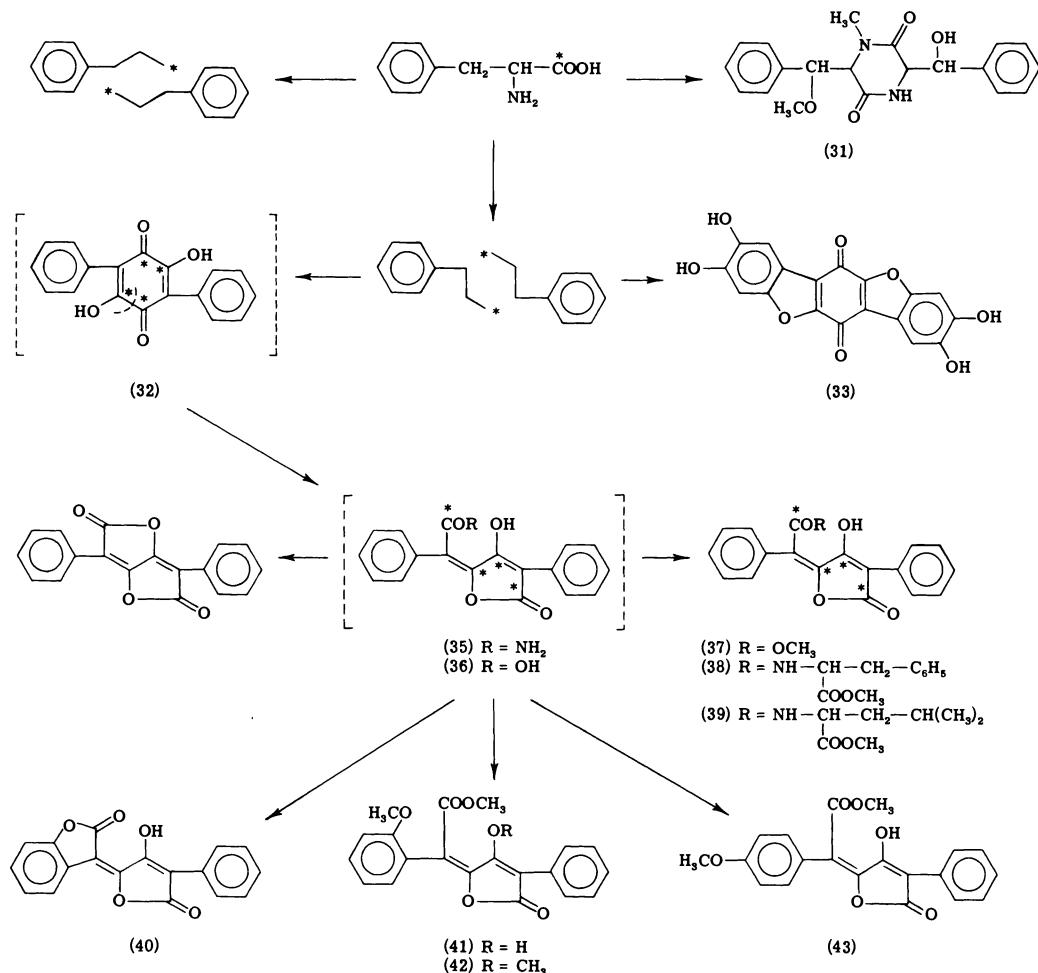
The triterpenes are all pentacyclic; noteworthy is the rare five-membered ring E found in zeorin (30) and leukotylin. Zeorin ($6\alpha,22$ -dihydroxyhopane), the most frequently occurring lichen terpene, seems to be formed by the cyclization of squalene (29) (Fig. 11). A direct precursor of lichen triterpenes with an oxygen function in position 3 (as in friedelin) may be 2,3-epoxysqualene (Corey and Ortiz de Montellano, 1967). As many as seventeen different cyclases, effecting such cyclizations, have been found in a number of different organisms (Dean, 1973).

D. The Shikimate Group

DIKETOPIPERAZINES, TERPHENYLQUINONES, AND PULVIC ACID DERIVATIVES

The shikimate moiety is the progenitor of thirteen aromatic lichen compounds derived via the sequence: phosphoenolpyruvate + erythrityl-4-phosphate \longrightarrow shikimate \longrightarrow chorismate \longrightarrow prephenate \longrightarrow C₆–C₃ derivatives, e.g., phenylalanine and phenylpyruvic acid. One diketopiperazine, picroroccellin (31), two terphenylquinones, polyporic (32), and thele-

FIG. 12. Biosynthesis of aromatic lichen substances of the shikimate group from C₆ — C₃ fragments. In compounds, whose formation was demonstrated experimentally after administration of [1-¹⁴C] phenylalanine, the ¹⁴C-labeling obtained has been marked with an asterisk. (34) = pulvic acid dilactone, (35) = pulvinamide, (36) = pulvic acid, (37) = vulpinic acid, (38) = rhizocarpic acid, (39) = epanorin, (40) = calycin, (41) = leprapinic acid, (42) = leprapinic acid methylether, (43) = pinastric acid. Brackets indicate possible enzyme binding of the compounds.



phoric acids (33), as well as ten pulvic acid derivatives (34–43) have so far been characterized (Fig. 12).

Picroroccellin appears to be formed by direct condensation of two phenylalanine units and is thus a modified cyclic dipeptide. A cyclic tetrapeptide has recently been encountered yielding on hydrolysis L-proline and D- β -amino- β -phenylpropionic acid. Labeled phenylalanine has been shown to be incorporated specifically into the fungal terphenylquinones volucrisporin, a *m*-dihydroxyterphenylquinone (Read *et al.*, 1962; Chandra *et al.*, 1966) and phlebiarubrone, an orthoquinone carrying a terphenyl moiety and a methylene-dioxy group (Bose *et al.*, 1969). The biosynthesis of the lichen terphenylquinones probably also involves condensation of two C₆ – C₃ units likely to be phenylpyruvic acid or phenylalanine, the latter possibly as an enzyme-bound Schiff's base condensing with a CoA thiol ester of phenylpyruvic acid as has been suggested by Maass (1970a).

Whereas in lichens pulvic acid derivatives seem to outnumber the known terphenylquinones, the reverse is true in fungi, there being only three of the former, e.g., xercomic acid (Beaumont and Edwards, 1971) but about ten more of the latter, including the parent polyporic acid, isolated recently from cultures of *Polyporus nidulans* (Lindberg *et al.*, 1973). Oxidative ring cleavage of the quinone ring of free polyporic acid or its enzyme-bound equivalent leads to pulvic-acid derivatives with their characteristic 1,4-diphenylbuta-1, 3-diene system (Fig. 12). In this context it has been suggested that the newly identified pulvinamide (35) in its enzyme-bound form represents the immediate precursor of the pulvic acid derivatives (Maass, 1970a,b). Leaving the above hypotheses, the following events in the biosynthesis of these structures have been established using the radioactive precursors [1-¹⁴C]-DL-3-phenylalanine (Mosbach, 1964b; Maass *et al.*, 1964) and [¹⁴C]polyporic acid (Maass and Neish, 1967). (a) Two C₆ – C₃ units condense to form a terphenyl structure, which (b) subsequently undergoes ring fission to the different pulvic-acid derivatives.

E. Others

Besides the lichen substances discussed, a number of compounds have been isolated which simply represent general constituents of living cells, biosynthesis of which will not be taken up here. Sufficient to mention choline sulfate and ethanol sulfate esters, with which ³²S-labeling studies have recently been carried out (Feige and Simonis, 1969). Finally, attention should be drawn to a report by Jackson and Keller (1970) on the formation of an unusual ferric oxide mineral through the action by a tropical lichen on basalt as an example of "indirect" biosynthesis.

III. Conclusion: The State of The Problem

In conclusion, it appears appropriate to examine the question of what new information of value for the understanding of lichen biology in particular and biochemistry in general might be obtained from the biosynthetic studies discussed. First of all, in the examples given, such as for the biosynthesis of gyrophoric acid, usnic acid, and pulvic acid derivatives, all representing typical lichen substances, the information gained from the labeling patterns obtained is of value *per se*, giving insights into biosynthetic sequences involved. Future studies must be aimed at isolation and characterization of the participating enzyme systems, a number of which are certain to be new and not found elsewhere. It should be stressed, however, that great technical difficulties are likely to be encountered in such a project, as has already been experienced on preparing a cell-free "depside-synthesizing system" (Mosbach and Jakobsson, 1968). Some progress has also been made towards answering the fascinating question of whether the lichen symbionts collaborate in synthesis (anabolism) and breakdown (catabolism) of lichen substances. This problem is of general biochemical interest since, by looking at cells as small-scale symbiotic forms of life (Dubos, 1963; Mosbach, 1969), light may be thrown on general aspects of cell organization and metabolic control. In a number of cases it has been shown that the fungal symbiont alone can be made to produce lichen substances in culture, i.e., the anthraquinone parietin (Thomas, 1939), four pulvic acid derivatives (34, 36, 37, 40) (Mosbach, 1967), the dibasic fatty acid roccellic acid, and three chromones (Fox and Huneck, 1969), as well as usnic acid and the depsidone salazinic acid (Komiya and Shibata, 1969). In this connection it is of interest that vulpinic acid (37) has not been reported in the normal lichen association and can be described as an "induced" metabolite.

The case of depside formation is still unclear at present. Again, the fungal part may very well have all the enzymes that participate both in synthesis of the monocyclic units as well as in their subsequent coupling, possibly as activated thioesters such as orsellinyl-SCoA. The overall process could likewise take place in the fungus on a single multi-enzyme complex made up of two, three, or four orsellinic acid synthetase complexes associated in a way permitting direct condensation of the enzyme-bound monomers. It is also conceivable, however, that the algal part enzymically condenses the monocyclic units. This would be an analogy to the observed enzymic breakdown of depsides, for which the first step, hydrolysis of the depside by orsellinate depside hydrolase, possibly present in the algal symbiont, is followed by a decarboxylation step catalyzed by orsellinate decarboxylase, likely to be present in the fungal part (Mosbach and Ehrensvärd, 1966) (Fig. 5). More

detailed studies of these two enzymes isolated from the intact lichen revealed that both have remarkably high turnover rates or molecular activities of 1300 units/mg protein and 14.1 units/mg protein, respectively, which is rather unexpected for these slow-growing organisms (Schultz and Mosbach, 1971; Mosbach and Schultz, 1971). The successful and high degree of purification of these highly substrate-specific catabolic enzymes might open up new frontiers in lichen biochemistry. It is to be hoped that further such reports will soon be forthcoming.

Regardless of whether or not the algal component contains enzymes participating directly in the synthesis of lichen compounds, it should be stressed that it is the metabolic activity, on the part of the algal components and in particular the photosynthesis, that provides the prerequisites for the synthesis of lichen substances.

Finally one is faced with the question of why these compounds are formed and accumulate. High metabolite content is characteristic for most lichens. For instance, the depside lecanoric acid, a diorsellinic acid, has been found to constitute about 20% of dry weight of a lichen species (Seshadri and Subramanian, 1949). Such high levels are usually found in molds only under culture conditions with high glucose content in the medium. Since lichens are slow-growing organisms, probably as an adaptation to nitrogen deficiency, it appears that any overflow of carbon metabolites caused through the photosynthetic activity of the algal partner is channelled into formation of lichen substances. Likewise one would like to ascribe the fact that in lichens polyketides are mostly found as opposed to fatty acids, the content of which appears to be low, because of a lack of the reduced cofactor nicotinamide adenine dinucleotide phosphate (NADPH_2) required for fatty acid formation. That such a diversion can be established with molds has recently been indicated (Mosbach and Bävertoft, 1971).

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Chapter 17

ANTIBIOTICS IN LICHENS

K. O. VARTIA

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I. Folklore

Unofficially, in the folklore, the medicinal use of lichens has had a long history. *Evernia furfuracea*, for example, was apparently used for medicinal purposes in Egypt in the seventeenth and eighteenth centuries B.C., and is still brought to Egypt, in the present century, together with Iceland moss, as a foreign drug from Europe. Hippocrates recommended *Usnea barbata* for uterine trouble; the same lichen (*Usnea longissima*) was employed by the Chinese, under the name of "Sun-Lo," as an expectorant, and its surface powder for the treatment of ulcers. The Manchurian "Shihoa" medicine contained obtusatic acid of the depside type. The Malayans still employ the *Usnea* species medicinally for colds and as a tonic.

In the fifteenth century A.D. lichens constituted an important commercial article in Europe. In the eighteenth century *Peltigera canina* was sold as *pulvus antilyssus*, and the famous medicine *Lichen quercinus virides* contained mainly *Evernia prunastri*, *E. furfuracea*, and *Parmelia physodes*. *Lichen islandicus*, at that time, was a remedy held in high esteem, and *Mucus cranii humanii*, lichen grown on the human skull, "cost its weight in gold." Iceland moss and *Lobaria pulmonaria* were generally used for the treatment of catarrhal hemoptysis and pulmonary tuberculosis, e.g., in the form of cough-tea or "lichen chocolate," the former also being employed as a laxative. *Xanthoria parietina*, *Cladonia*, and *Pertusaria* species also "helped" in the most varied range

of diseases: fever, jaundice, epileptics, convulsions, "gout and other diseases," edema, etc. Gilg-Brandt (1922), Hoffmann-Krauer (1927, 1929–30) von Hovorka-Kronfeldt (1908, 1909), Jungbauer (1934), Lid-Storaker (1932), and Perez-Llano (1944).

In the Finnish folklore*—as an example which has been more carefully studied—the popular use of lichens as remedies seems to have been very widespread. Thus, for instance, yellow lichens (*Xanthoria parietina*, *X. polycarpa*, *Cetraria pinastri*) have been recommended for the treatment of jaundice: *similia similibus*. Obviously, because of the exterior resemblance, *Lobaria pulmonaria* has been used for pulmonary tuberculosis and cough and *Peltigera aphthosa* as remedy for infantile aphthae.

Lichens with the widest range of medicinal uses in Finland are probably the beard mosses (genera *Alectoria*, *Usnea*, and perhaps also *Ramalina thrausta*). The advice given is to place a bunch of lichen on a fresh or infected wound, for athlete's foot or other skin eruptions. Oral doses have been used against sore throat and toothache. *Cetraria islandica* and *Cladonia* species have been used as remedies for pulmonary tuberculosis and cough, usually taken in the form of so-called lichen milk. Notes on so-called cup-lichens (*Cladonia coccifera*, *C. deformis*, etc.) and stone mosses (*Parmelia*, *Stereocaulon*, etc.) are along similar lines.

The lichens have often been mentioned in cattle breeding, for instance, as a remedy against inflammation of udders.

The use of lichens in the folklore history of Sweden has been studied by Ahmadjian and Nilsson (1963).

As can be seen from the above, superstition and a primitive medical skill, based on nature and on rough empiricism, are always mixed up in the popular medicinal use of lichens. It is of interest to note that, in the use of lichens for coughs or expressly for pulmonary tuberculosis, old folklore exists from different parts of the globe and that present-day science, too, has found fairly strong antibiotics against the tuberculosis bacillus in the same species to some extent (*Usnea* sp., *Cetraria islandica*). As to the lichen species mentioned above, which have obviously been popular as medicines, no lichen acids proper have been found in *Peltigera aphthosa*, and the lichen substances contained in *Lobaria pulmonaria* and in the *Xanthoria* species have not been found to be active against many pathogenic bacteria and fungi; the attention devoted to these species, however, is readily explicable from the general rule *similia similibus*. But *Cladonia alpestris*, *C. silvatica*, *C. coccifera*, *C. deformis*, *Ramalina thrausta*, *Evernia prunastri*, and all *Usnea*-species contain active usnic acid; *Cetraria islandica* contains *d*-protoliche-

*Collection of Folklore Archives of the Finnish Literary Society and of the Finnish Dictionary Foundation.

sterinic acid; *Parmelia physodes*, and *Evernia furfuracea* contain physodic acid; and *Cetraria pinastri* contains vulpinic and pinastric acids (Zopf, 1907), all of which have been found to be chemically active. The concentration of the active substances, it is true, is not very high, but the effect is enhanced by the fact that the lichen substances primarily exist on the exterior surface of the thallus in the form of powder or crystals [the most common active substance, usnic acid is always there (Tobler, 1925)], and hence, although not readily soluble, they can diffuse into their surroundings.

II. Pharmacopoeias

Purely on the basis of their medicinal use in folklore, lichens have been listed in the pharmacopoeias. The oldest European pharmacopoeia (of 1546), however, includes no lichens among the drugs listed, whereas the "Pharmacopoeia Universalis" of 1846 lists a great number of lichen medicines. The following species are mentioned: *Cetraria islandica*, *C. nivale*, *Cladonia coccifera*, *Cladonia pyxidata*, *Usnea plicata*, *Peltigera canina*, *P. venosa*, *P. horizontalis*, *P. polydactyla*, *Lobaria pulmonaria*, *Xanthoria parietina*, and *Evernia prunastri*. According to this work, Iceland moss has been included among the drugs listed in 50 contemporary pharmacopoeias or dispensaries, recommended primarily as a cure for coughs. Lichens have gradually disappeared from the list of official medicines, paradoxically, just before the discovery of antibiotic properties in lichens. However, at least the Japanese pharmacopeia of 1922, the Estonian of 1937, and the French, Swiss, and German pharmacopoeias that are valid at present include Iceland moss as a drug, the French one both as a paste and as a potion. *Lichen islandicus* is usually recommended for use as cough medicine and for its appetizing qualities. In Sweden about 600 pounds of dried Iceland moss are sold each year.

III. Lichen Substances

The explanation of the chemistry of lichens was begun by Pfaff (1826), when he found *eine eigentümliche Säure* from Iceland moss (*Cetraria islandica*). Pfaff's finding, evidently fumarprotocetraric acid (Hesse, 1904), was the beginning of the rapidly expanding field of lichen chemistry. Usnic acid was discovered in 1844 by Knop. The largest number of lichen substances has been isolated by Hesse (1861-1905) and Zopf (1907), and as a result of their investigations they totaled nearly 150 in number by 1907. Of this total, the structure of only a few had been studied; several of them later proved to be mutually identical or else impure.

The decisive work in the study of the structure of lichen substances has been carried out by Asahina and co-workers (1930–1942). Mainly on the basis of the classification worked out by Zopf, modestly termed by him as a “temporary one, to provide a general picture,” Asahina proposed a general system of known lichen substances as follows

1. Fatty acids and lactones
2. The zeorin group, neutral compounds not saponifiable by alkalis, the formulae of which are not well known
3. Pulvic acid derivatives
4. Cumarone derivatives, the only representative usnic acid with its derivatives
5. Depsides
6. Depsidones
7. Anthraquinone derivatives

In spite of the differences in structural formulae, lichen substances have a considerable number of properties in common. All lichen substances proper are crystalline and in most cases acid in character (which accounts for the widely used designation lichen acids), and even in the form of alkaline salts their solubility in water is very poor. Several lichen substances are optically active—this applies to all aliphatics; usnic acid even has an unusually high specific rotation. The color of the crystals varies from colorless to white and reddish yellow. Several lichen substances have a very bitter taste.

The amount of lichen substances contained in the different species varies greatly. From *Parmelia tinctorum*, 23.5% of lecanoric acid alone was obtained; from *Lepraria chlorina*, 10.5% of vulpinic acid; from *Aleatoria ochroleuca*, 5.5% of usnic acid (Zopf, 1907), whereas the content of the protolichesterinic acids in Iceland moss amounts to only a few promilles. The normal amount is possibly around 1%.

IV. Pharmacological Studies

Several lichen acids have been studied from the pharmacological point of view. Alms (1832) recommended picrolichenic acid (subsequently identified by Zopf) for the treatment of *Wechselfieber*, prompted in the first place by its taste, reminiscent of quinine, and similarly Lebail later (1853).

Ramm (1890) and Neuberg (1893) carried out animal tests with cetrarin, finding it relatively harmless for mammals (lethal dose 0.2 gm per kilogram of body weight). Injected in the form of sodium salt, the acid induced a specific acceleration in peristalsis, increased blood pressure, and secretion of

bile. According to Neuberg, accelerated peristalsis was also produced in the isolated bowels of a dog, and he recommended the substance as a remedy for anemia and lack of appetite. Later on, cetrarin was established to be ethylprotocetraric acid (Koller and Krakauer, 1929). Guesdon (1901) reported good results with cetraric and protocetraric acids as antiemetics in pregnancy and tuberculosis.

The vulpinic acid occurring in *Letharia vulpina*, used as a fox poison, has been repeatedly studied toxicologically. Kobert (1892) and Neuberg (1893) gave the lethal dose for mammals as 20–30 mg per kilogram of body weight; the same figure applies to the closely related pinastriac acid. The toxicity of vulpinic acid was later found to be lower; Santesson (1939) obtained 78.8 mg per kilogram of body weight as the lethal dose for a cat, the most noticeable symptom of poisoning being acute dyspnea. Brodersen and Kjaer (1946) reported the lethal dose for a mouse as 75.0 mg per kilogram of body weight.

Mikoshiba (1933) published some investigations on the pharmacology of usnic acid and its derivatives. According to his studies, usnic acid has a papaverine-like effect on the smooth muscles but is less poisonous than papaverine; the lethal dose for a mouse is 7.0 mg/10 mg subcutaneously and 0.25/20 gm intravenously applied (= 0.7 and 0.025 gm/kg).

Fischer and Toth (1938) investigated certain effects of lichesterinic acid. The hemolytic index obtained by them for defibrinated blood was 40,000; the index was found to drop to 5000 if equal amounts of lichesterinic acid and cholesterol were added. The lethal dose obtained for a frog was 200 µg/gm, and for a mouse, intravenously, 100 µg/gm (0.2 and 0.1 gm/kg); the fish index was 25,000, normal drop number of 186, and foam value 1:25,000. Of the greatest interest is the ability of the acid, as found by them, to promote resorption greatly: with a frog, the symptoms of strychnine poisoning were produced percutaneously by 1/2–1/2.5 of the ordinary amount with a small simultaneous administration of lichesterinic acid. The absorption-increasing dose for lichesterinic acid, in micrograms per grams of a frog, was 3 µg after 45 minutes, obtained from the curare test. They presume that part of the medicinal effects of Iceland moss is based on this property of lichesterinic acid.

Fuzikawa found (1939) that the phenols and their derivatives, components of lichen depsides and depsidones, possess remarkably strong antiseptic qualities.

V. Antibiotics in Lichens

The study of lichens and lichen substances, from the antibiotic point of view, started in 1944, when Burkholder *et al.* (1944) published the first qualitative study of the antibiotic properties of lichens. They tested 100 American

lichen species in relation to *Staphylococcus aureus* and *Bacillus subtilis*, by the Oxford Cup method, extracting a certain weight amount of lichen by phosphorus buffer solution. Fifty-two species (52%) prevented the growth of either one or both of the bacteria studied. With a few exceptions, the lichens studied had no effect at all on gram-negative bacteria. The usnic acid isolated from *Cladonia mitis* prevented the growth of *B. subtilis*, but not of the *Staphylococcus* or the colon bacillus. Stoll (1947) and Stoll *et al.* (1947) boiled some lichens for a short while with 5% alkaline glucose solution, and made corresponding plate tests with the extracts obtained: out of 58 Swiss species, 38 proved to be active against *Staphylococcus* (65.5%); clearly the most general active substance was usnic acid. In the plate tests with pieces of lichen by Vartia (1949, 1950a,b) 75 of 149 Finnish lichen species investigated revealed properties preventing the growth of various gram-positive bacteria 50%* (Fig. 1).

Bargellini *et al.* (1946) observed that usnic acid is active against the bacterial species *S. aureus*, *C. diphtheriae*, and *B. subtilis*, but did not inhibit gram-negative bacteria.

Quantitative studies of crystalline lichen substances were initiated by Barry (1946). He found that the chlorine-containing lichen substance diploicin, previously isolated from *Buellia canescens*, inhibited the growth of human tuberculosis and diphtheria bacillus 1:100,000, and that of *Mycobacterium smegmatis* 1:70,000 *in vitro*. He also pointed out that the only compound of the diphenyl ether type (such as the product of alkaline hydrolysis of diploicin) occurring in a normal organism is thyroxine, but he could not establish any definite physiological analogy between it and diploicin. Subsequent studies have primarily dealt with usnic acid, especially its inhibiting effect on the growth of *Mycobacterium tuberculosis*. Marshak (1947) isolated from the lichen *Ramalina reticulata* a crystalline substance, which he subsequently found to be usnic acid, completely inhibiting the growth of different strains of human tuberculosis 1:20,000–1:50,000, and weakening their growth 1:200,000–1:2,000,000. The growth of bovine tuberculosis was completely inhibited in 1:20,000, and at this titer two avian strains were only partly inhibited. The growth of *Staphylococcus*, *Streptococcus*, and *Pneumococcus* was inhibited in 1:20,000. The effect was evidently bactericidal. Stoll *et al.* (1947) obtained the following completely inhibiting titers for the usnic acid produced as by-product from *Cetraria islandica*: human tuberculosis 1:64,000–1:800,000, bovine 1:500,000, an avian strain 1:125,000, *Staphylococcus aureus* and *Streptococcus pyogenes* 1:100,000; no effect on colon bacillus, *Salmonella typhimurium*, and dysentery bacillus. In a third work

*Eight of 100 lichen species contained phytagglutinins; *Peltigera aphtsosa* agglutinated human red cells in titer 1:400 as saline extract (Estola and Vartia, 1955).



FIG. 1. Pieces of lichens on petri dishes inoculated with *Bacillus subtilis* and *Sarcina lutea*. Some lichens inhibit the growth of bacteria, others seem to improve it (from Vartia, 1949).

published the same year, Bustinza and Lopez (1946) reported complete inhibition by usnic acid isolated from *Usnea barbata* in the form of sodium salt on human tuberculosis cultivated on glycerine broth in 1:500,000, an avian strain 1:100,000. With Dubos' culture medium a 5–10 times greater concentration was necessary. Bustinza and Lopez tried to combine streptomycin and usnic acid into a salt but the result did not crystallize, possibly because it was a mixture of usnicates of very poor solubility. Shibata *et al.* (1948) Shibata and Miura (1948, 1949) compared the activities of usnic acid and its derivatives including didymic acid and related compounds. With the different optic forms of usnic acid and their sodium salts and didymic acids with derivatives, the inhibiting titer was, with an avian tuberculosis strain, 1:160,000 and with *Staphylococcus* 1:160,000–1/320,000. Acetylation of hydroxyl groups reduces the effectiveness from one-half to one-quarter of that of the existing one. Marshak *et al.* (1949) studied the activity of usnic acid and 33 related compounds, as well as of vulpinic acid: only monoacetyl usnic acid against human tuberculosis obtained the same titer as usnic acid: 1:100,000. Vartia (1949), for usnic acid isolated from *Cladonia alpestris*, obtained a completely inhibiting titer of 1:60,000, and a growth-retarding effect in 1:160,000. Klosa (1947, 1949) reported the inhibitory titer of 1:1,000,000 for usnic acid against *Mycobacterium tuberculosis*, *Streptococcus*, and *Staphylococcus*. Döpp and Bersch (1950) reported growth-weakening titer of 1:500,000 of usnic acid against the human tuberculosis bacillus.

With reference to other lichen substances, Burkholder *et al.* (1945) confirmed that atranorin and fumarprotocetraric acid are inactive. Asano (1945) determined the antibacterial action of lichesterinic acid and its derivative against *Staphylococcus*. Stoll *et al.* (1947) indicated that the effect of vulpinic, *d*-protolichesterinic, lichesterinic, dihydrolichesterinic, physodic, and diffratic acids was somewhat similar to that of the usnic acid. Cavallito *et al.* (1948) reported on a dilution series made with aliphatic lactones, including protolichesterinic, lichesterinic, and dihydrolichesterinic acids: *Mycobacterium tuberculosis*, 1:600,000–1:1,000,000; *Streptococcus pyogenes*, 1:300,000–1:50,000; *Staphylococcus*, 1:100,000; and *C. welchii*, 1:200,000–2,000,000. The results were in compliance with those obtained with other aliphatic lactones. Klosa (1949) reported the inhibitory titer of evernic acid against tuberculosis bacillus, *Streptococcus*, and *Staphylococcus* at 1:1,000,000. Shibata and Miura (1948) published results of testing 23 lichen substances against avian tuberculosis and *Staphylococcus aureus*: *l*-lichesterinic acid, *l*-dihydroprotolichesterinic acid and perlatoric acid inhibited growth of tuberculosis bacilli in 1:40,000–1:80,000 and, besides, protolichesterinic acid, spherophorin, divaricatic, anziaic acid, olivetoric, seki-kaic, ramalinolic, boninic, and lobaric acids had clear effects against staphylococci. Caperatic and rangiformic acid, zeorin, atranorin, thamnolic,

salazinic, psoromic, fumarprotocetraric acids, pannarin, and endocrin were inactive.

Vartia (1949–1950a,b) tested 20 lichen substances, of which five, lichesterlyc and pinastic acids, anilide of pulvic acid, and gyrophoric acid, were active against *Mycobacterium tuberculosis* and gram-positive bacteria. Further *d*-protolichesterinic, *d*-lichesterinic, divaricatic acids, atranol, physodic acid, and diploicin were active against several gram-positive bacteria. Vartia also proved that nine lichen substances have an inhibitory effect against several pathogenic fungi.

According to the investigations referred to above, the following conclusions can be made.

1. More than 50% of the lichen species have antibiotic properties.
2. The antibiotic effects are based on lichen substances of which the most effective are usnic acid, the lichesterinic acid group, as well as orcinol-type depsides and depsidones.
3. In the folklore, lichens have been used for thousands of years in several parts of the globe, to a remarkable extent quite logically.

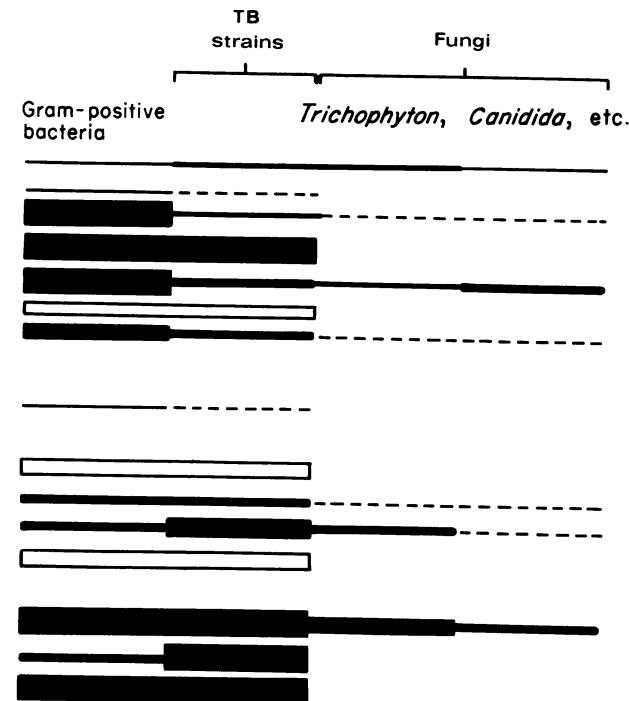
Figure 2 shows an overall view of antibiotics in lichen substances; it is approximate. One should note that the variable results, especially in the titers dealing with inhibition of tuberculosis strains, are due to many factors: the culture medium used, the inoculation type and amount, the cultivation time, etc. According to the schematic illustration, the main effect is on the rapidly growing gram-positive bacteria, *Mycobacterium tuberculosis*, and some pathogenic fungi.

In vivo tests—with animals or human patients—are the next step. Excluding folklore, the first report was made by Chiba (1898): he reported good results with *Usnea* tinctures taken per os with patients suffering from lymphadenitis tuberculosa colli. Shibata *et al.* (1948), Marshak (1947), Marshak *et al.* (1947, 1949), Marshak and Kuschner (1950) got somewhat contradictory results with inoculation of tuberculosis in guinea pigs treated with usnic acid. Pätiälä *et al.* (1948) and Jäntti (1952) found that usnic acid retarded the course and changed the type of tuberculosis in guinea pigs. In preliminary tests on human patients they found that a daily dose of 3 gm caused indefinite pains in the liver; 1 gm per day produced no symptoms of poisoning. Pätiälä (1949) has also published a case of lupus vulgaris treated with usnic acid.

The situation *in vivo* usually presents new problems and difficulties: questions of resorption, toxicity, effect of serum albumin, allergic reactions and so on. The fate of the lichesterinic acid group was disturbed *in vitro* when serum albumin was present. Tests with mice gave no positive result (Vartia and Tervillä, 1952).

In the case of usnic acid the problem has been its very poor solubility

Fatty acid-lactone group	<ul style="list-style-type: none"> Caperatic acid Rangiformic acid Protolichesterinic acid Dihydroprotolichesterinic acid Lichesterinic acid Dihydrolichesterinic acid Lichesterylic acid
Zeorin group	Zeorin
Pulvic-acid derivatives	<ul style="list-style-type: none"> Vulpinic acid Pinastric acid Anilide of pulvic acid Calycin
Cumarone derivatives (only distinctly active)	<ul style="list-style-type: none"> Usnic acid Diacytusnic acid Didymic acid deriv.



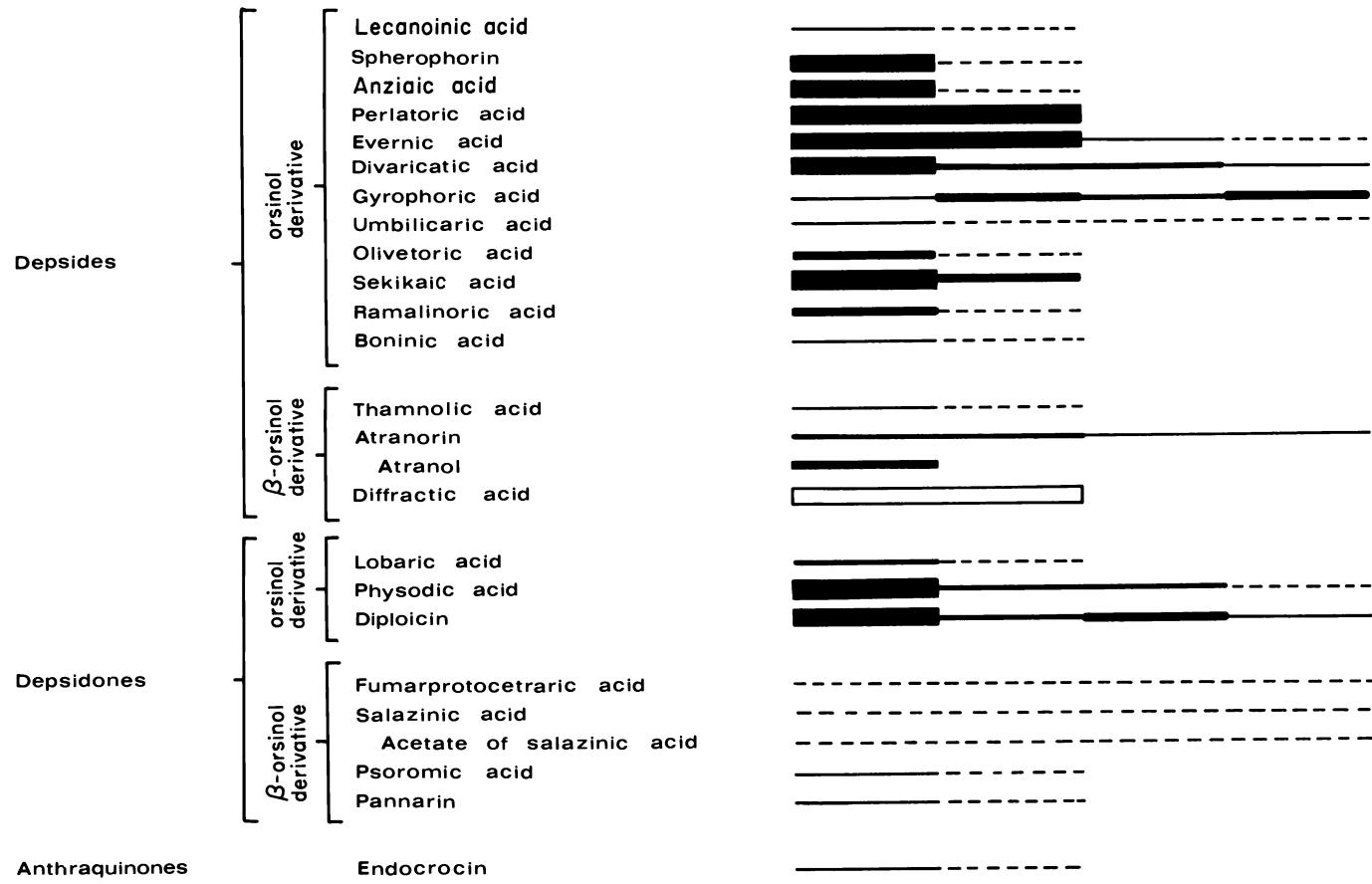


FIG. 2. Approximate diagram of the activity of the lichen substances studied on the different groups of microorganisms. The width of the ribbon symbolizes the strength of the antibiotic effect. ----- no effect.

(usnic acid dissolves in water less than 1:5 ml, its sodium salt at approx. 1:600) (Heilala and Siintola, 1949a). It has been used, however, as ointments in infectious skin diseases and also per os. [In Austria with the name Usniankin as wound salve and powder, in Germany Evosin I (usnic and evernic acids), Evosin II (usnic, physodic, and physodalic acids) and in Switzerland Lichusnin.] The Finnish team headed by Virtanen *et al.* (1954; Virtanen, 1954, 1955; Virtanen and Kärki, 1956; Virtanen and Niemi, 1955; Virtanen and Vähäatalo, 1956; Korlekangas and Virtanen, 1956) has done extensive work to render usnic acid soluble, especially by combining the acid with a suitable base compound. Having made more than a hundred combinations with several amino compounds, sulfonamides, and known antibiotic substances as isonicotinic acid hydrazide, streptomycin, *p*-aminosalicylic acid, neomycin, and cycloserin, they finally arrived at a compound as follows: benzylidimethyl-(2-[2-(*p*-1, 1, 3, 3)-tetramethylbutylphenoxyethoxy] ethylammonium hydroxide, which they named appropriately USNO. The LD₅₀ of USNO is 125 mg/kg for a white mouse. It has given good results in skin diseases (Kilpiö, 1956) and in veterinary use (mastitis in cows, Grignani and Tomaselli, 1957). Its antifungal effects are proved against yeasts (Capriotti, 1959, 1961) and in the treatment of *Trichophyton gallinae* (Virtanen and Kilpiö, 1957).

Neither Usno nor other lichen medicines have, however, gained a permanent position in the medical praxis: the fight for a place in the sun among antibiotics is too hard. Since the last decade the medicinal research of lichens has turned to new directions: the untiring Japanese group Nakazava, Komatsu, Hamada, Fujikawa, Hirai, Shibata *et al.* has studied the antitumor activity of lichens intensively since 1962. This activity obviously depends on the polysaccharide component in lichens, as found in *Umbilicaria*, *Lasallia*, *Cetraria*, *Cladonia*, *Parmelia*, and *Usnea*, and from psoromic acid.

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Part V

SYMBIONT INTERACTIONS

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Chapter 18

RESYNTHESIS OF LICHENS

V. AHMADJIAN

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I. Introduction

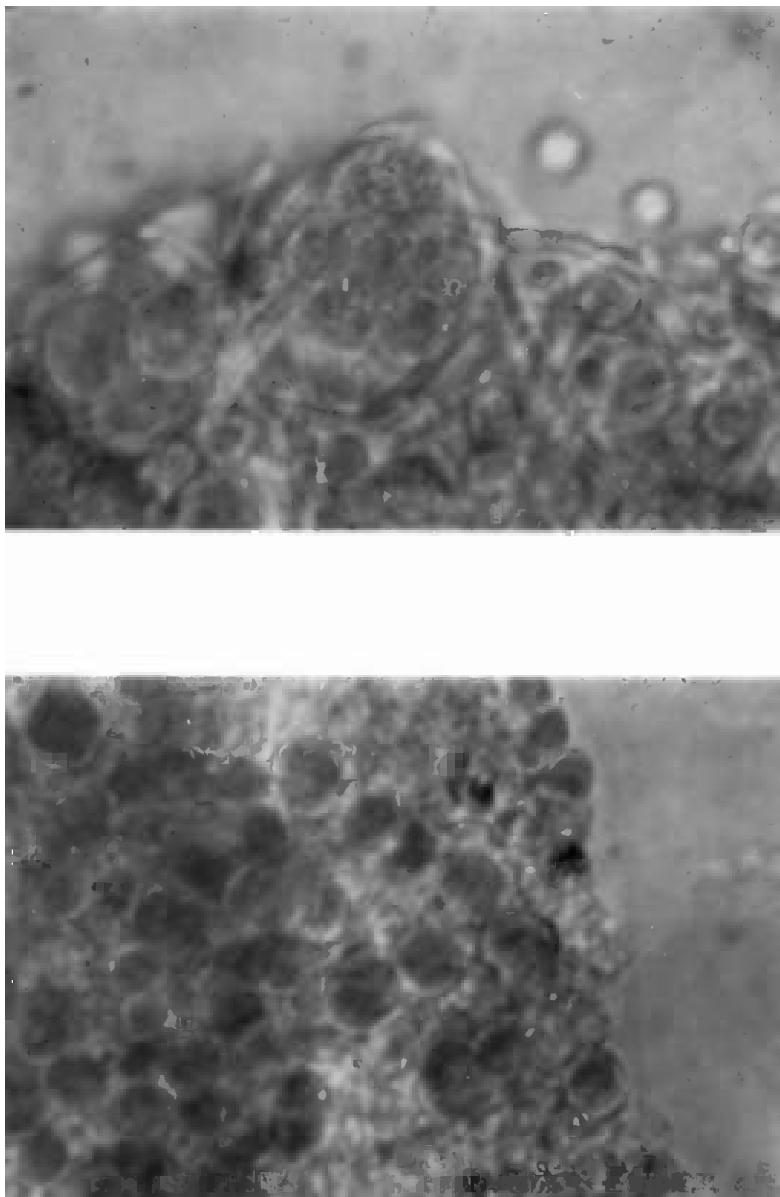
The resynthesis of a lichen has been a major goal in lichenology and a natural corollary of the theory that lichens are symbiotic associations of fungi and algae. The theory, proposed as a general one by Schwendener in 1868, split the lichenological community into bitter factions, and stimulated a series of resynthesis experiments that were intended to prove or disprove the theory. The attempts were not completely successful and the results were interpreted in different ways. Opponents of the theory noted the failure of the recombined symbionts to develop into a lichen thallus, while supporters considered achievement of the earliest stages of resynthesis as confirmation of their belief. Arguments proposed by one side were countered by the other. For example, observations that hyphae did not bud off the algal (=gonidia) cells as originally thought were countered by the argument (Krempelhuber, 1875) that the algae were formed only in advanced stages of thallus development or only by certain hyphae. Schwendener's theory finally was accepted, not because of the resynthesis studies, but by isolations of the algal symbionts and their correlation with free-living forms. Attempts at resynthesis persisted, however, even to recent times but still with limited success.

The reasons why the symbionts do not recombine readily to form a lichen are not understood fully. A general answer, proposed earlier by De Bary (1887), is that lichen algae have become so adapted to the symbiosis with fungi that they cannot live in the free-living state. Frank (1876) observed that algal cells engulfed by the marginal hypae of *Pertusaria* did not survive (see also, Ahmadjian, 1960) and concluded that algae in a lichen are specifically adapted to acting as symbionts. This adaptation becomes a limiting factor in resynthesis experiments. The success of algae and fungi as lichen symbionts implies a set of specialized and possibly irreversible characteristics. We know that removal of the alga from its special and integrative environment within the lichen causes significant changes within the algal cells. The success that has occurred in resynthesis studies has been due to maintaining the isolated symbionts under conditions that simulate their natural symbiotic environment.

II. Developmental Stages of Synthesis

The different developmental stages in the lichenization process have been elucidated in studies with *Acarospora fuscata* (Ahmadjian, 1962), *Cladonia cristatella* (Ahmadjian, 1966), and *Endocarpon pusillum* (Bertsch and Butin, 1967; Ahmadjian and Heikkilä, 1970). The results with *A. fuscata* revealed the stages in the development of the vegetative thallus, while the studies with *C. cristatella* showed the different steps in the production of fruiting bodies. Complete development of thallus and fruits occurred with *E. pusillum*.

The initial stage in the physical process of synthesis is envelopment of the algal cells by fungal hyphae (Fig. 1). It was thought that this initial fungal response was due to substances that diffused from the algal cells, but this seems unlikely in view of studies that indicate excretion of substances by the alga decreases rapidly after the cells are isolated from the thallus. In most of the synthesis investigations, the symbionts were cultured separately for a long time before the studies. Earlier studies reported that the fungal hyphae respond to the surface of the algal cells and that the hyphae would encircle any rounded object (cf. Ahmadjian, 1960). The initial response, therefore, is not specific. The fungus will encircle glass beads and rods as well as foreign algae. After envelopment of the algal cells, the hyphae divide to form a compact mass of cells that make up a type of tissue called a pseudoparenchyma. This tissue proliferates to encompass more algal cells (Fig. 2). This second step represents the first lichenized unit and a functional relationship between the symbionts has been demonstrated (Ahmadjian, 1962). The first two stages occurred on a nutrient-deficient substrate, i.e., either a natural substrate or purified agar. Development beyond the second stage



FIGS. 1-2. *Acarospora fuscata* resynthesis on purified agar. Fig. 1, envelopment of algal cells by fungal hyphae; Fig. 2, cells of *Trebouxia* phycobiont enclosed within fungal pseudoparenchyma.

occurred only when the units were exposed to drying. Stage three is the differentiation into a layered thallus and the formation of cortical, algal, and medullary layers.

A well-defined thallus, i.e., one that was comparable to the natural one, was achieved only with *E. pusillum*. *Acarospora fuscata* and *C. cristatella* formed rudimentary thalli, ones in which the upper cortex was not well formed. The natural brown pigmentation of *A. fuscata* and *E. pusillum* did not develop in the synthesis cultures probably because the light intensities under which the symbionts were grown were so low.

The sequential stages of lichen synthesis have been described also by Werner (1968).

The algal cells undergo changes in size after they become associated with the fungus. *Trebouxia* cells of *A. fuscata* become smaller by about one-half after being enclosed in the fungal pseudoparenchyma. In contrast, the cells of *Stichococcus diplosphaera*, the phycobiont of *E. pusillum*, enlarge two or three times after they are lichenized. For both algae, the chloroplast enlarges and fills more of the cell, the pyrenoid becomes larger and clearer and the mode of reproduction is altered.

Haustoria were evident in all three synthesized lichens. They were small and peglike in *A. fuscata* and *E. pusillum* and in the latter lichen they were observable only with the electron microscope (Ahmadjian and Jacobs, 1970).

Fruiting of the fungus was accomplished with *C. cristatella* and *E. pusillum*. With *C. cristatella* both juvenile apothecia and mature pycnidia (= spermatangia) were formed. The apothecia were borne on small podetia and showed the beginnings of the hymenial layer and the presence of ascogonial filaments and ascogenouslike hyphae. Asci were not present. The apothecia and podetia were small, 1–2 mm high, and did not develop beyond the rudimentary stages. In many respects the juvenile apothecia resembled protoapothecia of other ascomycetous fungi. This would explain why the development of these structures stops after the initial development phase. Further development of asci, spores, and maturation of the ascocarp depends on fertilization. There was some indication that this is a viable phenomenon in lichen fungi since trichogynes were seen protruding from the surface of the protoapothecia and pycniospores (= spermatia) were seen attached to the tips of the trichogynes. The development of the ascocarp as a somatic structure was indicated by the fact that the tissue could be cultured on an agar medium (V. Ahmadjian, unpublished results). Segments of protoapothecia that were placed onto a Lilly and Barnett agar medium ($\text{pH}=4.0$) grew well and produced a cluster of well-defined protoapothecia which resembled closely the natural apothecia. Fragments of these apothecia placed onto a malt–yeast extract agar medium developed entirely into vegetative hyphae.

Fruiting of the fungus occurred independently of the algal symbiont as well as the substratum. Low pH stimulated development of the fruits of *C. cristatella*. Drying also is a stimulatory factor in ascocarp development but it is more important for pycnidial development. Formation of juvenile apothecia was not as frequent as pycnidia and occurred on colonies that were not subjected to drying. *Endocarpon pusillum* developed perithecia and mature, functional spores after development of the thallus squamules with the alga. The fungus alone did not fruit.

III. Physiological Relationships between the Symbionts

Investigations with *A. fuscata* have shown that the functional interplay between the symbionts occurs during the second stage of synthesis, i.e., when the algal cells are enveloped by fungal pseudoparenchyma. A recent study (Hill and Ahmadjian, 1972) indicates that a relationship may exist even before there is an observable physical union between the symbionts.

In the synthesis of *A. fuscata* cells of the pseudoparenchyma formed water-soluble red pigments (Diner *et al.*, 1964) while fungal cells not in contact with algae did not form the pigments. Since these pigments were produced in culture by the isolated mycobiont only on media that contained organic supplements, this was indirect evidence that substances passed from the alga to the fungus. The pseudoparenchyma was also responsible for protecting the algal cells from high light intensities.

In a study of *C. cristatella*, Hill and Ahmadjian (1972) determined the incorporation of $H^{14}CO_3$ into a joint culture of the symbionts. The culture was grown on a malt-yeast extract medium which, according to previous studies, does not support lichenization. Thus, there were no physical contacts between the symbionts in this mixed culture. Analysis of the distribution of ^{14}C showed results that were intermediate between those obtained with the cultured alga and with the intact lichen. Specifically, the amount of ^{14}C incorporated into the ethanol-insoluble fraction was the same or more than that in the alga but the amount incorporated into lipid was closer to that of the lichen. Moreover, some ^{14}C occurred in mannitol which is formed only by the fungus in a lichen. The presence of [^{14}C]mannitol is an indication that ^{14}C was transferred from the alga to the fungus. The results from the joint culture indicated that some modification of the algal metabolism had occurred. The *Trebouxia* symbiont alone in culture incorporated most of the ^{14}C in sugar phosphates, amino and organic acids, and oligosaccharides and relatively little in ribitol. In the intact lichen most of the ^{14}C was in ribitol and arabitol and later in mannitol. On a nutrient-rich medium the alga will use the carbon fixed during photosynthesis for growth, but

with a limited supply of nutrients, as would occur in lichens, most of the carbon would be incorporated into carbohydrate such as ribitol, an excess of which would be used to supply the fungus. In the joint culture the metabolism of the alga was modified from its usual pattern on nutrient-rich medium and carbon became incorporated into carbohydrate instead of lipid and insoluble substances. It was not clear what caused this modification but it implies some type of influence by the fungus on the alga.

IV. Lichens Synthesized in Laboratory Cultures

A. *Endocarpon pusillum*

The first spore-to-spore synthesis of a lichen was accomplished recently with *E. pusillum* (Ahmadjian and Heikkilä, 1970). Fungal and algal symbionts were isolated from each other, grown separately, and then recombined to form all the lichenized stages including perithecia with functional spores. The complete synthesis occurred only on a soil surface that was subjected to alternate periods of drying, wetting, light, and darkness. The critical condition was drying and this was accomplished by allowing the small, clay flowerpots that contained the soil to dry out slowly after they had been allowed to soak thoroughly from water absorbed from the bottom of the flowerpot.

Why *E. pusillum* is the only lichen fungus known that can recombine successfully with its algal symbiont to form a complete lichen is not clear. It may be related to the nature of the algal symbiont and the presumably recent origin of this lichen. *Endocarpon pusillum* belongs to a group of lichens that have algal cells within the hymenium of the peritheciun. The algal cells are carried along with the spores during their discharge from the ascii. The effectiveness of this method of propagation was seen in the synthesis cultures where the soil surface after a few months was covered with squamules that developed from the spores and hymenial algae. The algal symbiont is *Stichococcus diplosphaera* and this alga undergoes a remarkable transformation when it becomes associated with the fungus. The cells increase in size, and the chloroplast and pyrenoid become greatly enlarged (Fig. 3). Squamule development is rapid and may occur within one month after the symbionts are mixed together on the soil surface (Fig. 4). The changes of the algal cells are not permanent and when the cells are freed from the fungal hyphae they resume after several divisions their original size and characteristics. The ability of *S. diplosphaera* to return to its original state explains the origin of the hymenial algae. Algal cells that become trapped within the developing peritheciun become freed of fungal hyphae and divide to form the hymenial colony.

Attempts to synthesize an *Endocarpon* fungus and the algal symbiont (= *Stichococcus mirabilis*) of *Staurothele clopima* and vice versa were not successful. This indicates a specificity among the symbionts.

The mycobiont of this lichen has more of a minimal requirement for growth than do those of other lichens. The fungus grew well on Bold's mineral-soil extract agar and formed a loose, mycelial growth. In fact, soil or soil extract was a growth requirement for this fungus.

B. Lichens with Trebouxia Phycobionts

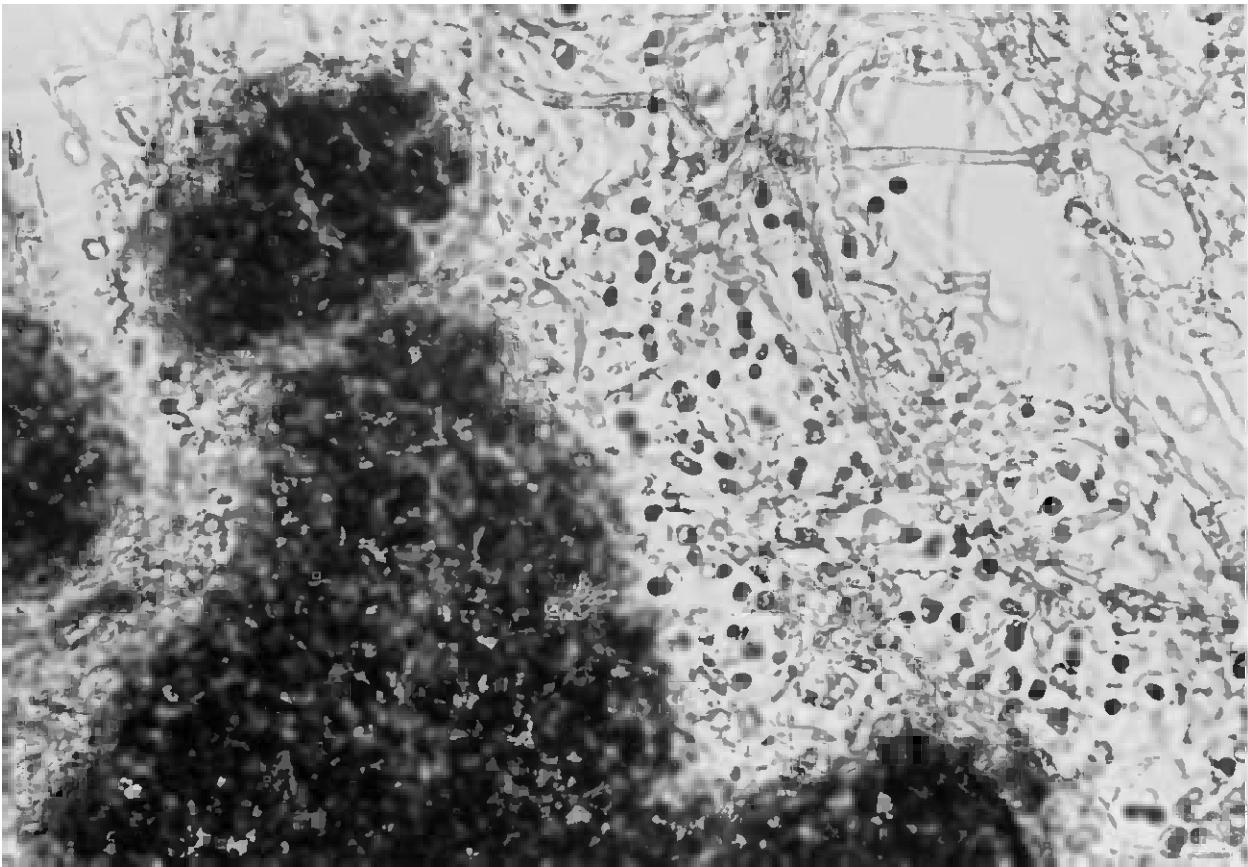
The resynthesis of lichens with *Trebouxia* as a phycobiont has not been completely successful. *Trebouxia* is the most common of the algal symbionts and is found in over half of the known lichen associations. The success of this alga as a symbiont implies a highly specialized nature; indeed, the alga has not been found in the free-living state. One theory is that *Trebouxia* is a genus whose traits developed in association with lichen fungi and that it now has evolved to a point where it has little relationship to other free-living forms or ancestral types (Ahmadjian, 1970). This type of specialization of characteristics would make synthesis experiments with this alga difficult. This theory, moreover, precludes natural resynthesis of lichens. If *Trebouxia* does not occur in free-living populations then there is nothing with which the fungus can recombine. What the fate is of the large numbers of fungal spores that are produced by lichens is unclear. A possibility is that the spores encounter isida and soredia and use the *Trebouxia* cells within these asexual propagules or that they establish a type of parasymbiosis with established thalli, and after a certain stage of development that includes incorporation of algal cells, they break off and develop independently.

A factor that greatly influences synthesis experiments is the changes that the *Trebouxia* symbionts undergo when they are isolated from the lichen thallus and cultured separately in organic media. These changes are morphological and physiological and they occur soon after isolation. The limited success of the synthesis experiments with *Trebouxia* has been due to bringing back the alga as close as possible to its natural state by using inorganic substrates such as purified agar or natural substrates such as wood fragments and pieces of stone. The changes that the *Trebouxia* phycobiont undergoes soon after isolation are significant and are listed in Table I.

C. Lichens with Filamentous Phycobionts

1. BLUE-GREEN ALGAE

There have been few synthetic studies of lichens with blue-green algae, undoubtedly because of the difficulty in separating the symbionts into axenic



Figs. 3–4. *Endocarpon pusillum* resynthesis on soil. Fig. 3, comparison of cells of the phycobiont *Stichococcus diplosphaera* enclosed in fungal hyphae with cells not enveloped by hyphae. Note the increased size of the algal cells that are lichenized.

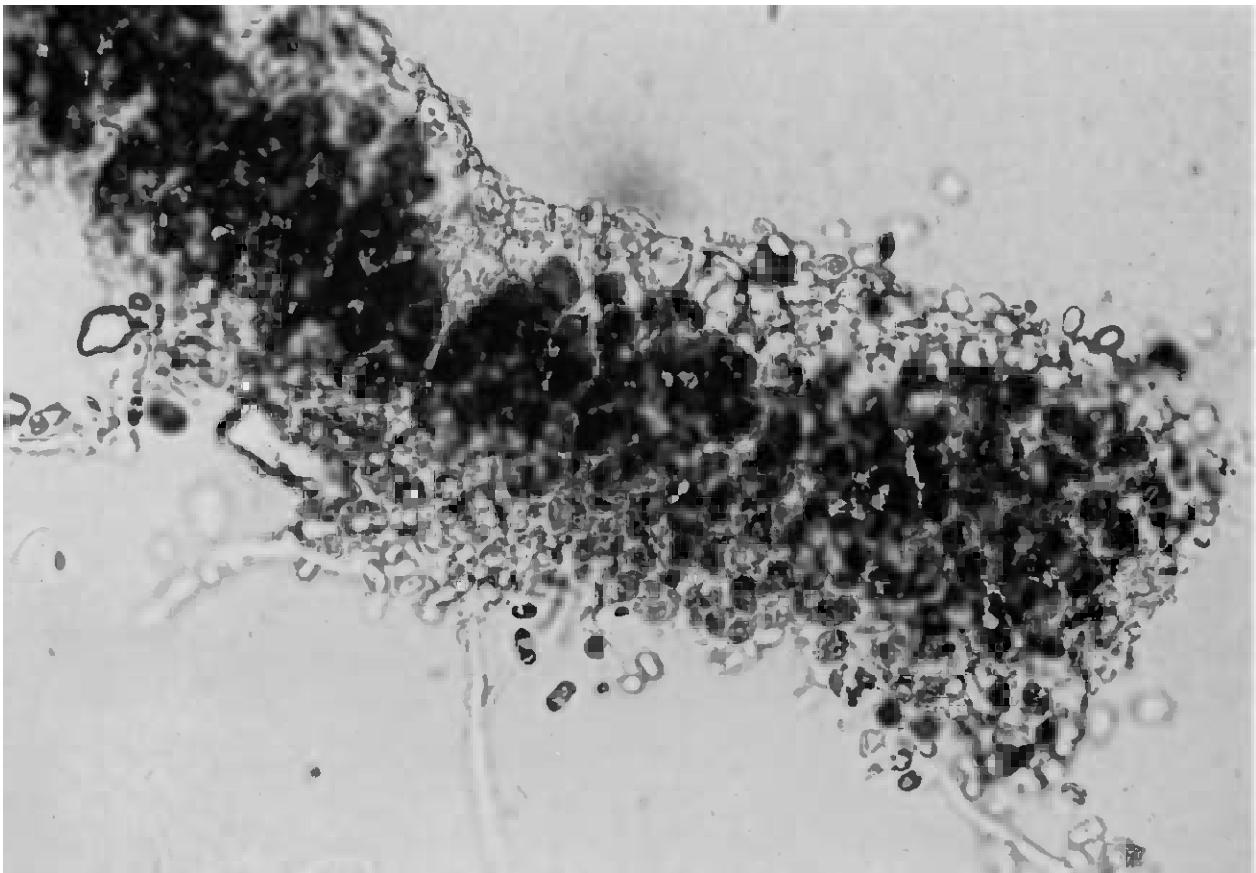


FIG. 4. Section through a resynthesized squamule showing upper cortex and algal layer.

TABLE I
CHANGES THAT A *Trebouxia* PHYCOBIONT UNDERGOES AFTER ISOLATION FROM THE THALLUS

Morphological changes

- a. Cells develop gelatinous sheaths
- b. Cells form a fibrillar sheath
- c. Cells become larger
- d. Cell walls become thinner
- e. Pyrenoglobuli are fewer in number and smaller in size
- f. Appearance of polyphosphate bodies and other storage bodies
- g. More starch in cells
- h. Pyrenoid less evident

Physiological changes

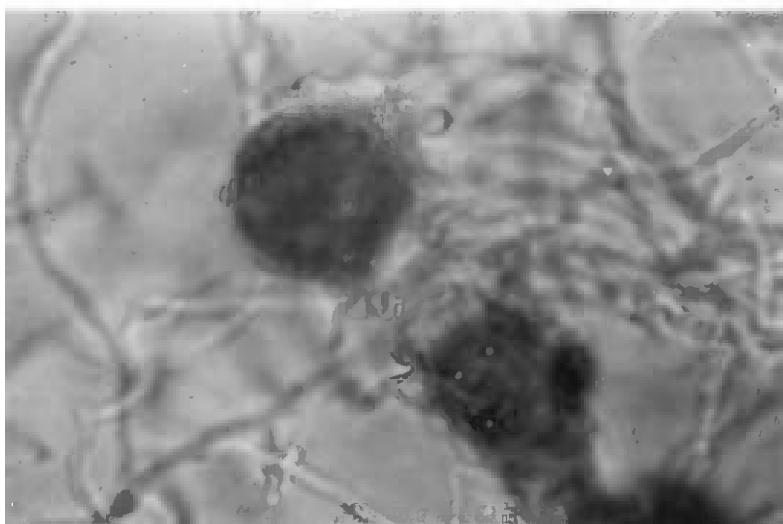
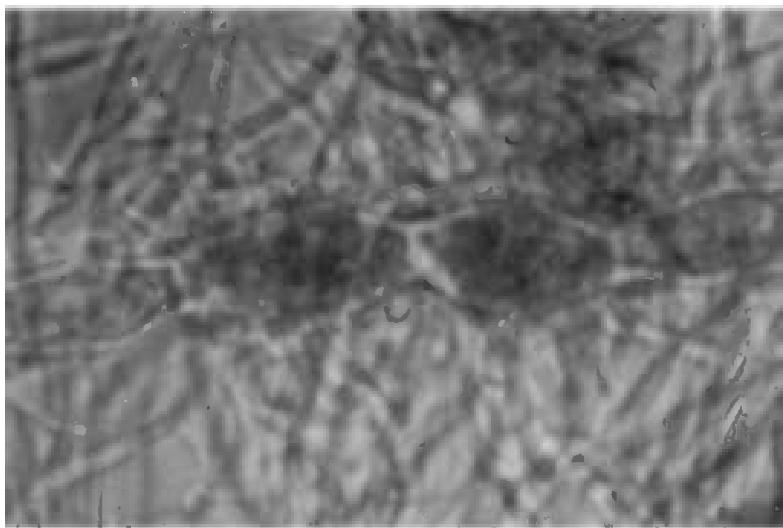
- a. Proportion of ribitol to sucrose diminishes
- b. More radioactivity is found in ethanol-insoluble compounds
- c. Only a trace of ^{14}C is incorporated during photosynthesis in ribitol
- d. Less photosynthate is released into the medium
- e. Types of compounds excreted are different

cultures. Only one successful cultivation of a fungus from a blue-green-containing lichen has been achieved and that was with *Collema tenax* (Henriksson, 1958). Early synthesis studies consisted of sowing spores of *Collema* onto colonies of *Nostoc* and observing proliferation of the fungal hyphae through the algal gelatinous mass and sometimes the transformation of the alga into the *Collema* thallus (Schwendener, 1872).

The development of cephalodia on lichen thalli represents a type of natural synthesis. When filaments of *Nostoc* become associated with some lichen thalli they provoke a specific response from the fungus that leads to incorporation of the alga and the formation of internal cephalodia, as in *Nephroma* species (Jordan and Rickson, 1971). Outgrowths of the internal cephalodia lead to the formation of a new thallus type (Jordan, 1972). The influence of the alga on the morphogenetic development of the fungus is clearly evident from several cephalodia studies (cf. Moreau, 1956). The fungus in association with a green phycobiont forms the *Lobaria* thallus, but in association with a blue-green symbiont it forms a totally different thallus, i.e., fruticose instead of foliose. Green algal cells that are pushed by fungal hyphae into the cephalodial outgrowths stimulate the fungus to form lobules similar to the foliose thallus.

2. TRENTEOPOHLIA

Hérisset (1946) placed on mineral agar spores of *Arthonia*, *Graphis*, and *Opegrapha* in contact with the free-living alga *Trentepohlia umbrina* and noted lichenized contacts between the hyphae and algal filaments after 3



FIGS. 5-6. Synthesis of *Trentepohlia* and *Phaeographina fulgurata* on purified agar. Fig. 5, envelopment of algal filament by hyphae; Fig. 6, cell that has been separated from the algal filament and has become rounded as a response to the fungal encirclement.

weeks. Algal cells surrounded by hyphae contained less β -carotene, indicating a functional relationship between the symbionts. After 5 months, there was growth of the mixed culture but no further differentiation.

Initial stages of synthesis were achieved also between the mycobionts *Glyphis lepida* and *Phaeographina fulgurata* and the isolated *Trentepohlia* symbiont of *Pyrenula nitida* (V. Ahmadjian, unpublished). The colonies of all three organisms were kept separately in distilled water for 22 days. Each fungus was then fragmented with part of the algal colony and a few milliliters of the suspension were then pipetted onto the surface of a 2% purified agar. Forty-five days later the fungal hyphae of both mycobionts were seen encircling the algal cells (Fig. 5). Haustoria also were evident and the enveloped algal cells had a reduced content of β -carotene. The hyphae broke up the individual algal cells from the filament and the cells became much enlarged and rounded (Fig. 6).

V. Factors that Influence Synthesis

A. Drying

How drying influences the symbionts towards lichenization is not clear, but evidence is accumulating that allows us a glimpse of the possibilities. The important factor appears to be the length of the drying period. Rapid, short-term, and complete drying of the symbionts has no effect on the lichenization process whereas a slow drying is effective. It seems likely that there is a buildup or development of substances or structures during the drying process that triggers the union of the symbionts.

There are several known ways in which drying influences lichens and their symbionts. Pueyo (1965) reported that lichens whose thalli were desiccated showed an increase in total soluble sugars. The increase was especially pronounced during the first two months of drying. Jacobs and Ahmadjian (1971) observed in *Trebouxia* a migration of pyrenoglobuli, lipid-containing storage droplets, to the outer parts of the pyrenoid when lichen thalli were dry and an absence of starch in the *Trebouxia* cells of dry lichens. The authors stated that starch stored by the alga during wet conditions was metabolized quickly during dry periods and the pyrenoglobuli were used after the starch was depleted. The authors did not specify the level of thallus wetness, but their methods indicate that the thalli were not fully saturated when they were examined.

Harris and Kershaw (1971) found that the *Trebouxia* phycobionts of *Parmelia physodes* and *Parmelia sulcata* stored starch only when the thalli were kept for several days in the light at low water contents or after the thallus dried slowly in the light. When the thalli were saturated with water,

either in light or dark, the starch grains disappeared and in the dark thalli there was a buildup of pyrenoglobuli. The conclusion of this study was that fluctuating wet and dry conditions combined with light and dark are necessary for thallus growth. The interactions of these conditions are as follows: At high levels of water saturation, in both light and dark, photosynthetic products move from the alga to the fungus and the rate of fungal respiration is limited by the carbohydrate supply. At low levels of water saturation (15–50% of the maximum) in the light the activity of the fungus is restricted by the lack of water. Thus, the alga can store its photosynthetic products and multiply as well. At low water levels in the dark the stored starch is respired by the alga and the fungus remains in its substrate-limited and water-inhibited condition. According to the authors, if a thallus is kept excessively wet or dry for prolonged periods the alga will die because of the loss of its storage products. Similar results were found in an earlier study by Pearson (1970).

Smith and Barrett (cf. Richardson *et al.*, 1968) indicated that the production of polyol in lichens increased as the degree of water stress rose. Hill (1970) found that *Trebouxia* in culture incorporated more ^{14}C into ribitol after a period of drying.

Ellipsoidal bodies, unique structures found only in the hyphae of lichen fungi, are especially prevalent in lichens from harsh, dry habitats such as deserts and alpine regions. These bodies have not been found in the hyphae of mycobionts grown in isolated culture except in one instance where the fungus was induced, by drying, to form reproductive structures. Ellipsoidal bodies were not found in *Hydrothyria venosa*, a freshwater lichen that is always inundated (Jacobs and Ahmadjian, 1973) (see Chapter 5).

B. Nutrient-Deficient Conditions

Synthesis will not occur on media that supports the independent growth of the symbionts. This fact has been established from a number of studies. An established lichen that is placed onto a nutrient medium will dissociate into its separate symbionts. The nutrient-deficient condition is similar to that found in most lichens whose habitats provide only a limited supply of nutrients. The changes that a *Trebouxia* phycobiont undergoes when cultured in organic medium are significant and relate to promoting the growth of the algal cells. With a sufficient supply of nutrients the alga incorporates carbon into compounds needed for new cells, i.e., proteins, polysaccharides, nucleic acids, pigments, lipids, and intermediates in biosynthetic pathways (Hill and Ahmadjian, 1972). If the nutrient supply is limited, the cells will not divide as rapidly and much of the carbon would be incorporated into carbohydrate such as ribitol. The excess carbohydrate then becomes avail-

able to the fungus. Culture studies have demonstrated that extremely slow growth on inorganic medium is a common attribute of *Trebouxia*.

In synthesis studies the symbionts are generally taken from cultures that had grown on organic media. This leads to a considerable lag in initiating the synthesis because of the time required for the alga to adjust to the conditions of inorganic culture. For example, in the synthesis of *A. fuscata*, distinct lichenized unions occurred 30 days after the symbionts were placed together. One way to reduce this lag time is to keep the symbionts in distilled water or a mineral solution for a week or longer prior to the synthesis attempts.

VI. Conclusions

Although we know the factors that influence lichen synthesis and the developmental stages associated with this symbiosis, it still is not a procedure that can be accomplished routinely. An exception to this is *E. pusillum* where the flowerpot-soil combination provides optimum resynthesis conditions. Perhaps the most profitable avenue for future studies would be to examine lichens other than those with *Trebouxia*, ones whose algal symbionts have not as yet reached a high degree of specialization. Such synthesis systems and the possibility of their being done under axenic conditions will answer many questions such as the role of bacteria in the symbiosis and the range of specificity of a fungus to an alga.

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Chapter 19

EVOLUTIONARY ASPECTS OF SYMBIOSIS

G. D. SCOTT

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I. Introduction

Of all the life systems known to biologists, symbiosis can be singled out as unique. Depending on how widely one interprets symbiosis it encompasses all shades of physiological and behavioral associations. In the physiological sense, symbiotic systems are life-support systems. They have evolved into systems which are physiologically so closely unified that each participant necessarily is life supported by, as it supports the life of, its coparticipant. This is a step further than mutual aid which has long been the criterion of numerous classes of symbiosis. Mutual aid is a misnomer as a generalized description of the mutualistic type of symbiosis for there is implicit, in many symbiotic systems, a deeper physiological interdependence than "aid." The mutual-aid phase in the evolutionary development of symbiotic systems is transitory. It is but one phase in the progression from casual association toward the physiologically highly integrated, obligatory association exemplified by the lichen symbiosis and several plant-animal systems.

The ultimate in the evolution of symbiotic systems—one organism—one physiological entity—has apparently not been achieved; or perhaps it has in the guise of the chlorophyllous terrestrial plant. Where two recognizable organismal entities remain, however, in any symbiotic system, the highest known degree of integration is still well short of the physiologically unified individual organism.

A retrospective consideration of symbiosis serves not only to indicate the probable pathways along which evolutionary progression or retrogression has taken place but to indicate the probable ways in which physiological association has developed and to indicate the probable stresses that have contributed to or, indeed, have been the dominant guideline, of, this development.

Perhaps the greatest misfortune for the study of symbiotic evolution is the paucity of fossilized remains that can be clearly categorized as symbiotic. In particular, there is little conclusive evidence of the lineage of the lichen symbiosis. A fossil record, however, would not help to elucidate the physiological circumstances surrounding the development and progression of symbiosis. Past probabilities in this respect can only be evaluated from existing symbiotic systems, their physiological differences and such lines of physiological development as can be seen within the various types.

In prospect, our thinking might be governed by the pattern of development which we can discern in present symbiotic systems (Henry, 1966, 1967; Cheng, 1970). A rational survey of these systems and of the interrelations between the symbionts—of the pressures that impinge on these developing symbiotic systems, of the general direction in which symbiotic systems are evolving toward mutual life support and composite autotrophy—leads unerringly to the conclusion that the concept of symbiosis can well provide a basis for future research into the capacity of the earth's productive surfaces sustain its ever-increasing heterotrophic population.

II. Symbiosis in Retrospect

A. *The Free-Living Organisms*

Separate existence preceded symbiosis. We accept this as fact because we cannot conceive of two organisms, which we know by experimentation to be genetically different, evolving from a common ancestor at one and the same time in one and the same space as a physiologically interdependent unit. Rational proof can never be provided, however, so we state categorically that all symbiotic systems known to us at the present time did not formerly exist as such, and that the individual symbionts were at one time free-living organisms. But there is much more to symbiosis in retrospect than this statement of what we take to be fact. When considering the lichen symbiosis in particular, it is apparent that numerous physiological and ecological characteristics have been responsible, both jointly and separately, for the discontinuance of the free-living status of certain algae and fungi. Whether such organisms are to be regarded as degenerate or opportunistic is a matter of opinion. We can only assemble here some of the relevant facts and suppositions.

1. PHYSIOLOGICAL AND ECOLOGICAL CONSIDERATIONS

All free-living organisms are either heterotrophic or autotrophic. The evolution of autotrophic plants is to be correlated with the existence of carbon chemically combined with oxygen as carbon dioxide, and hydrogen chemically combined with oxygen as water. Air is present over the entire surface of the earth, in every space that is not occupied by solid or liquid matter. This implies that CO₂ is the most readily available source of carbon to all plants. Those which can use this source of carbon have a clear advantage over those which cannot.

Liquid matter, and particularly water, because of high surface-tension forces, tends to occupy that shape and volume consistent with the dissipation of the least surface energy. Gases, however, are relatively unrestricted in their flow by surface-tension forces and therefore tend to occupy all available space. Thus we have two contrasting characteristics of the "parent compounds" of carbon and hydrogen. Carbon dioxide diffuses throughout the maximum possible space and is therefore of continuous distribution over the earth's surface while water is confined to the least possible space and is of discontinuous distribution.

Any plant that has attained the capacity to assimilate carbon from carbon dioxide and hydrogen from water will have the greatest possible chance to colonize all spaces on the earth's surface from which it is not barred by other physiological or ecological deficiencies. Such a plant is, of course, a chlorophyllous or autotrophic plant. We should more correctly term such plants autotrophic for carbon because autotrophism for both carbon and nitrogen is displayed by the blue-green algae, certain species of which are lichen phyco-bionts. So the autotrophic plant is one which we should expect would colonize those areas in which no more than a minimal demand on the nutritional capacity of the substratum can be met.

This is in sharp contrast to the situation for heterotrophic organisms. The physiological demands of heterotrophs on the substratum are heavy and may be termed absolute, except for oxygen and relatively small amounts of CO₂ involved in acid fixation. All heterotrophic organisms depend on the previous existence of one or more species of autotrophic organism at or near to the point of their colonization. This dependence is manifest in the absolute sense in all parasitic heterotrophs. They are what one might term physiological appendages of the host organism.

The spatial distribution of the parasite coincides at all times with that of the host and therefore the survival of the host determines the survival of the parasite. This is a physical contact relationship which is attended by a high degree of physiological adaptation on the part of the parasite.

In general terms, host species selectivity and physiological adaptation show high correlation in parasites. If the host is an autotroph, there is

a close link between the heterotroph and the carbon supply from the atmosphere. It is evident, however, that there is, in the host-parasite relationship, an effective barrier to the direct extraction of carbon compounds by the parasite from the host chloroplasts. Whether or not chloroplasts are derived from a symbiotic system, they are the seat of carbon assimilation. It is rather surprising that no parasite has gained, through evolution, the supreme advantage of direct association with the chloroplasts of the autotrophic host.

In contrast, the saprophytic organism is dependent only on the remote presence of the living autotroph and there is necessarily a time lag of at least one generation between the two. There is little or no direct physical contact and no direct physiological relationship. The nutritional requirements of the saprophyte are met from a heterogeneous mass of decaying vegetable or animal matter. This mass is discontinuous in time and in space and therefore the greater the degree of discontinuity the less is the chance of survival of the saprophyte.

A chain of numerous links between the living autotroph and the heterotroph, be it parasitic or saprophytic, is thus a physiologically inefficient system. With the elimination of each successive link, efficiency is increased until the point is reached at which there is direct physical contact between the heterotroph and the host chloroplast. This near ultimate in proximity of association appears to have been approached only by the lichens and a few other symbiotic systems.

2. PROGRESS TOWARD ASSOCIATION

Symbiotic, parasitic, and saprophytic fungi have been labeled with different degrees of physiological efficiency according to their physical closeness to chloroplasts of the "host" organism. Such a categorization is not intended to indicate a series of evolutionary advances, nor does it indicate that one group is ecologically more successful than another. What is indicated is the existence of numerous examples of different levels of association between fungi and other organisms—mostly autotrophic plants.

The saprophyte, dependent on the previous presence of an autotroph, and the parasite, dependent on the immediate presence of an autotroph, are no less dependent than is the lichen mycobiont on the phycobiont. Both examples of association can be regarded as representing probable stages in the convergence of autotroph and heterotroph which led to the evolution of symbiotic systems.

From the least specialized saprophyte to the most highly specialized parasite, there runs a common thread of increasing dependence on specific organic substances of autotroph origin. The ultimate in specialization is

complete dependence of the parasite on the metabolic products of a single species of autotroph (Quispel, 1951). At this level, a relatively stable state has been reached in which the defense-reaction mechanisms of the host are sufficiently powerful to prevent self-elimination but yet are insufficiently powerful to effect the elimination of the parasite. Physiological symbiosis, in the sense of mutual life support, is only one step further. The circumstance can easily arise, and presumably has arisen, in which the host organism through mutation develops a metabolic deficiency which is made good by the associated organism. The host becomes dependent on the parasite just as the parasite is dependent on the host, and a state of symbiosis ensues.

It is probable that the green lichens evolved by such a process, starting with the casual association of fungal hyphae and free-living green algal cells, and continuing through stages of progressive adaptation of both fungus and alga to the conditions of the new environment thus created. The present-day green lichens represent, so far as we are aware, those stages in symbiosis evolution at which the autotrophic symbiont has become fully dependent, ecologically and perhaps physiologically, on close association with the heterotrophic symbiont.

Although this may have been the general pattern of evolution of the lichen symbiosis, it is clear that not all present-day examples have reached comparable stages in the development of interdependence. The phycobionts of many blue-green lichens, for example, appear to be facultative symbionts. There is no concrete evidence that any such phycobiont cannot lead an independent existence from one generation to the next. It is evident, too, that the secondary phycobionts of cephalodial lichens, such as *Stereocaulon*, *Peltigera* (*Peltidea*), *Nephroma*, etc., are facultative associates only. Whether these blue-green lichens are to be regarded as intermediate stages in the progression to obligatory association is a matter for conjecture. The only pertinent evidence in favor appears to be the fact that *Nostoc*, and perhaps other blue-green phycobionts, are nitrogen fixers. So long as this attribute remains inherent to the species, there is only a slender chance of physiological adaptation to exclusive utilization of nitrogenous metabolites of the mycobiont.

The lichen symbiosis is thus a classic example of the progression from facultative to total interdependence of the symbionts. It is certain, however, despite the lack of evidence, that there is a stage of independence prior to the initiation of every individual symbiosis. This may be, and perhaps usually is, brief, but no symbiosis arising from a mycobiont ascospore can be initiated without the previous independent existence of the phycobiont. This is one instance when the lichenologist should not be confounded by the mathematical nicety that one plus one equals one—itself a fair, if not very illuminating, definition of symbiosis.

B. *Incipient Symbiosis*

In the course of the history of the lichen symbiosis and, indeed, in the histories of all symbiotic systems, there must, because of the trial and error nature of evolution, have been numerous examples of incipient symbiosis. Interaction between various algae and fungi at the unicellular and multicellular level is a matter of common observation, particularly with reference to "algal covers" on tree trunks. But we still cannot identify a single example of early interaction between alga and fungus as a case of incipient lichen symbiosis.

There are many recorded instances of fungal hyphae associating with unicellular algae under cultural conditions but again we are largely ignorant of the physiological interrelationships that exist in these ephemeral associations. At best, we can say that numerous observations indicate that some of them could represent early stages of symbiosis. We need a great deal more information before we can arrive at any tenable conclusions. In the meantime, we may look closely at the circumstances that perhaps contribute to the evolution of symbiotic systems, and attempt to derive what we might call the laws of symbiosis.

1. THE "LAWS" OF SYMBIOSIS

In order to fully appreciate the complexities of the circumstances surrounding the evolution of lichen symbiotic systems, we must attempt to "think" for evolution. Evolution is not a random process in the sense that it is by pure chance that any pathway is followed. If we accept the basic premise that the physiologically and ecologically more successful plants are those that have attained the autotrophic state and those whose reproductive systems expose the filial generation to the least vulnerability before "safe" establishment, then we can perhaps establish criteria by which these ends have been achieved.

The simplest definition of a lichen is that it is a photosynthetic fungus. This definition would not be accepted by the purists among lichenologists and mycologists, but the fact remains that the principal feature of distinction between fungi and lichens is that the latter are photosynthetic.

It seems evident that among the 16,000–18,000 distinct species of lichens there has been a "common pathway" in the sequence of evolutionary events. This common pathway is most certainly the advance of certain groups of fungi toward the autotrophic state (Chadefaud *et al.*, 1968). Underlying this move of heterotrophic systems toward autotrophy must be a basic factor, presumably physiological, which has "steered" the numerous paths of evolution in this common direction. The nature of this factor is one of the

major questions confronting those who are concerned with this aspect of symbiosis.

Let us consider the numerous physiological and ecological characteristics of the fungi which might be contributory factors in this evident move toward the autotrophic state by way of symbiosis. For the purpose of this exercise, we must think of the primitive fungus from the physiological point of view. The individual concept of the primitive organism, of course, can only be highly subjective in nature. If this fact is recognized, however, there is every excuse to consider the primitive fungus as being an organism of a diffuse mycelial nature and a saprophytic type of physiology.

Among saprophytic fungi there are several well-recognized types of physiology which center around the presence of particular constitutive or adaptive enzyme systems. For example, there are saprophytes that can metabolize simple carbohydrates only, such as glucose; those that can metabolize higher carbohydrates, such as starch or other polysaccharides, and those that, although they may normally metabolize the simple carbohydrates, seem to have an adaptive enzyme system that can incorporate a polysaccharide substrate if simpler forms of carbohydrate are not available.

One might suppose that one way in which fungi were induced toward symbiosis may have been because of a purely ecological situation, i.e., the scarcity in the gross medium of the carbohydrate to which a species was physiologically adapted. Under these circumstances one can visualize the possible gradual elimination of that fungal species.

All organisms have an exosphere of varying dimension—a sphere of influence which is exerted through organic or inorganic molecules exuded from, or accumulated on the surface of the organism. The limits of the exosphere are defined by factors that include the following: the intensity or concentration of exudation; the degree of volatility of the exudate; the physical nature of the medium in which the organism is growing, be it soil, water, air, or other substrate; the presence of barriers to diffusion such as the electrical properties of the medium and of the organism itself; the occurrence of flow patterns of air or water in the vicinity of the organism.

The exosphere of motile organisms in a liquid medium will thus be very small indeed if the organism is of fairly large proportions and is capable of considerable movement through the medium. If the organism is small or microscopic and unable to progress significantly against any current in the medium, it will be relatively large and uniform. In both instances, frequent violent movement of the liquid medium, such as occurs by wave action, will tend to erase the exosphere even of microscopic organisms. Despite these physical circumstances, however, chemotactic responses are indispensable stages in the life cycle of many aquatic organisms.

So far as static terrestrial organisms are concerned, the dimensions of the exosphere frequently vary with changes in the water tension of the soil, in the physical characteristics of the soil, in air movement over aerial plant organs, in relative humidity, and in relative proportions of the constituent gases of the air.

It follows that the chance growth of one organism within the exosphere of another will result in a physiological and probably an orientation reaction. The extent and biochemical composition of the exosphere will profoundly influence the intensity of such reactions. As an example, the presence of unicellular algae might, through exosphere exudation, create a localized increase in the population density of a saprophytic fungal species. Adaptation to utilization of the exosphere exudations of the algae might take place and the distribution of the fungal species would gradually coincide with that of the algal species with which it has become associated. This is the physiological threshold of symbiosis.

The motivating force behind this apparent move toward autotrophy through symbiosis might, of course, be looked at from an entirely different point of view. Carbohydrates and other organic nutrients in the soil are derived principally from autotrophic plants. The distribution of such plants over any substrate is discontinuous in space and in time. Thus the distribution of derived organic matter must also be so discontinuous. This being so, it is obvious that there is an unfavorable comparison between the soil or other substrate environment and the environment within the exosphere of unicellular algae. In the latter, there is a degree of constancy that is unparalleled by any situation which could conceivably occur within the organic matter fraction of any soil. Fungal hyphae growing within soil organic matter will continue to produce new growth so long as the supply of the requisite organic nutrients is available. When this is depleted, as may frequently occur, that particular individual of the fungal species must die unless some of its hyphae have been able to bridge the gap between the original locus of colonization and another pocket of organic matter.

If by chance, however, the fungal species can colonize the exosphere of unicellular algae through an ability to use the exudation products as total or partial nutrition, its survival is more assured. The algal colonies, although of sporadic occurrence, are self-perpetuating with a constancy that is characteristic of the living organism. This is patently not the case for any saprophytic system that is dependent for its supply of energy on the chance deposition of litter.

The total nutrition of unicellular algae is normally inorganic. These organisms are autotrophic—their only disability relative to the saprophytic chain of events in the soil is that colonization of a substrate is confined to regions where light is available. Thus, within these limitations, the fungal

species can continue living and reproducing so long as the colony of algae does likewise.

2. A "MODEL" OF EVOLUTION

We can perhaps better understand the evolution of the lichen symbiosis if we consider various aspects of the colonization of available space and attempt to draw some conclusions on the theoretical progress of evolution.

All plant species have the potential to colonize all available space on the earth's surface because every plant produces more than one diaspore. The ability of the individual species to compete for the available space is a function of its growth habit and this, together with environmental factors, tends to prevent universal coverage. So we have the model situation in which the progress of colonization is governed on the one hand by the endogenously controlled factor of growth habit and, on the other hand, by the numerous exogenous factors of the environment. The interplay between these results in limitations of varying degree on the colonization of available space by different species.

Many of these exogenous factors are of too obvious function to warrant mention here, but there are several that may not be quite so obvious. An autotroph, for example, cannot colonize the light-facing surface of another autotroph without interfering with the photosynthetic capacity of the host. But an autotroph can colonize the light-facing surface of a heterotroph without causing such interference. The importance of these basic observations, in relation to the numerous forms of lichen symbiosis, can be readily appreciated.

Following this concept we can see that, unless there is a marked difference in size, it is virtually impossible for any foliose lichen to colonize the surface of another foliose lichen without the eventual destruction, through lack of light, of the species serving as the substrate. Equally, it is impossible for an autotrophic lichen to colonize the ventral surface of either an autotroph or a heterotroph because insufficient light is available for growth. But it is perfectly possible for any heterotrophic species to colonize the ventral surface of an autotroph, i.e., the ventral surface of any foliose lichen is freely open to colonization by heterotrophic species. Here, of course, the limitations on colonization lie in the normally low availability of organic carbon supply.

As a general concept, each type of surface colonized can be related to the nature of the organism and to the nature of the interference with neighboring organisms. It is against this background that we may profitably look at various facets of the lichen symbiosis as a means of building up a "model" of evolution within this large group of plants.

A cursory survey of existing symbiotic systems reveals the fact that one of the major trends is toward the attainment of the autotrophic state by the holobiont, even though one of the symbionts is heterotrophic. We can include within this trend the entire lichen symbiosis and all other systems involving an autotroph. It is also evident in the cross-kingdom symbioses, i.e., associations between unicellular chlorophyllous organisms and protozoans or molluscs. Symbiosis, exemplified by the "attachment" of a heterotroph to an autotroph, is manifest in every phylum of chlorophyllous plants. None has escaped the "attempt" of heterotrophic organisms to associate with them in their evolutionary progress toward autotrophism by the frequently spectacular but effective back door of symbiosis.

Further elucidation of symbiosis is probably to be found in an examination of the possible ways in which one of the symbionts has influenced the behavior, or the physiology, of its partner. The casual relationship, dwelt upon earlier, is fairly obviously a "forced association" of a heterotrophic organism with an autotrophic partner. This is because the association is favored by the fact that the autotroph presents an alternative and perhaps "easier" source of nutrition.

If we accept that the heterotrophic organism is the driving force in the association, it is not too difficult to accept that it will preferentially associate with specific individuals of the autotrophic symbiont. This may be promoted by genetic variation in either symbiont. The process of natural selection is thus evident at two distinct phases of the life history of every symbiotic unit. Genetic recombination takes place during reproduction of the individual symbionts and phenotype recombination occurs during reconstitution of the symbiosis. This implies that change in genetic makeup, and hence in morphological and physiological characteristics of the holobiont, might take place more rapidly than in nonsymbiotic organisms.

Taking this a step further, it is quite possible that the heterotrophic symbiont might be the cause of variation within the autotrophic symbiont. Any such change which leads to a physiological or morphological variation in the autotrophic symbiont might confer a growth advantage and thus an enhancement in distribution and survival of the holobiont.

This is the successful line which develops through normal evolution and it is perhaps thus that the autotrophic symbiont has gained success in ecological amplitude, but at the expense of free existence (Ahmadjian, 1970). In other words, evolutionary advantage and ecological amplitude have become dependent on symbiosis. Reversion of the symbionts to the free-living condition would quickly result in their elimination.

This line of thought is in accord with the concept that all symbiotic systems had their origin as a system of host versus parasite and that this antagonistic system has gradually developed towards a system of true symbiosis. It is

essential in this scheme to regard the heterotrophic partner as the dominant force in the evolutionary pathway of the holobiont. Physiologically, it is always the partner at the greatest disadvantage. One can only marvel at the remarkable evolutionary "guile" of the heterotroph in acquiring the permanent services of a CO₂ fixation system of its own by the ingenious method of inducing such profound physiological change in the autotroph that it can no longer continue as a free-living organism.

Thus we have one version of the model or template by which symbiotic systems have perhaps evolved. Implicit in this model is the fact that physiological necessity is the driving force, as it is in all living systems. Physiological independence, on the part of the autotrophic symbiont, has been bartered for the evolutionary advantage of wider ecological amplitude and increase in population density—the inevitable law of nature. The heterotrophic symbiont, be it the macro- or microsymbiont, in relation to its autotrophic partner, is clearly the *force majeur* in the ascent of the unending evolutionary stairway.

III. Symbiosis Achieved

If the achievement of the symbiotic state is accepted as being the result of a sequence of chance processes, it might be considered that the pathway toward symbiosis would be strewn with the remnants of unsuccessful attempts. That this is not so is indicative of the fact that either the determination of success takes place at a very early stage in the evolution of association, or the participants in abortive efforts at symbiosis are quickly eliminated.

Among the lichens there are very few examples indeed of what could be called half-way stages towards symbiosis. Even in the least sophisticated associations, there is a degree of constancy that warrants the designation of symbiosis. Facultative symbiosis, so far as mycobionts are concerned, appears to be unknown in the lichens. It is thus evident that there is a large gap in the evolution of this particular symbiotic system; we are presented with a series of *faits accomplis* with but little hint of what has gone before. We can, however, gain some idea of immediate past trends and perhaps also of future trends by an examination of the status of present-day lichens in relation to other symbiotic systems.

Apart from the fact that the majority of lichens can be categorized as either basidiolichens or ascolichens, and the numerous sterile species that have obvious affinity with the latter, lichen taxonomy is very much an arbitrary system. Classifications so far developed are artificial and based, to a large extent, on seemingly constant morphological characteristics or more recently on chemical constituents (Culberson and Culberson, 1970) It is

impracticable to apply to lichens the criteria that are used to distinguish between, for example, the primitive and the advanced angiosperm.

Even if it were possible to adopt a natural classification of lichens, such a system would have to be based on mycobiont characteristics. This would tell us very little, if anything, of the evolution of symbiosis, for it would take no account of any contribution that the microsymbionts have made to the evolution of the holobiont. Further, it is evident that there is strong selection against increase in species populations by recreation of the symbiosis from ascospore and algal cell. The dissemination of ascospores from any fertile lichen has one chance in several million of resulting in the initiation of symbiosis with a physiologically compatible phycobiont colony. In the immediate neighborhood of the parent thallus, or of a distant individual of the same species, if the ascospores are disseminated by wind or water, the chance will be considerably higher. But it must still be negligible in comparison with the chance of reproduction by thallus fragmentation.

All lichens with the possible exception of certain aquatic and marine species—and even they are doubtfully excepted—are exposed to various patterns of change in moisture content depending on their growth habitat. Whatever this habitat, there is no significant physiological control of moisture loss although there is evidence of a purely fortuitous control resulting from unequal rates of moisture loss from dorsal and ventral surfaces. This causes a certain amount of inrolling of the thallus and consequent protection of the vulnerable phycobiont layer.

Lichens are very brittle when they are air-dry. In this condition, which frequently persists for a significant part of the daytime, they are exposed to environmental abrasive forces. Even windblown sand particles can cause the removal of sizable fragments of lichens such as *Sphaerophorus* and *Stereocaulon*. Fissuring of lichen thalli by hygroscopic flexing is a standard feature and is perhaps the primary cause of fragmentation. Saxicolous lichens exhibit a strong tendency toward erosion of their own substrate with consequent dispersal of the loosened fragments. Corticolous species likewise have a limited existence which depends on the morphology and longevity of the bark substrate. In both cases, dissemination by thallus fragmentation is a corollary of their existence on these particular substrata.

Since minute particles from any part of a lichen thallus are theoretically capable of species regeneration, provided they contain both symbionts, it follows that clonal reproduction is characteristic of most lichen species and probably far surpasses the extent of reproduction from ascospores. It is thus to be expected that, although there is an obviously large gene pool in the lichen symbiosis, rates of gene interchange will be very slow compared with free-living fungi or higher plants. This leads to the conclusion that the whole lichen symbiosis, with perhaps certain exceptions, is a relatively stable system whose evolution, like the rates of growth, is very tardy.

There are, nevertheless, numerous indicators of evolutionary advance within certain groups of lichens. The primitive symbiosis is presumably of the type represented by any one of the numerous species with little or no tissue differentiation and with a fairly well circumscribed distribution. They are usually confined to habitats such as calcareous rocks, permanently shaded damp situations, or to specific microhabitats on trees. Narrow ecological amplitude is a characteristic of these lichens even though some are of world-wide distribution wherever these specialized habitats occur. Relative to the majority of lichens, inefficient protection of the phycobiont is to be viewed as a factor prevailing against widening amplitude.

In contrast, development of heteromerous dorsiventral construction, with confinement of the phycobiont layer to a subcortical position, is perhaps the key to the wide distribution of lichens in habitats exposed to high insolation. It compensates the lichen symbiosis for the lack of a root system and a transpiration stream. Additionally, the vulnerable area of high physiological activity, represented by the phycobiont layer, is rendered secure from excessive insolation by the dorsal cortex functioning as a light barrier in the low moisture condition.

The relegation of the phycobiont layer to the subcortical position is probably correlated in part, at least, with the morphogenetic effect of light. With the exception of pigmented species such as *Trentepohlia*, neither green nor blue-green algae are notably resistant to high insolation. It is therefore to be expected that a significant growth advantage accrues to lichen phycobionts under the screen provided by the cortex. Light transmission by the cortex is proportional to moisture content and inversely proportional to the density of colored lichen acids and other pigments. *Xanthoria*, *Peltigera*, *Umbilicaria*, and many others are singularly low in acid content when growing in shade, but are deeply colored in high insolation habitats.

These two control mechanisms undoubtedly act in unison to effect a coarse and a fine adjustment of the amount of light reaching the phycobiont layer. The lichen-acid screen contributes a seasonal control while variation in moisture content of the cortex exerts a variable diurnal control within the limits of light transmission set by the lichen-acid screen. The dorsal limit of the phycobiont layer can be considered to coincide with that part of the thallus above which the majority of the phycobiont cells cannot survive because of excessive light. Similarly, the ventral boundary indicates the limit of light intensity below which the majority of cells cannot survive.

There is an interesting comparison, in relation to the light susceptibility of the phycobiont, between the heteromerous lichen construction and that of other symbiotic systems involving algae as autotrophic symbionts. Three major groups of living organisms that have effected symbiosis with algae are the protozoa, coelenterates, and molluscs. In these, the phycobiont although strictly endosymbiotic, is afforded little protection from excessive

sunlight by ectodermal tissue. A protective mechanism does operate, however—one that is a consequence of symbiosis and is not displayed by the asymbiotic zoobionts. The physiological activity of the phycobiont confers, in an imperfectly understood manner, the property of light sensitivity on the holobiont. This is related to tentacle orientation in anemones and to locomotory adjustments toward positions of tolerable light intensity in both anemones and molluscs.

The most interesting feature of this light sensitivity is that compensatory movements are effected in terms of minutes or hours. The deleterious effect of failure to compensate would not, however, be evident for a considerably longer period. That is to say, the lethal effect of excessive insolation of the phycobiont is measurable in days rather than in hours, particularly in the marine environment of many of these organisms. It is therefore necessary to postulate a further factor in these symbiotic systems. This is most likely to be a waste-product feedback reaction which triggers a locomotory response.

In coelenterates, the spatial orientation of the phycobiont is maintained at the optimum level of light intensity by movement of the holobiont up or down the light intensity gradient. The phycobionts of these organisms gain protection against excessive insolation through autonomous movement of the whole organism while, in the lichens, protection is achieved by a particular spatial disposition of the phycobiont within the thallus. These two types of symbiosis, one locomotory, the other static, have achieved the same end by radically different means. The important point, however, is not the means toward the end but the end itself. It is highly significant that the most successful symbiotic systems have become dependent for survival on the evolution of intricate and sensitive light-control mechanisms.

Parallel mechanisms to these are to be found in chlorophyllous vascular plants in which discoid chloroplasts show change in spatial orientation within the palisade cells of the leaf in relation to incident light intensity. The fact that a means of ensuring maintenance of a safe environment for chloroplasts is essential to survival of the nonsymbiotic higher plant is sufficient indication that no autotrophic symbiotic system could have evolved without the evolution of a comparable system built into the symbiosis.

Whether or not these theories provide the total answer, it is clear that there can be no genetic factor controlling the position of the phycobiont layer. We are dealing with two genetically distinct organisms and this precludes any direct morphogenetic interaction at gene level. Morphogenesis there must be, but it can only be brought about indirectly either by symbiont exudations or by environmental factors.

There is ample evidence of progression of the lichen thallus from the dorsiventral squamulose type to the erect or ascending foliose type. The existence of such development series indicates that, as in marine molluscs

and coelenterates, the presence of an autotrophic symbiont has the effect of imparting morphological change in the heterotrophic symbiont.

The same physiological principles apply to this situation as apply to the chlorophyllous vascular plant. Efficiency is increased by the orderly arrangement of the photosynthetic tissues in space. Dissection and elevation in the vertical plane are primarily advantageous to optimum utilization of energy for carbon fixation and secondarily to efficient dispersal of diaspores. This is no less true for the lichen symbiosis than for the higher plant in which these traits are so evident.

These facts render the comparison between the lichen thallus and the green leaf even more valid. In both cases the morphogenetic effect of light is twofold. It controls the distribution of chlorophyll within the tissues over short time sequences, and its distribution in space by stimulating physiological responses which lead to elevation and orientation.

The most successful photosynthetic organism, be it higher plant or symbiotic system, is that which succeeds in orienting its chlorophyll in the optimum position in space. Numerous species of *Sticta*, *Cladonia*, *Stereocaulon*, *Ramalina*, and various others represent close approaches to this optimum. It would appear, in fact, that there is considerable truth in imitation being the highest form of flattery.

IV. Symbiosis in Prospect

It would be very difficult to find a single example of a lichen which shows an advanced state of integration of the symbionts. But, just as in the case of the highly specialized parasitic fungi such as the rusts, it is evident that integration in some lichens may have progressed further than that shown by the majority. The number of lichen mycobionts successfully isolated in culture runs into the hundreds, although genera such as *Lobaria*, *Peltigera*, and *Sticta* have been notably resistant to isolation. The probability in these genera is that the mycobiont has become so specialized in its nutrition, as a result of symbiotic association, that the spores are unable to germinate except in the presence of the phycobiont. This can perhaps be interpreted as a move toward closer physiological integration than in other lichens. But it is no more of a step in this direction than is evident in *Endocarpon* and allied genera in which phycobiont cells are ejected with the ascospores, thus ensuring elimination of the most hazardous phase in the life history of the species.

Perhaps, in a rather negative sense, we can say that those lichens, for which no sexual reproductive system is known, have attained a higher degree of integration than any others. In the total absence of a mycobiont reproductive

system, integration is complete in the sense that the holobiont is entirely confined, in its dissemination mechanisms, to thallus fragmentation and perhaps the dispersal of soredia or isidia. Phenotypic recombination in such lichens is only possible if the propagules are exposed to an environment conducive to breakdown of the symbiosis and outgrowth of the mycobiont. This would create a situation in which a new symbiosis might be initiated with a different strain of phycobiont.

These levels of integration within the lichen symbiosis, however, are a long way from that to be found sporadically in other systems. The relationship between the symbionts in certain flagellates and rhizopods might be taken as representing a probable future stage in evolution of the lichens. The *Cyanophora* phycobiont, for example, shows little semblance of a cell wall. It is the nearest approach in any symbiotic system to reduction of the autotrophic symbiont to the function of a chloroplast. Some hint of a development in this direction is evident in the lichen symbiosis, however. Higher membrane permeability and thinner cell walls, compared to the free-living condition, are characteristic of some phycobionts in symbiosis. These differences may represent the initial stages of development through which *Cyanophora* has already passed.

The difficulties attending this supposition, of course, appear to be great. In flagellates, rhizopods, and other plant-animal symbiotic systems, the dissimilarity in size of the symbionts is compatible with ingestion of the autotrophic microsymbiont. In these cases the microsymbiont probably responds to a chemotactic stimulus to make contact with the macro-symbiont. There is no known instance, however, of a motile algal cell actively penetrating the cell wall or membrane of another species. A parallel can be drawn with the fusion process of motile unicells such as *Chlamydomonas*. Here, there is mutual attraction through the medium of chemotactic stimulus and response, but this only serves to bring the unicells into contact. Subsequently, there is only localized dissolution of the cell wall and passive fusion of the protoplasts.

This reveals the barrier to further integration of the symbionts in lichens. In terms of size, green phycobiont cells are usually considerably larger than the diameter of the mycobiont hyphae. This raises an immediate mechanical barrier to possible inclusion of phycobiont cells within the mycobiont hyphae. Just as the small size of the higher plant chloroplast, relative to the diameter of pathogenic fungal hyphae, seems to be one of the principal factors in their freedom from direct parasitization by pathogens, so does the size difference noted for lichen symbionts present an effective barrier to evolution toward the level of symbiotic flagellates and rhizopods.

We have noted that, in the fusion of motile unicells, there is no active penetration of one into the other. Similarly, there can be no active penetra-

tion of the phycobiont cell into the mycobiont hypha. The former is normally spherical, and linear growth is not one of its characteristics. It is thus impossible for any phycobiont cell to "grow" into a mycobiont hypha.

There are, of course, exceptions to this general statement, the most notable being blue-green phycobionts. *Nostoc* and related species have the property of "pseudolinear" growth, i.e., new cells are added by cell division thus increasing the length of the filament. Such filaments are motile at a certain stage of their life history, and they are more closely related to the size of the mycobiont hypha than is the green phycobiont cell. Mechanically, this line of development is possible in the blue-green lichens.

In the cycad symbiosis, *Nostoc* filaments penetrate fractures in the root cell walls to form an intracortical phycobiont layer similar in form to that of heteromerous lichens. It is conceivable, therefore, that the blue-green phycobiont might become incorporated in the mycobiont hyphae even though the present morphology of the phycobiont cells seems to preclude this. They are not known to be motile within the thallus and are usually much enlarged in size and aggregated compared with the motile free-living condition.

The mechanical construction of the green lichen thallus is more compatible with the incorporation of the mycobiont hyphae within the phycobiont cells—appressoria and haustoria being of frequent occurrence. This is the normal parasitic trend in which penetration of the host is achieved by enzymatic dissolution and mechanical rupture of the host cell wall by growth in length of the parasite hyphae.

There are thus two opposing trends to be seen within the green lichens at the present time. The parasitic tendency of the mycobiont hyphae to penetrate the phycobiont cells can be viewed as a retrogressive feature in that it tends to preserve the status of parasitism rather than symbiosis. On the other hand, the tendency toward elimination of the phycobiont cell wall and increase in membrane permeability can be viewed as a progressive step if we can legitimately consider such trends to be homologous to those evident in other symbiotic systems.

I have already remarked on the similarity of the foliose lichen thallus to the angiosperm leaf and particularly on the subcortical location of the phycobiont layer. There is little doubt that, physiologically, one is closely mirrored by the other. To dwell on the probabilities and patterns of further evolution of the green leaf is an instructive exercise and not wholly irrelevant to the foregoing discussion, but it goes somewhat beyond the intention of the present chapter. It is thus concluded with a brief but pertinent query into the future. Since the course of evolution has succeeded in producing the lichen symbiosis from a template remarkably similar to that from which the green leaf has been molded, can we except further convergence toward the green-leaf pattern?

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Appendix A

CLASSIFICATION

JOSEF POELT

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I. Early Lichen Systems

Space does not allow a survey of the many lichen systems that have already been proposed. Von Krempelhuber (1867–1872) summarizes these up to 1870. Fries' proposed system, based for the most part on type of alga, was unfortunately never completed. Vainio (1890) and Reinke (1894–1896) laid the groundwork for a system later used and popularized by Zahlbrückner (1907, 1926). Vainio developed his ideas further, as in his divergent conception of grouping the pyrenocarpous lichens (1921) and Coniocarpineae (1927). He placed the most highly differentiated genera at the beginning of his system and regarded less differentiated groups as reduced forms. Watson

(1929) achieved a more natural system for a number of groups but took a step backward when he emphasized spore type. Choisy (1949–1953) proposed some broad divisions based on rather obscure details where structure of pycnidia played an important role. Räsänen (1943) built on Vainio's system, presenting brief synopses and keys for all known genera and the most important sections but using characters that are not regarded as basic ones today. In the meantime, Nannfeldt (1932) had proposed a fungal system where two divisions were made, Ascoloculares and Ascohymeniales. These groupings have retained a considerable degree of validity up to the present time. A number of authors, in particular Luttrell (1951) and R. Santesson (1952, 1953), have made further subdivisions into ascohymenial and unitunicate versus ascolocular and bitunicate groups and proposed somewhat modified systems of classification (R. Santesson, 1952, 1953; Hale, 1967; Hale and Culberson, 1970) which are still far from being acceptable "natural" ones.

Even a superficial analysis of any of the past systems will show that we cannot really speak of a true integration of lichenized fungi in any presently accepted fungal system. Lichen systematists have hardly ever been really familiar with the corresponding fungal groups, and mycologists have had enough difficulties with their own groups without bringing in the lichenized fungi. We are still far from a final natural system for lichen fungi. Much remains to be done on important diagnostic characters in all groups.

II. Parasitic Lichens, Parasympbionts, and Lichen Parasites

As symbiotic organisms lichens are by definition autotrophic. It must seem strange then that we still find species containing algae which regularly parasitize other lichens or, more rarely, mosses. This concept of parasitism is taken here in the broadest sense. There are actually numerous species which live as parasites in various degrees. References to these are scattered through the literature of the nineteenth century (see, for example, Fries, 1874, p. 343; Arnold, 1874, p. 86; Malme, 1892). Most such species have by definition been excluded from lichens proper. Their names accordingly do not usually appear in Zahlbrückner's *Catalogus*. In the past decade many new species in this biological group have been described. They are parasitic but also have their own algae-containing thalli so that they may be assigned to definite lichen genera (Magnusson, 1929, p. 38; Lamb, 1953, p. 405). The group has received more recent attention (Poelt and Doppelbaur, 1956) and an incomplete survey for Europe (Poelt, 1958) indicates 54 species among the discolichens. More parasitic species are being described (Poelt, 1963; Hertel, 1970b; Poelt and Steiner, 1971). By contrast, the pyrenocarpous parasitic lichens have attracted very little attention. Steiner (1896, 1898) long

ago discussed pertinent species in the genus *Verrucaria* and proposed a separate genus *Verrucula*.

The biological relationships of the species involved are often very different. In addition to the facultatively parasitic species, as the group in *Rhizocarpon geographicum*, one also finds a widely dominant group of obligate parasites which, because of their dependence on parasitism, are often reduced in size and thallus structure when compared to related non-parasitic species. This form of parasitism is from all existing observations genetically conditioned. The parasitic lichen species appear to have arisen from true autotrophic associations.

The majority of organisms that are usually designated as lichen parasites are probably derived from parasitic lichens. For example, one can find in *Buellia* not only many undoubted independent parasitic species that still form their own thallus, but other species which have hyphae entering a secondary symbiotic association with the host algae without forming a morphologically independent thallus. These organisms have been referred to as parasymbionts since Zopf (1897). We can cite as an example *Buellia destructans*, a parasitic species which injures its host and ultimately grows as a saprophyte on wood. Another direction has been taken by a species group in *Arthonia* (Hertel, 1969), the most extreme being *A. intexta* Almqu., the ascii of which are imbedded in the hymenium of the host rather than in its own fruiting body. Most of the parasites such as this one are biologically rather strongly bound to a definite host but the poverty of good characteristics makes them difficult to study taxonomically.

Other so-called lichen parasites are clearly derived from nonlichenized fungi, for example, the Nectriaceae (Santesson, 1953). We are not concerned here with groups of lichen parasites without a lichenized thallus. The greater part appear to be very closely related to lichens but have received little attention from mycologists. They have almost exclusively been the province of lichenologists.

Arnold (1874) drew up a comprehensive list of these organisms using works by Tulasne (1852), Lindsay (1869), and Koerber (1855, p. 452). Zopf (1896a,b, 1897, 1898), Tobler (1911), Bachmann and Kotte (for citations, see Keissler, 1930, pp. 17, 20, and 24) dealt with the biology of this group. Systematic lists for finite regions were published by Zopf (1896b), Olivier (1905–1906, 1907), Vouaux (1912–1913, 1914), and Watson (1948). The foundation of this branch of lichenology was laid by Keissler in his treatment of lichen parasites in Rabenhorst's *Kryptogamenflora*. Nomenclature and species concepts, however, are in need of considerable revision (R. Santesson, 1960; Vězda, 1963, 1969, 1970). Progress in the taxonomy depends more on an understanding of the biological relationships, much as in autotrophic groups.

III. Lichenized and Nonlichenized Fungi

As just mentioned, one is faced with great difficulties in any attempts to fit lichenized fungi into any system proposed for the nonlichenized fungi. The latest synopsis of the discomycetes (Kimbrough, 1970), in an all too characteristic way, omits the lichen fungi completely.

Many authors consider that the Lecanorales represent a very special group of almost exclusively lichenized fungi whose nonlichenized representatives are partly, if not completely, secondarily nonsymbiotic. Earlier workers called them "Lichenes athall," "Lichenes parasitici," or "Pseudolichenes." Some poorly known genera such as *Mniaecia* and *Epiglia* occupy the transitional area with the Lecanorales. Likewise, the Helotiales, which contain no typical lichenized species, include a number of atypical genera not previously positively classified (*Odontotrema*, *Beloniella*, *Tryblidium*, etc.), which resemble lichens in habit with perennial thalli easily discoloring the substrate, long-lasting fruiting bodies, etc. This group should receive more attention. It may be closely related to the Lecanorales.

The families grouped under the Arthoniales below in our proposed classification also seem to represent an evolutionary line wholly dominated by lichenized fungi with a number of species occurring as parasites on lichens. The boundary with nonlichenized forms is of course harder to draw (*Opegrapha*-*Hysteriales*?) and satisfactory delimitations will require much more study. One should consult the discussions of the Arthoniaceae by Müller and von Arx (1962, p. 223).

The lichenized Dothideales appear to be a small relatively recent group with a very large number of nonlichenized forms. Many form a "lichenoid" type but the boundary between lichens and lichenoid fungi and fungi which are perennial is especially difficult to draw in this group.

The Caliciales [according to Kimbrough (1970) a family in the Helotiales] are in our opinion basically lichenized fungi although various parasites and delichenized forms are known. It is difficult to decide whether the Mycocaliciaceae as studied by Schmidt (1970) should be considered as primary nonsymbionts or an offshoot of a lichenized group.

The Verrucariales also comprise an order of obviously primary lichenized fungi. See the discussion by Müller and von Arx (1962, p. 802). The lichen parasites *Tichothecium* and *Pharcidia* may or may not belong here.

The Pyrenulales are far too little known to be discussed intelligently. We assume that the nonlichenized relatives would be found in the tropics, if at all. Mycological literature dealing with comparable groups is of no help. We would tentatively regard them as a somewhat heterogeneous group of primarily lichenized fungi. The same may hold for the Ostropales, which have few nonlichenized species and some are partially lichenoid (*Stictis*). The

Graphidales are very doubtfully circumscribed at this time, and it is not possible to consider further the families of uncertain position here.

IV. Taxonomic Categories in Lichenology

A. Taxonomic Ranks

There is a very large body of literature on taxonomic ranks, dealing mostly with flowering plants (see Du Rietz, 1930; Davis and Heywood, 1963). The population concept in biotrophic groups has gained a good deal of attention in fungi (cf. Johnson, 1968). These concepts have played only a minor role in lichenology except for the arguments surrounding "chemical" populations. The following discussion will touch briefly on taxonomic ranks being used today and their probable value.

1. FORMA

Lichen (as well as bryophyte) floras, identification manuals, and lists are filled with "formae," 95% of which are merely modifications in the broadest sense. They do not really exist and should be dropped. The range of variation in the phenotype of a population is better expressed in a short description.

The concept of "forma" can be reserved for a slightly genetically deviating population which occurs dispersed over the area of the parent population and does not form a larger population and unique area. Examples of this are probably the mutant populations of *Solorina crocea* and *Parmelia centrifuga*.

2. VARIETY

This is used interchangeably for the term "forma" with many varieties of lichens. It is also used chiefly for more strongly divergent but still environmentally determined phenotypes without systematic value. Lichenologists have not agreed on a uniform interpretation of this category. It is often applied to insufficiently known populations or for phenotypes where the true basis of the deviation, environmental or genetic, remains unproved.

3. SUBSPECIES

Lichenologists have made little use of this rank. Runemark (1956) employed it for the difficult complex of yellow *Rhizocarpon* species in morphologically weakly differentiated populations with distinct centers of distribution. Imshaug and Brodo (1966) used it chiefly for chemical races of different geographical origin. Subspecies rank should be reserved in the future for

populations without strong morphological differences but with very clear chemical differences as well as distinct distributions or divergent ecological requirements.

4. SPECIES

It is unrealistic to discuss the rank of species in detail here. It is used for all distinct populations that are different from others and are based on morphological traits. When combined with geographic differences, a decisive chemical character alone may be sufficient for completely sorediate and usually widespread apomictic species.

5. GENUS

The concept of genus in lichens is now in a state of flux. Outside of a series of long-known strongly isolated and often species-poor genera, the rank of genus has long been used for large schematically delimited generic groups, while in other areas of cryptogamic botany there is a tendency to use genus for smaller species groups that are not always well delimited and without transitional areas but that are still natural. It would seem essential to examine the genus concept in nonlichenized fungi and to standardize it in lichens for smaller more natural groups.

No attempt will be made here to discuss ranks above genus.

B. Use of Chemical Criteria

No field in lichenology is as controversial as the use of lichen substances in taxonomy. Chemistry was introduced by Nylander in 1867 for color-spot tests that have a real diagnostic value. An enormous mass of chemical data for lichens has accumulated since then and several excellent compilations are available (Asahina and Shibata, 1954; C. Culberson, 1969, 1970). Recent lichenologists often specify the lichen substances in a species as a matter of course, as one would cite spore characters, etc.

Many papers have also been written in the last 20 years on the systematic value and meaning of lichen substances (Thomson, 1963; Hale, 1966, 1967; W. Culberson, 1969; Culberson and Culberson, 1970), both at the species level and in terms of generic and familial relationships. Nevertheless, dispute continues as to the systematic relevance of chemistry, although agreement is gradually being reached on the most important criteria for evaluating this character (J. Santesson, 1970).

Mention should be made of the systematic importance of the iodine test which has been used so often in lichenology. Unfortunately, little is known about the structure of substances where the reaction occurs and the chemical

and physiological basis for it. The iodine test is very dependent on the concentration of the solution, temperature, and other conditions and should therefore be carried out under comparable terms (Ziegenspeck, 1924; also Lettau, 1932, p. 15).

Iodine reactions have been given for the medulla of lichens (for example, isolichenin in *Cetraria islandica*) but only rarely for the cortex. In many groups such as *Lecidea* section *Silaceae* the medullary reaction (usually indicated as I+ blue but mostly a question of violet hues) has been found to be a remarkably constant and useful character for definition of species and even species groups. There are of course cases where a transient very slight coloration occurs and this should not be confused with a true test. The I+ red medullary reactions in the *Collemataceae* (Dughi, 1942) are meaningless.

Iodine reactions have been reported for fruiting bodies and used systematically for the cortex of certain crustose lichens [e.g., in the *Caloplaca ferruginea* group by Magnusson (1944), or in species of *Rinodina* by Magnusson (1947)]. The reactions are sometimes rather weak and should be followed with great care. The medullary reactions in ascocarps most often correspond to the medullary reaction of the thallus and are quite valid. The hymenia have truly diverse reactions; those in the subhymenium and parathecium are less studied. Various parts of asci also react [amyloid structures or "lichenin" of Ziegenspeck (1924) also defined as isolichenin]. Colloids or gelatin in the hymenium deposited by asci or hymenium colloids in general also react, as do, in a relatively smaller number of cases, the spores (for example, *Diploschistes*, *Graphis*, *Pachyascus*), but the intensity may vary according to population or species. A transient blue is replaced by a yellow or red hue in many groups. These reactions still demand a more exhaustive study.

V. Proposed Classification: Ascolichens

The following system is a composite drawn from numerous scattered articles. A number of colleagues have offered suggestions and help, including in particular Dr. A. Henssen, Dr. H. Hertel, and Dr. A. Vězda. This system, based partly on my own preliminary studies of ascus structure, is offered primarily as a help and stimulant for other investigations, not as a final solution to the problem of lichen classification.

Nomenclature of families follows Cooke and Hawksworth (1970) and for genera Ainsworth and Bisby (Ainsworth, 1971) was used. The number of species in each genus was taken for the most part from Ainsworth and Bisby and it should be realized that this number is at best a rough approximation. The morphological terms are those already defined in my chapter on taxonomic characters (Chapter 3) and in the chapter by Jahns (Chapter 1).

A. Order Arthoniales

Thallus crustose, more rarely distinctly lobed, predominantly fruticose in the Roccellaceae. Ascocarps round to lirelliform pseudothecia, solitary or rarely grouped, either lacking a receptacle or with a special often carbonized and frequently reduced receptacle, surrounded by the thallus. In some genera asci lying loosely on the mycelium and indistinctly grouped. Paraphysoids thick, branched in gnarls to anastomosing netlike. Asci typically bitunicate, nassaceous, rarely annelaceous. Spores two-celled to many-transversely septate to muriform and then with angular spore cells. Pycnospores rod-shaped to filamentous. Algae trentepohlioid to protococcoid. A series of species parasitic to parasymbiotic. Widespread but most commonly developed in the tropics.

1. ARTHONIACEAE REICHENB.

Thallus crustose. Ascocarps round to irregularly undulate and branched, without or with only a rudimentary receptacle. Asci globose to broadly clavate, united in lax groups or defined. Hypothecium sometimes carbonized. Thallus lichenized or saprophytic or parasitic to parasymbiotic on lichens. Widely distributed but richly developed in the tropics.

Genera: *Arthonia* Ach. (500), *Arthothelium* Mass. (80), *Cryptothecia* Stirt. (17), *Stirtonia* A. L. Sm. (6). This family is unrelated to the Myriangiales.

2. OPEGRAPHACEAE STIZB. EX TUCK.

Thallus crustose. Ascocarps sessile with a distinct receptacle or immersed and then often the receptacle reduced, clustered in stromata in some genera, lirelliform, simple to branched. Hymenium remaining as a slit or later opening widely. Asci more or less clavate. Thallus lichenized or parasitic to parasymbiotic on lichens. Widely distributed but chiefly tropical.

Genera: *Chiodecton* Ach. (175), *Dictyographa* Müll. Arg. (5), *Enterographa* Féé (30), ? *Enterostigma* Müll. Arg. (1), *Mazosia* Mass. (9), *Minksia* Müll. Arg. (3), *Opegrapha* Ach. (300), *Sclerophyton* Eschw. (8).

3. LECANACTIDACEAE STIZB. EX TUCK.

Thallus crustose. Ascocarps sessile with a distinct receptacle or immersed with a reduced receptacle, round. Asci more or less clavate. Spores transversely many septate. On bark and rock, chiefly in warmer regions.

Genera: *Lecanactis* Eschw. (90), *Melampygium* Stirt. (1), *Schismatomma* Mass. (85); ? *Pseudolecanactis* Zahlbr. (1). This family should perhaps not be segregated from the Opegraphaceae.

4. ROCCELLACEAE CHEV.

Thallus corticate, rarely crustose, mostly fruticose, round to flattened in cross section, rarely distinctly foliose, with a very irregular shape in many genera. Ascocarps sessile to immersed, rounded to irregularly sinuous, arranged in groups in many genera with a distinct receptacle or surrounded by thallus. Spores transversely many-septate, colorless to brown. Algae trentepohlioid. On rocks, rarely on wood, principally along coastlines in the tropics and subtropics.

Genera: Crustose: *Dirina* Fr. (14), *Dirinastrum* Müll. Arg (1), *Lobodirina* Follm. (1), ? *Cyclographa* Vain. (1). Fruticose—foliose: *Combea* de Not. (1), *Darbshirella* Zahlbr. ex Darbish. (1), *Dendrographa* Darbish. (2), *Dolichocarpus* R. Sant. (1), *Gorgadesia* Tav. (1), *Hubbsia* W. Web. (1), *Ingaderia* Darbish. (1), *Pentagenella* Darbish. (1), *Reinkella* Darb. (2), *Roccella* DC. (35), *Roccellaria* Darb. (1), *Roccellina* Darb. (1), *Roccellodea* Darb. (1), *Roccellographa* J. Steiner (1), *Sagenidium* Stirt. (1), *Schizopeltie* Th. Fr. (1), *Simonyella* J. Steiner (1).

5. CHRYSOTRICHACEAE ZAHLBR.

Thallus granular—cottony, white or brightly pigmented. Ascocarps sessile to immersed and then covered by thallus, white or brightly pigmented. Spores transversely many-septate, colorless. Algae protococcoid. On organic substrates in warmer regions.

Genera: *Byssocaulon* Mont. (6), *Chrysotricha* Mont. (1), ? *Temnospora* Mass. (?) consisting of very densely branched, thick-walled, anastomosing hyphae. The delimitation of this family is unsettled.

B. Order Dothideales

Thallus crustose. Ascocarps mostly perithecialike ascolocular pseudothecia with a loculus opening with a pore. Rarely several loculi present with pores or a fissured opening or growing together in stromata. Paraphyses filamentous, persistent or gelatinizing. Asci typically bitunicate, nassaceous, I—. Spores often asymmetric.

The nonlichenized fungi make up the bulk of this order (see, for example, Müller and von Arx, 1962, p. 262). The relationship of lichenized forms will remain unclear until the systematics of the order as a whole is clarified.

PLEOSPORACEAE WINT. (INCLUDING ARTHOPYRENIACEAE W. WATS.)

Pseudothecia sessile to immersed, free, with a single loculus (rarely several) or several growing in immersed to sessile stromata. Walls extremely thick. Pores and fissures apical, bare or provided with periphysoids. Asci

mostly eight-spored. Spores transversely two- or more-septate to muriform, colorless to dark. Many lichenlike forms are only doubtfully lichenized. The algae have usually proved to be *Trentepohlia* in those cases where algae can be demonstrated. Chiefly corticolous.

Genera: *Arthopyrenia* Mass., incl. *Lejophloea* S. Gray and *Xanthopyrenia* Bachm. (?200), *Dermatina* Almq. (30), *Leptorhaphis* Koerb. (36), *Microthelia* Koerb. (70), *Microtheliopsis* Müll. Arg. (1), *Mycomicrothelia* Keissl. (3), *Mycoporellum* Müll. Arg. (7 + ?6), *Mycoporopsis* Müll. Arg. em. Riedl (6 + ?3), *Polyblastiopsis* Zahlbr. p.p. (?), *Sporoschizon* Riedl (1), *Tomasellia* Mass. (50).

C. Order Verrucariales

Thallus crustose or foliose, gray to brown, lacking typical lichen substances. Ascocarps perithecia, structurally similar to the apothecia of the order Lecanorales, sessile or more or less immersed; wall colorless to carbonized, often surrounded by an annular to closed, appressed to spreading carbonized involucrum, and with persistent periphyses inside at the ostiole. Paraphyses very soon gelatinizing and deliquescent. Asci thick-walled, with a corona but nonfissitunicate, I- or I+ blue. Spores usually comparatively large, thin-walled. Pycnidia immersed; fulcra with long cells, the pycnospores threadlike. Algae protococcoid. Predominantly on inorganic substrates outside of the tropics.

VERRUCARIACEAE ESCHW. (INCLUDING DERMATOCARPACEAE ECHW.)

Characters as described for the order.

A. VERRUCARIOIDEAE. Hymenial algae absent. Thallus crustose to squamulose or peltate-foliose.

The division into genera in all previously proposed systems is quite artificial. One can find transitional stages in spore septation, in the presence or absence of involucral formation, and in growth form of the thallus.

Genera: *Dermatocarpon* Eschw. (80), *Leucocarpia* Vězda (1), *Placiopsis* Beltr. em. Servít (10), *Polyblastia* Mass. (120), ? *Sarcopyrenia* Nyl. (1), *Thelidium* Mass. (100), *Trimmatothele* Norm. (5), *Verrucaria* Schrad. (300).

B. STAUROTHELOIDEAE (STAUROTHELEACEAE SERVIT). Hymenial algae present. Thallus crustose to squamulose-foliose.

Genera: *Endocarpon* Hedw. (30), *Staurothele* Norm. (40).

D. Order Pyrenulales

Thallus crustose. Ascocarps perithecia with a typical ostiole, solitary or in groups. Asci more or less distinctly bitunicate, thick- or rarely thin-walled,

I-, with or without a corona or a nonamyloid less distinct ring structure. Spores symmetrically transversely septate, two- or more-celled or muriform. Paraphyses more or less filamentous, forming a net, or remaining free. Fulcra mostly simple. Algae predominantly trentepohlioid. On various substrates, principally in warmer regions. The following arrangement is strictly provisional. The divisions into families are taken chiefly from Vězda (in litt.).

1. PYRENULACEAE ZAHLBR.

Perithecia solitary or grouped with upright, oblique, or horizontal ostioles. Paraphyses branched and anastomosing. Asci mostly cylindrical with a corona and well-developed endoascus. Spores transversely several septate to muriform, with thickened walls and rhombic lumina, colorless or brown. Mainly corticolous in warmer regions.

Genera: *Anthracothecium* Hampe ex Mass. (93), *Astrothelium* (Eschw.) Trevis. (40), *Cryptothelium* Mass. (15) *Parathelium* (Nyl.) Müll. Arg. (?15) *Parmentaria* Müll. Arg. (25), *Plagiotrema* Müll. Arg. (2), *Pleurotheliopsis* Zahlbr. (7), *Pseudopyrenula* Müll. Arg. (45), *Pyrenastrum* Eschw. (22), *Pyrenula* Ach. (190).

2. TRYPTHELIACEAE ESCHW.

A number of perithecia produced in a stroma. Paraphyses branched and joined in a net. Asci cylindrical-clavate with a well-developed endoascus and less distinct ring (constant?). Spores transversely several septate with thick walls and more or less lens-shaped lumina, colorless. Chiefly tropical in distribution.

Genus: *Tryptothelium* (100).

3. LAURERACEAE VĚZDA AD INT.

Perithecia solitary or more commonly growing together in stromata. Paraphyses branched and netlike. Asci cylindrical-clavate with a well-developed endoascus and less distinct ring. Spores muriform, thin-walled, the lumina angular. Chiefly corticolous in the tropics.

Genera: ?*Bottaria* Mass. (6), *Campylothelium* Müll. Arg. (9), *Phyllobatellum* (Müll. Arg.) Müll. Arg. (3), *Laurera* Reichenb. (28), some species in *Polyblastiopsis* Zahlbr.

4. STRIGULACEAE ZAHLBR.

Perithecia solitary, erect, or lying oblique or horizontal and with oblique or lateral ostioles and then often growing together in groups. Paraphyses simple to sparsely or richly branched and netlike. Asci thick-walled, the endoascus continuous. Ring structure present or lacking. Spores trans-

versely two- to many-septate, colorless, the lumina angular. Algae mostly trentepohlioid, rarely protococcoid. On various kinds of substrates, primarily in warmer regions, with many foliicolous species in the tropics.

Genera: *Acrocordia* Mass. (?), *Aspidothelium* Vain. em. R. Sant. (4), *Lithothelium* Müll. Arg. (2), *Phylloblastia* Vain. (1), *Pleurotrema* Müll. Arg. (10), *Raciborskiella* Höhnel (2), *Strigula* Fr. (12).

Perhaps the family should be further divided into the Strigulaceae sens. strict. and the Acrocordiaceae.

5. CLATHROPORINACEAE VĚZDA AD INT.

Perithecia solitary, upright. Periphyses lacking or weakly developed. Paraphyses mostly simple. Ascii thin-walled, the endoascus reduced to a thickening of the ascus tip. Spores transversely septate to muriform, colorless, thin-walled. On various substrates, principally in warmer regions with many foliicolous species in the tropics.

Genera: *Belonia* Koerb. ex Th. Fr. (12), *Clathroporina* Müll. Arg. (60), *Porina* Müll. Arg. (300), *Trichothelium* Müll. Arg. em. R-Sant. (6).

The following pyrenocarpous family has not yet been assigned a definite position:

6. MICROGLAENACEAE SERVÍT.

Thallus crustose. Perithecia solitary, upright. Periphyses lacking. Paraphyses thin and filamentous, simple to branched and netlike. Ascii thick-walled, I+ light blue, the tips with a strongly I+ blue apical cushion or plug. Spores transversely septate to muriform, colorless, the walls or at least the septa thin. On various substrates, most common in cooler regions.

Genera: *Geisleria* Nitschke (3), *Microglaena* Koerb. (40); *Thrombium* (?) with one-celled spores could tentatively be placed here. *Thelenidia* also falls somewhere near here. (Vězda, in litt.).

E. Order Caliciales

Thallus crustose-squamulose to foliose and fruticose. Ascocarps apothecia with closed or more often open discs, most with distinct, more rarely reduced stipes. Ascii with an apical thickening, the spores released in the usual form; normally uniformly thin-walled, disintegrating at maturity or breaking up into spore-bearing fragments. Spores, fragments of ascii and paraphyses mostly forming a powdery mass, the so-called mazaedium. Spores normally weakly to strongly pigmented, often ornamented. Algae quite variable. Common on wood and bark, rarer on rocks, with some species parasitic or algae-free saprophytes.

This order appears to be very uniform. Included here as well are the nonlichenized populations except for *Caliciopsis*. There is no obvious connection, as previously assumed, to the Coryneliales.

1. MYCOCALICIACEAE A. SCHMIDT

Thallus thin, "crustose." Apothecia almost always distinctly stipitate. Ascii thick-walled or with an apical wall thickening, not breaking up into a mazaedium. Paraphyses simple or furcate. Spores more or less brown. Saprophytes, lichen parasites, or (?) lichens.

Genera: *Chaenothecopsis* Vain. (8), *Mycocalicium* Vain. (?), *Phaeocalicium* A. Schmidt (3), *Stenocybe* Nyl. ex Koerb. (5), *Strongyleuma* Vain. (4).

2. CALICIACEAE FÉE

Thallus crustose, squamulose to effigurate. Apothecia stipitate, sessile, or immersed with a rudimentary conical stipe in the thallus. Ascii thin-walled, disintegrating into a mazaedium. Spores colorless or brown. Fulcra simple. Pycnospores short or filamentous.

Two families were formerly recognized: Caliciaceae (apothecia stipitate to sessile) and Cypheliaceae (apothecia sessile to immersed). This division is certainly not a natural one. Possibly some tropical genera with filamentous pycnospores could be segregated as a distinct family, but this would be impossible in the light of our knowledge today.

Genera: *Allophoron* Nádv. (1), *Calicium* Pers. (100), *Carlosia* Samp. (1), *Chaenotheca* Th. Fr. (23), *Coniocybe* Ach. (24), *Coniocybopsis* Vain. (4), *Cyphelium* Ach. (30), *Cypheliopsis* Vain. (2), ?*Farriolla* Norm. (1), *Pyrgidium* Nyl. (1), *Pyrgillocarpon* Nádv. (2), *Sphinctrina* Fr. (20), *Schistophoron* Stirt. (1), *Stephanophoron* Nádv. (2), *Texosporium* Nádv. (1), *Tylophorella* Vain. (1), *Tylophoron* Nyl. (12).

3. THOLURNACEAE RÄS. (ILLEG.)

Thallus foliose, consisting of cylindrical hollow podetia coming out from the lobes and bearing immersed apothecia on the contracted tips. Spores two-celled, dark. Fulcra short-celled. Algae protococcoid. Growing on thin twigs.

Genus: *Tholurna* Norm. (1)

4. SPHAEROPHORACEAE FÉE

Thallus fruticose, branched, the branches flattened to round, solid in cross section, uniform or differentiated into an axis and phyllocladia, with or without cephalodia. Apothecia marginal to terminal, enveloped by the

thallus. Spores one- to mostly two-celled, pigmented. Fulcra short-celled. Algae protococcoid. In cool moist regions and centered in the Southern Hemisphere.

Genera: *Acroschyphus* Lév. (1), *Pleurocybe* Müll. Arg. (1), *Pseudosphaerophorus* Sato (1), *Sphaerophorus* Pers. (incl. *Calycidium* Stirt.) (7), *Thysanophoron* Stirt. (1).

The family is not at all homogeneous. *Acroschyphus* is a strongly isolated genus that could be placed in its own family. *Pleurocybe* also diverges from the type, while *Sphaerophorus*, *Pseudosphaerophorus* and *Thysanophoron* are very closely related.

F. Order Ostropales

Thallus crustose. Ascocarps round apothecia with punctiform to open ostioles, deeply immersed to sessile. Receptacles consisting of interwoven hyphae without forming a radiating amphithecum, colorless or dark. Periphysoids arising from the innermost receptacle layer. Paraphyses simple, sometimes anastomosing, not or only somewhat thickened apically. Ascii thick-walled but nonfissitunicate, cylindrical to clavate, I-, with or without apical rings and corona structure. Hymenial gelatin not rarely I+ blue. Spores mostly transversely several-septate to muriform, rarely one- or two-celled, often I+ violet. Fulcra usually long-celled. Pycnospores short. Algae predominantly trentepohlioid, occasionally also protococcoid. On various substrates in warmer regions.

1. OSTROPACEAE REHM

Apothecia at most barely surrounded by thallus. Ascii narrowly cylindrical. Spores extremely long and narrow, transversely many-septate to weakly muriform, thin-walled with angular spore cells. Algae protococcoid. On bark.

Genus: *Conotrema* Tuck. (5). The other members of the family are non-lichenized saprophytes.

2. THELOTREMATACEAE ZAHLBR. (INCLUDING DIPLOSCHISTACEAE)

Ascocarps round, mostly distinctly surrounded by the thallus. Ascii more or less clavate. Spores ellipsoid to long ellipsoid with lens-shaped or angular cells, colorless or brown. Algae trentepohlioid or more rarely protococcoid. Some of the genera are divided rather artificially on spore characters.

Genera: *Chroodiscus* (Müll. Arg.) Müll. Arg. (2), *Diploschistes* Norm. (35), *Gloeolecta* Lett. em. Vězda (2), *Leptotrema* Mont. et v.d. Bosch (65), *Ocellularia* Mey. (120), *Petractis* Fr. (5), *Phaeotrema* Müll. Arg. (30), *Polystroma*

Clem. (1), *Ramonia* Stiz. (10), *Thelopsis* Nyl. (6) *Thelotrema* Ach. (200), *Tremotylium* Nyl. (6).

The relationship of this order to the Graphidales which follow needs further study. They should perhaps be combined.

G. Order Graphidales

Thallus crustose. Ascocarps round or lirelliform apothecia with punctiform, fissured, or broadly opening discs. Receptacle consisting of thick, interwoven hyphae which do not form a radiating amphitheciun. Periphysoids lacking. Paraphyses simple or branched to netlike, very flexible, with more or less free thickened tips. Asci thick or less commonly thin walled, I- or I+ blue, a tholus present or not, I-. Spores often halonate. Algae protococcoid or trentepohlioid. On various substrates and best developed in warmer regions.

1. GRAPHIDACEAE DUMORT.

Ascocarps lirelliform, sparingly bordered by the thallus or emarginate. Receptacle frequently carbonized. Asci not amyloid. Spores ellipsoid to long ellipsoid, transversely several septate to muriform with lens-shaped cells, colorless to pigmented, I+ violet. Algae protococcoid or trentepohlioid. Chiefly on bark in warmer regions.

Genera: *Acanthothecis* Clem. (4), *Aulaxina* Fée (8), *Graphina* Müll. Arg. (270), *Graphis* Adans. (300), *Glyphis* Ach. (30), *Helminthocarpon* Fee (15), *Medusulina* Müll. Arg. (6), *Phaeographina* Müll. Arg. (90), *Phaeographis* Müll. Arg. (200), *Sarcographa* Fee (70), *Sarcographina*, Müll. Arg. (8).

The division into genera in this family is in part artificial.

2. MELASPILEACEAE W. WATS.

Ascocarps lirelliform, not bordered by thallus. Receptacle often carbonized. Asci not amyloid. Spores two-celled, thin-walled, colorless to mostly colored, often verrucose, the cells not lens-shaped. Pycnospores long. Algae trentepohlioid. On various substrates.

Genus: *Melaspila* Nyl. (60)

3. ASTEROTHYRIACEAE W. WATS. EX R. SANT.

Ascocarps round, immersed to sessile. Receptacle proso- to paraplectenchymatous, well-developed or rudimentary, barely carbonized. Spores transversely septate to muriform, colorless, with angular cells. Algae protococcoid. Mostly folicolous on evergreen trees in the tropics.

Genera: *Asterothyrium* Müll. Arg. (8), *Calenia* Müll. Arg. em. R. Sant. (10),

Echinoplaca Fée (97), *Gyalectidium* Müll. Arg. (3), *Psorotheciopsis* Rehm. em. R. Sant. (3), *Tricharia* Fée em. R. Sant. (8).

Vězda has recently assigned the following nonfoliicolous, largely calciphilous genera to this family: *Absconditella* Vězda (5), *Gyalidea* Lett. em. Vězda (13), *Petractis* Fr. em. Vězda (5), *Sagiolechia* Mass. (2), *Solorinella* Anzi (1).

4. GYALECTACEAE ZAHLBR.

Thallus crustose. Apothecia immersed to sessile, disc concave with a thick paraplectenchymatous proper margin covered by a layer of thallus. Paraphyses simple, more or less thickened knoblike at the tips. Asci uniformly thin-walled, I+ blue, without a tholus. Spores transversely several-septate to muriform with angular cells, colorless. Pycnospores short. Algae mostly trentepohlioid. On various substrates, common in moist habitats and often on limestone.

Genera: *Coenogonium* Ehrenb. ex Nees (15), *Dimerella* Trevis. (25), *Gyalecta* Ach. (34), *Gyalectina* Vězda (8), *Pachyphiale* Lönnr. (6).

The order Graphidales is still in need of critical study. The Gyalectaceae should perhaps be set apart more definitively.

H. Order Lecanorales

Thallus crustose, foliose, or fruticose. Apothecia usually solitary with open round discs and a clearly developed rarely absent radiate amphithecum in most species. Paraphyses most often with thickened knoblike tips. Asci normally I+ blue and thick-walled. Spores polymorphous. On various substrates in all zones. Most species are lichenized, some are parasitic on lichens, and a very few are saprophytic.

1. LICHININEAE

Thallus crustose-squamulose, dwarf fruticose to foliose, often peltate, with blue-green algae as the symbiont. Hyphae with rather large cells, at least in growing thalli. Apothecia immersed or sessile, frequently arising from pycnidia, initially and also later remaining closed by a pore or finally expanding. Receptacle usually present. Epihymenium brown, rarely green. Asci comparatively thin-walled. Spores 8 or many, always one-celled, colorless, roundish or ellipsoid. Paraphyses of many different shapes. Pycnospores short to filamentous.

a. LICHINACEAE NYL. (Including Ephebaceae Th. Fr. and Pyrenopsidaceae Th. Fr.). Thallus more or less gelatinous, mostly blackish or dark

green to red brown, granular, crustose-squamulose, foliose, or tiny fruticose. Apothecia variable in shape. Hymenium gelatinous. Symbionts unicellular or filamentous blue-green algae which are attacked by haustoria. Principally on stones, some of the time in very wet habitats, many on limestone. Sorediate species lacking.

Genera: *Anema* Nyl. (7), *Calotrichopsis* Vain. (3), *Ephebe* Fr. (12), *Gloeohepnia* Gyel. (1), *Gonohymenia* J. Steiner (8), *Lemmopsis* Zahlbr. (10), *Lempholemma* Koerb. (30), *Lichina* C. Ag. (7), *Lichinella* Nyl. (3), *Lichinodium* Nyl. (3), *Phylliscidium* Forss. (1), *Phylliscum* Nyl. ex Mass. (6), *Peccania* Mass. ex Arnold (9), *Poroscyphus* Koerb. (7), *Psorotichia* Mass. (50), *Pterygiopsis* Vain. (2), *Pyrenopsidium* (Nyl.) Forss. (8), *Pyrenopsis* Nyl. (40), *Rechingera* Serv. (1), *Synalissa* Fr. (5), *Thermitis* Fr. (1), *Thyrea* Mass. (20), *Zahlbrucknerella* Herre (2).

The generic divisions in this family are most unsatisfactory and will probably undergo considerable change in the future.

b. HEPIACEAE ZAHLBR. Thallus nongelatinous, squamulose to peltate-foliose or peltate-fruticose, distinctly corticate at least on the lower surface, occasionally continuously cellular. Apothecia immersed, initially closed and then opening wide or open from the beginning, frequently filling up the areoles on which they are borne. Paraphyses unbranched. Asci with an I+ blue tholus. On soil and rocks in hot dry regions, normally in habitats receiving occasional wetting. Some sorediate species.

Genera: *Heppia* Naeg. (1), *Peltula* Nyl. (18).

2. PELTIGERINEAE

Nongelatinous or only weakly so, foliose and often attaining large size, or small fruticose or granular-squamulose with usually large-celled paraplectenchyma in the cortex. Algae blue-green or green, but if green, then cephalodia with blue-green algae commonly present. Apothecia immersed to sessile, more or less distinctly hemiangiocarpous. Paraphyses mostly unbranched and free. Asci either anellascaceous with an I+ blue ring, or less commonly I- and nassascaceous. Spores mostly transversely many-septate, often brown when mature. Fulcra long- or short-celled. On various substrates but frequently preferring wet or at least moist habitats. Many sorediate species.

a. PLACYNTHIACEAE DAHL. Thallus crustose-squamulose to tiny fruticose, mostly with a blue-green algal symbiont. Apothecia at maturity sessile, either biatorine to lecideine with a well-developed amphithecum or pseudoeoxiciple or lecanorine with a mostly large-celled paraplectenchymatous

cortical structure. Asci with an I+ tholus. Spores eight, rarely simple, usually two or more transversely septate. Chiefly on stones, soil, and mosses, more rarely on bark.

Genera: *Epiphloea* Trevis. (1), *Koerberia* Mass. (2), *Massalongia* Koerb. (2), *Moelleropsis* Gyel. (1), *Placynthium* (Ach.) Gray (25), *Polychidium* (Ach.) Gray (5), *Psoroma* (Ach.) Michx. (35), ? *Steinera* Zahlbr. (1), *Vestergrenopsis* Gyel. (2).

This delimitation follows Henssen loc. div.

b. PELTIGERACEAE DUMORT. Thallus foliose, upper surface or in part both surfaces corticate paraplectenchymatously with large cells, lower surface often veined, rhizinate or indistinctly umbilicate. Apothecia distinctly immersed and hemiangiocarpous, marginal or laminal, emarginate. Asci I+ blue with a distinct I+ blue ring-shaped apical apparatus. Spores transversely two- or more- septate, colorless to mostly brown at maturity. Fulcra long- celled. Algae either of the *Nostoc* type or protococcoid but in addition with blue-green algae in the cephalodia. Predominantly occurring on soil.

Genera: *Hydrothyria* Russ. (2), *Peltigera* Willd. (30), *Solorina* Ach. (?10).

c. NEPHROMIACEAE MOREAU. Thallus foliose, paraplectenchymatously corticate on both surfaces. Apothecia immersed, distinctly hemiangiocarpous, produced on the lower surface of the thallus lobes which turn up, leaving the hymenium finally lying on the functional upper surface. Asci nassaceous, without a ring, I-. Spores four- or more- septate transversely, brown. Fulcra short-celled. Algae belonging to *Nostoc* or *Coccomyxa* but in either case with *Nostoc* present in the cephalodia. On many kinds of substrates in moist regions.

Genus: *Nephroma* Ach. ex Luyken (35).

d. LOBARIACEAE CHEV. (STICTACEAE ZAHLBR.). Thallus foliose, large, with a large-celled paraplectenchymatous cortex on both surfaces, lower surface with cyphellae, pseudocyphellae, or more or less distinct lenticellularlike areas. Apothecia marginal or laminal, sessile, not distinctly hemiangiocarpous, biatorine to lecanorine with large-celled receptacle. Asci I+ blue without an apical ring. Spores transversely two- or more-septate, brownish at maturity. Fulcra short-celled. Algae often belonging to *Nostoc* but if protococcoid then often with blue-green algae in addition in cephalodia. On various substrates in humid areas, especially in tropical mountains and in the Southern Hemisphere.

Genera: *Lobaria* (Schreb.) Hoffm. (80), *Pseudocyphellaria* Vain. (200), *Sticta* (Schreb.) DC. (200).

3. FAMILIES WITH BLUE-GREEN SYMBIOTS: CLASSIFICATION NOT SETTLED

The following families in the Lecanorales s. ampl. have blue-green algal symbionts, but their position in the system is still not settled.

a. COLLEMATACEAE FÉE. Thallus gelatinous, usually blackish, foliose to more or less fruticose, rarely nearly crustose, isidiate in many species. Apothecia mostly densely sessile, formed of the ascogonium and its stipe cells, usually with a closed cup-shaped exciple, lecideine, biatorine, or lecanorine, hyphae in the receptacle anticlinal or periclinal. Paraphyses simple or branched, strongly gelatinous. Asci clavate with an amyloid tholus, the ascus gelatin I+ blue. Spores normally eight rarely fewer, one-celled, transversely two- or more-septate to muriform, colorless. Fulcra short-celled. Pycnospores ± oblong. Algae belonging to *Nostoc*. On various substrates in rather moist habitats, worldwide. No sorediate species but many with isidia or phyllidia.

Genera: *Collema* Wigg. (80), *Homothecium* Mass. (3), *Leciophysma* Th. Fr. (2), *Leightonella* A. Henssen (1), *Leptogium* (Ach.) Gray (50), *Physma* Mass. (10), *Ramalodium* Nyl. ex Cromb. (3).

b. COCCOCARPIACEAE HENSSSEN. Thallus dwarf fruticose to foliose, attached with anchoring hyphae. Apothecial primordium with many upright ascogones formed in association with isodiametric cells. Apothecia sessile, dark, lecideine. Proper exciple barely seen. Receptacle cellular, the hymenium gelatinous. Paraphyses simple, thick, and rigid. Asci with a large I+ blue tholus. Spores unicellular, colorless. Fulcra short-celled. Pycnospores short, bacilliform. Algae filamentous blue-green. Occurring in habitats that are quite moist at least part of the time or in humid regions.

Genera: *Coccocarpia* Pers. (25), *Spilonema* Born. (4).

These two genera have very different structure.

c. PANNARIACEAE TUCK. Thallus squamulose to foliose, not gelatinous, lower surface densely covered with tomentum, at least the upper surface corticate with anticlinal hyphae. Apothecia immersed to mostly sessile, with a normally large-celled paraplectenchymatous receptacle, biatorine to lecanorine. Asci strongly amyloid with an amyloid tholus. Paraphyses rigid. Spores mostly unicellular, ellipsoid fusiform. Fulcra short-celled, divided, pycnospores long. Algae of the *Nostoc* or *Scytonema* type. In moist habitats and humid regions on noncalcareous substrates. Many species sorediate.

Genera: *Erioderma* Fée (16), *Pannaria* Del. (80), *Parmeliella* Müll. Arg. (50).

d. ARCTOMIACEAE TH. FR. Thallus granular crustose to rosettiform, dark red-brown to olive, rhizinate, corticate with one or more cell layers. Apothecia broadly sessile, lecideine, arising from a generative structure, with a large-celled receptacle. Paraphyses netlike, with strongly thickened ends. Asci thick-walled, strongly amyloid. Spores colorless, transversely several-septate. Fulcra short-celled. Pycnospores bacilliform. Algae *Nostoc* filaments lying in gelatinous spheres. On mosses and plant debris in the Arctic.

Genus: *Arctomia* Th. Fr. (2)

4. LECANORINEAE

Thallus crustose to squamulose, foliose with or without rhizines, to fruticose and bearded. Apothecia sessile, rarely immersed, normally with a distinct amphithecum, biatorine or lecideine, or with a thalline margin. Paraphyses free, simple or branched or netlike interwoven. Asci with a tholus which is at least in part distinctly I+ blue. Spores mostly eight, predominantly unicellular and colorless, also sometimes transversely two- or more-septate and muriform, occasionally pigmented. Fulcra formed in various ways. Algae almost always protococcoid, usually *Trebouxia* in the more highly developed forms, in a few cases trentepohlioid. On various substrates, worldwide. Many species sorediate.

The group outlined here comprises the nucleus of the Lecanorales. It includes, on the one hand, the crustose forms with lecideine and biatorine receptacles (Lecideaceae), still poorly understood, and those with a lecanorine receptacle (Lecanoraceae; see Eigler, 1969); and on the other hand derivatives to foliose and fruticose forms which are all obviously from the lecanorine state and are more closely allied to the Lecanoraceae than the Cladoniineae are to the Lecideaceae. Since the Lecideaceae and Lecanoraceae cannot now be separated significantly, it would seem best to propose the interim arrangement given below. The two families form conglomerates of crustose forms with a great many species that are not further divisible at this time, while the following families are much more narrowly defined and are probably more natural.

a. LECIDEACEAE CHEV. Thallus crustose to effigurate or squamulose, provided with hyphae or rarely weakly differentiated rhizines below. Apothecia sessile, biatorine, or lecideine, the margin sometimes differentiated into medulla and cortex. Other characters variable, as given in the diagnosis of the suborder. Algae protococcoid. On various substrates, cosmopolitan in distribution.

Genera: *?Aglaothecium* Groenb. (1), *Bacidia* de Not. (400), *Byssolecania* Vain. (2), *?Byssoloma* Trevis. (6), *Catillaria* Mass. em. Th. Fr. (300), *Crocynia*

(Ach.) Mass. (?), *Gomphillus* Nyl. (1), *Lasioloma* R. Sant. (4), *Lecidea* Ach. em. Th. Fr. (800), *Lecidella* Koerb. em. Hertel & Leuckert (16), *Lopodium* Koerb. (70), *Megalospora* Mey. et Flot. (50), *Mycoblastus* Norm. (9), *Phyllopsora* Müll. Arg. (35), *Psorella* Müll. Arg. (14), *Rhizocarpon* Ram. ex DC. (200), *Schaerera* Koerb. (1), *Sporopodium* Mont. em. R. Sant. (9) *Tapellaria* Müll. Arg. em. R. Sant. (8), *Toninia* Mass. em. Th. Fr. (80).

b. LECANORACEAE FÉE. Thallus crustose to effigurate, squamulose and peltate-foliose, provided with hyphae, rhizinal strands, or umbilicus below, lacking rhizines. Apothecia sessile, rarely somewhat sunken, brightly pigmented, with a lecanorine receptacle. Paraphyses mostly free, occasionally anastomosing somewhat. Spores colorless, unicellular to transversely two-or-more-septate. Fulcra and pycnospores extremely variable.

Genera: *Haematomma* Mass. (30), *Lecania* Mass. (90), *Lecanora* Ach. ex Luyken (400), *Physcia* Tuck. (2), *Solenopsora* Mass. (13), *Squamaria* Poelt (18).

c. ASPICILIACEAE AD INT. Thallus crustose to effigurate, even foliose and fruticose, not strongly pigmented, without rhizines. Structure often distinctly paraplectenchymatous, especially in the cortex. Apothecia more or less immersed, cryptolecanorine, light or blackish. Paraphyses often at least in part divided moniliform, mostly flaccid. Asci thin-walled, flaccid, with various I+ blue structures in the ascus tip. Spores large, up to eight but often fewer, with very flexible walls. Fulcra long-celled. Pycnospores short to long filamentous. Algae protococcoid or trentepohlioid. Predominantly saxicolous in cool or warm xeric regions.

Genera: *Aspicilia* Mass. (100), *Hymenelia* Krempelh. (3), *Ionaspis* Th. Fr. em. G. Eigler (25), *Lecanorella* Frey (1).

It may be that this family is not at all closely related to the Lecanoraceae even though *Aspicilia*, the basis for the family name, has long been treated as a section of *Lecanora*.

d. HYPOGYMNIACEAE AD INT. Thallus distinctly radiate foliose to subfruticose, corticate on both surfaces, with haptera below, gray but rarely yellow green, usually with a very lax medulla, the cortex consisting of anticlinal hyphae. Rhizines lacking. Apothecia wide, substipitate. Asci small, amyloid with a strongly amyloid ring in the tholus. Spores small and unicellular, up to eight, or fewer and larger. Fulcra moderately long-celled, with bayonet sterigmata. Pycnospores bacilliform. Algae belonging to *Trebouxia*. On various acidic substances in boreal and subantarctic regions or cool mountains and islands.

Genera: *Cavernularia* Degel. (2), *Hypogymnia* Nyl. (40), *Menegazzia* Mass. (30), *Pseudevernia* Zopf (4).

The family forms a definite phylogenetic entity. The genera had previously been placed in the heterogeneous family Parmeliaceae. It seems best to segregate them, for intermediates are lacking or at least very doubtful.

e. PARMELIACEAE ESCHW. Thallus foliose, corticate on both surfaces, provided with rhizines below, rarely umbilicate, very rarely without rhizines (reduced?), in some forms fruticose-suberect but mostly dorsiventral in cross section, often brightly pigmented. Apothecia narrowly sessile, more rarely sunken, laminal, or rarely marginal. Ascii with a strong amyloid tholus. Spores frequently small but unusually large in a few groups. Fulcra moderately long-celled, pycnospores mostly short bacilliform, rarely long filamentous. Algae belonging to *Trebouxia*. Widely distributed on various substrates, the various natural groups having quite different centers of geographic origin.

Genera: *Asahinea* Culb. et Culb. (3), *Cetraria* Ach., incl. *Nephromopsis* Müll. Arg (45), *Cetrelia* Culb. et Culb. (14), *Dactylina* Nyl. (19), *Omphalodium* Mey. et Flot. (3), *Pannoparmelia* (Müll. Arg.) Darbish. (5), *Parmelia* Ach. (600), *Parmeliopsis* (Stizb.) Nyl. (7), *Platismatia* Culb. et Culb. (10).

The family is still not homogeneous even with this delimitation.

f. USNEACEAE ESCHW. Thallus fruticose to beardlike, radial in cross section but sometimes dorsiventral, erect or pendulous, often with a basal attachment or dying away from below in those species growing erect on soil, usually strongly pigmented. Medulla often lax but traversed by one or more cords. Apothecia lateral or terminal constricted sessile, lecanorine. Spores unicellular and colorless, rarely dark and muriform. Fulcra of various structure. Algae belonging to *Trebouxia*. On various substrates, chiefly in humid regions.

Genera: *Alectoria* Ach. ex Luyken (40), *Bryopogon* Link em. Bystrek (60), *Cornicularia* (Schreb.) Ach. (16), *Evernia* Ach. ex Luyken (6), *Everniopsis* Nyl. (1), *Himantormia* M. Lamb (1), *Letharia* (Th. Fr.) Zahlbr. (2), *Neuro-pogon* Nees et Flot. (10), *Oropogon* Th. Fr. (4), *Sulcaria* (Mot.) Bystrek (2), *Usnea* P. Browne ex Adans. (600).

The family is not homogeneous but the relationships of the individual genera are much too poorly known to permit a better division. Perhaps *Alectoria* should comprise a separate family with *Bryopogon*, *Oropogon*, and *Sulcaria*, perhaps with inclusion of certain parts of *Parmelia*. The other genera are close to the Parmeliaceae group.

g. RAMALINACEAE AG. Thallus erect to pendulous fruticose, rarely foliose, more or less compressed, rarely round in cross section, but hardly zygomorphically structured, gray-green to yellow, the cortex mostly cartilaginous with thick-walled anticlinal or periclinal hyphae, often also with

mechanical tissues under the cortex. Apothecia for the most part lateral. Spores two-celled, ellipsoid-fusiform, often somewhat curved, colorless. Fulcra long-celled, pycnospores bacilliform. Algae belonging to *Trebouxia*. On bark and noncalcareous rock, largely distributed in warmer regions. Genera: *Desmaziera* Mont. ex Gay (13), *Ramalina* Ach. ex Luyken (200), *Ramalinopsis* (Zahlbr.) Follm. et Huneck (1), ?*Speerschneidera* (Stizenb.) Tuck. (1).

This group of genera (except for *Speerschneidera*?) forms a natural entity. Relationships to other families (*Himantormia*) are not clear.

h. ANZIACEAE SATO. Thallus foliose with deeply divided, often somewhat articulated lobes, upper surface gray, the cortex composed of anticlinal hyphae, the medulla below turning into a thick layer of tomentum consisting of anastomosing pale to mostly dark hyphae, and individual stout rhizines coming from the medulla. Apothecia laminal. Ascii with a large amyloid tholus. Spores numerous, unicellular, colorless, curved in a crescent shape. Pycnospores short. Algae belonging to *Trebouxia*. On bark in oceanic areas, lacking in Europe.

Genus: *Anzia* (28).

Anzia is so aberrant from the Parmeliaceae, as well as other groups in the suborder, that it must be segregated as a distinct family. *Pannoparmelia* (see Parmeliaceae), while superficially similar, has nothing to do with *Anzia*.

5. CLADONIINEAE

Thallus differentiated into a crustose to squamulose-foliose horizontal thallus, which may be lacking or disappear with age, and a vertical simple or branched, solid or hollow structure (podetium or pseudopodetium) which in turn can be covered with squamules. Thallus with hyphae on the lower surface, or lacking attachment organs. Apothecia biatorine, light-colored, or rarely lecideine and dark. Paraphyses simple or branched. Spores unicellular to transversely two- or more-septate, rarely muriform, colorless. Fulcra long-celled. Pycnospores bacilliform to filamentous. Algae apparently always belonging to *Trebouxia*. On soil and stones, predominantly on non-calcareous substrates.

The families brought together in this suborder have some sort of podetia. Their ancestors have been placed in the "family" Lecideaceae. We still do not know the extent to which the suborder as comprehended here is polyphyletic.

a. STEREOCAULACEAE CHEV. Pseudopodetia of thalline origin present, generative tissues arising at the tips, horizontal thallus squamulose or scarcely developed. Pseudopodetia solid, either holostelide, arising by

elongation of a complete thallus squamule and sharing the same algal layer, or enteropodial, arising from the medullary layer, the algal layer being carried upward and developing into the more or less foliose assimilatory organs, the phyllocladia. Cephalodia usually present, external or rarely internal. Apothecia biatorine or lecideine. Asci I+ blue with an I+ strongly blue tholus. Spores several-septate to muriform. Saxicolous and terricolous, exclusively on acidic substrates, chiefly in cool humid regions, boreal or in warmer areas montane, a primitive group centered in the Southern Hemisphere.

Genera: *Argopsis* Th.Fr. (1), *Compsocladium* M. Lamb (1), *Pilophorus* Th.Fr. (10), *Stereocaulon* (Schreb.) Hoffm. (120).

b. CLADONIACEAE REICHENB. Podetia consisting of generative tissue ("true" podetia), but intergrading to pseudopodetia or with the primary thallus itself forming the stipe, hollow or solid with or without a central cylinder, mostly covered with a corticate algal layer. Cephalodia lacking. Asci with an amyloid tholus. Spores unicellular, rarely multicellular. Mainly terricolous on acid substrates, most of the genera occurring in warmer areas but the large genus *Cladonia* cosmopolitan.

Genera: ?*Cladia* Nyl. (5), *Cladonia* (Hill.) (300) ?*Cladoniopsis* Zahlbr. (1), *Glossodium* Nyl. (2), *Gymnoderma* Nyl. (2), *Heteromyces* Müll. Arg. (1), *Neophyllum* F. Wils. (1), *Pycnothelia* (Ach.) Duf. (1), *Sphaerophoropsis* Vain. (2), *Thysanothecium* Mont. et Berk. (4).

The foliose genus *Heterodea* Nyl. (1) may be related to *Cladia*.

The family is probably not homogeneous.

c. BAEOMYCETACEAE FÉE. Apothecia solitary on short unbranched stipes which make up true podetia and can be surrounded by algae-containing tissues. Apothecia biatorine to lecanorine. Ascus wall with a weak apical thickening, not amyloid. Spores multicellular. Cephalodia lacking. Mainly terricolous on very acidic substrates, centered in tropical-subtropical mountains.

Genera: *Baeomyces* (44), *Icmadophila* Trevis (?2)

Chadefaud (1960, p. 545) has recently assigned *Baeomyces* to the Helotiales near *Leotia* ("Leotiales") on the basis of similarities in the form of the fruiting bodies and in ascus structure. We cannot support this disposition from the knowledge of the groups presently available.

d. SIPHULACEAE REICHENB. Thallus consisting of prostrate to erect, cylindrical or flattened, solid, elongate or mostly branched lobes or vermiciform and hollow, gray-white, the cortex paraplectenchymatous. Algae protococcoid. Apothecia unknown. Widely occurring on soil and rocks in cool moist regions.

Genera: *Endocena* Cromb (1), *Siphula* Fr. (25), *Thamnolia* Ach. ex Schaeer. (2).

These three genera had customarily been assigned as *incertae sedis* in the Usneaceae, where they do not belong. Their chemistry is closer to that of the Cladoniaceae, near which they are provisionally placed.

6. UMBILICARIINEAE

Thallus foliose, umbilicate, corticate on both sides, whitish-gray to blackish, never greenish or yellow, lower surface bare or provided with medulla-containing rhizines (not functioning as attachment organs), very rarely attached in crustose way. Apothecia sessile, sometimes sunken in depressions or substipitate, anatomically strongly differentiated, lecideine to superlecidideoid, frequently umbilicate to furrowed and deeply divided, with a colorless to dark hypothecium. Paraphyses simple, lax, slightly thickened. Asci with relatively little thickened tips that are I+ strongly blue with I+ violet ascal gelatine. Spores unicellular, transversely two- or more-septate to muriform, colorless to brown. Fulcra short-celled. Algae protococcoid. Almost exclusively occurring on noncalcareous rocks.

UMBILICARIACEAE FÉE. Characters as given for the suborder. Mainly large lichens on noncalcareous rocks but rarely also on hard wood or bark.

Genera: *Lasallia* Mér. (8), *Umbilicaria* Hoffm. (45).

This seems to be an ancient family set apart from others by sharp discontinuities. It does not fit in closely with the Lecideaceae, and it seems more convenient to give it a separate place in the classification.

7. ACAROSPORINEAE

Thallus crustose, squamulose, foliose-peltate, or distinctly dwarf fruticose. Apothecia sunken or more rarely sessile, open or rarely closed in the manner of perithecia. Paraphyses simple, free, rarely netlike interwoven. Asci mostly thick-walled, I+ weak or barely turning blue, the tholus also only I+ pale blue. Ascal gelatin usually strong I+ blue. Spores always very numerous, unicellular, colorless. Fulcra long-celled. Pycnospores short bacilliform. Algae protococcoid. On various substrates but predominantly saxicolous, weakly nitrophilous or not so. There is only one rather heterogeneous family.

ACAROSPORACEAE ZAHLBR. Thallus as given in the suborder diagnosis, little to distinct differentiation into medulla and cortex, the cortex usually with a distinctly developed paraplectenchyma. Apothecia solitary or several sunken in areoles, or sessile, with well-developed, more or less open or rarely punctiform disc. Spores ellipsoid to rarely globose, unicellular (but

often appearing two-celled). Pycnidia frequently multichambered. The various genera have quite different centers of origin.

Genera: *Acarospora* Mass. (300), *Ahlesia* Fuckel. (3), *Biatorella* de Not. (50), *Glypholecia* Nyl. (1), *Maronea* Mass. (13), *Maronella* M. Steiner (1), *Sarcogyne* Flot. (30), *Sporastatia* Mass. (4), *Thelocarpon* Nyl. (12).

The family is distinguished by production of numerous spores per ascus, this being correlated with other characters. There are no isidiate or sorediate species. There is some question on the derivation of *Thelocarpon* which should be related to *Acarospora (Xanthothallia)* by way of *Ahlesia* and some of the yellow species assigned to *Biatorella*.

8. PERTUSARIINEAE

Thallus crustose to dwarf fruticose, white, gray, or yellowish-green, frequently with a pale prothallus. Apothecia sessile or sunken, the disc open or closed as in perithecia with a pore opening, solitary or produced several at a time in more or less distinct stromata. Hymenium high. Paraphyses strongly netlike branched. Amphithecum poorly developed. Ascii thick-walled with several layers, I+ blue, with corona and amyloid tholus. Exoascus at maturity bursting in layers. Spores large to very large, polyenergide, walls often structured. Fulcra elongated. Pycnidia often multi-chambered, pycnospores filamentous. Algae protococcoid. On various substrates but rare on limestone.

PERTUSARIACEAE KOERB. Characters as in the suborder description.

Genera: *Coccotrema* Müll. Arg. (6), *Melanaria* Erichs. (15), *Ochrolechia* Mass. (40), *Pertusaria* DC. in Lam. et DC. (250), *Varicellaria* Nyl. (3).

The family, which includes many isidiate and sorediate populations, is recognized under a separate suborder because it has so many characters that set it apart from other families. A special characteristic is the co-occurrence of species with fruiting bodies remaining open and closed within certain species groups.

9. BUELLIINEAE

Thallus crustose, squamulose, foliose, or fruticose, variously colored. Apothecia sessile, rarely sunken, biatorine to lecideine or lecanorine with a more or less distinct amphithecum. Paraphyses simple or branched, as a rule with thickened tips, often anastomosing. Ascii more or less clavate, moderately thick-walled, I+ blue, with a distinct I+ blue tholus. Spores normally to eight, mostly two-celled, less commonly multicellular and muriform, unicellular only in a few at least partially derived groups, often thick-walled with complex wall structure. Fulcra short-celled. Pycnospores short

and straight, rarely filamentous. Algae protococcoid. Weakly to often strongly nitrophilous lichens on various substrates, cosmopolitan.

a. CANDELARIACEAE HAKULINEN. Thallus indistinct or crustose-squamulose, foliose, or appearing fruticose, pigmented yellow as in the apothecia with pulvinic acid derivatives except for a few species. Apothecia sessile, biatorine to lecanorine. Thallus and apothecial structure very distinctly paraplectenchymatous. Asci with a large tholus. Spores up to eight or numerous, one- to two-celled, often somewhat asymmetric. Predominantly strongly nitrophilous lichens, worldwide and occurring in many kinds of habitats.

Genera: *Candelaria* Mass. (7), *Candelariella* Müll. Arg. (40), *Candelina* Poelt (3), *Placomaronea* Räs. (2).

The family is hardly a typical division of this suborder but is best placed here because of ascal structure, type of paraphysis, and ecological relationships.

b. TELOSCHISTACEAE ZAHLBR. (INCLUDING CALOPLACACEAE ZAHLBR.). Thallus crustose to foliose or fruticose and beardlike, usually containing a yellow to red anthraquinone pigment. Thallus and apothecial structure usually rather indistinctly paraplectenchymatous. Apothecia sessile, rarely sunken, biatorine to lecanorine, barely distinctly lecideine, frequently pigmented by an anthraquinone, rarely brown or black, and without a K reaction. Spores up to eight but sometimes more, colorless, typically polaridiblastic, rarely unicellular, two-celled, or with several cells, connected by a canal. Moderately to strongly nitrophilous lichens, developed chiefly in dry warm regions.

Genera: *Brigantiaeae* Trevis. (?), *Caloplaca* Th. Fr. (450), *Fulglesia* Mass. et de Not. (10), *Protoplastenia* J. Steiner, (11), *Teloschistes* Norm. (30), *Xanthopeltis* R. Sant. (1), *Xanthoria* (Fr.) Th. Fr. (15).

c. PHYSCIACEAE ZAHLBR. (INCLUDING BUELLIACEAE ZAHLBR.). Thallus crustose, foliose, or fruticose, gray or rarely brown, yellow, or yellowish green, anthraquinones lacking in the cortex. Apothecia sessile, more rarely sunken. Spores eight or rarely more, two- or rarely more-celled or unicellular, at maturity gray-green and soon brown to blackish brown with frequently regularly or irregularly thickened walls and septa. Predominantly more or less nitrophilous lichens on various substrates.

Genera: *Anaptychia* Koerb. (9), *Buellia* de Not. (600), *Buelliastrum* Zahlbr. (2), *Dimelaena* Norm. (3), *Diploicia* Mass. (?1), *Dirinaria* Tuck. (10), *Heterodermia* Trevis. (60), *Orphniospora* Koerb. (1), *Physcia* (Schreb.) Michx. (150), *Physciopsis* Choisy (5), *Physconia* Poelt (15), *Pyxine* Fr. (35), *Rinodina* (Ach.) Gray (200), *Tornabenia* Trevis. (2).

d. DERMATISCACEAE AD INT. Thallus foliose, umbilicate, corticate on both surfaces. Apothecia sunken, cryptolecanorine. Spores two-celled. brown. Fulcra long-celled (?). On noncalcareous rocks.

Genus: *Dermatiscum* Nyl. (?1).

This genus has long been classified in the Umbilicariaceae. Its natural affinities lie with the Physciaceae because of spore type and ascus structure. It is probably better treated as a separate family because of the strong discontinuities; it is obviously not derived from *Rinodina* as are the other foliose and fruticose Physciaceae.

10. FAMILIES WITH NO CLEAR RELATIONSHIPS

Families falling within the order Lecanorales but lacking any clear relationships that can be determined at this time. Arranged alphabetically.

a. ARTHRORAPHIDACEAE AD INT. Thallus crustose, in the host or later independent. Apothecia sessile or occurring between thallus areoles (on prothallus?), black, very fine celled, more or less strongly permeated as the hymenium with greenish-brown excretions. Receptacle externally dark. Paraphyses thin filamentous. Ascii uniformly thin-walled without indication of a tholus, I-. Spores transversely several-septate, colorless, filled with oily drops. Algae protococcoid. Normally parasitic on *Baeomyces* on noncalcareous substrates.

Genus: *Arthroraphis* Th.Fr. (4).

This genus bears no relation to *Bacidia* and the Lecideaceae. It is obviously isolated and cannot be put in any of the existing families.

b. HARPIDIACEAE VĚZDA AD INT. Thallus crustose, homoiomerous with rather large cells, brown. Apothecia sunken, bordered by thallus, the proper exciple barely developed, pale. Paraphyses strongly moniliform. Ascii with an I+ blue tholus. Spores eight, crescent-shaped, colorless, thin-walled. Pycnospores short. Algae protococcoid. On noncalcareous rocks.

Genus: *Harpidium* Koerb. (1).

This is an extremely isolated genus, unrelated to the Lecanoraceae. It should be recognized as a separate monotypic family.

c. LITHOGRAPHACEAE AD INT. Thallus crustose. Apothecia lirelliform with a usually carbonized receptacle. Paraphyses netlike interwoven. Ascii clavate, amyloid, with a distinct I+ blue tholus. Spores one- or two-celled, colorless or with a dark wall layer. Algae protococcoid. On rocks and bark.

Genera: *Encephalographa* Mass. (?9), *Lithographa* Nyl. (14).

Genera with lirelliform apothecia occur in the Arthoniales, the Ostropales, and, judging from the ascus structure, also in the Lecanorales where

they are best tentively treated as a single, possible heterogeneous family. Some of the smaller genera in the Graphidaceae *sensu* Zahlbrückner cannot be disposed of at this time.

d. MICAREACEAE VĚZDA AD INT. Thallus crustose, at least in one part goniocyste-like in form. Apothecia sessile, emarginate, more or less arched, very variable in color. Receptacle little developed, formed of paraphysislike hyphae. Paraphyses strongly netlike interwoven. Ascii thick-walled with a simple I+ blue tholus and an I+ blue external layer and ascal gelatin. Spores unicellular to transversely several septate, colorless, I-. Algae "micareoid." On acidic substrates but otherwise not strongly substrate-specific.

Genera: *Micarea* Fr. em. Koerb. (30), *Roccellinastrum* Föllm. (1), *Scolicosporum* Mass. (?).

The Lecideaceae in all likelihood contain other groups that could be classified here.

e. PACHYASCACEAE AD INT. Thallus crustose, Goniocystis-like. Apothecia sessile, emarginate, convex, not black. Receptacle little developed, composed of paraphysislike hyphae. Paraphyses strongly netlike interwoven, forming a separate netmantle around each ascus. Ascii very thick-walled, amyloid, with a distinctly I+ blue tholus. Spores two- or more-celled, colorless, I+ blue or I-. Fulcra long-celled. On mosses.

Genus: *Pachyascus* Poelt et Hertel (2).

At best this genus is related to the Micareaceae.

f. PHLYCTIDACEAE AD INT. Thallus crustose, pale. Apothecia round, sunken, without parathecium or amphitheciun. Paraphyses simple or branched and anastomosing. Ascii amyloid, tholus scarcely developed, I-. Spores mostly few, colorless, muriform many-celled. Pycnospores long. Algae protococcoid. On bark, mainly in warmer regions.

Genera: *Phlyctidia* (Vain.) Müll. Arg. (2), *Phlyctis* (Wallr.) Flot. (10). *Phlyctella* Krempelh. (15) does not seem to belong here.

Phlyctis is remote from the Pertusariaceae, but cannot be assigned to any other families.

g. STENHAMMARELLACEAE AD INT. Thallus crustose. Ascocarps round, with a receptacle which begins development as an involucellum, next becoming positioned at the hymenium, which is almost without a receptacle, and merges with the darkening subhymenium. Paraphyses simple to anastomosing. Spores unicellular with a halo. Algae protococcoid. On calcareous schists in cool moist habitats.

Genus: *Stenhammarella* H. Hertel (1).

This genus occupies a very isolated position according to our present knowledge. It may be related to the order Graphidales.

h. TRAPELIACEAE H. HERTEL. Thallus crustose to squamulose and distinctly effigurate, grayish. Apothecia sessile, rarely sunken, pale, never carbonized, with a biatorine or lecanorine receptacle. Paraphyses simple or branched, at times amastomosing, filamentous. Asci more or less cylindrical, normally not amyloid. Spores eight, unicellular, colorless, fairly large. Algae protococcoid. On rocks and soil.

Genera: *Orceolina* H. Hertel (1), *Placopsis* Nyl., (34), *Trapelia* Choisy (12).

Other groups long assigned to the Lecideaceae might belong to this recently narrowly delimited family.

i. AGYRIACEAE CORDA. Thallus crustose, little differentiated. Apothecia sessile, roundish to elongate, not marginate or with a not well defined differentiated margin composed of paraphysislike hyphae, not carbonized. Paraphyses mostly free. Asci with apical wall thickening, I+ bluish, then immediately green, without an I+ blue tholus. Spores unicellular, colorless. Pycnospores ellipsoid. Algae protococcoid. On wood and bark.

Genus: *Xylographa* Fr. (6).

The genus *Xylographa* is very much isolated, disregarding the allegedly nonlichenized *Agyrium* Fr., which is possibly generically identical. There is no basis for combining it with other genera in the Graphidinae. It might have a place with forms related to the Lecideaceae in a broad sense.

Finally, many genera of ascolichens cannot be integrated in the system of classification outlined above because their descriptions are inadequate and no material is available for study. This is especially true for the Moriolaceae and Epigloeaceae which I have not been able to characterize.

VI. Proposed Classification: Basidiolichens

For many years the only lichenized basidiomycetes were thought to be represented by the small, much discussed groups known in the tropics. In the past few years it has been shown that some lichenized species occur in temperate regions and have different relationships. Since this group does not contain a very large number of species and a discussion of the complex systematics of the pertinent fungi is out of order here, only the briefest outline will be given. See Oberwinkler (1970) for fuller discussions.

A. Aphyllophorales

1. DICTYONEMATACEAE TOMASELLI (CORTICIACEAE HERTER IN PART)

Fruiting body crustose to bracket-shaped, with a cutaneous, smooth hymenium or one broken into plates. Hyphae unpigmented, smooth, thin- or thick-walled. Basidia clavate to cylindrical. Spores ovoid to cylindrical,

colorless, smooth-walled. Symbionts single-celled or filamentous Cyano-phyceae deposited in the trama of the fruiting bodies. On various substrates, distributed chiefly in tropical mountainous areas.

Genera: *Athelia* Pers. (only some weakly lichenized, some parasitic on algae, most saprophytic), *Dictyonema* C. Ag. ex Kunth (including *Cora* Fr., *Corella* Vain., and *?Wainiocora* Tomaselli).

The lichenized forms have no relationship at all to the Thelephoraceae in a modern sense.

2. CLAVARIACEAE CHEV.

Fruiting bodies clavarioid, simple or divided, pale or pigmented. Basidia clavate-cylindrical. Spores four or more, ovoid to cylindrical, colorless, smooth. Symbionts green algae of the *Coccomyxa* or *Chlamydomonas* type, or blue-green algae surrounded in groups by mycelial hyphae and with them forming the thallus. On soil and wood, tropical and extratropical.

Genus: *Multiclavula* Petersen (in part) [1967; not recognized by Corner (1970), who divides the lichenized species into *Clavulinopsis* Corner and *Lentaria* Corner].

B. Agaricaceous Fungi

TRICHOLOMATACEAE ROZE

The complex diagnosis of this family can be found in Singer (1962, p. 201). On raw humus and peat in cooler regions (as far as lichenized).

Genus: *Omphalina* Quel. Several species (6) of this genus are associated with algae of the *Coccomyxa* type. The mycelial hyphae surround groups of algae with spherical coverings ("Botrydina") or form foliose long-lived thallus squamules in which the algae are restricted to a layer ("Coriscium"). The generic arrangement of the lichenized species is not yet clarified by Singer (1962, p. 263).

VII. Lichenes Imperfeci

(Fungi imperfecti lichenisati, Deuteromycetes lichenisati, Deutero-lichenes, Hemilichenes): There are a number of lichen species which have never been found with ascocarps. They may be found eventually in some. In a few cases these sterile lichens can be assigned to specific genera (*Umbilicaria*, *Parmelia*, *Caloplaca*) on the basis of certain vegetative characters (thallus structure, chemistry). In other cases a tentative assignment can be made (*Pertusaria*). Most, however, lack any suitable criteria. They are simply put into artificial genera according to thallus structure (e.g., *Lepraria*

structure) or type of vegetative diaspore (e.g., *Phyllophiale*). While improved analysis of characters may result in reassignment of some species to definite genera, the greater part of the forms under *Lepraria* will never be referred to sexual groups. The Lichenes Imperfecti do not form a natural group, although it includes some smaller natural relationships.

The fruticose genera *Siphula*, *Endocena*, and *Thamnolia* have often been cited in the Lichenes Imperfecti but they are better placed in a separate family after the *Cladoniaceae* because of their chemistry.

Genera: *Cystocoleus* Thwait. (1 or 2), *Lepraria* Ach. (20), *Leprocaulon* Nyl. ex Lamy (7), *Lichenothrix* Henssen (1), *Normandina* Nyl. (1), *Phyllophiale* R. Sant. (1), and others.

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Appendix B

IDENTIFICATION AND ISOLATION OF LICHEN SUBSTANCES

JOHAN SANTESSON

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I. Identification without Previous Isolation

A positive identification of lichen substances usually requires isolation of the compounds and comparison with authentic samples. However, there exist microchemical methods by which more or less reliable identifications can be achieved without the use of too much effort or lichen material.

Color tests and fluorescence analysis give indications of which groups of compounds might be present in a lichen sample, while microcrystallization, chromatography, and lichen mass spectrometry lead to tentative identifications of the compounds.

A. Color Tests

Four color tests for lichen substances are used routinely in lichenology. The C, K, and KC tests were discovered by Nylander (1866a,b) and the PD

(or P) test was introduced by Asahina (1934). During the last decades, some other tests have also been suggested. The color tests can be carried out by applying the appropriate reagent to a lichen fragment by means of a pointed glass rod. The color changes are best observed under a binocular lens. Cortex and medulla should be tested separately. If carried out on the same lichen fragment, the PD test should be done before the C test.

Color reactions are often more easily observed if the tests are carried out with the filter paper method (Santesson, 1967a). A lichen fragment is pressed down in the middle of a piece of filter paper, and the lichen substances are extracted by treatment with 10–20 drops of acetone. Each drop of acetone is allowed to evaporate, leaving the extracted substances in a ring around the lichen fragment. The fragment is removed and tests are carried out upon the "extract ring."

TABLE I
COLOR REACTIONS OF LICHEN SUBSTANCES

Reaction	Compound
Pigments	
K+ red to violet	Anthraquinones, bisanthraquinones, terphenylquinones, naphthoquinones, pyxiferin
K+, Dimroth+, KC+, K+	Xanthones, sordidone
K+, TiCl ₃ +, PD+, K+	Usnic acids
Colorless compounds	
PD+, K+	<i>Depsides</i> : alectorionic acid, atranorin, baeomycesic acid, barbatolic acid, chloroatranorin, decarboxythamnolic acid, haemathamnolic acid, nephroactin, thamnolic acid, <i>Depsidones</i> : constictic acid, fumarprotocetraric acid, norstictic acid, physodalic acid, protocetraric acid, salazinic acid, stictic acid, virensic acid
PD+, K-	Pannarin, psoromic acid, fumarprotocetraric acid, protocetraric acid, virensic acid
PD-, K+, C+	Cryptochlorophaeic acid, hiastic acid, hypothamnolic acid (K+ violet), merochlorophaeic acid, paludosic acid, ramalinolic acid, scrobiculin
PD-, K-, C+ red	Anziaic acid, 4-O-demethylbarbatic acid, erythrin, ethyl orsellinate, gyrophoric acid, lecanoric acid, methyl 3,5-dichlorolecanorate, methyl-β-orsellinate, montagnetol, olivetoric acid, siphulin
PD-, K-, C+ greenish	Didymic acid, pannaric acid, porphyrylic acid, strepsilin
PD-, K-, C+ blueish	Diploschistesic acid
PD-, K-, C-, KC+	Alectoronic acid, α-collatolic acid, glomelliferic acid, lobaric acid, 4-O-methylphysodic acid, microphyllinic acid, norlobaridone, physodic acid, picrolichenic acid

All color test reagents (except C) must be handled with care and should not come into contact with skin, herbarium envelopes, etc. If spilled, PD can be (incompletely) removed by washing with very dilute acetic or hydrochloric acid.

Table I summarizes the color-test reactions of some lichen substances. Color tests are only indicative for groups of compounds, and must be supplemented with other tests if positive identification of a compound is sought.

It should be noted that the color reactions of a compound are to a certain extent dependent on concentration and localization in the thallus. Thus, a C+ or K+ red compound might in low concentrations give a C+ or K+ faint orange color. (In the same way, thallus color due to presence of a certain pigment might differ considerably from one lichen specimen to another for the same reasons. Pure parietin is red, but *Xanthoria parietina* might have any shade varying from greenish-yellow to deep orange. *Alectoria fremontii* is dark brown but still contains yellow vulpinic acid.)

1. THE C TEST

As reagent, a saturated aqueous solution of calcium hypochlorite [bleaching powder, $\text{Ca}(\text{OCl})_2$] or a dilute aqueous solution of sodium hypochlorite (NaOCl) is employed. Some commercial bleaching fluids, containing "active chlorine," can be used as substitutes. The $\text{Ca}(\text{OCl})_2$ and NaOCl solutions must be prepared daily, since they decompose within 24–48 hours. In sunlight, they are stable for less than 1 hour. The stock chemicals are best stored in a cool dry place. Light, heat, humidity, and carbon dioxide hasten their decomposition.

Aromatic compounds, having two free hydroxyl groups *meta* to each other, give a positive pink to red C test (Fig. 1a). If the position between the hydroxyls is substituted by a side chain, as in β -orcinol derivatives, the reaction often fails. Halogen substitution (as in methyl 3,5-dichlorolecanorate) does not interfere with the test. Hydroxyl substitution (diploschistesic acid) may change the response to a blue color. The majority of the lichen dibenzofuranes, having at least one free hydroxyl group, give a positive green C test, often difficult to observe.

The colored reaction products obtained in the C test are unstable and easily destroyed by an excess of the reagent. Hypothamnolic acid (albeit substituted at the position between the *m*-hydroxyls) gives a red color with C which disappears within seconds. The chemistry behind the C test has not been elucidated. Possibly a combination of chlorination and oxidation reactions leads to monomeric and/or dimeric quinonoid structures. Only one colored compound has been isolated from the reaction of orcinol with calcium hypochlorite: the yellow tetrachlorodihydroorcinol (L. Gren and J. Santesson, unpublished).

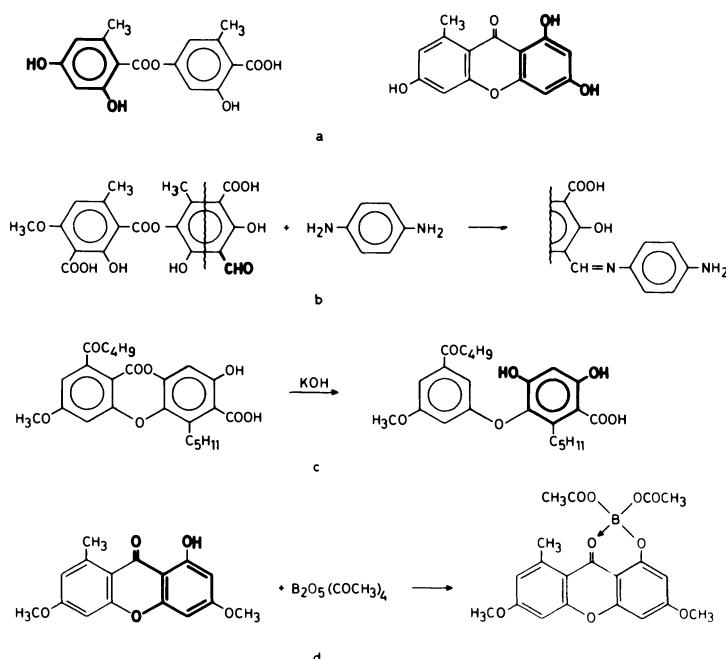


FIG. 1. Color reactions of lichen substances. The structural part responsible for the color reaction is drawn in heavy lines. (a) C+ compounds: lecanoric acid (left) and norlichexanthone (right); (b) PD+ thamnolic acid reacting with *p*-phenylenediamine; (c) KC+ lobaric acid hydrolyzed with KOH to yield a compound with 2 free *m*-hydroxyls; (d) lichexanthrone reacting with Dimroth's reagent to give a fluorescent compound.

2. THE K TEST

A 10–25% aqueous solution of potassium hydroxide is used as the reagent. The solution is stable, but etches glass vessels slowly. Quinonoid lichen pigments (anthraquinones, naphthoquinones, terphenylquinones) give a positive dark red to violet K test, whereas pulvinic-acid derivatives, xanthones, and usnic acids do not respond. Some depsides (e.g., atranorin, thamnolic acid) and many β -orcinol depsidones exhibit yellow to red colors with K.

The K test depends upon salt formation and requires the presence of at least one acidic functional group in the molecule. Thus, fully *O*-methylated phenolic quinones (not yet found in lichens) would not react. The chemical structures of colored potassium salts of K+ depsides and depsidones are not yet known.

3. THE PD TEST

The commonly used reagent is a 1–5% ethanolic solution of *p*-phenylenediamine that will keep for about a day. A more stable reagent has been

described by Steiner (1955): 1 gm *p*-phenylenediamine, 10 gm sodium sulfite and 1 ml of a neutral, liquid detergent are dissolved in 100 ml of water. It can be used for at least a month.

p-Phenylenediamine reacts with aromatic aldehydes, giving yellow to red Schiff bases (Fig. 1b). Most β -orcinol depsidones, as well as some β -orcinol depsides, give a positive PD test. Aromatic diamines other than *p*-phenylenediamine also react with aromatic aldehydes. Asahina (1934) found that benzidin gives less intensely colored reaction products than *p*-phenylenediamine. Santesson (1966) compared some diamine reagents and suggested the use of *o*-dianisidine (OD) as a PD substitute. *o*-Dianisidine is more sensitive, more stable, and more toxic than PD, but not as corrosive. The color reactions obtained with OD are not always identical with those of PD.

4. THE KC TEST

K is first applied to the lichen, then immediately followed by C. The potassium hydroxide hydrolyzes depside and depsidone ester bonds, and if the phenolic hydroxyl group released is in a position *meta* to another hydroxyl, an orange to red color will be obtained as C is applied (Fig. 1c). Some *meta*-depsides (e.g., cryptochlorophaeic acid, scrobiculin), which exhibit a fleetingly red C reaction, are KC+ more persistently red, although the freed hydroxyl is located between two free *meta* hydroxyls. Usnic acids give a deep yellow KC test. If a strong red color is already produced by K or Calone, the KC test is meaningless and superfluous.

5. OTHER COLOR TESTS

A 5% aqueous solution of chloramine-T gives a yellow color with usnic acids (Mitsuno, 1953). An 8% aqueous solution of titanium trichloride ($TiCl_3$) produces a yellow-green color with usnic acids (Bendz *et al.*, 1967).

Dimroth's reagent is rather specific for xanthones (Santesson, 1968). It is prepared by adding 10 gm of boric acid to 100 ml hot (100°C) acetic anhydride and allowing the solution to cool. The color test is carried out in UV light, and an intense yellow fluorescence, stable for at least 1 minute, suggests the presence of xanthones. The test depends upon the formation of boro-acetates (Fig. 1d). Some chromones give a positive test. As a substitute for Dimroth's reagent, an alkaline beryllate solution can be used (Santesson, 1969c).

A methanolic solution of magnesium acetate has been suggested as a reagent for 1-hydroxylated anthraquinones (Shibata *et al.*, 1950). An orange to red color, appearing after a few minutes at 90°C, indicates a positive response. The test is best used in combination with the filter paper method.

Aromatic *o*-hydroxyaldehydes (PD+ compounds) can be detected by the use of a solution of 5 gm hydrazine sulfate and 10 gm sodium acetate in

100 ml water (Feigl and Anger, 1966). A yellow to orange fluorescence (in UV light) appears within a minute.

A solution of 0.2–0.5 gm iodine in 100 ml aqueous 0.5% potassium iodide is often used as a reagent for certain polysaccharides in lichens (the I test). The reagent is susceptible to air oxidation and should be renewed when the brownish color fades. Isolichenin, but not lichenin, will give a blue color. The chemistry of the color reaction is probably the same as that for the well-known iodine test for starch. The reaction is reversible (the color disappears upon dilution with water).

Dilute nitric acid (HNO_3) is sometimes used as a color test reagent in lichenology. It is not known what types of compounds are actually detected in this test.

B. Fluorescence

Many lichen substances fluoresce in long-wave (366 nm) ultraviolet light. Examination of lichen specimens in UV light can thus provide valuable clues to the presence or absence of certain compounds (Černhorsky, 1950; Ozenda, 1951; Hale, 1956a). Anthraquinones appear brick red to vermillion, pulvinic-acid derivatives yellowish and xanthones bright yellow to orange red. Some depsides and depsidones fluoresce bright white to bluish or greenish white, e.g., alectoronic acid, divaricatic acid, lobaric acid, sphærophorin, and squamatic acid. Addition of a drop of alkali (e.g., K reagent) will often change the fluorescence.

C. Microcrystallization

In 1936, Asahina introduced microcrystallization as the first generally applicable method for tentative identification of lichen substances on a micro scale (Asahina, 1936, 1937, 1938, 1939, 1940). The method rapidly gained acceptance among lichenologists and has been used extensively for chemical studies in connection with taxonomic work, e.g., on *Cladonia* (Evans, 1943; Thomson, 1967), *Parmelia* (Krog, 1951; Hale, 1965; Hale and Kurokawa, 1964), and *Cetrelia* and *Platismatia* (Culberson and Culberson, 1968).

Crystal tests require no specialized equipment. One or a few lichen fragments are placed on a microscope slide and the lichen substances present extracted by dropwise treatment of the fragments with a suitable solvent, usually acetone. After evaporation of the solvent, the fragments are removed, leaving a more or less crystalline residue of lichen substances on the slide. A drop of a suitable crystallizing solvent mixture is added to the residue and a coverslip added. The slide is heated gently over a tiny flame or on an

electric plate. Upon cooling, substances present may appear in crystalline form and can then be identified from crystal shape and color. The crystals are best observed in polarized light under low magnification ($\times 100-\times 400$).

The most frequently used crystallizing reagents are: GE, glycerol:acetic acid, 1:3; GAW, glycerol:ethanol:water, 1:1:1; GAoT, glycerol:ethanol: *o*-toluidine, 2:2:1; GAQ, glycerol:ethanol:quinoline, 2:2:1 (all parts by volume). If kept in stoppered bottles, the solutions are stable for at least a month.

Tests with the GE and GAW solutions are simply recrystallizations, and the volume of the added reagent should be kept at a minimum. The GAoT and GAQ tests depend upon salt formation and—in the case of aromatic aldehydes—possibly also upon condensation reactions. Characteristic crystalline salts are also formed with some inorganic reagents. An aqueous solution of potassium carbonate and potassium hydroxide (10% of both) will precipitate the potassium salt of norstictic acid as red needles. A saturated solution of barium hydroxide produces easily recognized barium salts with many desides, notably atranorin.

Photographs of microcrystals of various compounds are scattered in numerous papers (for references, see Culberson, 1969, 1970a). Hale (1967) illustrates the crystalline appearance of 24 compounds, Taylor (1967) 18 compounds, and Thomson, (1967) 22 compounds of known structure. The latter book also contains an extensive discussion of the microcrystallization methods.

This method is best suited for the identification of depsides, depsidones, and dibenzofuranes. It is less suitable for aliphatic acids and terpenes (except zeorin and ursolic acid) and cannot be used for pigments (except usnic acid). The sensitivity is high enough to allow the identification of microgram amounts of compounds (Culberson, 1963).

D. Chromatography

Only paper and thin-layer chromatography have been used to any larger extent for the identification of lichen substances. Column and preparative-layer chromatography have been employed for isolation of lichen substances (see below). Gas-liquid chromatography (GLC) has been applied in a few cases. The separation of triterpenes was studied by Ikekawa *et al.* (1965), Shibata *et al.* (1965), and Yosioka *et al.* (1969). Gas-liquid chromatography of the aliphatic lichen acids protolichesterinic acid and lichesterinic acid (as methyl esters) was reported by Bloomer *et al.* (1970a,b). Published data on high-pressure liquid-liquid chromatography of lichen substances are just beginning to appear (Culberson, 1972).

1. PAPER CHROMATOGRAPHY (PC)

Paper chromatography was introduced into lichenology independently by Wachtmeister (1952) and Mitsuno (1953). Further studies have been published by Mitsuno (1955), Wachtmeister (1956), and Hess (1958). A useful review has also appeared (Wachtmeister, 1959). Most PC analyses, however, can advantageously be replaced by thin-layer chromatographic methods.

In PC of lichen compounds polar solvent systems are used for the most part. Typical examples are *n*-butanol:concentrated ammonia (4:1, parts by volume), *n*-butanol:acetone:water (5:1:2), and *n*-butanol:ethanol:water (4:1:5). Better R_f values and less trailing can sometimes be obtained if the chromatographic papers are buffered with phosphate (Na_3PO_4 or Na_2HPO_4) (Wachtmeister, 1956). Depsides and depsidones should be chromatographed both before and after microhydrolysis of the extracts (see below).

2. THIN-LAYER CHROMATOGRAPHY (TLC)

The sensitivity, rapidity, general applicability, and simplicity of equipment needed makes TLC one of the best microchemical methods for the systematic botanist. Good texts are available on the general aspects of TLC techniques (e.g., Stahl, 1969; Randerath, 1966; Truter, 1963).

The first TLC separation of lichen substances was reported by Stahl and Schorn (1961). Some papers giving TLC data on one or more groups of lichen substances are listed in Table II. Especially extensive tabulations of R_f data are found in the works of Santesson (1967a), Huneck (1968) (a review with many previously unpublished data), and Culberson and Kristinsson (1970). Numerous publications contain data on TLC of single compounds (for references, see Culberson, 1969, 1970a).

Both "laboratory-made" and precoated TLC plates have been used for separations. The adsorbent is almost always silica gel, although polyamide has been used in TLC of anthraquinones (Chan and Crow, 1966).

Numerous solvent systems have been described. Almost all used for TLC of depsides and depsidones contain an acid (acetic or formic acid) to prevent trailing. Pastuska's mixture (benzene:dioxane:acetic acid, 90:25:4 parts by volume) is especially useful for the separation of acidic aromatic lichen substances.

A number of methods are available for visualizing the spots after chromatography. If the adsorbent is impregnated with a fluorescence indicator, most aromatic compounds will be visible in UV light. Even on ordinary adsorbents (e.g., silica gel G) many aromatic compounds will exhibit a characteristic fluorescence in UV light. Nearly all lichen substances can be made visible by spraying the chromatographed plates with a 10% solution of sulfuric acid

TABLE II
PUBLICATIONS ON THIN LAYER CHROMATOGRAPHY OF LICHEN SUBSTANCES

Compound class; references	Remarks
Aliphatic acids	
Bendz <i>et al.</i> (1966)	11 Acids
Santesson (1967a)	9 Acids, precoated plates
Depsides	
Bachmann (1963)	
Ramaut (1963b)	
Santesson (1965)	6 PD+ depsides
Ramaut (1967a,b)	
Santesson (1967)	30 Depsides, precoated plates
Huneck (1968)	17 Depsides
Culberson and Kristinsson (1969)	7 Depsides, 11 hydrolysis products, precoated plates
Culberson and Kristinsson (1970)	40 Depsides, precoated plates
Depsidones	
Bachmann (1963)	PD+ Depsidones
Ramaut (1963a)	PD+ Depsidones
Santesson (1965)	PD+ Depsidones
Santesson (1967a)	15 Depsidones, precoated plates
Culberson and Kristinsson (1970)	23 Depsidones, precoated plates
Dibenzofuranes	
Santesson (1967a)	Precoated plates
Culberson and Kristinsson (1970)	Precoated plates
Usnic acids	
Bendz <i>et al.</i> (1967)	Separation of optical antipodes
Ramaut (1967b)	
Santesson (1967a)	Precoated plates
Nuno (1968)	Separation of usnic and isousnic acids
Xanthones	
Santesson (1967a)	Precoated plates
Santesson (1969c)	11 Xanthones, precoated plates
Culberson and Kristinsson (1970)	Precoated plates
Anthraquinones	
Chan and Crow (1966)	Polyamide layer
Santesson (1967)	Precoated plates
Bohman (1968)	Precoated plates
Piatelli and Guidici de Nicola (1968)	Three different layers
Shibata <i>et al.</i> (1968)	Chrysophanol, skyrin, rugulosin
Yoshioka <i>et al.</i> (1968)	Also bisanthrones
Culberson and Kristinsson (1970)	Precoated plates
Santesson (1970a)	Skyrin, oxyskyrin, skyrinol
Terpenes	
Huneck (1962)	A _{1,0} , layer
Culberson and Kristinsson (1970)	Precoated plates
Pulvinic acid derivatives	
Bendz <i>et al.</i> (1965b)	7 Compounds
Harper and Letcher (1966)	8 Compounds
Santesson (1967a)	7 Compounds, precoated plates
Culberson and Kristinsson (1970)	8 Compounds, precoated plates

and heating at 110°C for a few minutes (e.g., Culberson and Kristinsson, 1970).

Phenolic compounds can be detected by diazonium reagents (also useful in paper chromatography). The most widely used are *bis*-diazotized benzidine, Echtblaualsz B, and Echtblaualsz BB. The benzidine reagent consists of two solutions (solution A: 5 gm benzidine and 14 ml concentrated hydrochloric acid in 1000 ml water; solution B: 100 gm sodium nitrite in 1000 ml water) of which equal amounts are mixed just before use. The ready reagent mixture is stable for less than 1 hour. The Echtblaualsz reagents can be used as 0.01–0.1% aqueous solutions either alone or followed by a 1% potassium hydroxide solution. Heating the plates for a short time might reveal additional spots.

A solution of 0.5 ml anisaldehyde and 1 ml concentrated sulfuric acid in either 25–50 ml glacial acetic acid or methanol will give colored reaction products with many phenols after 1 to a few minutes at 100°C. Aromatic aldehydes are most conveniently detected by spraying the plates with a very dilute (0.01–2%) ethanolic solution of *p*-phenylenediamine or *o*-dianisidine. Triethylamine, used in neat form, will produce intense colors with quinonoid compounds. A saturated solution of antimony pentachloride in chloroform or a 10% solution of chlorosulfonic acid in glacial acetic acid will, for example, reveal terpenes after heating the plates to 100°–120°C.

Aliphatic acids can be visualized by the use of a 0.04% solution of bromocresol green in 0.01 M sodium hydroxide or by simply spraying the plates with distilled water (the silica gel is less wetted where aliphatic acids are present).

More complete details on the different reagents have been given by, e.g., Wachtmeister (1959), Santesson (1967a), and Huneck (1968).

The sensitivity of the TLC method is usually higher than that of the PC or microcrystallization methods. Microgram quantities of substance are nearly always sufficient, and under favorable circumstances, less than 100 ng of pulvinic-acid derivatives can be identified (Santesson, 1967b).

An important standardized TLC method for the identification of lichen substances has been described by Culberson and Kristinsson (1970). The chromatography is carried out in three solvent systems (solvent A, Pastuska's mixture; solvent B, hexane:ethyl ether:formic acid, 5:4:1; solvent C, toluene:acetic acid, 85:15) on precoated plates. Atranorin and norstictic acid are cochromatographed on all plates as controls. The spots of unknowns are assigned to R_f classes defined by the R_f values of the control substances. Tentative identification can then be achieved by checking punched cards containing data on R_f classes (and other microchemical properties) for all compounds previously studied. Since "unidentified substances" are sufficiently characterized to allow recognition if encountered again, it would be useful if all reports on the occurrence of such compounds in lichens in-

cluded data on R_f classes (and color reactions, etc.) according to the Culber-
son and Kristinsson system.

E. Lichen Mass Spectrometry (LMS)

Usually, only a very few secondary metabolites are present in lichens in appreciable quantities. These compounds may sublime if the lichen is heated at very low pressure. This is the basis for lichen mass spectrometry (Santesson, 1969a).

A small lichen sample is introduced into a mass spectrometer by a direct inlet system. The sample is heated, and many lichen substances sublime readily at the very low pressure (about 10^{-7} torr) in the mass spectrometer. Mass spectra of the subliming compounds may then be recorded and used for tentative identification. (For a general introduction to mass spectrometry, see Beynon *et al.*, 1968).

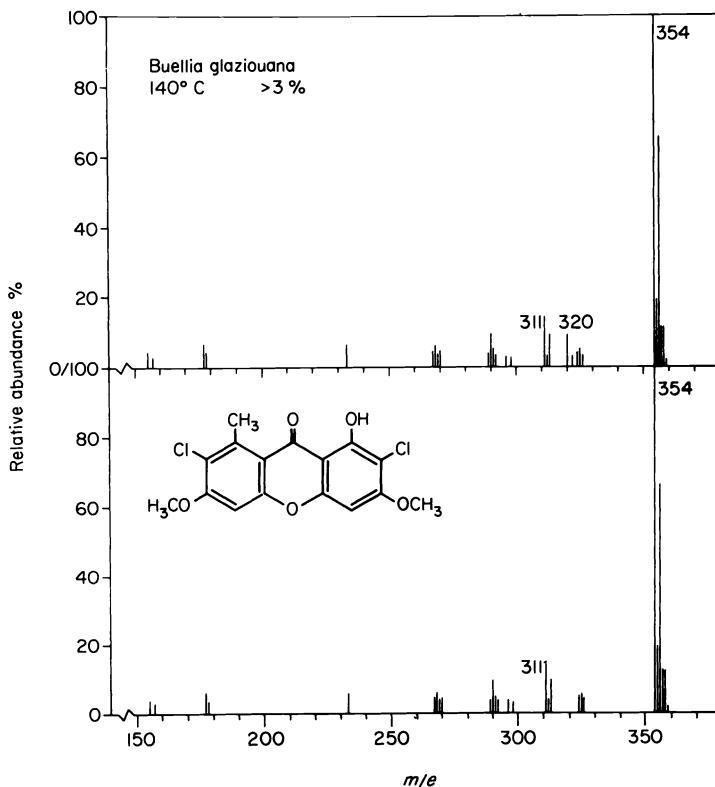


FIG. 2. Lichen mass spectrum of *Buellia glaziouana* and the mass spectrum of 2,7-dichloro-lichexanthone which occurs in the lichen thallus of this species.

The "lichen mass spectra" obtained in this way are as a rule very similar to the spectra of the corresponding pure compounds (Fig. 2). In the low mass region (up to about m/e 150), however, many peaks due to thermal decomposition of the lichen may appear, and hence this region of the lichen mass spectrum may not be very useful for the identification of the vaporized lichen substances.

The method is especially well suited for the study of lichen pigments. These are generally vaporized at moderate temperatures (100°–150°C) and give very prominent parent ions, thus facilitating the interpretation of spectra. Some pigments also give characteristic fragment ions of high intensities, e.g., usnic acids (m/e 233 and 260) and pulvinic acid derivatives (m/e 145 and 290). Table III lists m/e values for parent ions and important fragment ions of most lichen pigments.

The presence of many types of compounds other than pigments may also be recognized from lichen mass spectra (zeorin and dibenzofuranes: Santesson, 1969a; atranorin: Santesson, 1969c; picrolichenic acid: Santesson, 1969d).

When several compounds are present in a lichen sample, the spectra obtained are usually a superposition of the spectra of the individual compounds (Fig. 3). In some cases a certain amount of fractionation is possible by the recording of spectra at several different temperatures.

Very little plant material is required. Theoretically, less than 50 ng would sometimes suffice. A single apothecium is usually more than enough. In many cases type specimens may be chemically examined by LMS without serious loss of material. The histological distribution of the compounds can also be studied.

Typical applications of LMS are studies of xanthones in *Lecanora* (Santesson, 1969c) and *Pertusaria* (Santesson, 1969d) and a survey of anthraquinones in *Caloplaca* (Santesson, 1970b).

F. Quantitative Determination

Most published quantitative data are based on isolation of the compounds, and thus represent minimum values. Colorimetric and spectrophotometric methods for quantitative determination of a few substances (without isolation) have been described. Ramaut *et al.* (1966) and Rundel (1969) determined usnic acid spectrophotometrically. Laasko and Gustafsson (1952) determined usnic acid as the FeCl_3 -complex, and Jayasankar and Towers (1968) used the reaction product of usnic acid with Ehrlich's reagent for the determination. Determination methods for parietin (Hill and Woolhouse, 1966; Richardson, 1967) and atranorin (Vainshtein and Ravinskaya, 1971)

TABLE III
PARENT PEAKS AND CHARACTERISTIC FRAGMENT IONS OF LICHEN PIGMENTS

Parent peak (<i>m/e</i> , No. of Cl)	Other characteristic peaks (<i>m/e</i>)	Compound
254		Chrysophanol
258	229	Norlichexanthone
270	213	Emodin
284	241, 255	Parietin
286	286	Citreorosein
286	243, 257	Lichexanthone
290		Pulvinic dilactone
292	145	Polyporic acid
292, 1 Cl		2-Chloronorlichexanthone
298	297	Fallacial
300		Teloschistin
300		Xanthorin
300	256	Emodic acid
304	260, 302, 306	Haemoventosin
304, 1 Cl	276	1,3,8-Trihydroxy-2-chloro-6-methylanthraquinone
306	161	Calycin
306, 1 Cl		Vinetorin
308	145, 290	Pulvinic acid
314	284	Parietinic acid
314	284	Endocrin
318, 1 Cl	272, 289, 300	1,3-Dihydroxy-8-methoxy-2-chloro-6-methylanthraquinone
318, 1 Cl	275	Fragilin
320, 1 Cl		Paulosin
320, 1 Cl		1,3,5,8-Tetrahydroxy-2-chloro-6-methylanthraquinone
322	145, 290	Vulpinic acid
326, 2 Cl		2,4-Dichloronorlichexanthone
326, 2 Cl		2,7-Dichloronorlichexanthone
332, 1 Cl	286, 303, 314	1-Hydroxy-3,8-dimethoxy-2-chloro-6-methylanthraquinone
332, 1 Cl	286, 300, 314	8-Hydroxy-1,3-dimethoxy-2-chloro-6-methylanthraquinone
338, 2 Cl		1,3,8-Trihydroxy-2,4-dichloro-6-methylanthraquinone
340, 2 Cl		3-O-Methyl-2,5-dichloronorlichexanthone
340, 2 Cl		Thiophaninic acid
344	233, 2600	Usnic acids
352	145, 175, 320	Leprarinic acid
352	145, 264, 320	Pinastrinic acid
354, 2 Cl	311	2,5-Dichlorolichexanthone
354, 2 Cl	311	2,7-Dichlorolichexanthone
360, 3 Cl	325, 331	Arthothelin
360, 3 Cl	325, 331	2,5,7-Trichloronorlichexanthone
366	219	Leprarinic acid methyl ether
370	299, 327	Norsolorinic acid
374, 3 Cl		3-O-Methyl-2,5,7-trichloronorlichexanthone
374, 3 Cl		Thuringione
384	313, 341	Solorinic acid
394, 4 Cl		Thiophaninic acid
435	145, 290	Epanorin
469	145, 290	Rhizocarpic acid
638	579	Secalonic acid A

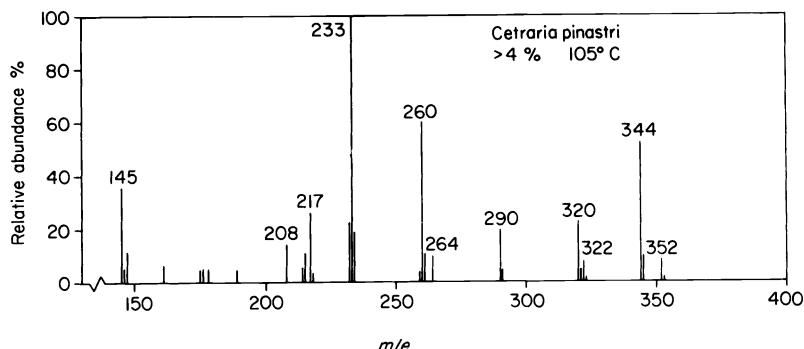


FIG. 3. Lichen mass spectrum of *Cetraria pinastri*. Usnic acid (peaks at m/e 233, 260, and 344), vulpinic acid (m/e 145, 290, and 322), and pinastropic acid (m/e 145, 290, 320, 322, and 352) occur in this lichen.

have also been reported. A polarographic method for determining usnic acid has been described by Hakoila (1970).

II. Isolation

Most lichen substances are stable to air oxidation and ordinary light. Usually no special precautions such as a nitrogen atmosphere, darkness, or low temperature are necessary during isolation procedures. Carotenes and unsaturated fatty acids constitute the main exceptions.

Isolation of lichen substances without extraction is only rarely possible. Parietin might be obtained from *Xanthoria parietina* by microsublimation (Heyl and Kneip, 1913). (-)-16 α -Kauranol occurs as moldlike crystals on old herbarium specimens of some *Ramalina* (*Desmaziera*) species and can be collected in a very pure state simply by brushing the specimens (Bendz *et al.*, 1965a).

A. Preparation of Lichen Material for Extraction

The lichen material should be free from impurities and homogeneity ascertained by inspection in both visible and UV light. If a compound present in large amounts (at least a few percent of the dry weight) and previously known from the species under study is being isolated, small amounts of foreign material (other lichens, mosses, soil, etc.) do not usually interfere and can be tolerated.

The lichens should be dried and pulverized before extraction. Air-drying is almost always adequate, but desiccator-drying and oven-drying have been used for quantitative studies. Only previously air-dried material should be

dried at elevated temperatures. Apothecial pigments are best isolated if detached from the thallus. Basal parts of some fruticose lichens might contain decomposition products which can interfere with purification of lichen substances.

B. Extraction

Only organic solvents which do not react with lichen substances should be used. Methanol and ethanol may cause (trans-)esterification and/or hydrolysis of many compounds, e.g., depsides and pulvinic-acid derivatives (ethanol is often present in chloroform). All solvents must be dry, and ethyl ether should be peroxide-free as well.

The use of a few different solvents in succession will often effect a certain degree of separation of the constituents. The series benzene-ethyl ether-acetone is commonly used. Most lichen substances will appear in benzene and ether extracts, but certain compounds (e.g., erythrin, thamnolic acid, β -orcinol depsidones) will only be extracted by acetone. Polyols (erythritol, arabinitol, mannitol, etc.) are usually found in the acetone extract, but saccharides (especially polysaccharides) have to be extracted with alcoholic solvents or water after prior removal of other constituents (cf. Lindberg *et al.*, 1953; Lewis and Smith, 1967).

Continuous extraction procedures (Soxhlet extractors) are usually preferred. The extraction may be complete after a few to 24 hours, but longer extraction times are sometimes necessary. The "unextractable pigment" of *Mycoblastus sanguinarius* (Zopf, 1899) was isolated by a 2 weeks' extraction procedure (Bohman, 1970). In some cases, prolonged extraction might lead to the formation of artifacts. After one week in refluxing acetone, thamnolic acid may be decarboxylated to the extent of 5–10%.

C. Working-Up Procedures

In many cases the main substance will separate out in crystalline form as the extract cools. Separation of carboxylic acids, other rather strong acids (e.g., halogenated phenols), weak acids, and neutral compounds can be achieved by shaking the water-immiscible extract consecutively with aqueous solutions of sodium hydrogen carbonate, sodium carbonate, and sodium hydroxide. In some cases buffer solutions are best used. The aqueous solutions are then acidified and extracted with ether. The operations should be carried out rapidly using ice-cooled solutions. Acetone extracts may be fractionated in the same way if first diluted with 4–5 times their volume of ether.

Many compounds are best isolated by column chromatography. Depsides and depsidones can be eluted from a silica-gel column with benzene-ethyl

ether mixtures (Culberson, 1966, 1967, 1970b) or benzene-acetone mixtures (Komiya and Kurokawa, 1970). Elution of a silica gel column with chloroform will separate pulvinic acid derivatives (Maas and Neish, 1967). Anthraquinones have been chromatographed on magnesium carbonate (Murakami, 1956) and on silica gel (Yosioka *et al.*, 1968).

Preparative-layer chromatography has been used in some cases, especially when small quantities of substance are involved. Culberson and Kristinsson (1969) separated some depsides on silica gel plates with Pastuska's mixture. Piattelli and Guidici de Nicola (1968) isolated anthraquinones, Santesson (1970a) isolated bis-anthraquinones and Santesson (1969b) isolated xanthones, in all cases on silica-gel plates. Bloomer *et al.* (1970a,b) reported on preparative-layer chromatography of some aliphatic lichen acids and Aberhart *et al.* (1970) isolated portentol and its acetate by PLC.

The final purification of isolated lichen substances is usually achieved by recrystallization, but many anthraquinones are purified by high-vacuum sublimation. No special precautions are necessary for storage of purified substances.

III. Identification after Previous Isolation

Only the identification of known compounds will be discussed here. Structural determination of novel compounds is beyond the scope of this book. Illustrative examples on the use of "classical techniques" are given by Asahina and Shibata (1954). Huneck (1968) reviews the application of spectroscopic methods.

Generally, an isolated lichen substance is identified by comparison of selected physical and chemical properties with recorded data. In many cases, a direct comparison with an authentic sample of the compound is necessary to make positive identification.

A. Melting Points

In most cases melting-point values are of great assistance in the identification of isolated lichen substances, and a mixed melting point determination may furnish nearly conclusive proof of the identity. However, mp's might be uninformative for some phenolic carboxylic acids.

Many β -orcinol depsidones discolor and decompose slowly without melting at temperatures above 240°–250°C, the decomposition being dependent upon the heating rate. For physodalic acid a decomposition range of 230°–260°C has been given, for salazinic acid 260°–280°C, and for fumarprotocetraric acid 245°–260°C. Presence of the solvent of crystallization may alter the mp drastically. Alectoronic acid (from benzene) melts at 193°C,

whereas the hydrate (from ethanol-water) melts at 120°–121°C (then resolidifies at 140°C and remelts at 193°C).

B. Spectral Properties

1. INFRARED (IR) SPECTRA

Very few complete IR spectra of lichen substances have been published, but selected values of absorption frequencies are listed by Huneck (1968) and Culberson (1969, 1970a). If an IR spectrum of an unidentified compound is identical with that of an identified, authentic sample, recorded under the same conditions, this usually constitutes sufficient proof of the identity of the compound. However, optical antipodes of a compound [e.g., (+)- and (−)-usnic acid] will give identical spectra, and the dissimilarities between spectra of pairs of homologue aliphatic acids (e.g., protolichesterinic acid and nephrosterinic acid) are usually too small to be noticed.

2. MASS SPECTRA

An extensive discussion of mass spectra of aromatic lichen substances has been published (Huneck *et al.*, 1968). Identification by comparison of mass spectra is usually only possible if the spectra have been recorded on the same instrument under identical conditions (cf., e.g., Beynon *et al.*, 1968). The mass spectra of some isomeric pairs of substances (e.g., 2,4-dichloronorlichexanthone and 2,7-dichloronorlichexanthone) are almost indistinguishable and thus unsuitable for the purposes of final identification.

3. ULTRAVIOLET (UV) AND NUCLEAR MAGNETIC RESONANCE (NMR) SPECTRA

UV spectra are very useful for a determination of main structural features of an isolated compound but cannot be used for final identification. Brief discussions have been published by Hale (1956b) and Huneck (1968).

Nuclear magnetic resonance spectra can conveniently be used as proofs for the identity of a compound, especially in connection with IR or mass spectra. Extensive data on NMR spectrometry of depsides and depsidones are presented by Huneck and Linscheid (1968), for xanthones by Santesson (1969e). Culberson (1969, 1970a) also lists data on many single compounds.

C. Chromatographic Comparisons

Although not a full proof for the identity of a compound, a chromatographic comparison of an isolated compound and a sample with known identity can provide very good supplementary evidence. Chromatographic

comparisons are best made by TLC in at least two or three solvent systems, where the R_f values are in the range 0.2–0.8 and where the compound does not travel with any “secondary solvent front.” Preferably the comparisons should be done with cochromatography. Three spots are applied at the starting line: the unidentified sample, a known sample, and an equal mixture of the unidentified and the known samples. All the spots should contain approximately equal amounts of material. A depside can sometimes be chromatographically identified without access to an authentic sample of the compound. Hydrolysis of the depside will give the “acid part” and the “alcohol part” of the ester (often also the decarboxylated “acid part”). The same parts might be obtained by hydrolysis of other depsides that are available. The depside halves can be chromatographically identified by this means and the identity of the depside deduced.

Hydrolysis is performed ideally by dissolving 0.1–5 mg of the depside in 0.05–1 ml of concentrated sulfuric acid at -10° – 0° C, and after 10–30 minutes adding crushed ice. The hydrolysis products are extracted in ether, and the ethereal extract can be used directly for chromatography.

For examples of microhydrolyses, see Culberson (1967) and Culberson and Kristinsson (1969). Wachtmeister (1959) discusses both acid and alkaline hydrolyses.

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Appendix C

METHODS OF ISOLATING AND CULTURING LICHEN SYMBIOTS AND THALLI*

V. AHMADJIAN

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I. Fungal Symbiont

A. Isolation Techniques

1. SPORES

The best way to isolate a lichen fungus is by its spores. The ascocarp is allowed to discharge its spores onto a sterile surface, the spores are collected, either before but preferably after germination, and transferred in groups or singly to a suitable culture medium. The ascocarps may be soaked in water before being used or they may be placed directly onto a moist filter paper in the discharge chamber. Excess water should be removed from the surface of the apothecium. Spores are discharged within a few hours, and discharge from one ascocarp may occur continuously for days. One apothecium of *Cladonia cristatella* discharged spores for 13 continuous days after which the experiment was stopped (V. Ahmadjian, unpublished results). Discharge was determined by placing the petri-dish cover with the apothecium each day over a new agar surface.

*For additional information on this subject see Ahmadjian, V. (1967). "The Lichen Symbiosis," Ginn (Blaisdell), Boston; and Richardson, D. H. S. (1971). Lichens. In "Methods in Microbiology," pp. 267-293. Academic Press, New York.

Spores may appear on the agar surface within minutes or several hours after the ascocarp is placed into position. Spores may be caught on the following surfaces: (a) *Agar*—an ascocarp is fixed by means of petroleum jelly to the inside cover of the top of a petri dish. The top cover is then placed over the bottom half which contains a thin layer of nonnutritive or mineral agar. Discharged spores fall onto the agar surface either singly or in groups. A variation of this method is to have the petri dish with its top cover down and allow the spores to discharge up onto the agar surface. This way prevents debris and foreign organisms from contaminating the agar. Care must be taken to ensure that the distance between the ascocarp and the agar is within the spore's normal discharge range (maximum recorded = 45 mm). The duration of discharge can be determined either by examining each day whether spores were discharged onto a fresh agar surface or by placing the ascocarp near the outer rim of the petri-dish cover and rotating the cover at periodic intervals. Each impact area below the fruit can be circled with a crayon. To observe the spores without opening the petri dish place the dish in an inverted position on a microscope stage and focus on the agar surface.

When the spores have germinated small blocks of agar that contain spores are excised and transferred to a culture medium. Since the spores of many lichen fungi remain together in groups of two–eight, and the discharge area soon becomes crowded, the isolation of single spores is difficult. To facilitate single spore isolation it is necessary to have the spores separated as far as possible from each other. This can be done by reducing the discharge time, thus allowing only a relatively few spores to be discharged onto the agar and to increase the distance between the ascocarp and the underlying agar surface. Drifting of the spores will occur over the greater distance and the impact area will be widened considerably. When a spore has been sighted that is well separated from others, the area above the dish is marked with either a fine crayon if the dish is glass or, if the dish is plastic, with a sharp implement. Under a dissecting microscope the petri-dish cover is removed, the mark on the dish is located, and the spore, along with a small piece of agar, is removed with a fine needle.

(b) *Glass*—spores can be discharged onto a glass slide that is either suspended over or is directly below an ascocarp. Discharge is effected in a damp chamber apparatus. More sophisticated techniques include passing a row of glass slides at a known rate over the ascocarps. Studies on spore discharge have provided information on the duration and periodicity of spore discharge and how factors such as light, temperature, humidity, and pH affect discharge (Kofler and Bouzon, 1961; Pyatt, 1968, 1969; Garrett, 1971). To determine the distance of spore discharge an ascocarp is fixed on the vertical surface of a block at the end of a marked glass slide. The spores discharge along the length of the slide (Bailey and Garrett, 1968) (See Chapter 4).

(c) *Parafilm*—quantities of spores may be collected by having them discharge onto a parafilm surface and then washing them off with distilled water. The parafilm is sterilized with ultraviolet light, laid across the opening of the bottom of the petri dish and then the cover of the dish is set in place. Apothecia are placed on the bottom of the dish on moist filter paper. The suspension of spores can be maintained as stock material for use in future investigations. Tests have shown that such stocks still germinated after 1 month's storage (Kofler, 1970).

2. HYPHAL FRAGMENTS

Fragments of hyphae, either teased from the medulla, or those attached to algal cells, when placed onto a culture medium may grow into fungal colonies. The fragments should be small enough to minimize the chances of contamination. Sufficient numbers should be attempted to ensure that the fungal growth obtained is likely to be that of the mycobiont and not a foreign fungus growing on or in the thallus. The element of doubt with this technique is so high that it should be used only if spore isolations are unsuccessful.

B. Culture

When the spores have germinated, a process that takes several hours for some mycobionts and several days for others, they are transferred to a nutrient agar contained in a test tube, glass bottle, or Erlenmeyer flask. The medium that supports the strongest growth for many mycobionts is malt-yeast extract (malt extract, 20 gm; yeast extract, 2 gm; agar 20 mg; distilled water, 1000 ml). When grown on a solid medium the colonies that develop are compact and elevated above the agar surface. Mycobionts of some filamentous lichens may form long extensions and even lobelike structures. Not all lichen fungi do well on nutrient agar. Some, like *Endocarpon pusillum*, grow well only on media with limited nutrients, i.e., soil-extract agar (Bold's mineral solution, 960 ml; soil-water, 40 ml; agar, 15 gm). Malt-yeast extract inhibits the growth of this fungus. Incubation should be at about 20°C. After 6–8 weeks, small colonies of the fungus should be visible. If fungal growth occurs before this time the changes are high that the growth is that of contaminant molds.

Because lichen fungi grow slowly, cultures can be kept for months or even several years before they are transferred. Vegetative mycelia have been lyophilized successfully and stock cultures of representative lichen fungi are being maintained by the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

To obtain large quantities of mycelium for chemical analysis special laboratory-scale growth apparatus have been developed (Bloomer, 1968a,b).

II. Algal Symbiont

A. Isolation Techniques

1. MICROPIPETTE

The surest but most tedious way to isolate a lichen alga is with a micropipette. This method minimizes accidental isolation of a foreign alga that may be an epiphyte on the thallus and provides high assurance that the alga isolated is the true symbiont. All glassware and implements are sterilized before use, and if possible the slides should be soaked first in an acid bath and then washed well with distilled water. The reason for this is that the cells of many lichen algae adhere to a glass surface, particularly if the surface is dirty. Small thallus fragments are picked out from a clean part of the thallus and placed into a drop of water on a glass plate. These fragments are gently grinded with a glass slide until the water turns green. Fragments adhering to the slide are rinsed with a few drops of water over the stock suspension. The suspension is then placed into a small glass vial.

Micropipettes are made as follows: 9-inch segments of 3–4-mm glass tubing are plugged with cotton at both ends and drawn in the middle over a bunsen burner. The drawn tubing is separated into two gross pipettes either with a file or by melting the glass in the middle of the tubing. The latter method is preferred if the pipettes are to be stored since the burner can seal the openings and the pipettes can remain sterile indefinitely. A gross pipette is drawn into a micropipette over a flame that must be small enough not to heat the glass too rapidly. Such a small flame can be obtained by connecting to the gas supply tube a segment of glass that is tapered at one end. The tapered end of a gross pipette is grasped with a forceps, held over the flame until the glass softens and then pulled smoothly at the same time as the pipette is withdrawn from the flame. The softened glass will stretch into a thin tube. The opening of the micropipette should be about 50–75 μm in diameter. If the pipette is drawn too much the sealed end may be removed with a sharp pull from a fine forceps. Micropipettes that are not suitable, either because their openings are too large or their tips jagged, should be saved and repulled later. Because these pipettes are so fragile, about a dozen should be made before beginning the isolations.

A drop of the stock suspension is placed on one end of a glass slide and examined with a microscope at low power ($\times 100$). About 15 algal cells, preferably those with bits of the fungus adhering to them, should be transferred from this drop through 3 or 4 new drops of water on the same slide. By the fourth drop the cell is usually removed from other impurities and can be then transferred to the surface of a nutritive agar slant. The pipette is drawn slowly along the agar surface and the liquid is blown out until air bubbles

appear. The tubes are incubated at 15°–20°C under dim illumination or in darkness for 6–8 weeks. Single cells or those in groups of two or three may be isolated.

A length of rubber tubing added to the micropipette facilitates its use. The tubing is placed in the investigator's mouth during the isolation process and it helps to increase the natural capillary action of the micropipette. The worker can apply suction or blow out the contents of the pipette.

2. THALLUS OUTGROWTHS

Small pieces of a thallus are placed into a mineral solution or onto an agar surface and incubated under light. After several weeks, or even longer with blue-green phycobionts, the algal cells will grow out of the confines of the fungal hyphae. The thallus fragments may have to be transferred to fresh media several times because of the dense growth of foreign algae. Identification of the phycobiont can be made from observations of the fragments at a stage where the algal cells still are loosely associated with fungal hyphae or, with filamentous algae, where the filaments can be traced back to fungal tissue.

3. CENTRIFUGATION

This technique is used to obtain quantities of algal cells that will be more or less free of fungal filaments. Lichen thalli are ground up with a mortar and pestle in distilled water and the resulting suspension then centrifuged at the following speeds: (a) *Blue-green algae*—375 g for 3 minutes; the green algal zone of the precipitate is resuspended and centrifuged at 125 g for 30 seconds. This procedure is repeated several times with greater speeds up to 90 seconds. (b) *Green algae*—60 g for 10 seconds; the supernatant is recentrifuged at 375 g for 5 minutes. The precipitate is resuspended and centrifuged for another 5 minutes. These speeds are guidelines and will vary slightly according to the different types of lichens (Richardson, 1971). Lichens that have algal symbionts of small size, i.e., species of *Peltigera* that have *Coccomyxa* as phycobiont, are especially suited to this technique because of the wide differences in size between the algal cells and fungal hyphae.

B. Culture

Lichen algae can be separated into two groups on the basis of their cultural requirements, i.e., *Trebouxia* phycobionts and other phycobionts. Most strains of *Trebouxia* grow well only on a medium supplemented with organic

compounds. On such a medium they grow well even in complete darkness. A basic mineral medium with additives (per liter) of glucose (20 gm), proteose peptone (10 gm), and even coconut milk (140 ml) will support strong growth. *Trebouxia* is sensitive to high light intensities and should not be grown under light intensities of more than 150 ft-c. Other lichen phycobionts grow well on organic medium but they will develop also on inorganic media.

III. Lichen Thallus

Culture of the whole thallus is difficult and complicated by the sensitivity of lichens to air pollution, their need for varying environmental growth conditions, and the contaminant organisms that grow on the thallus. In recent years, because of increased studies in whole thallus physiology, greater attention has been focused on cultivating thalli for long periods of time. A special controlled environmental lichen growth chamber was developed by Kershaw and Millbank (1969) that kept thalli of *Peltigera aphthosa* in a healthy condition for 6 months and Dibben (1971) achieved new growth of 5 terricolous lichens under Phytotron conditions. Other studies on whole lichen culture are those of Tobler (1939), Pearson (1970), and Galun *et al.* (1972). Kershaw and Millbank (1970) studied the development of isidia excised from *Peltigera*.

Alternate drying and wetting are essential for successful lichen growth as they are also for the recombination of the separated symbionts (see Chapter 18). Sustained moisture conditions bring about contamination and dissociation of the symbionts. The substrates on which lichens have been cultured include soil, sand, silica gel, and moist filter paper on a sandy surface. The essential property of these substrates has been their ability to dry slowly.

Axenic cultures of lichen thalli are not possible because of the bacterial flora on the thalli. Bacterial cells are found lodged in chinks and cracks of the lichen thalli and embedded within the extracellular polysaccharide material found throughout the thalli (Jacobs and Ahmadjian, 1971). Attempts to sterilize thalli with plasmolyzing agents or radiation have not been successful. The only possible way to achieve lichen thalli that are free from contaminants is to begin with the separate symbionts and recombine them under sterile conditions. Such a possibility now exists with *Endocarpon pusillum* (Ahmadjian and Heikkilä, 1970).

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