

[*Discovery Reports.* Vol. XXX, pp. 301-408, Plates VII-XXVIII, August, 1960].

## STUDIES ON *PHYSALIA PHYSALIS* (L.)

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# STUDIES ON *PHYSALIA PHYSALIS* (L.)

## PART 2. BEHAVIOUR AND HISTOLOGY

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(Plates XXVI to XXVIII, text-figures 1-6)

### INTRODUCTION

THE morphological complexity of the siphonophores is well known. It has engaged the attention of a great number of distinguished biologists, and it is only comparatively recently that the true interrelationships of the group have been satisfactorily established. However, preoccupation with gross morphology has led most workers to neglect certain important aspects of siphonophore organization, particularly in the fields of physiology and behaviour.

The group is unique amongst the Coelenterata in the capacity shown by some of its species to secrete gas into a float and to regulate density (and hence to achieve vertical migration) by adjusting the amount of enclosed gas; and yet, in recent times only one worker (Jacobs, 1937) has investigated this phenomenon. The small size and delicacy of most siphonophores makes them exceptionally difficult animals to handle, but, if the various technical difficulties could be overcome, the rewards of a thorough physiological investigation would certainly be rich. Certain members of the Calycophora, such as *Hippopodius*, can perform density regulation *without* the aid of a gas-filled float. The mechanism for this is completely unknown. Furthermore, there is virtually no published information on the extent of nervous co-ordination between the different parts of a siphonophore colony. These are but two of the outstanding problems in the realm of functional organization.

An investigation of the behaviour and reactions of the siphonophores was begun by the author in 1954, but it soon became clear that the significance of the behavioural findings would be hard to establish unless more was known of the histology. Although we are still far from achieving a full understanding of the structural and functional organization of the siphonophores, it has been possible to make some progress by means of this twofold method of investigation. In the following account, an attempt will be made to describe the chief activities of one siphonophore, *Physalia*, together with the extent of co-ordination between the different parts and the microscopic structure of the component tissues.

The material used in the investigation came from three sources. The bulk of it was collected at Lanzarote in the Canary Islands during the spring of 1955, and the behavioural observations were made at that time. Some material was fixed for the author by Miss Elaine Robson from a specimen captured near Plymouth in November 1954. Finally, some material from the 'Discovery' collections was examined.

The author wishes to acknowledge his particular debt to Mr A. K. Totton, who conceived the idea of a joint expedition to the Canary Islands and whose advice has been a great help, both then and since. Certain of the results reported here were incorporated in the author's Doctor's thesis, which was presented, under the supervision of Dr W. Holmes, at Oxford in 1956. It is a pleasure to acknowledge the help of Professor Hardy, Dr Holmes and others at Oxford, and of the late Professor R. B. Miller in whose department at Edmonton the work was completed. The expenses of the project were met by a Research and Maintenance grant from the Department of Scientific and Industrial Research (which included an additional sum of money for the Canary trip) and by grants from the General Research Fund of the University of Alberta, and from the National Research Council of Canada.

## BEHAVIOUR

### 1. INTRODUCTION: INDIVIDUALITY IN SIPHONOPHORES

It is customary to speak of siphonophores as colonies consisting of modified polypoid and medusoid individuals, all attached to a common stem.

This simple picture is satisfactory enough where the medusoid individuals are concerned. In the colonial Hydrozoa nectophores and gonophores are always easy to recognize as medusoids, both in their manner of development and in their mature structure. The remaining parts of a colony are not always so easy to classify. For example, in both the sessile *Corymorphida* and its pelagic relative *Veabella*, the sexual medusoids are borne upon protuberances from the body-wall which are alike and homologous in every respect, except that in *Corymorphida* they are mouthless, while in *Veabella* they have mouths and ingest food. In *Veabella* they are known as 'gonozoooids'. Taking a rigid view of the concepts of the colony and the individual, two interpretations are possible for this state of affairs. Either the gonozoooids represent true individuals (as their name implies) and function as such in *Veabella* while becoming reduced in *Corymorphida*; or they have originated in phylogeny from hydranth outgrowths, and such individuality as they have acquired is secondary. If the first interpretation is correct, then *Corymorphida* must be regarded as a reduced colony. If the second is correct, then the structures in question should not be called *gonozoooids*, unless it is made clear that they have acquired their individual status secondarily. The latter interpretation is preferable and it leads to the conclusion that individuality can be complete or partial; that it can probably also be lost or gained; that it is, in fact, an unreliable concept.

If the siphonophores are considered from the functional point of view, the distinction between the colony and the individual becomes even harder to define. In many siphonophores, one must recognize that a new, communal individuality has emerged from the ancestral assemblage. The organism acts like a well-integrated individual. Whatever their origins, the component parts have now achieved the status of organs in an individual. In a certain sense, the siphonophores represent a method of escaping from the limitations of the diploblastic pattern. Another more successful method of escape involved the development of a third germ layer. In the Triploblastica, organ systems develop within the individual. In the Siphonophora, individuals become organs.

The problem now to be considered concerns the extent to which *Physalia* has progressed beyond the condition of an assemblage of autonomous individuals toward the status of a new individuality.

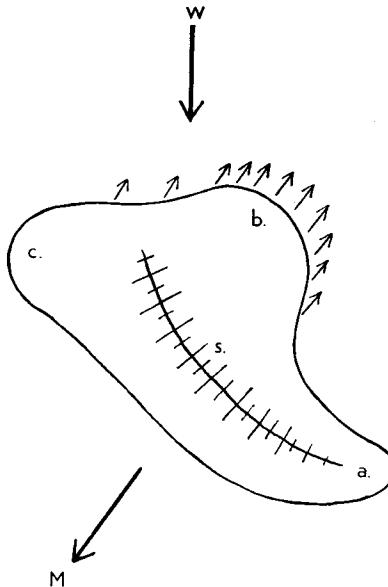
### 2. BEHAVIOUR OF THE FLOAT

The outline of the float (seen from above) is shown in Text-fig. 1. The main groups of appendages are situated around the bulge (*b*). When the float is being driven before the wind the drag is concentrated in this region and, as a result, the float automatically assumes the orientation shown. The shape of the float is such that when orientated for sailing it does not move directly down-wind, but to one or other side of the down-wind direction, according to the mirror-image dimorphism (Woodcock, 1944, 1956; Totton and MacKie, 1956).\*

In adopting a sailing posture the float responds actively by erection of the crest or sail (*s*); at the same time the apical pore-end (*a*) curves round toward the windward side. Bigelow (1891) found that

\* In my thesis (submitted July, 1956, at Oxford) I suggested that the advantages inherent in dimorphism are not to be explained by reference to local phenomena such as distribution of Sargasso weed, islands, etc., but should be thought of in terms of world distribution. In any given ocean one dimorphic form will presumably be better fitted to survive than the other, but it will not always be the same form, and, for the species as a whole, it is not important which form has the advantage. The important thing must be that by virtue of the dimorphism the species is fitted for life in *any* ocean. Woodcock (publ. August, 1956) adopts a rather similar view.

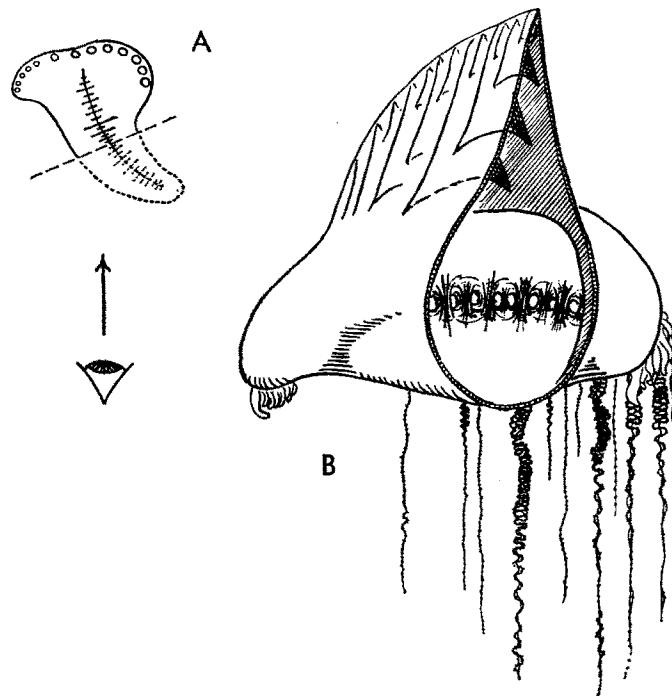
erection of the crest took place when air was blown against the float with bellows. Wilson (1947) found that splashing seawater on the float was also effective, although according to Bigelow only rain-water elicits the response. In the Canary Islands it was observed that in specimens brought into the calm of the laboratory, the crest usually collapsed and the float became flaccid. In specimens exposed to the wind, on the other hand, the crest became erect. The erect condition of the crest is shown in Pl. XXVI, fig. 1.



Text-fig. 1. Diagram of *Physalia*, seen from above, to show the sailing posture. *M* = direction of movement, *W* = wind direction, *a* = apical pore end of float, *b* = bulge, *c* = oral end, *s* = sail (crest). The arrows around the bulge (*b*) represent the forces of drag caused by the tentacles.

The way in which the wind acts has not been determined. Nerve-cells equipped with sense-hairs (to be described below) occur in the ectoderm of the float, and may be concerned in the reception of wind stimuli. Alternatively the buffeting and agitation caused by the wind or spray, or a combination of such factors may evoke direct responses in the float musculature, leading to crest erection. It should be explained that erection of the crest occurs automatically whenever the float musculature achieves a certain degree of tension. Any stimulus evoking an overall tightening of the float musculature will therefore normally lead to crest erection. The mechanism is surprisingly simple. The float consists of two containers: an outer muscular case, the codon (strictly: 'pneumatocodon') and an inner air bladder, the saccus ('pneumatosaccus'). The saccus lies free in the coelenteron, being surrounded by the codon and attached to it only in the region of the apical pore. The saccus is an inert, pliable structure whose shape is determined by that of the enclosing codon. Electric shocks applied to its wall were found to evoke no visible response, either local or general. Along its upper side it bears a row of thin-walled outpushings which become inflated whenever the air in the saccus as a whole is sufficiently compressed. At other times, the outpushings are collapsed and inconspicuous. This can be demonstrated experimentally by removing a saccus intact from a specimen, ligaturing its apical connection with the codon as a precaution against loss of air, and placing it under pressure by means of weights. In Pl. XXVI, fig. 2 a saccus is shown under pressure, with the outpushings inflated. In Pl. XXVI, fig. 3 the same saccus is shown without the weights. In normal circumstances, the necessary pressure is engendered by contraction of the enclosing codon. The dorsal processes of the saccus fit into pockets (Text-fig. 2) in the roof of the codon causing them to fill out, and stand erect. The arrangement is reminiscent of a pneumatic tyre, with inflatable inner tube. The enclosed air functions, indeed, as a 'pneumatic skeleton', comparable in some ways to the 'hydrostatic skeleton' of actinians (Chapman, 1949).

Many observers have commented on the strange rolling-activity and somersaulting performed by the float of *Physalia* in calm weather. Wilson (1947) suggests that this activity serves to keep the float moist. This would be particularly important in calm conditions in tropical waters. Perhaps a better interpretation of the activity is that proposed by Totton (personal communication). The upright position of the float, with sail 'trimmed to the wind' is inherently unstable, and can only be maintained when the wind is exerting a certain pressure against the side of the float. If there is little wind, the instability of the float in the sailing posture becomes manifest, and the float collapses on to its side. The float responds to collapse by movements which have the effect of restoring the upright position. In the upright position again, a new attempt at the sailing posture takes place, which, if the wind is still insufficient, results in another collapse.



Text-fig. 2. A, View of the float from above, to show the angle from which B is seen. The apical pore end (dotted) has been removed at the level indicated by the broken line. The openings around the bulge region and the side lead into the groups of appendages. B, View into the interior of a float from which the apical pore end and the whole saccus has been removed. For convenience, the float and crest are drawn as if they were still supported from within by the saccus, but if an animal were actually opened in this way, the float would collapse completely. Note the vaulting in the roof, forming pockets into which the saccus outpushings would normally fit.

The author's observations, considered in retrospect, support Totton's interpretation. It was several times observed that a sudden drop in the wind would cause sailing specimens of *Physalia* to fall over on to the windward side. Specimens were much less apt to fall on to their leeward sides; to do so would mean hauling a considerable weight of tentacles up out of the water. Bigelow (1891) was the first to draw attention to this fact. The float is precariously balanced, and slight local contractions of the muscular wall can bring about shifting in the centre of gravity and consequent rolling into a new orientation. Rolling occurs mainly about the *a-b* axis (Text-fig. 1), sometimes about the *b-c* axis. In the latter case, the apical pore end may rear up high in the air, and a complete somersault take place. These variations are understandable when one considers that all three sides of the float are contractile and that contraction can be localized in particular areas. A considerable range of body form can be assumed, in all of which the pattern of rolling will be different. It was found possible, by electrical stimulation of appropriate regions of the float wall, to evoke local contractions of sufficient power to bring about shifting of the centre of gravity and various kinds of rolling.

In the laboratory, the animals' own efforts are not always sufficient to achieve righting; in the natural environment, however, wave action may assist the process as in *Porpita* (Mackie, 1959). One laboratory specimen made unsuccessful attempts at righting for more than 10 min. The attempts occurred at intervals of 25–35 sec.

The righting activity, whether successful or not, consists of leeward side contractions. The repeated performance of righting movements may be of assistance in developing the asymmetry of the young animal. It is not known how the primary asymmetry arises, but it is a reasonable assumption that once a leeward side and a windward side have been established, however tentatively, the normal activity of the float will tend to fix and even to enhance it.

The contraction of the codon musculature during the righting movements is not a smooth, steady increase in tension; it is a succession of jerks, sometimes barely perceptible, which follow one another at intervals of 1½–2 sec. After each jerk there is some slight relaxation of the muscle, but this is more than made good by the next jerk. In most specimens where rolling-behaviour was studied, five or six jerks were sufficient to bring about the necessary degree of contraction for righting to take place. In the specimen referred to above, which had difficulty in righting, ten or fifteen jerks were observed in each unsuccessful attempt. If a kymograph record of the reaction could be obtained, the tracing would be a 'staircase'. We are probably dealing with a phenomenon in the same class as the stepped contraction of the sphincter-muscle of *Calliactis*, or of the circular swimming-musculature of *Aurelia*. Such responses are explained on a basis of neuromuscular facilitation (Pantin, 1935).

### 3. BEHAVIOUR OF THE TENTACLES

In a medium-sized *Physalia* (gas-gland diameter = 1·4 cm.) observed when sailing freely outside the harbour at Arrecife, the longer tentacles were found to extend to distances of 8–10 m. Following stimulation of the float, the tentacles were drawn in, achieving what appeared to be full contraction after about 1 min. The tentacles then measured 12–15 cm. These findings accord with Parker's statement that a tentacle may contract to 1/70 its full length (Parker, 1932).

As it contracts, a tentacle is thrown into lateral loops and then into primary and secondary coils. The coiling seems to be a straightforward mechanical accompaniment of shortening, and is not to be thought of as a device for ensnaring prey. The tentacles cannot 'writhe' or wrap themselves actively round objects. The prey is secured to them solely by the nematocyst-filaments, acting as harpoons.

The process of fish-capture has never been closely observed in natural conditions, although specimens holding prey in their tentacles have been studied in the laboratory (Wilson, 1947). Under normal environmental conditions, contact of a tentacle with any solid object such as a fish will presumably evoke nematocyst discharge. The object, if animate, will suffer a temporary paralysis from the action of the toxic substance in the nematocysts. It will be held against the tentacle by the nematocysts, and its weight, or the increased drag it causes, will stretch the tentacle and thus evoke contraction. Gradually the fish will be brought up to the region of the gastrozooids which will apply themselves to it, and begin to digest it.

Laboratory observations made in the Canary Islands suggest that any abrupt mechanical stimulation applied to the tentacles, whether causing stretching or not, will evoke contraction. For instance, it was observed that a captured fish would be held passively in the tentacles for quite a long time unless it became active, and struggled to escape. If this happened, the tentacles would begin to shorten again. Any tactile stimulus, such as pinching with forceps, or striking with a glass rod caused some degree of contraction either local or general. Bigelow (1891) found that there was little, if any, sensitivity to food substances in solution.

The most characteristic activity of the tentacles is a rhythmic shortening and lengthening. On the other hand, tentacles, whatever their rate of contraction, can remain completely motionless for long periods. Bigelow was the first to describe the shortening and lengthening of the tentacles. He did not regard it as sufficiently regular to be called 'rhythmic'. Parker (1932), however, found evidence for rhythmicity in the movements, the interval between successive performances being 30–75 sec. In the present investigation a definite rhythm was observed. It was sometimes a very loose one as in the cases studied by Parker, but in many examples, particularly where fresh healthy specimens were under observation, a much more regular rhythm was in evidence. It was found that the rhythm was slower in small tentacles than in large ones, but that in all tentacles, whatever their size, the frequency depended on the general degree of contraction of the tentacle within which the smaller rhythmic changes in length were taking place. In one case a small moderately extended tentacle was timed over 23 min. The contractions followed one another every 10–17 sec. The specimen was then transferred to another tank and, as invariably happens following such disturbance, the tentacles shortened considerably. When the rhythmic activity emerged again in the original tentacle studied, the contractions were coming at 5–10-sec. intervals. Gradually the overall length of the tentacles increased again, and the frequency of the rhythm declined.

Rhythmic shortening and lengthening seems as a rule to accompany gradual changes in overall length and it may, in fact, be the basis for such changes. In this there is a superficial resemblance to the rhythm in *Metridium* (Batham and Pantin, 1950). However, in *Metridium* the rhythm is slow, sigmoid and distinct from the facilitated responses evoked by mechanical stimulation, while in *Physalia* the contraction phase of the cycle is rapid, 'stepped', and indistinguishable from a mechanically evoked contraction. The 'steps' are much more noticeable in the contraction of the tentacles than in contraction of the float because the amplitude of the contractions is much greater. Each 'step' may shorten the tentacle by several inches. Not more than three or four 'steps' are distinguishable in the contraction phase of the rhythmic movements. The elongation of the tentacle following one of these stepped contractions is smooth and gradual. It is probably passive, being caused by gravity or drag. Endodermal circular muscle is present in the tentacles, but there is no evidence that it assists elongation. It is more likely that it serves for movement of fluids in the coelenteron.

Rhythmic shortening and lengthening is carried on independently by individual tentacles, its rate depending on their sizes, and states of contraction. We are evidently dealing with a well-developed local action-system, such as frequently characterizes structures with a high degree of autonomy (Pantin, 1952). It was observed in the laboratory that rhythmic movements had the effect of unravelling any tangles which had occurred among the tentacles. This, however, may be no more than an incidental occurrence.

#### 4. THE BEHAVIOUR OF THE REMAINING APPENDAGES

The gastrozooids show a considerable amount of activity in some specimens. This consists of random 'searching' movements. On touching a solid object they apply themselves to it, the mouths spreading out over any area up to 1 cm. in diameter. Although Bigelow (1891) stated that attachment to inorganic objects was of brief duration, it was observed in the Canary Islands that gastrozooids sometimes remained attached to the polythene wall of the tank for periods of more than an hour. It is, however, too early to say whether or not the feeding response involves chemical as well as tactile stimuli, as in *Hydra* (Semal, 1954b).\*

The appearance of a fish enshrouded by feeding gastrozooids is well-known from Wilson's account (*op. cit.*). The edges of the zooid-mouths spread out until they touch and then press up against one

\* It does (see footnote on p. 308).

another forming a seam. Thus the whole fish, or a large part of it, becomes enclosed in an improvised stomach, or extension of the coelenteron. Fish in various stages of digestion were collected and preserved on the Canary expedition. The largest, which was an unidentifiable specimen 9 cm. long, had been caught by a fairly small *Physalia* (gas-gland diameter = 1 cm.). Another specimen collected by the author is the shapeless lump figured by Hardy (1956, page 120). It is not known how much of a fish is usually digested and how much rejected. A partially decomposed fish might serve as bait to lure other fish into the tentacles, but this is a matter for speculation.

No original observations were made on the remaining appendages. According to Brooks (cited by Steche, 1907), the nectophores can perform pumping-movements. The author has observed nectophores in *Forskalia* pulsating while little more than bell-buds. Like immature heart muscle, the subumbrellar muscle of medusae is active long before its activity is required. Steche's suggestion that the nectophores come into their own only when the whole gonodendron is shed is therefore still tenable. The function of the nectophores, according to Steche, would be to keep the detached gonodendron in motion and well-oxygenated. It is certainly hard to see what function they could have while the gonodendra are still attached to the parent colony.

##### 5. CO-ORDINATION

For the most part, the appendages behave independently. The capture of prey by one tentacle does not visibly affect the activities of other tentacles or of the gastrozooids. Although the gastrozooids apply themselves eagerly to any object they encounter, the presence of a fish struggling in the tentacles a few centimetres away causes not the slightest alteration in their random movements. Whether this is still true when some of the gastrozooids have begun to digest the fish, is not known. The rolling behaviour of the float is not affected by the activities of the tentacles or gastrozooids. It continues without interruption during feeding. In fact, *Physalia* usually acts like an assemblage of autonomous individuals. The behaviour of the individuals is linked in terms of functional significance but not in terms of direct nervous integration.

There is, however, one response in which nervous integration can be seen. When an unusually strong stimulus, such as firm pinching with forceps, is applied to the float, a general contraction follows immediately, or so quickly that no time lag is visible to the naked eye. This contraction involves a sudden shortening of all or a proportion of the appendages. Appendages near to and remote from the site of stimulation are affected equally and, as far as can be seen, simultaneously. The stems of the gonodendra also shorten. The contraction is brief, and the appendages soon fall back into their autonomous activities again.

This response has the appearance of a nervous through-conduction reaction. Parker (1932), in his valuable physiological study of the nervous system, overlooked this reaction. He also overlooked the earlier account of Bigelow (1891). In it, Bigelow describes how squeezing the float or applying some strong stimulus to the base of the tentacles may evoke a general contraction of the colony. In natural conditions, it would seem likely that very turbulent surface conditions or abrupt collision with any floating object, such as a clump of Sargasso weed or another *Physalia* would evoke this response. This might be of value in preventing tangling of the tentacles.

The co-ordination observed is of a most elementary type. It is interesting in that it points to the existence of nervous communication between the different members of the organism. A somewhat similar response takes place in *Alcyonium* (Horridge, 1956b), where, likewise, the response is apparently protective in nature. In the physonectid siphonophore, *Forskalia*, the author has observed similar violent overall contractions of the siphosome appendages following abrupt stimulation of the stem, a closely comparable situation to that found in *Physalia*. Here, however, a more elaborate form

of integration (which has still to be fully analysed) is in evidence. It appears that *Forskalia* can swim forwards or backwards by altering the shape of the mouths of the swimming bells; the shape of the bell mouth in *Forskalia* appears to depend on the degree of contraction in two groups of radial muscle fibres in the velum, an arrangement unique to this group. Phases of activity or inactivity are common to the whole assemblage of swimming bells, but when active the bells beat at different frequencies after the first one or two beats. Certain observations point to the possibility of inhibition as a co-ordinating mechanism in the activities of the swimming-bells (Mackie, cited by Hardy, 1956). The observations in question were, however, made on a few, rather imperfect specimens, at a time when the author was not fully aware of the issues involved, and it would be of great interest to repeat the work.

In the case of certain Calycophora (for example, *Hippopodius*, *Chelophyses*), histological study provides clear evidence that in contrast to the arrangement in *Forskalia*, where two median exumbrellar nerve tracts connect the marginal rings of the bell with the stem, there is in these genera no direct nervous connection between the stem and bells. What is remarkable, however, is that in spite of the histological absence of nerve connectives, observations on living calycophores suggest that some sort of integration does, nevertheless, exist.

The problems of nervous co-ordination in the siphonophores are therefore considerably more complex than study of a simple form like *Physalia* would tend to suggest.

## HISTOLOGY

### I. INTRODUCTION: THE SIPHONOPHORA COMPARED WITH OTHER HYDROZOA

The main features in the histology of the siphonophores were established by the end of the nineteenth century. Good comparative accounts were given by Chun (1897, 1902) and Schneider (1902), the two workers who contributed most to the actual investigations. There has been little subsequent work of importance.

In their basic microscopic structure, the siphonophores bear a close similarity to other Hydrozoa. However, the tissues are often brought to a higher degree of elaboration and histological perfection than elsewhere in the Class. This is true of the striated muscle in the swimming-bells of certain Calycophora, where Q, J and H bands are clearly distinguishable; of the nervous system in the bells of Physonectae, where exumbrellar tracts are developed; and of the mesogloea in genera such as *Hippopodius*, *Chelophyses* and *Diphyes*, where the exquisite moulding of the swimming bells is achieved by means of an elaborately orientated system of mesogloal fibrils. One must also mention the complicated mechanism of the nematocyst batteries in such forms as *Praya* sp.n.\* (Korotneff, 1884), which is without parallel in the entire Phylum.

In addition to the tissues common to all hydrozoans, the siphonophores (except for the Calycophora) possess gas-secreting tissue, for which the only known counterpart in the Class is the basal disk in *Hydra* (Kepner and Miller, 1928; Kepner and Thomas, 1928). The Calycophora show two tissue regions for which no counterpart is known: the capillary network in the radial canal system of the swimming bells, and the sack-like 'somatocyst', whose cells show a great capacity for changes in volume. The functions of these regions are unknown.

There is one other striking feature in the histology of the siphonophores which should be mentioned. Although the two marginal nerve-rings are present, the swimming bells lack a subumbrellar nerve-plexus. Conduction across the muscle-sheet must therefore be myoid. This and certain other evidence suggest the possibility that, in hydrozoan medusae generally, the swimming beat, though

\* Identified by Totton as *Stephanophyses superba* Chun.

originating in the marginal nerve rings, may be conducted across the muscle-sheet independently of the nervous system, the latter serving simply to negotiate radial responses between the margin and the manubrium. The absence of the subumbrellar plexus in swimming-bells and in such forms as *Eucopella* (Lendenfeld, 1883), may be correlated with the absence of a manubrium. A nerve-plexus has never been found in the velum of a hydrozoan, and here too the muscle must presumably conduct the impulse for its own contraction. Horridge (1955) has found that in *Geryonia* radial responses involving movements of the proboscis can take place at the same time as rhythmic pulsation of the circular swimming muscle, which suggests that one or other of the responses is independent of the nervous system. The histological study of Krasinska (1914) indicates that the nerve-plexus is connected not with the circular muscle but with the radial. Thus, a variety of evidence points to the independent myoid conduction of the rhythmic swimming impulses in certain, if not in all, Hydrozoa. In the Scyphozoan medusae, where two nerve-plexuses are present (Horridge, 1956a), the situation is probably different.

*Physalia*, by virtue of its large size and sturdy construction, is a good object for histological study. All other common siphonophores are extremely delicate, and it is with justice that Bolles Lee (1900) wrote of them: 'This group contains some of the most difficult forms to preserve that are to be found in the whole range of the animal kingdom.' The tendency toward fragmentation is often a severe handicap to the investigator. In *Physalia*, the gonodendra tend to break off when the animal is fixed, but otherwise the parts remain intact.

In the account which follows, reference will be made to the work of previous authors where appropriate, but it may be stated at this point that the basic work on *Physalia* is Chun's section in Bronn's *Thier-Reich* (Chun, 1897, 1902). The present account is, in the main, supplementary to it.

## 2. HISTOLOGICAL TECHNIQUES

Specimens of *Physalia* quickly deteriorate in the laboratory, unless the water in which they are kept is well oxygenated. Only freshly caught specimens were used in this investigation.

Pieces of tissue were removed, washed briefly in distilled water, and placed in one or other of the following fixatives: Flemming's fluid without acetic acid ('F.W.A.'), Baker's formaldehyde-calcium (Ca-formaldehyde), both made isotonic with the seawater (Pantin, 1948); Zenker's and Helly's Fluids (Baker, 1950); Bouin's and Carnoy's Fluids (Pantin, *op. cit.*), formaldehyde-sublimate-seawater, being a mixture of ten parts formaldehyde with ninety parts saturated mercuric chloride in seawater.

If the float was to be fixed, the fixing-fluid was injected directly into the coelenteron between the codon and saccus, the whole float being immersed in the fixative at the same time. The intact float can be fixed with virtually no shrinkage or distortion by this method, so long as the air inside is not allowed to escape. When injecting the fixative care is therefore needed to avoid puncturing the saccus. The fixative was injected with a hypodermic syringe, or in the case of fixatives containing mercuric chloride, with a fine glass pipette.

Wherever possible, whole strips of material were examined. Paraffin sections are hard to make, more subject to distortion and altogether less revealing than thin strips of intact epithelium. In certain cases, as in the study of the nervous system, sections were found to be almost useless. The strips were prepared from fixed material under a binocular dissecting microscope, the tissue being lightly stained beforehand in some dye, such as gentian violet, which could be washed out afterwards in alcohol. The special staining techniques will be referred to where particular tissues are described. Unless otherwise stated, Heidenhain's iron haematoxylin (Pantin, 1948) was used. This method was found to be most generally useful, giving incomparably the best results with Flemming material, as other students of the Coelenterata have found.

Stained preparations were mounted in Canada balsam or in some other alcohol- or xylene-miscible medium. As a check on the amount of shrinkage caused by dehydration and clearing, control strips or gelatin sections were mounted in an aqueous medium (usually Farrant's) where shrinkage is negligible. Measurements of cellular and epithelial dimensions were made on preparations which had never been subjected to alcohol higher than 70%.

### 3. THE MUSCULAR SYSTEM

In the tentacles, gastrozooids, palpons and float of *Physalia*, both ectodermal and endodermal muscle fibres are present. As in *Hydra*, the endodermal fibres run in a circular direction, the ectodermal in a longitudinal.

In the tentacles and codon, the ectodermal muscle is very strongly developed. The ridges of mesogloea which support the muscle fibres are thrown into deep folds, particularly when the organism as a whole is contracted. There appear to be no radially orientated muscle fibres in any region. The endodermal system is less well-developed than the ectodermal, except in the codon. There is no endodermal muscle in the medusoid members, and the ectodermal system is only properly developed in the asexual nectophores. The subumbrellar muscle of these members has been examined in several specimens, but no striations are visible. This probably indicates that the medusoids examined, though the most advanced specimens obtainable from attached gonodendra, were not yet mature when fixed.

The musculature of the float has been studied in greater detail than that of other regions because it is technically easier to prepare. However, muscle throughout the animal shows the same histological characteristics.

A longitudinal section through a young *Physalia* is given by Okada (fig. 156D in Hyman, 1940). The inner chamber of the float (the saccus) develops as an invagination, the region of invagination becoming almost occluded in later life; the only trace of it is the apical pore. Okada's section does not go directly through the apical pore but slightly to one side of it, so that the opening from the inside of the saccus to the exterior is not shown. In actual fact, the tissues never grow together in this region, and the pore is not obliterated. It is sometimes possible to squeeze out a bubble of air, but only by vigorous pressure. Normally the pore is tightly constricted and it is unlikely that in natural conditions any leakage of air takes place. There is no other opening out of the float chamber, such as occurs in forms like *Physophora* (Leloup, 1941).

The saccus, being an invagination of the codon, has the same tissue layers as the latter, but they are 'inside out'. The ectoderm of the saccus is the innermost layer of the float. Like the ectoderm of the codon, it secretes a chitinous cuticle, but this has the special title of 'pneumatocyst'. It was first described by Schneider (1898).

The cross-section through the float-wall given by Chun (1902, fig. 79) correctly shows the relative thicknesses of the codon and saccus, and their general structure. The figure omits certain features, the chief of which are: the cuticle, the pneumatocyst, the nerve-plexus in the codon-ectoderm, the muscle fibres of the codon-endoderm and of the saccus-ectoderm. These omissions have been corrected in Text-fig. 3 accompanying this text. The ectodermal fibres run along the length of the float, parallel to the crest. The endodermal fibres run round the float, in a circular direction. Thus in Text-fig. 3, which represents a section at right angles to the longitudinal axis, only the cut ends of the ectodermal fibres (*m.ec*) are shown, while the endodermal fibres (*m.en*) appear in side view. The septa which divide up the roof of the codon into pockets (Text-fig. 2) are folds of endodermal tissue and mesogloea drawn out from the body-wall. The inflatable processes of the saccus, described on page 373, fit into these pockets. As the animal grows, a regular subdivision of the pockets proceeds, new septa appearing between those already formed.

Throughout the animal, the muscle tissue shows uniformity in its fine structure, although there are marked regional differences in fibre density, and in the degree of flattening of the layers. The flattened regions are particularly easy to examine in whole-strip preparations.

For showing the nuclei and cell membranes as well as the muscle fibres, iron haematoxylin is to be recommended; to stain the nuclei against a clear background, thionin and toluidin blue are suitable; and for staining the fibres, leaving the interfibrillar substance and cell membranes clear, Newton's crystal violet gives good results. The latter stain is often used for chromosomes, but in coelenterate material, where muscle fibres are ubiquitously present, it is not to be recommended. Thionin gives sharp and precise chromosome staining in F.W.A. and Zenker material. Chromosomes can also be studied 'in negative', that is they show up as light bodies against a darkly staining nuclear sap in silver preparations of Ca-formaldehyde material.

The float musculature consists of simple undifferentiated sheets, one cell thick. There are no special muscle groups. The fibres run parallel to one another in either a longitudinal or a circular direction, except at certain angles where the muscle sheet is 'tailored' into a triangular pattern, similar to that described and figured for the subumbrellar muscle sheet of *Forskalia* swimming-bells (Schaeppi, 1898, p. 536). In the region of the gas-gland the saccus-ectoderm is almost devoid of muscle fibres.

In cross-section the muscle layer can be seen to consist of a cell body layer containing the nuclei and a fibre layer in contact with the mesogloea (Text-fig. 3). The cell boundaries are not usually visible in sections, and are omitted from the figure. Seen in surface view (in strip-preparations) the cellular outlines can be made out (Pl. XXVI, fig. 4). The cells are usually five- or six-sided, the nucleus (or nuclei) lying towards the centre. Binucleate cells occur in all four muscle sheets of the float, but without regularity. Polyploid cells also occur, and there is reason to believe that they arise from binucleate cells by metaphase combination of the chromosomes, as in certain mammalian tissues such as the liver (Beams and King, 1942). The data obtained on polyploidy and the cytology of *Physalia* in general will be presented in more detail elsewhere. The cells shown in Pl. XXVI, fig. 4, with the exception of a probable tetraploid on the extreme right, are diploids.

The chromosome number most commonly encountered in diploid float-cells is twenty (Pl. XXVII, fig. 1). There are no constrictions visible along the length of the chromosomes which would correspond to centromeres or to nucleolar organizer regions. The chromosomes are simple rods, three pairs of which are particularly long ( $4.5 \mu$  in a typical late prophase), the remaining seven pairs grading down in length (from  $3.0 \mu$  to about  $1.8 \mu$  in such a case). Because of the flattening of the layer in which they lie, the chromosomes of the saccus epithelia are spread out in prophase, as if they had been treated by a squash-technique. If the prophase chromosomes lie on their flat sides, that is with both chromatids in the plane of the muscle sheet, they show little lateral curvature. If, however, they lie on their narrow edges, vertical to the plane of flattening, they are usually bent. Examples of both these conditions are shown. None of the chromosomes is intrinsically V-shaped, although all are susceptible to distortion in the flattened epithelia.

The nuclei contain a variable number of nucleoli, as can be seen in Pl. XXVI, fig. 4, but if there are many of them, the individual nucleoli are small. Planimeter estimations, using camera lucida tracings of 112 nuclei and their nucleoli, have been carried out, and it has been found that whatever the ploidy of a cell (which may be up to  $32n$ ), the total of the surface areas of the nucleoli is between  $1/12$  and  $1/19$  the surface area of the nucleus in which they occur. The tissue used was the saccus-endoderm, which is fairly evenly flattened over wide areas. A similar finding is reported for *Rana* by Beatty (1949).

The nuclei in the more flattened regions, particularly in the saccus-ectoderm which may be less than  $2 \mu$  thick, are often so compressed within the narrow confines of the layer that they assume very irregular shapes, frequently becoming elongated in the grooves between the muscle fibres. Under these conditions fragmentation appears to take place. Pieces of nuclear matter become lodged on

either side of a muscle ridge and, though sometimes remaining connected by tenuous tubes of nuclear membrane, they frequently separate completely. This phenomenon is to be regarded as a cytological accident rather than as a regular, significant process that could be dignified by the title 'amitosis'. Chromosomes lodge in the troughs between muscle-fibres and fail to reach the spindle at metaphase; the daughters of such divisions are therefore deficient. Chromosomes break transversely but, in some cases at least, the fragments take their place on the spindle and divide normally. This is interesting as it suggests that the spindle attachment is not a single, localized one, but is of the diffuse or polycentric type. There is evidence too that the nucleoli, like the nuclei, can be squeezed out and caused to divide if subjected to exceptional mechanical stress.

The occurrence of mitosis in the nuclear layer has no visible effect on the underlying fibre layer of the epithelium. This is also the case in the *Hydra* ectoderm, although in the endoderm the fibres are said to be absorbed during mitosis and resecreted following its completion (McConnell, 1932). Electron-microscope studies by Hess, Cohen and Robson (1957) show that in *Hydra* (both in the ectoderm and endoderm) the muscle fibres are contained within basal outgrowths of the epithelial cells and that the fibres do not fuse with one another, although they may come into close proximity. There is no evidence therefore in *Hydra* for true anastomosis in the fibre sheet; and the fact that fibres are not affected by the mitosis of their nuclei cannot serve as a demonstration of any breakdown in the simple cell-fibre relationship.

In *Physalia* the fibres form what at first sight appears to be an anastomosing network. Individual fibres can be traced for distances of over 300  $\mu$  without interconnections. Few fibres end freely; they may vary in thickness, becoming thin and inconspicuous, but usually they can be traced back into the 'net' again. Where two fibres come together, the *appearance* is one of direct fusion (Pl. XXVI, fig. 5). In actual fact true anastomosis (that is, direct confluence of fibre material) may never occur. Instances have been found where what appears to be a single fibre in the net has become bent or buckled at some point, revealing itself as two closely applied fibres (Pl. XXVI, fig. 6). The 'anastomosing net' may be no more than an array of discrete but closely juxtaposed fibres, as in *Hydra*.

Although in many preparations the nuclear layer appears syncytial, staining with iron haematoxylin invariably shows up the cellular boundaries. A typical cell of the saccus-endoderm may have ten or fifteen fibres running under it, some of which can be followed beneath six or eight other cells. Attempts to relate particular fibres to particular cells have not been successful, even in regions where the fibres are strongly contracted (Pl. XXVI, fig. 7). Although we cannot at this stage determine in any case which fibres 'belong' to particular cells, we have no definite grounds for abandoning the classical view of the muscle sheet as divisible into territories referable to individual cells.

#### 4. THE NERVOUS SYSTEM

Chun (1882) was the first to identify nerves in *Physalia*, in the ectoderm of the gastrozooids. In the related *Rhizophysa*, but not in *Physalia* itself, Chun discovered nerves in the float. With the exception of Parker (1932) whose studies revealed that conduction in the tentacles is neuromuscular, not simply muscular, no other workers on *Physalia* refer to the nervous system. Nerves have never been identified histologically in the tentacles.

An attempt to stain the nerves in *Physalia* was made in the present investigation. It is possible to make out the general distribution of the nerves in lightly stained carmalum and haemalum preparations, and Sudan-black staining of Ca-formaldehyde material sometimes colours the fibres fairly well. However, the author's main efforts have been directed towards making silver preparations, since these, when successful, are much the most revealing. Holmes's method (1947), which has been used

successfully on a variety of coelenterate material, was used to stain paraffin sections and whole strips of tissue taken from *Physalia*. Results have been only partially successful. In many preparations, the nerves show up quite well, but there is generally too much background staining (particularly in the region of the muscle-mesogloea interface) for the nerves to stand out in fine detail. This fault could probably be rectified by further trial and error adjustment of the staining schedule. In paraffin sections, nerves have not been positively identified, partly because the formaldehyde-sublimate fixative recommended for the sharpest nerve staining is rough in its action on the tissue generally, and partly because the imbedding technique further distorts the tissue relationships, where much muscle and collagenous mesogloea are present. Nerves have been found only in the strip preparations.

Chun's finding of nerves in the ectoderm of gastrozooids has been confirmed. A continuous ectodermal plexus extends throughout gastrozooids, codon, palpons and gonophores. In Pl. XXVII, fig. 2, a portion of the nerve-plexus from the codon-ectoderm is shown. A tripolar neuron (centre) and a bipolar (lower right) can be seen. The dark bands crossing the field from top to bottom are the longitudinal muscle-ridges of the ectoderm. In the codon-ectoderm there were estimated to be about 140 nerve-cells per sq. mm. In the gastrozooids, there appear to be about half this number. Tripolar neurons predominate; out of fifty studied in a particular area, thirty-two were probably tripolar, the remainder being bipolar. A few multipolar cells (4- and 5-polar) were also seen.

Bi-nucleate neurons also occur sparsely in the float. This is not a unique finding; Hertwig and Hertwig (1878a) found bi-nucleate neurons in *Cunina*. Nervous tissue in coelenterates is evidently post-mitotic in the main but, where bi-nucleate neurons occur, it may be that mitosis has taken place in the nucleus of a young neuron after differentiation has proceeded too far for the cytoplasm also to divide.

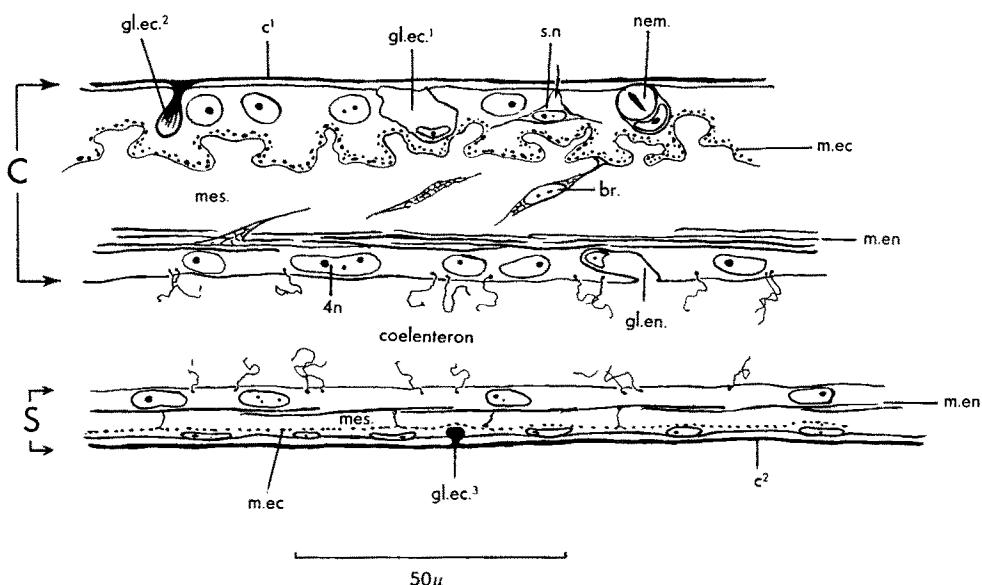
The exact character of the inter-neural associations has not been determined. It is often difficult to decide where a fibre from one nerve ends and another begins, but the preparations are not stained sufficiently precisely for accurate observations on the fine structure to be possible. Fibres have been found to run for distances of up to 100  $\mu$  without branching or associating with processes from other nerves.

In no region examined was there any hint of an elaboration of the plexus into orientated tracts. Pieces of tissue were examined from several regions of the float, and it is unlikely that tracts, such as those which occur in the stem-bladder of *Physophora* (Korotneff, 1884, Taf. 15, fig. 26) would have escaped observation. The plexus is distributed evenly in all regions examined.

In some of the preparations, sense-hairs (in groups of two or more) can be seen emerging from the surface of the ectoderm (Text-fig. 3). In most such cases, a nerve-cell (*s.n.*) can be found in the region underlying these sense-hairs. In a few cases a conical projection of the nerve-cell can be seen passing up to the superficial region, the sense-hairs being embedded in it. Around the roots of the sense-hairs, the cytoplasm of the conical projections shows a dense aggregation of very fine granules. Thus, although the evidence is incomplete, it is probably true to say that the sense-hairs belong to the nerve-cells, not to the surrounding muscle-cells, and that the majority of the nerve-cells possess sense-hairs. Where one or other component is apparently absent, this can usually be attributed to vagaries in the staining technique. It was found that some gastrozooids fixed in Ca-formaldehyde showed the sense-hairs and their relationships with the nerve-cells quite clearly, when Peter's glycine developer (Peters, 1955) was used. It is not quite clear whether the sense-hairs penetrate the cuticle, but it would seem reasonable to suppose that they do.

Attempts to demonstrate nerves in the tentacles have so far been unsuccessful. It is highly probable that nerves do exist here, particularly in view of Parker's physiological findings (Parker, 1932). The exceptionally muscular nature of the tentacles makes them very difficult to examine histologically.

Thin strips of ectoderm are hard to obtain because of the deep folding of the layer. It would be of great interest to obtain histological data about the nerves in these regions, because Parker's finding of a high conduction rate (120 cm./sec. at 26° C.) makes it probable that the neurons would show a longitudinal orientation as they do in the mesenteries of *Calliactis* (Pantin, 1952).



Text-fig. 3. Transverse section through adjacent portions of the codon (C) and saccus (S). *br* = bridge cell, *c*<sup>1</sup> = cuticle of codon, *c*<sup>2</sup> = pneumatocyst, *gl.ec*<sup>1</sup> = ectodermal gland cell, *gl.ec*<sup>2</sup> = root-like cuticular body believed to represent site of extinct gland cell, *gl.ec*<sup>3</sup> = similar body attaching pneumatocyst, *gl.en.* = endodermal gland cell opening into the coelenteron, *mes.* = mesogloea, *m.ec*, *m.en.* = muscle fibres of ectoderm and endoderm respectively, *nem.* = nematocyst, *4n* = tetraploid nucleus, *s.n.* = nerve-cell with sense hair.

An examination of the sexual medusoids (gonophores) from formol-fixed 'Discovery' material revealed the presence of nerves in the ectoderm of both ex- and subumbrellar surfaces. These nerves have been overlooked by previous workers using the sectioning technique (for example Steche, 1907). In the present investigation, they were studied in strips of tissue peeled off from the mesogloea, and stained in Hansen's trioxyhaematein. The nerve-cells are distributed sparsely but evenly. A point of interest is that there is no differentiation of the plexus into marginal nerve-rings. In medusae and swimming-bells where rhythmic pulsations take place, two marginal nerve-rings, one subumbrellar and one exumbrellar, are found in the ectoderm at the base of the velum. In *Physalia* the development of the gonophore is halted in the stage known as 'eumedusoid' (Hyman, 1940); the gonophore probably has no capacity for movement, judging from the almost complete absence of muscle fibres from the subumbrellar. One assumes that the ancestor of *Physalia* had free-swimming gonophores and that the captive, reduced condition is a secondary one. But it is not easy to say whether the diffuse, undifferentiated arrangement of the nervous system is degenerate, or whether it represents the ancestral pattern, or only a stage in the development from the ancestral pattern. At all events, it represents a *hypothetical* ancestral condition, from which the nervous systems of all hydrozoan medusae and medusoids can be held to derive. The chief specialization of the original diffuse plexus has, of course, been the development of the marginal rings, but evolution has led also to the complete loss of the plexus from certain regions (for example, the subumbrella of swimming-bells) and its elaboration into exumbrellar tracts (for example, in the swimming-bells of certain Physonectae). It may one day become possible to give a complete and coherent explanation for these developments, in terms both of form and function.

The arrangement of the nervous system in the asexual medusoids (nectophores) of *Physalia* has not been ascertained. The epithelia are hard to peel off for examination as strip preparations. No well-fixed examples of larger medusoids were available for study, and sections of specimens fixed in museum formalin have revealed nothing definite.

Certain regions of *Physalia* show no trace of nervous tissue. Intensive studies have been made, prompted by a feeling that nerves 'ought' to be present throughout the animal, but the only result has been to fortify the author's opinion that they are frequently absent. One of these regions is the saccus-ectoderm, a tissue extending over an area of several square inches in medium-sized specimens. As noted earlier, it appears to be inert, and incapable of active movements. It is so thin that nerves, if present, would stand out clearly. Indeed, if nerves were present, this would be an ideal situation in which to study their fine structure. In the codon-ectoderm, which is much thicker, and consequently more difficult to treat, nerves can nevertheless be identified by a variety of staining methods. Thus their absence from the saccus is all the more striking. It is possible to imagine a situation where very long, fine nerve processes, sparsely distributed, proceed across the saccus from cell-bodies located in the codon, but the improbability of such an arrangement will be apparent to anyone familiar with the histology of the coelenterates.

Nerves have not been found in the endoderm of any part of *Physalia*. It is possible, but not likely, that they could have escaped observation in the gastrozooids, palpons and tentacles, for these regions (particularly the last) are hard to examine by the strip technique. But in the endoderm of the codon and saccus, where conditions for observing nerves would be nearly ideal, their absence can be vouched for. It is worth noting that only rarely have students of the Hydrozoa found clear evidence for the existence of endodermal nerves. They seem to be present in *Hydra* (Semal, 1952b and earlier workers), but this is something of an exception.

##### 5. THE MESOGLOEA

The mesogloea in most regions of *Physalia* functions as a *Stützlamelle*, that is, as a support for the muscle fibres. When the fibres contract the mesogloea falls into folds; when they lengthen the folds are smoothed out (though they may not completely disappear). The mesogloea is noticeably thickest where the musculature is most powerful (that is, in the tentacles and codon), and in such regions permanent folds develop. It was at one time held that this folding of the mesogloea in coelenterates was simply and solely the result of muscle activity in regions of high fibre density but Krasińska (1914) argues convincingly against this view.

In the float, tentacles, and gastrozooids, and in the stems of the gonodendra, strands of cytoplasm stretch across the mesogloea from endoderm to ectoderm. In the codon and tentacles (where the mesogloea is thick) these cytoplasmic bridges are often nucleated, and consist of complete cells (bridge cells). Some are shown in Text-fig. 3 (br) and Pl. XXVII, fig. 4. In older specimens the bridges are thick, and contain several nuclei. Cell boundaries are hard to distinguish here, and the bridges often look syncytial; polyploid cells are common in them. The cytoplasmic bridges crossing the mesogloea elsewhere are not nucleated.

The bridges probably originate from the endoderm. Their exact limits are hard to define, as they emerge from among a mass of muscle fibres on one side of the mesogloea and disappear into another mass of muscle fibres on the other. Of all structures tending to obscure cellular relationships, muscle is the chief offender. A sufficient number of cases has been observed, however, where cytoplasmic bridges seem to arise directly from the endoderm, for the arrangement to be depicted as in Text-fig. 3. Chun (1902) was also of the opinion that the bridges were endodermal.

The bridges are often drawn out into long, thin strands crossing the mesogloea at very acute angles

(down to 10°). The oblique orientation of the bridges is constant over wide areas, and may, in part, represent tensions in the mesogloea set up by contraction in the two muscle sheets.

In the tentacles and codon (and possibly elsewhere), the endodermal bridges have little blind projections which pierce the mesogloea on either side. In the tentacles these projections form a cluster around each bridge-cell where it leaves the endoderm. In the codon the projections occur along the whole length of the bridge-cell. It is not known whether these outgrowths have pseudopodial properties, but they look like pseudopodia. As to their function, that is a matter for speculation. No amoebocytes have been observed and the bridge-cells may fulfil the function of transporting nutritive substances across the mesogloea; their outgrowths could serve to distribute the substances to the mesogloea. On the other hand they might serve to collect metabolic waste, like the outgrowths from the flame-cells which penetrate the parenchyma of planarians. It is not intended to suggest that the bridge-cells are homologous with flame-cells; on the contrary, the presence of 'pseudopodial' outgrowths may indicate that they represent amoebocytes which have become fixed in permanent positions.

Large pieces of mesogloea, freed from muscle-fibres, can be prepared from the codon and saccus by careful dissection. The tissue is first briefly immersed in gentian violet; then it is placed in a petri-dish of distilled water under a low-power binocular microscope and the muscle fibres are stripped away with watch-maker's forceps. The sheet of mesogloea (pure, except for the bridge-cells) is then washed in 70% alcohol to remove the stain. It can be restained in any way desired. In the present investigation, iron haematoxylin was found to show up the fine structure well, if differentiation was not carried too far.

Study of stained sheets of mesogloea, both by teasing in glycerine and by examination intact at high magnifications, reveals that the mesogloea has a laminated structure. The laminae are broad, flat sheets which cross one another at a variety of oblique angles, without any apparent regularity in orientation. The mesogloal substance is finely fibrillated. This, and the laminated structure, can be seen in Pl. XXVII, fig. 3. The laminae do not appear to be regularly interwoven. In *Calliactis* and other actinians, on the other hand, the mesogloea has a lattice-like structure of 'undulating sheets' as in a woven fabric (Chapman, 1953), and the warp-and-weft arrangement is regular in character, with a definite orientation in particular regions. It is thus more highly organized than the mesogloea of the float in *Physalia*.

The periodic acid-Schiff technique has been applied to sections of *Physalia* material. The mesogloea in all regions gives a strong positive reaction (indicating abundant polysaccharide), particularly near the muscle interface. This can be seen in the transverse section of a palpon (Pl. XXVII, fig. 6), where the mesogloea forming the central core of the villus has a definite, dark outer ring. X-ray diffraction analysis of strips of mesogloea (prepared by the method described above) was carried out by Dr K. M. Rudall. Collagen, as recognized by its wide-angle diffraction pattern was found in abundance in the thick mesogloea of the codon. It was also found, but less abundantly in the mesogloea of the saccus. For a survey of the distribution of collagen and chitin in coelenterates, Rudall (1955) should be consulted.

The mesogloea in the nectophores and jelly-polyps ('Gallertpolypoide') is of the gelatinous, distended type characteristic of medusae and medusoid members. Such mesogloea seems to serve primarily for buoyancy (Jacobs, 1937). In many medusae it is fortified by a system of orientated fibrils (Alvarado, 1932) and this is true of the swimming-bells in siphonophores such as *Hippopodius* (Mackie, unpublished). However, the nectophores and jelly-polypoids of *Physalia* have not been investigated from this point of view. The functional significance of the jelly-polypoids remains problematical. One is at a loss to know whether they serve for flotation or food storage or whether indeed they have a function at all, or are merely vestigial.

### The cuticle and pneumatocyst

The pneumatocyst is the lining of the saccus cavity. It can be regarded as an internal cuticle, for it is secreted by invaginated ectoderm and remains continuous with the rest of the cuticle via the apical pore, which is the site of invagination. It is a regular feature of the float in Cystonectae and Physonectae. Usually, as in *Physophora* (Leloup, 1941), it is a thick conspicuous structure, evidently having a skeletal function, whereas the external cuticle is either absent or so thin that it escapes observation. In *Physalia* the pneumatocyst (first described by Schneider, 1898) is a transparent membrane about  $\frac{1}{4}\mu$  thick in a young specimen. The external cuticle is probably much thinner.

It seems possible to distinguish two clear types of cuticle in the Hydrozoa. The first type of cuticle, found in *Hydra fusca* (Schulze, 1871) and *Cunina* (Hertwig and Hertwig, 1878b) is apparently not secreted by specialized gland-cells. It is a very thin membrane attached to the ectoderm by means of numerous small bulbous protuberances, many of which are found embedded in each ectodermal cell. Such a cuticle is probably secreted by the whole ectoderm. In the second type, seen in *Eucopella* (Lendenfeld, 1883), the cuticle is secreted by specialized gland cells distributed throughout the ectoderm. This type of cuticle is often very thick and may be laid down in layers as the perisarc or theca. The cuticle of *Physalia* is here considered to belong to the second type, for the evidence strongly suggests that it is associated with special gland-cells.

In dealing with cuticles of the second type, particularly where a perisarc is formed, it should be noted that the concepts of the production of the perisarcal substance as a viscous secretion from ectodermal gland cells containing refringent inclusions, its subsequent hardening, and its reabsorption in budding regions are at least seventy years old, inherent in the writings of Weismann, von Lendenfeld and other workers of that period. Berrill (1949), in his studies on *Obelia*, appears to have arrived more or less independently at the same conclusions. The term 'polymerization' is now used to describe the process of hardening in the cuticular substance (Hammett, 1943).

Weismann's work has been criticized by Berrill (1949, p. 235) on the grounds that his illustrations, though beautiful and accurate 'were interpreted in an extremely static manner remote from any concept of a living organism'. This may be true to some extent of the particular publication that Berrill refers to, but in justice to Weismann we should note that he also provided us with a detailed account (Weismann, 1881) of active processes in living hydroids somewhat along the lines of Berrill's own studies. Again, in the particular case where Berrill (p. 245) finds it necessary to interpret 'at face value' some illustrations which seem to show that the perisarc can be laid down and dissolved 'under certain more or less obscure conditions', it would be more appropriate to consult Weismann's account of the process in *Plumularia* (Weismann, 1880).

The distribution of the cuticle in *Physalia* has been investigated by teasing fixed material under the dissecting microscope. The earlier workers, for instance, Schulze (1871), used maceration techniques on fresh material. After fixation in Bouin, the cuticle can be lifted or scraped away with fine needles and its distribution can thus be determined. By means of this technique the cuticle has been found to extend over the float, gastrozooids, tentacles, ampullae, palpons and gonodendra. It has not been found in the gonophores, nectophores or in the jelly-polyps, but in these regions, a very thin cuticle might escape detection by the method used. For studying the fine structure of the cuticle, it was found that certain silver preparations served the purpose. These preparations were some of the numerous 'failures' from attempts to stain the nervous system, and the exact conditions under which the cuticle becomes impregnated have not been worked out. No other methods proved to be of any use; the cuticle is normally transparent, and shows no particular affinity for acidic or basic coal-tar dyes, carmine or haematoxylin. No greenish coloration was produced by treatment with chlorazol-

black such as occurs in the arthropod cuticle; however, the value of the latter stain in the histochemical identification of chitin is doubtful.

The main value of the silver preparations has been to show how the cuticle is attached to the underlying epithelium. In certain of the silver preparations of whole strips of codon-ectoderm, the cuticle shows up well as a dark sheet of granular material, split in places and flaking away, revealing the ectoderm underneath. Around the edges where the cuticle is damaged, one can see dark strands of cuticular substance passing down into the ectoderm. In places where the cuticle has disintegrated more completely, these strands are often all that remains of the cuticle. They are funnel-like structures, rooted in the epithelium by means of a basal swelling. They may run almost perpendicularly from the cuticle proper down into the ectoderm, but more often they run obliquely. In such cases, they can be seen to good advantage in surface view, that is to say in the strip-preparations. Pl. XXVII, fig. 5 shows two of these funnel-like cuticular tubes running down into the ectoderm, where their swollen basal parts are rooted. The details of the ectoderm cells are not shown in this photograph, as the silver has precipitated heavily and coarsely over the whole exposed surface. The basal swellings are light in colour, and appear to be hollow. Sometimes the stalk or funnel region is also hollow in appearance.

These structures appear to consist of solidified streams of cuticular substance running from extinct gland cells (represented in outline by the swollen bases) up into the cuticular sheet. This was also von Lendenfeld's interpretation of a closely similar histological picture in *Eucopella* (Lendenfeld, 1883). Apart from these structures, no connections between the cuticle and ectoderm have been found.

In the case of the pneumatocyst, or cuticle of the saccus, the means of attachment to the ectoderm again appears to consist solely of swollen portions of the cuticle embedded in the ectoderm, and again it is probably fair to say that these represent the sites of extinct gland-cells. The globular thickenings are black in unstained preparations, and can be seen without difficulty. They proceed directly from the underside of the cuticular sheet, and there is no stem or funnel region as in the codon. The only part of the saccus not lined by the pneumatocyst is the gas-gland. This becomes explicable if it is assumed that the pneumatocyst has the properties of an insulator against gaseous diffusion; in the main part of the saccus its function would be to prevent loss of gases by diffusion, but in the region of the gas-gland, where gases enter the float-chamber, its absence is a functional necessity. Certain other findings support the view that the pneumatocyst may function as an insulator (see page 389).

The chemical composition of the cuticle or pneumatocyst in the Siphonophora has never been satisfactorily determined. In *Velella*, which is not a true siphonophore (see Totton, 1954), it is clear that the substance lining the concentric float-chambers is chitin (Leuckart, 1852; Henze, 1908; Rudall, 1955), but apart from some unsatisfactory early work by Leuckart on *Physalia*, nothing has been done on the siphonophores. We know from an earlier paper (1851) that Leuckart thought the saccus to be a homogeneous, 'structureless' substance, like the chitinous skeleton of *Velella*, whereas, in fact, it is living tissue, composed of ectoderm, endoderm and mesogloea, with only a thin membrane of what might be and, as we shall see, actually is chitin. Thus when Leuckart (1852) reported that the saccus consisted of chitin, we cannot place much value on his finding. Nearly all the saccus dissolves in caustic potash, as he would surely have noticed if, as he claimed, he used this reagent.

In the present investigation a modification of Campbell's chitosan method (Richards, 1951) was employed to test for the presence of chitin in the pneumatocyst. A whole saccus was used in one test, and the dissected pneumatocyst in another.

The tests gave a positive result. It was necessary to shorten the period in caustic alkali to 2 min., as after this the membrane tended to disintegrate (but not to dissolve) at 160° C. This curtailed treatment has a precedent in Richards's method for butterfly wing scales, which are also thin and fragile. In the

test where a whole saccus was used, all the tissue dissolved, except for the pneumatocyst. Subsequent treatment with iodine and then with sulphuric acid gave results consistent with the other evidence that the material is chitinous.

A further investigation of the chemical nature of the cuticular material of the float has been carried out by Dr K. M. Rudall, using the X-ray diffraction technique. A characteristic chitin pattern was obtained. The pattern could not be defined as typically  $\alpha$ - or typically  $\beta$ -chitin. In addition to the chitin, another substance soluble in benzene was found to be present; it is probably lipid in nature. No other coelenterate cuticle has yet been shown to contain lipid material. No trace of it was found in *Veabella* by Rudall (1955) using the X-ray diffraction technique.

It is too early to say much on the significance of the lipid-like material, but if we are looking for some substance which would provide the cuticle with a capacity to resist diffusion (chitin alone being useless from this point of view) then a lipid would certainly meet our needs. In *Veabella*, where the stigmata, float-chambers and tracheae are believed to serve as a route for gaseous exchange (Chun, 1888), diffusion taking place directly through the walls of the tracheae, it would be surprising indeed to find lipids associated with the chitin, for what is needed here is permeability to gases rather than impermeability. Thus the presence of the lipid in the one case and its absence in the other accords well with the presumed functional requirements.

With regard to the secretion of the cuticular substances, the evidence is very circumstantial and incomplete. Gland-cells occur in the ectoderm, and their distribution corresponds to a large extent with the distribution of the cuticle. The gland-cells show many features in common with gland-cells which, in other hydrozoans, are believed to secrete the cuticle (see, for instance, Berrill on *Obelia*). However, it is a regrettable fact that none of the author's preparations simultaneously show both distinct and recognizable gland-cells and an intact, unmistakable cuticle. The bulbous swellings at the bases of the cuticular funnels (Pl. XXVII, fig. 5) probably represent the sites of gland-cells, and the tattered fragments of material overlying the ectodermal gland-cells in sections of a palpon (Pl. XXVII, fig. 6) probably represent the cuticle, but in neither case are the relationships well-enough shown to be regarded as conclusive. The gland-cells *might* have another function, or only a proportion of them might be concerned in cuticle secretion, or some might secrete chitin and others lipid. We do not yet know. A thorough cytological and cytochemical examination is needed.

Ectodermal gland-cells occur in the codon, tentacles, ampullae, gastrozoids, palpons (where they are very abundant) and stems of the gonodendra. Steche (1907), referred to similar gland-cells in the palpons of *Rhizophysa*, and figured one (Taf. xi, fig. 4). He stated that they are also present in *Physalia* but did not say where; they do not appear in his drawings of the medusoids of *Physalia*, but this is in accord with the present finding that gland-cells are absent from these members, and from the jelly-polyps. The cuticle is also absent from these regions, as far as we can tell.

The absence of gland-cells from the ectoderm of the saccus is puzzling, when it is considered that the cuticle here (pneumatocyst) is fairly thick. The author's tentative explanation is that like the cuticle elsewhere in the animal, the pneumatocyst is secreted by gland-cells, but that secretion stops at a fairly early stage; the sites of the extinct gland cells would be shown by the knob-like thickenings referred to above. Possibly, by virtue of its completely protected situation inside the air bladder, the pneumatocyst is not subject to erosion or damage, and a continuous supply of the material is not needed; the exposed parts of the cuticle on the other hand, would need replenishment from time to time. If this is true, the pneumatocyst would keep pace with growth of the float not by uptake of new material but by stretching to cover a wider area.

Although a detailed study of the gland-cells and their secretion (or secretions) has not been undertaken, certain information is nevertheless available. The gland-cells give a strong P.A.S. reaction

(Pl. XXVII, fig. 6). The reaction appears to take place both in the granules and in the matter interspersed among them. The secretion generally shows considerable tenacity towards haematoxylin, as noted by Berrill (1949) in the case of similar cells in *Obelia*. There appears to be little if any metachromasia with toluidin blue. In some gelatin sections of Ca-formaldehyde material a diffuse background coloration was obtained with Sudan black. This is of interest in view of the finding that a lipid-like material occurs in the cuticle.

The gland-cells present a fairly uniform appearance throughout the animal and it has not been possible to distinguish more than one type of cell. It is of interest in this connection to note the discovery by Mettey and Hamon (1949) of two types of gland-cell in the ectoderm of *Abylopsis tetragona* (Calycophora). No hint is given of their functional significance, but one of them ('cellule à substance hyaline') has histological features in common with the gland-cells in *Physalia*. In *Physalia*, if secretion of substances other than those of the cuticle were involved, mucus would merit consideration. Specimens kept in captivity sometimes produce a glutinous substance, but this seems to come from the endodermal gland-cells of the gastrozooids.

Not only in the Siphonophora, but in the Hydrozoa generally, cuticle secretion presents many problems. Bonner (1955) found that, in the planula of *Phialidium*, gland cells giving a positive P.A.S. reaction are concentrated in the anterior pole, where a chitinous secretion appears at the time when the larva fixes itself. As with *Physalia*, the chitinous secretion has not been definitely traced to the gland-cells. Bonner further found that the gland-cells disappear after fixation, although the perisarc continues to form around the column of the young hydroid. Although this might suggest that the gland-cells are only needed when a copious amount of chitin is to be produced (that is, for attachment), it might also mean that the cells produce some substance which mixes with the chitin at the time of attachment but is not produced at other times. In the actinula larva of *Tubularia larynx* Pyefinch and Downing (1949) found evidence for an 'extra-chitinous cement' substance in the attachment region. However, a cuticular sheath is also present in this region, and Ciamician (1879) reported that in *T. mesembryanthemum* such a sheath (early perisarc) is produced by gland cells in the aboral ectoderm.

Thus, although there is a possibility that the gland-cells, which are found in the aboral ectoderm of planula and actinula larvae, produce something other than chitin, this is unlikely. Bonner's description of the gland-cells in *Phialidium* accords with the present writer's findings on *Physalia*; in both cases the major part of the evidence points toward chitin as the substance secreted.

#### 6. THE GAS-GLAND

The gas-gland occupies a circular thickened area on the lower surface of the pneumatosaccus, toward the side *b-c* (Text-fig. 1) of the float.

The length, breadth and depth of the float may vary considerably according to the posture of the animal, but the diameter of the gas-gland (*g*) is less variable, and can be used as a rough index of the size (and, perhaps, age) of the specimen. Collapse of the float leads to very little alteration in *g*. It would be of interest to calibrate degrees of morphological complexity in the budding pattern against *g* values.

According to Haeckel (1888) the gas-gland of the largest specimens of *Physalia* may measure 10–15 cm. across. In a large 'Discovery' specimen in the University Museum, Oxford, *g*=5·25 cm.; in the largest 'Discovery' specimen in the British Museum, *g*=4·0 cm. Compared with these, the specimens collected in the Canary Islands were small: in the largest *g*=2·5 cm., and, in the smallest, *g*=0·35 cm.

The histology of the gas-gland in *Physalia* has been studied by Dahlgren and Kepner (1908). A section through the gas-gland of a very young specimen is given by Okada (reproduced by Hyman,

1940). In the present investigation gas-glands from seven specimens of *Physalia* were examined, either in sections or in whole mount preparations. Some new observations have been made, but interpretation has been difficult, and a further analysis is needed. The appearance of the cells in the gas-gland varies markedly from one specimen to the next, and it is not clear to what extent this variability is due to differing ages of the specimens, differing physiological states at the time of fixation or to differing methods of fixation. A new investigation should take account of all these factors.

In a young specimen ( $g=0.6$  cm.) the gas-gland achieves a thickness of  $60\ \mu$ . The surrounding saccus has a thickness of about  $10\ \mu$ . The gas-gland consists of ectoderm, mesogloea and endoderm, all of which are continuous with but clearly distinguishable from the corresponding layers of the surrounding tissue. Ectoderm and endoderm consist of tall columnar cells. They are separated by a thick mesogloea; elsewhere in the saccus the mesogloea is thin, and the cells flat. Muscle fibres are present in both ecto- and endoderm, but are infrequent in the ectoderm. They run for the most part circularly in the ectoderm and radially in the endoderm. Cytoplasmic processes cross the mesogloea here, as elsewhere in the saccus. No nerves are present.

The endoderm of the gas-gland consists of cells of one type, resembling the cells of the surrounding regions in size, number of nucleoli and staining properties of nucleus and cytoplasm, but they are unflattened and closely packed together forming a columnar epithelium. They each bear about 4–10 flagellae arising from basal bodies located in their distal tips. Around the edge of the gas-gland the endoderm merges into the flattened cells characteristic of the remainder of the saccus. The endoderm of the gas-gland, then, appears to be an unspecialized tissue, differing only from the general endoderm of the float in being columnar, rather than squamous. There is no reason to suppose that it functions actively in gas secretion.

The ectoderm of the gas-gland shows a greater degree of specialization than the endoderm. This is also the case in the other Siphonophora which have been examined. The cells of the gas-gland ectoderm are sharply delimited from those of the surrounding regions. At least three types of cell have been identified, each with cytological characteristics distinct from those of the remaining saccus ectoderm cells. The cuticle (pneumatocyst) is absent over the gas-gland ectoderm, and the musculature reduced.

The three types of cell occurring in the gas-gland ectoderm are: (a) tall columnar cells forming the bulk of the tissue; (b) giant cells, scattered sparsely throughout the tissue, being particularly evident in younger specimens; (c) a third type ('islet cells') occurring in clusters of three and upwards, characterized by their denser cytoplasm and darkly staining, deep-lying nuclei. Of these three types, only the first has hitherto been described in *Physalia*. Giant cells have been described in various Physonectae and in other Cystonectae. Islets cells have no known counterpart in other Siphonophora.

#### (a) *The columnar cells*

Dahlgren and Kepner's description of gas-secreting cells in *Physalia* refers to cells of this type. According to these authors a gaseous secretion develops in the region of a 'chromatic vacuole' distad of the nucleus, originating from granules which swell and become filled with gas. Gas-bubbles rupture the cell-wall and break into the gas-chamber.

A study of the Canary material has revealed the existence of chromatic vacuoles in only one specimen, a young one ( $g=0.8$  cm.) fixed in Ca-formaldehyde. The gland was post-chromed for 36 hr., sectioned in paraffin and stained in iron haematoxylin. The chromatic vacuoles are not as regular in size and shape as those figured by Dahlgren and Kepner, but they are recognizably the same structures.

They are present in the large majority of interphase cells, but appear to be absent in cells undergoing mitosis, where the nucleus or spindle lies up near the free end of the cell. The chromatic vacuole consists of blobs or crescents of darkly staining matter deposited irregularly around the walls of a clear vacuole. Fixation and subsequent treatment were compatible with the preservation of lipid. It is, however, too early to say what relation, if any, these structures bear to the Golgi element.

The chromatic vacuole has not been identified in the remaining specimens, which were fixed in Zenker, Helly and F.W.A. (without post-osmification). Whether this is because the fixatives were unsuitable, or because the gas-glands were older or inactive at the time of fixation has yet to be determined. Dahlgren and Kepner did not give the size of their specimens, nor did they mention the histological techniques they employed.

With regard to the secretory activity described by Dahlgren and Kepner, bubbles or vacuoles have from time to time been observed in the distal part of these cells. Such cases are rare in the author's material. Granules have not been seen, and the distal region typically shows a homogeneous or finely fibrillar content, representing normal fixed cytoplasm. However, the histological picture would be expected to vary according to the physiological state of the gland when fixed, and Dahlgren and Kepner's material may have been in a more active secretory state than the author's. The fact that bubbles were found in the Canary material, however infrequently, does constitute evidence in support of Dahlgren and Kepner's general hypothesis.

Dahlgren and Kepner figured and described bundles of mesogloea matter interspersed amongst the bases of the gas-gland cells. Such bodies have not been observed in the author's material.

Groups of columnar cells have been found which, from the size of their nuclei, would appear to be tetra- or octoploids. The cells are in other respects indistinguishable from the remaining columnar cells. This condition has already been encountered in the general ectoderm and endoderm of the float, and no particular significance need be attached to it. It appears to be fortuitous, and the result of metaphase fusion in binucleate cells.

#### (b) Giant cells

In all of the specimens examined, isolated giant cells occur in the gas-gland ectoderm. They occupy a sub-epithelial position, being covered by slanting columnar cells. In young gas-glands they are fairly evenly distributed; their nuclei are rounded and compact, and stain densely in Feulgen preparations (Pl. XXVIII, fig. 1). In older specimens, the giant cells are separated from one another by wide expanses of columnar epithelium. Evidently, while the latter multiply by regular mitosis, causing expansion of the gas-gland, the giant cells do not divide. Even in the most mature examples studied, the majority of the giant cells occur singly. In the older gas-glands, the giant cells show considerable lobulation and distortion of the nucleus. There is no sign, however, that they are degenerate or pyknotic. A similar distortion characterizes normal muscle nuclei in young, healthy specimens, and this too increases with age. A large number of nucleoli may be present, and the cells vary considerably in size, the larger ones having the greater numbers of nucleoli. It has not yet been possible to establish clear polyploid groupings on a basis either of nuclear volume or number of nucleoli, but it seems very probable that we are dealing with a case of 'endopolyploidy' ('*endomitotische Polyploidisierung*', Geitler, 1953). It is interesting to find that in the salmonoid genus *Argentina* the gas gland also contains giant cells with deformed, fragmented nuclei (Fänge, 1958).

#### (c) Islet cells (Pl. XXVIII, fig. 2)

Cells of this type have been found only in one specimen ( $g=0.8$  cm.). The specimen was fixed in F.W.A., which gives a most faithful and delicate fixation, well-suited to the detection of fine cytological detail. The islet cells show up well in iron haematoxylin, thionin and Feulgen preparations.

The cells have been found to occur in clusters (islets) of from three to eighteen; in some cases, they are distributed around a narrow but well-defined intercellular space. Like the giant cells, they occupy a sub-epithelial position. They have sharp outlines, by virtue of their condensed and fibrous cytoplasm, and a small, densely staining nucleus near the base of the islet. The nuclei show varying degrees of staining intensity with iron haematoxylin. Some are so dark that the nucleolus (which normally stains much more deeply than the rest of the nucleus) is not separately distinguishable. In Feulgen preparations, the nuclei stain brilliantly. This is sometimes a sign of pycnosis (Alfert, 1955), but there are no other symptoms of degeneration in the islets.

The islets occur without apparent regularity throughout the gland. They are frequently, but not in the example shown in Pl. XXVIII, fig. 2, associated with the giant cells, clustering around the latter. Mitosis has not been observed in them. From their frequent association with the giant cells, it might be thought that they contribute in some way to the latter's formation, but there is no direct evidence for this.

With regard to the physiological aspects of gas-secretion in the Siphonophora, very little is known. In certain forms, such as *Physophora*, there is abundant evidence that gas-bubbles can be emitted from the float, causing the animal to sink in the water. The gas can be regenerated in a few minutes (Keferstein and Ehlers, 1861, and subsequent workers). Jacobs (1937), in an important paper, described a similar process in *Stephanomia* (= *Nanomia*) *bijuga*. Gas-secretion was observed by this worker in an isolated float after some of the contents had been discharged; it involved the appearance of small bubbles in the region of the gas-gland, their rapid increase in size and fusion together, and their final merging with the air already in the float.

It has yet to be shown which of the various cells of the gas-gland are actually responsible for the secretion. Schneider (1902) maintains that the giant cells in *Rhizophysa* and *Physophora* are responsible, but Dahlgren and Kepner's observations on *Physalia* point to the columnar epithelium as the source. The present investigation has given some support to the latter view. The chemistry of gas secretion, and the character of the stimulus or stimuli evoking it or inhibiting it are completely unknown.

Some workers have held that in *Physalia*, as in *Rhizophysa*, etc., gas may be emitted from the float causing the animal to sink below the surface. Observations in the Canary Islands in no way support this view. Specimens were never observed to liberate gas spontaneously. It is very difficult to squeeze even a few small bubbles out through the apical pore by manual pressure. Nothing in the structure of *Physalia* suggests that it is adapted for submarine existence. It is a robustly built animal adapted for sailing on the surface, and there is no shred of evidence to show that it ever leaves the surface during its adult life.

The float probably loses gases slowly by diffusion in spite of its insulation (the pneumatocyst), and as the animal grows the volume of gas in the float must steadily increase. A steady demand for gas is therefore to be expected, but there is no reason why sudden demands for large quantities of gas need normally occur. The persistence of the gas-gland in the adult, and its growth with the rest of the float tissues, as well as its small size relative to the float as a whole are just about what one would expect to find.

A series of experiments was undertaken to test the capacity for gas-secretion. The volume of the float was estimated by water displacement in a measuring cylinder. Known values of gas were then withdrawn with a syringe, and the volume of the float measured again at intervals. However, this rather clumsy technique failed to yield any significant data. It was found, moreover, that the specimens deteriorated quickly in the laboratory, and both controls and experimental animals tended to

Table 1. *Analysis of the gases filling the float in Physalia  
(previous work summarized from Winterstein, 1921)*

	Oxygen (%)	Carbon dioxide (%)	Nitrogen and other gases (%)
De Quatrefages	17.22-17.78	—	The remainder
Schloessing and Richard			
(a)	12.2	—	The remainder
(b)	15.1	1.7	The remainder, of which 1.18 is argon
This paper			
Sample (a)	17.24	0.071	The remainder
Sample (b)	17.71	0.033	The remainder
Normal air	20.95	0.03	The remainder, of which about 1% is argon

lose volume after a few hours. Large tanks with circulating water are needed to keep *Physalia* in good condition. Bigelow kept specimens in this way for up to a week at Woods Hole. Frequent changing of the water in a small tank is laborious and has the disadvantage of disturbing the animals. An attempt to keep specimens in a plastic cage anchored in the bay at Arrecife was unsuccessful as the wave motion battered the animals against the walls of the cage, and damaged their appendages.

Two samples of gas were collected in tubes by displacement of liquid paraffin, the gas being withdrawn from the float with a syringe and injected into the tubes (capacity about 30 c.c.) equipped with self-sealing rubber diaphragms. The results of the analysis, made two months later by Miss Ann Sweeney of the Department of Physiology, Oxford, together with some earlier records, are given in the accompanying table (Table 1).

#### 7. NEMATOCYSTS

Weill (1934) describes two sorts of nematocyst in *Physalia*: atrichous isorhizas in the tentacles, stenoteles in a certain (not clearly specified) region of the gonodendra. The present study has confirmed the existence of two types of nematocyst, and it is now possible to add some further details on their structure and distribution.

First, with regard to structure, examination of the discharged filaments of the isorhizas, both large and small types, reveals the presence of small teeth on three spiral ridges. An early illustration by Murbach (cited and reproduced by Will (1915)) hints at this feature. Hardy (1956, fig. 24E, F) in a drawing based on a preparation by the present author shows the teeth and ridges. Thus it is clear that Weill's description of the isorhizas as 'atrichous' should be amended to 'holotrichous'. Pl. XXVIII, figs. 5, 6 show the appearance of the teeth under phase contrast.

In this investigation a convenient technique for preparing isolated nematocysts from fixed tissue was found to be as follows: The tissue is washed well to remove the fixative. Then it is placed in 1% solution of pepsin in 0.1 N-HCl at 37° C. After an hour or more the tissue is removed and without washing is dabbed gently on a slide. Nematocysts come loose forming a smear. When the smear is nearly dry it is exposed to the vapour of 40% formaldehyde for 5 min. Then it is transferred to 90% alcohol. Staining can be carried out in Unna's orcein or the smear may be carried through to absolute alcohol, cleared and mounted and examined under phase contrast. In the latter case a mounting medium with a low refractive index such as G. Gurr's XAM (R.I. = 1.491) offers certain advantages.

In many tentacular preparations isorhizas with incompletely discharged filaments were found (Pl. XXVIII, fig. 5). Picken (1953) and Robson (1953) should be consulted for a detailed analysis of the process of discharge and the structure of the filament in *Corynactis* which, like *Physalia*, has large holotrichous isorhizas.

An interesting feature of the isorhizas in *Physalia* is the elaborate fibrillar 'basket' occurring in the surrounding cnidoblasts. The general appearance of this fibril-complex is well known from Will (1909).

Reproductions of two illustrations showing the fibrils in large and small cnidoblasts are given by Will (1915) and of the small type only by Hyman (1940). The peptic digestion technique has the notable advantage of dissolving away the cnidoblast completely except for these fibrils. Where a pepsin smear is allowed to dry before being placed in the alcohol, violent surface-tension forces accompanying the last moments of evaporation draw the nematocysts together in clumps, often removing them from their 'baskets'. In the mounted preparation intact isolated 'baskets' can thus be studied. The appearance of such a 'basket', originally surrounding a large isorhiza, is shown in Pl. XXVIII, fig. 7. It will be seen that the fibrils, far from tailing-off towards the apex as Will suggests, continue and form an elaborate fibrillar reticulum around a hole through which, in the intact cell, the nematocyst would discharge. The fibrillar 'basket' in the cnidoblast containing the small type of isorhiza has a similar reticular structure at its apex and resembles the large type except that, as Will shows, the fibrils intertwine in the basal region forming a single, helically wound stalk.

The fibrils are thought to be contractile. The effect of magnesium salts and chloretoe in preventing nematocyst discharge in an electrical field was attributed by earlier workers (and more recently by Parker and Van Alstyne, 1932) to anaesthesia of these fibrils. The fibrils stain strongly with iron haematoxylin after coagulant fixation in a way similar to muscle fibres. Like fixed muscle, they resist peptic digestion. Whereas chitin and collagen are laid down extracellularly in Coelenterata, muscle is probably always intracellular, like these fibrils. However, if muscular, these fibrils are unique in certain respects, chiefly in their tendency to intertwine in a helical fashion. Attempts to stain the fibrils with Unna's orcein, known to colour elastic tissue, failed completely, though the nematocyst capsules took the stain. While it is possible that the fibrils are indeed muscular and assist discharge in some way, another possible function is worth mentioning: that they serve to strengthen the wall of the cnidoblast and to retain the capsule *after* discharge has occurred. 'Harpooned' fish have been seen to struggle violently but unsuccessfully after capture by the tentacles. The discharged nematocysts are the only means by which the fish is secured to the tentacles. The fibrils in the cnidoblasts are rooted in the mesogloea and may thus prevent the nematocysts (which they enclose) from tearing loose while a captured fish is being carried up to the digestive organs.

With regard to distribution, Weill correctly states that the tentacles contain numerous large and small isorhizas. They do not form an even series from large to small; the intermediate sizes are rare. The range of variation in capsule diameters in the material studied was 9–30  $\mu$  (Weill gives 15–40  $\mu$ ).

Nematocysts also occur in the gastrozooids (as Huxley, 1859, pl. x, fig. 4, discovered). They tend to occur in groups arranged at regular intervals around the lip region. These nematocysts resemble the small isorhizas of the tentacles and like them lie in cnidoblasts with long basal stalks. In addition to isorhizas, stenoteles occur in the gastrozooids; they are scattered sparsely over the organ. The butt region (Weill's *hampe*) can be clearly seen in the undischarged capsule. For some reason only one discharged stenotele has been found in the author's material. The everted butt is 14  $\mu$  long and lacks lateral spikes or barbs. Measurements of stenoteles from a gastrozooid give typical diameters of 17–19  $\mu$ . The isorhizas in this preparation are 11–15  $\mu$ .

In the codon-ectoderm, stenoteles occur sparsely, either independently or in small groups. However, in very young specimens of *Physalia*, this region appears to be much more generously equipped with nematocysts.

In mentioning the occurrence of stenoteles in the gonodendra Weill was probably referring to the palpons as the specific site. Stenoteles are very abundant in the distal region of the palpons and spread back along one side for a distance of about one-third the length of the whole organ. They are evenly matched for size and achieve diameters of 21–25  $\mu$  (Weill gives 35–40  $\mu$ ).

It will be noted that Weill's micron-estimations are higher than those given here both for stenoteles and isorhizas. This may reflect true variation between individuals or populations of *Physalia*. Semal (1954a) found such variation between different populations of *Hydra*. Even in an individual *Physalia* the typical capsule diameter of the large isorhizas may differ by as much as  $5\mu$  between two tentacles.

Developing stages can easily be obtained by peptic digestion from the ampullae connected with the bases of the tentacles. The young nematocysts nearly always exhibit what Weill has shown to be the prematurely discharged condition. Earlier interpreters of nematocyst development have been hampered by failing to appreciate this fact. In the present study, using only fixed material, the author has not attempted to reinterpret the development picture. The most recent account referring to the process in *Physalia* is that of Will (1929).

Developing nematocysts are found in the float, gastrozooids and palpons as well as in the ampullae. In silver preparations the capsules and discharged tube (Will's 'zuführender Kanal') stain heavily in silver preparation (Pl. XXVIII, fig. 4). The group shown is typical in that it consists of a small, even number of developing stages. Groups of two, four and eight are so common that one can probably assume that such groups derive from one or two primordial cnidoblasts which divide *in situ* by mitosis before capsule secretion begins. The close grouping of the cnidoblasts can often be attributed to the presence of bridges of cytoplasm holding the cells together. Such bridges have been described in the older literature (for example, in *Physalia* by Goto, 1895) and more recently in *Hydra* (Hess, Cohen and Robson, 1957). In silver preparations one can often make out, especially in the younger stages, a strand of fibrous material running between the two nuclei within the cytoplasmic bridge (Pl. XXVIII, fig. 3). This fibre strand is in all probability a mitotic spindle relic—the 'fusom' of J. Hirschler (1935). In certain 'fusoms' chromatin material lodging in the interchromosomal connectives during anaphase gives to the persistent 'fusom' a nuclear reaction. Such cases have been interpreted as stages in amitotic division. Some earlier students of cnidoblast development appear to have been misled by this 'pseudoamitosis'.

In the more mature cnidoblast groups the 'fusom' appears to degenerate but the cytoplasmic bridges persist longer, and are sometimes seen in the fully mature groups. Hirschler (1955) describes a case (egg nurse-cell complexes in the Arthropod ovary) where the 'fusom' provides a means whereby physiological co-operation between members of the group can take place. It would be interesting to ascertain whether any such co-operation were possible within the cnidoblast groups in *Physalia*. In cases where the group remains interconnected in maturity one might look, for instance, for some capacity for synchronized development or simultaneous discharge. A 'fusom'-like structure also occurs in interstitial cells of *Hydra* (McConnell, 1937). In *Physalia*, the 'fusom' is not confined to the cnidoblast groups. It occasionally occurs in the float musculature but is only easily detectable where it contains Feulgen-positive material. This is probably matrical matter from the chromosomes which has become lodged in the interzonal connectives during anaphase. Examples of such anaphases also occur in the float.

Some simple experiments were carried out on living material in the Canary Islands to determine some of the factors influencing nematocyst discharge.

Parker and Van Alstyne (1932) mention that the tentacle nematocysts in *Physalia* discharge in an electrical field, and that discharge is inhibited by magnesium anaesthesia. The author independently arrived at the same results. An attempt was made, by controlling the 'electrical field', to compare discharge thresholds in different regions and under various conditions. The electrodes were placed at a fixed distance from the tentacles, and the strength of the shock was increased until the nematocysts discharged *en masse*. A 2 V. accumulator was used, giving make-and-break shocks through an induction

coil. The distance between tentacles and electrodes was selected arbitrarily, but was kept as constant as possible throughout the experiments. The results may be summarized as follows:

(1) Nematocysts from large and small tentacles, from all animals tested, discharged at approximately the same threshold.

(2) The threshold appeared to be unaffected by the state of contraction in the tentacle.

(3) Nematocysts from the basal growing region of the tentacles required strong shocks to discharge, or were undischARGEable.

(4) Weak solutions of chlorethane, chloral hydrate and magnesium sulphate all inhibited discharge partially or completely, depending on their concentration. Solutions too weak to produce total anaesthesia produced definite levels of anaesthesia, which were quickly reached and were maintained with little perceptible change over periods of hours.

(5) Strong solutions of many chemical substances, including anaesthetics, had an irritant effect, and caused discharge, along with contraction of the tentacles.

(6) Elevation and depression of the temperature caused depression and elevation respectively of the threshold.

(7) Following anaesthesia, the normal discharge level was restored when the tentacle was transferred to fresh seawater.

(8) Segregated nematocysts (teased on a slide) failed to discharge in the electrical field.

In all these experiments seawater controls were maintained. The method was too rough and ready to be used for absolute determinations, but for comparative studies of the type carried out it gave consistent results.

The reaction to anaesthesia and to cold resembles that of muscle and, as the cnidoblasts contain fibrils similar in appearance to muscle fibres, Parker and Van Alstyne's suggestion that it is these fibrils whose reactivity determines the discharge level is a reasonable one (see, however, p. 395, para. 2).

The high threshold of discharge in the basal regions of the tentacles indicates that the nematocysts or their cnidoblasts are immature here. Histological examination confirms this. The immature region extends a few centimetres below the tip of the ampulla in large tentacles; exact data were not obtained.

It is interesting to consider the case of the fish *Nomeus* which lives in commensal association with *Physalia* and appears to escape injury from the nematocysts. The author has not observed *Nomeus*, and the records do not clearly show whether the fish survives because it avoids being stung, or because, when stung, it is immune to the poison. To the author it seems likely that the fish avoids being stung and has innate or acquired behaviour patterns directed to this end. The situation in which it lives is not really so hazardous as it might appear. The tentacles are only dangerous if contact is made with them. No searching or exploratory movements of the sort performed by the tentacles of *Velella* take place. To escape injury, all that a fish will require is small size, agility and an avoidance reaction.\*

The toxic and distressing nature of the sting to human beings has been the subject of frequent comment (see particularly Phisalix, 1922). In the present study, the matter was not deliberately investigated, although the author was frequently stung. The pain and shock produced are evidently strictly proportional to the number of nematocysts discharged. Probably several hundred need to penetrate the skin to produce really severe discomfort in a human.†

## 8. HISTOLOGY OF THE DIGESTIVE REGIONS

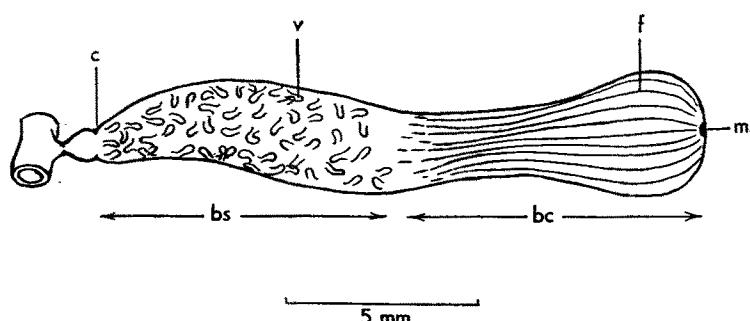
Our knowledge of digestion in the siphonophores is rather scanty. Most of the information available is reviewed by Yonge (1930, 1931) in discussions of digestion in the Coelenterata generally. In those species which have been most thoroughly investigated, the evidence shows that a preliminary extracellular digestion, of proteins only, takes place in the enteron, this being followed by intracellular

\* See Totton, Part I, p. 309.

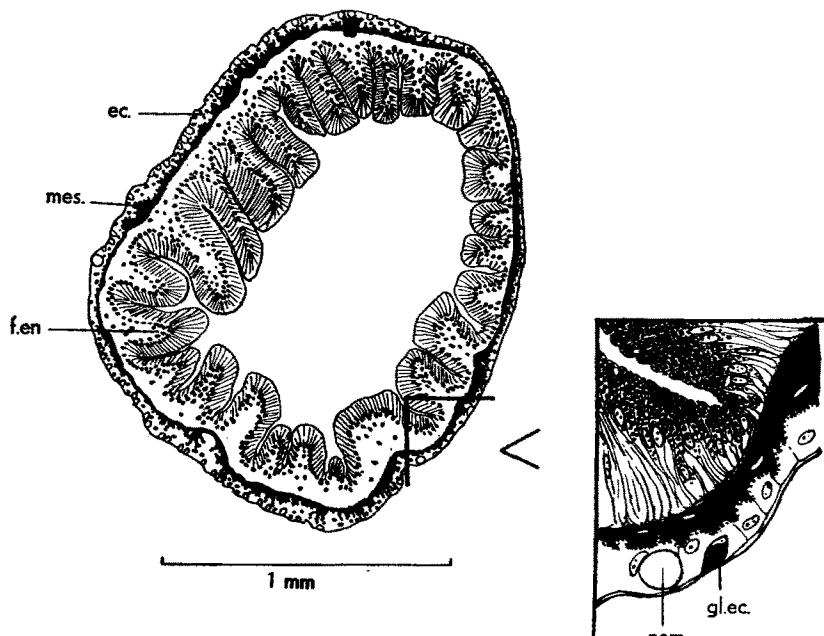
† Investigation of the Loggerhead turtle, which feeds on *Physalia*, reveals no immune bodies in the blood (Dodge and Lane, 1960. Nature 185, pp. 330-331). Probably the nematocysts cannot penetrate the reptile's thick hide.

digestion by the endoderm cells of proteins, fats, and, to a lesser degree, of some carbohydrates. Whether this is true of the Siphonophora in general has yet to be shown, but there are good reasons for supposing that the two phases of digestion, extra- and intracellular, take place in *Physalia*.

The gastrozooids are, of course, the main digestive units. They spread out over the body of a fish, and the surface of the fish dissolves; this can only be the result of extracellular digestion. A partially digested broth then passes into the enteron. In a later stage of digestion, gastrozooids are found with



Text-fig. 4. Sketch of a gastrozooid showing the main regions. *bc* = buccal region, *bs* = basal region, *c* = valve-like constriction near base, *f* = fold, *m* = mouth, *v* = villus. The endodermal folds and villi are seen through the transparent wall of the zooid.



Text-fig. 5. Transverse section through a gastrozooid in the buccal region. Inset: an enlarged region. *ec* = ectoderm, *f.en* = folded endoderm (secretory cells), *glec* = ectodermal gland cell, *mes* = mesogloea, *nem* = nematocyst.

their mouths constricted, and their interiors filled with food matter. For reasons given below, intracellular digestion can be stated to take place at this stage. We know from Bodansky and Rose (1922) that various enzymes are produced in the gastrozooids, but we are still far from knowing which cells produce which enzymes. In addition to the gastrozooids, we have to consider the palpons, which, from their structure, are clearly concerned in digestion at some stage in the life-cycle, even though they may not play an important role while the gonodendra are still attached to the parent colony.

The gastrozooid of *Physalia* consists of two well-defined regions (Text-fig. 4) which will be referred to as the 'buccal' and 'basal' regions. The buccal region, consisting of the whole distal half of the zooid, can be spread out as a flat disk in feeding. The basal half is characterized by conspicuous pro-

jections of the endoderm into the enteron known as 'villi', which have often been described in the earlier literature (for example, Huxley, 1859). In young gastrozooids the basal region is longer than the buccal, but in mature examples the two regions are approximately the same length when the zooid is relaxed. The gastrozooid has considerable powers of expansion and contraction, tending to elongate during exploratory activity.

The buccal region (Text-fig. 5) in a relaxed zooid shows a regular folding of the endoderm into longitudinal ridges. These ridges are visible to the naked eye in ordinary preserved material. In zooids where the buccal region was fixed in an expanded state, the ridges are inconspicuous or absent, and it may be concluded that the folding is a mechanical device making possible the accommodation of the bulky endoderm cells when contraction takes place.

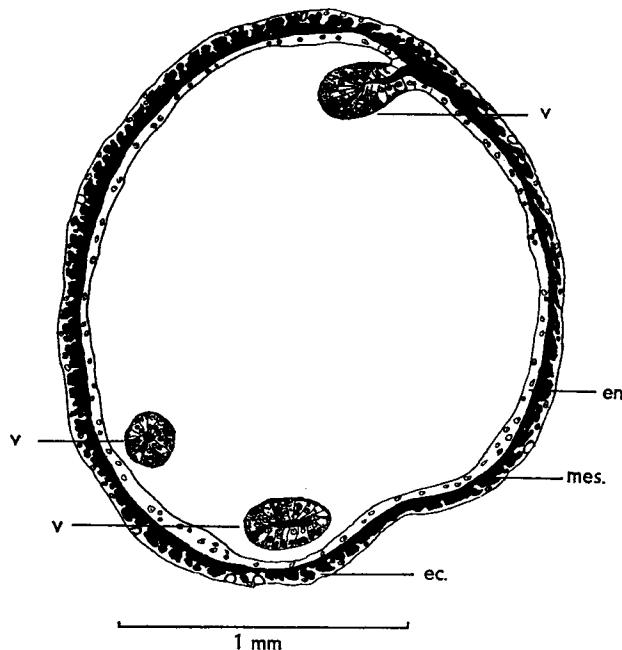
The cells of the buccal region are secretory. The secretory matter fills the distal part of the cells and takes the form of rounded droplets, or, in some cases, of a finer, more diffuse substance. The droplets may measure  $10\ \mu$  in diameter. They are well seen in unstained F.W.A. material, where they show an amber coloration. In gelatin sections of Ca-formaldehyde material, stained in Sudan black, the droplets show little if any darkening, and can therefore be said to contain little lipid material. They stain strongly with eosin in Zenker material, and with iron haematoxylin in Helly. The P.A.S. reaction is strong in the region of the secretion, but the secretion droplets do not stain any more strongly than the matter surrounding them. Protein tests have not been carried out, but are clearly indicated for any future study, in view of the probably enzymatic character of the secretion. Where the secretion is more diffuse, we may be dealing with cells in the process of elaborating their product, this not yet having coalesced to form the droplets. Alternatively, the cells with the diffuse secretion might constitute a distinct type, like the '*cellules spumeuses*' in *Hydra*, while the cells with the droplets would correspond to the '*cellules sphéruleuses*' (Semal-van Gansen, 1954b). A further investigation along the lines of Semal-van Gansen's beautiful study is required.

In gastrozooids fixed when not feeding, the buccal cells are packed with secretory matter. In one specimen, fixed while actually attached to a captured fish, many of the cells appear empty, although it is difficult to compare the appearance of cells in the expanded, flattened specimens and in the folded, contracted ones. If correct, however, this observation would indicate that the buccal cells tend to discharge their product during feeding, and it would seem likely therefore that the secretory matter consists of the enzymes responsible for the preliminary extracellular digestion. It was mentioned above (page 390) that a glutinous substance produced by captive specimens of *Physalia* seems to originate from the gastrozooids, and the author is inclined to suppose that this substance may derive from the buccal gland cells by degenerative breakdown in conditions of oxygen shortage. However, Mettey and Hamon (1949) hold that the buccal gland-cells in the gastrozooid of *Abylopsis* secrete a viscous substance as their normal product, and that it serves for the agglutination of prey.

In *Hydra*, the cells concerned in the production of proteolytic enzymes undergo a series of secretory cycles, discharging their product when food is ingested, and resecreting it again rapidly, until they reach a stage of senescence, when they quit the endoderm and drift out into the enteron. It is too early to say whether, in *Physalia*, the buccal cells undergo cycles of secretion or, if they do, how many cycles can take place, but there is evidence that cells or large portions of cytoplasm containing secretion-droplets are shed from the endoderm and mix with the food matter undergoing digestion. Such objects, sometimes apparently consisting of whole buccal endoderm cells, have been found free in the enteric fluid. Loose droplets are also found, and these sometimes appear to have fused together into large globules, or to have formed aggregates. Parallels to these observations are recorded by Semal-van Gansen for *Hydra*.

In *Hydra* the gland-cells usually have two flagella projecting into the enteron from their free edge.

In *Physalia* two sorts of flagellum occur, and they are found in large numbers. They are best seen in gastrozooids fixed while feeding, the buccal endoderm being spread out as a flat sheet (Pl. XXVII, fig. 7). They occur in rows. The shorter flagella (about  $10 \mu$  in length) occur in groups of about a dozen. The long flagella (about  $25 \mu$  in length) are more closely bunched together, and occur in groups of about half a dozen. These numbers are rather variable. The short flagella seem to arise from the centre of the cells, the larger from the sides. Both sorts of flagellum can probably occur in one and the same cell, but this is not always the case, and groups of the longer sort are in a minority. The blepharoplast in both types is single, not double as in *Hydra*. The endoderm throughout *Physalia* is flagellated, but the bunches of long flagella are confined to the buccal cells of the gastrozooids.



Text-fig. 6. Transverse section through a gastrozooid in the basal region. *ec* = ectoderm, *en* = endoderm, *mes.* = mesogloea, *v* = villi, cut at various angles.

Turning now to the basal region of the gastrozooid, we find a more complicated organization of the endoderm. The most conspicuous features are the villi. These are finger-like projections from the lining of the zooid, consisting of endoderm cells covering a central core of mesogloea (Text-fig. 6; Pl. XXVII, fig. 6); the ectoderm is not involved. The villi can be seen through the transparent wall of the zooid (Text-fig. 4).

The endodermal cells of the villi are probably all of one type; they are quite clearly active in intracellular digestion. In certain specimens, which had been feeding shortly before fixation, the enteron is filled with a mixture of partly-digested flesh, pigment, both dispersed and in aggregates, and nematocysts, discharged and undischarged. The same objects have been identified in vacuoles in the endoderm cells of the villi, and it may therefore be stated with confidence that these cells engulf whole particles of food, and enclose them in digestive vacuoles. A variety of stages in the breakdown of food can be found amongst the vacuoles.

The pigment in the food is probably melanin, guanin or some other pigment originating from the dermal chromatophores and iridocytes of the captured fish. Part of it may, however, derive from broken down haemoglobin originating in the blood of the fish. The pigment evidently resists digestion in the vacuoles fairly successfully, for the cells of the villi nearly always contain some, whether feeding has taken place recently or not. Presumably it is eventually ejected, and some of the dispersed

pigment found in the enteron may have been excreted in this way; the aggregates of pigment, however, are clearly derived directly from the food, and are sometimes surrounded by tissues from the fish. The pigment found in the endoderm of *Hydra* is also probably melanin derived directly from the food, and in this case originating in the eyes of the *Daphnia* on which *Hydra* preys (Semal-van Gansen, 1954b). It was earlier supposed to derive from the chromatin of broken-down nuclei (Schlottke, 1930).

The occurrence of nematocysts in the cells of the endoderm and in the enteric contents is at first rather surprising, although a similar finding has been recorded for *Halistemma* (Claus, 1878). The nematocysts are clearly undergoing digestion, for they have no surrounding cnidoblasts. In the villi irregular, amber-coloured lumps are sometimes found which probably represent nematocysts in advanced stages of digestion, but more often intact, spherical nematocysts are seen, and these may lie in vacuoles situated well down towards the base of the cells.

It might be supposed that these nematocysts are senile ones which have been removed from the ectoderm, crossed the mesogloea to the endoderm and have then passed to the gastrozooids for digestion. If this were so, one would expect to find nematocysts in stages of migration across the mesogloea. However, study of numerous sections from various parts of many specimens has revealed no evidence, direct or indirect, that this takes place. The nematocysts in question are the large and small isorhizas characteristic of the tentacles. What probably happens is that the gastrozooids apply themselves to the captured fish while some of the tentacles are still attached to it, and tentacular matter is ingested together with the tissues of the fish. Only in this way does it seem possible to account for the presence of undischarged capsules in the enteric contents, and, later on, the villi. It is interesting to note that the cells of the villi can engulf objects such as the largest nematocysts, whose diameter is typically about  $25\ \mu$ .

In the general endoderm of the basal region of the gastrozooid (that is, in the parts lying between the villi) three main cell types are distinguishable. The first type, which is in a majority, is apparently non-glandular, and is probably simply absorptive. Cells of the second type are closely similar to the buccal gland-cells, and may even be identical to them. Cells of the third type are evidently glandular, but they differ from the cells of the buccal region. They resemble more the '*cellules glandulaires moyennes*' which Mettey and Hamon (1949) described in *Abylopsis*. In the latter form, these cells have a region of very basophil, sudanophil cytoplasm ('chromatoplasm') around the nucleus: they are believed to produce a diastase. In *Physalia* the cells thought to be comparable have a similar region of basophil cytoplasm around the nucleus; this area also stains strongly in P.A.S. preparations. The cytology of these regions has not yet been investigated in detail, and no further observations can be added.

The palpons, as stated above, probably do not play an important role in digestion while the gonodendra are still attached to the parent colony; indeed, the younger animals manage without them. In the structure of their endoderm they resemble young gastrozooids. The villi are well developed, but the buccal region lacks the heavy concentration of glandular cells found in the gastrozooids and, probably as a result of this, is not thrown into folds. In the distal extremity, where the mouth 'should' be, an indentation can sometimes be seen, but sections have not revealed an actual opening here. If tightly constricted however, a mouth or incipient mouth, might not be easy to detect.\*

The villi in a number of cases have been found to contain food vacuoles, with nematocysts and pigment matter in them, but palpons have not been observed feeding, and it seems unlikely that they do feed at this stage; the food matter could reach them from the general enteron, having been taken in via the gastrozooids in the first place. Bits of semi-digested food and pigment have been found elsewhere in the enteron, for instance in such remote regions as the ampulla of a large tentacle.

\* Totton (Part I, p. 354 and Plate XXV, figs. 2, 3) shows that openings do exist.

Throughout the Siphonophora, palpons show a perplexing array of possible functions. In *Apolemia* (Willem, 1894) and *Forskalia* (Neppi, 1921) they seem able to eject waste matter through a terminal orifice. In *Physophora*, however, they are sensitive, prehensile effector organs (Totton, 1954). In *Physalia*, their precise function is not known, but it seems probable that they are concerned with the feeding or defence of the gonodendra, particularly if the latter drop away into deep water, as Steche (1907) suggests. Alternatively, they may be vestigial structures.

Only two clearly distinct cell types have been found in the endoderm in regions other than the palpons and gastrozoooids. The first of the two types is a gland cell (Text-fig. 3, *gl.en*); its nucleus is a thin curved disk pushed away to one side of the cell by the pressure of the secretory mass. The secretion apparently consists not of granules or globules (although fixation may sometimes give the cytoplasm a reticular appearance), but of a homogeneous substance. It is P.A.S. positive. In whole pieces of codon-endoderm fixed in F.W.A. and stained in iron haematoxylin a clear area, evidently an opening, can be seen at the free surface of each of these gland cells. The gland cells usually occur singly, and mitosis has not been observed in them. Their function is not known for certain, but from their structure it would seem reasonable to suppose that they are mucus-secreting ('goblet') cells. They very closely resemble goblet cells of the type shown in fig. 64 of Hertwig (1895).

The second cell-type from the general endoderm shows no secretory inclusions and no digestive vacuoles. It is probably simply absorptive. Mitosis takes place in these cells with approximately the same frequency as in the ectoderm; the descriptions of polysomaty, the binucleate condition, and nuclear fragmentation under stress, etc. (see under 'Muscle') apply to both ecto- and endoderm.

In the spadix of the gonophores, the endoderm cells are exceptionally tall and columnar; they show a multinucleate condition, the nuclei undergoing amitosis (Perez, 1929). The significance of this is not clear. In the ampullae, the endoderm also shows regional specialization, the cells along one side being very small and closely packed together; again, we cannot suggest an explanation.

The probable sequence of events in feeding and digestion may be reconstructed as follows. On making contact with a fish, the gastrozoooids apply themselves to it, spreading out the whole buccal region as a flat disk. The buccal gland-cells then discharge their contents (?proteolytic enzymes), whose powerful corrosive action dissolves away the surface of the fish. Detached particles of food and dissolved substances are then swept back by the rows of long and short flagella towards the basal region of the zooid, where they accumulate. After an unknown time the gastrozoooids detach, their mouths close, and the matter in the enteron is subjected to further digestion, under the action of secretions from cells of the buccal type, and from another type of cell located in the basal region. At the same time, solid particles of food are engulfed phagocytically by the cells of the villi, and digested in vacuoles. Further steps in the process are less clear. The gastrozoooids have a valve-like structure near the base (Text-fig. 4c) and this is presumably shut during digestion. After a time, it would probably open, allowing the digested matter to disperse throughout the enteron, where dissolved substances would be absorbed by the general endoderm. The bulk of the solid matter which resists extracellular digestion in the enteron of the gastrozoooids would be dealt with intracellularly by the cells of the villi. Whether the products of digestion in the villi are set free again, or are used locally we do not know.

Ejection of waste matter has not been observed, and nothing is known about the time necessary for various stages of digestion nor about the variations in pH during digestion. If a further investigation should become possible, feeding experiments with pH indicators and with substances in suspension like carmine, Indian ink, etc., would be of value in solving many of these problems.

## DISCUSSION

From what has been said on the structure and activities of *Physalia*, it will be seen that the outstanding need is for further physiological and experimental work on living animals. This need is very apparent where the functions of gas-secretion and digestion are concerned, but I would like to comment particularly on the organization of the nervous and muscular systems.

We know from observation of living specimens that a rapid, probably through-conducted, contractile response takes place throughout the organism following strong tactile stimulation, and that at other times the members behave independently, but histological study reveals no differentiation of the nerve plexus into two systems, one of which could serve for through-conduction, the other for local conduction. Even more puzzling is the absence of nerves from three of the four float epithelia. In the case of the ectoderm and endoderm of the saccus, the histological absence of nerves fits in well with the apparent incapacity for active response, but in the case of the codon endoderm, where the musculature must contract and relax in harmony with that of the ectoderm to produce the characteristic postural changes, the absence of nerves is surprising. One can only suppose that the muscle response here is a direct one, possibly to tensions set up in the mesogloea by contraction in the ectoderm, possibly to forces negotiated via the 'pneumatic skeleton'.

It is easy to make the mistake of attributing to siphonophores more refined physiological mechanisms than their basically simple structure can allow. It must be borne in mind that there are few structures here which could be called organs, that there are no muscles in the sense of groups of fibres or fibre bundles having localized origins and insertions, and that there are no ganglionic aggregations of nerve elements which could function as centres of direction and co-ordination. To think of the integration of the organism in terms of motor versus sensory pathways, reflexes, pacemakers etc., is fraught with danger. The nervous system is dispensed with completely in many regions, for example, the gas-gland, which by analogy with vertebrates one would expect to be well innervated.

One other topic calls for comment. In studying the histology of *Physalia*, one finds signs that the large size of the animal sometimes places demands upon the component tissue elements which can be met only with difficulty and with deleterious side effects. One recalls particularly the cytological aberrations referred to on pp. 381, 382, some of which appear to be caused by excessive flattening of the epithelia and mechanical stress. If the epithelia were able to proliferate in depth and to differentiate into further cell types, such mechanical difficulties would not arise, but this ability would require the existence of a third germ layer, that is a mesoderm. Some writers deny that the distinction between mesoderm and mesogloea is a valid or significant one. However, mesoderm, as a source of cells, can relieve the ectoderm and endoderm of many functions and allow an overall increase in tissue specialization. Mesogloea, however elaborate, provides no such benefits.

## SUMMARY

### A. BEHAVIOUR

- (1) The float of *Physalia* responds actively to wind by adoption of a characteristic 'sailing posture' together with erection of the crest. Crest erection results automatically from an increased pressure on the enclosed air ('pneumatic skeleton') whether caused by a general increase in muscular tone, as in natural conditions, or by experimental manipulation. Observations on rolling behaviour support the view that these movements represent attempts to adopt the sailing posture in conditions of calm.
- (2) Muscular contraction, both in the float and tentacles, is stepped.

(3) The tentacles show an inherent rhythmic shortening and lengthening behaviour. The frequency depends on the general degree of contraction within which the rhythmic movements take place, being more rapid when the tentacle is contracted than when extended. Tentacles perform rhythmic behaviour independently.

(4) When extended, the larger tentacles of a medium-sized *Physalia* were estimated to measure 8–10 m.; following stimulation they contracted within a minute to 12–15 cm.

(5) The gastrozooids perform random, searching movements. The presence of food in the tentacles does not appear to affect this behaviour. Gastrozooids respond to physical contact by spreading out their buccal regions over the object encountered.

(6) Strong stimulation applied to the float results in general contraction of all appendages. This reaction is very rapid and, unlike the other reactions, appears to be through-conducted.

## B. HISTOLOGY

(1) Muscle is present in all four epithelia of the float but nerves are present only in the codon ectoderm.

(2) Polyploid cells, probably deriving from binucleate cells by combination of the two sets of chromosomes during mitosis, are found throughout the float epithelia. Their distribution is random.

(3) The diploid chromosome number is 20, but mitotic aberrations are common and may lead to the production of cells with abnormal chromosome complements.

(4) Fragmentation of nuclei is demonstrable in the saccus-ectoderm and is attributable to the excessive flattening and stretching of the layer.

(5) Muscle-fibres are not visibly affected during mitosis in the nuclear layer.

(6) The nerve-plexus has been demonstrated throughout the ectoderm except in the tentacles, where technical difficulties have prevented effective study, and in the saccus where nerves are absent. Nerves have not been located in the endoderm of any region.

(7) The plexus is a diffuse one. Tripolar neurons predominate over bipolar. Many (possibly all) nerve cells bear conical projections extending to the surface from which emerge two or more hairs, presumed to be sensory.

(8) Cellular processes or whole cells cross the mesogloea of *Physalia* from endoderm to ectoderm. In certain regions they have short lateral diverticula.

(9) The mesogloea has a laminated structure. Broad flat sheets cross one another. X-ray diffraction tests point to the collagenous nature of the mesogloea.

(10) A thin cuticle has been identified covering the ectoderm in many parts of *Physalia*. Evidence is given for the secretion of this material from ectodermal gland-cells.

(11) The chitosan test identifies the cuticular material as chitinous. X-ray diffraction tests agree and, in addition, reveal the presence of a benzene soluble material, possibly lipid, in the cuticle lining the air-sac.

(12) In the ectoderm of the gas-gland three types of cell have been found. Results from an analysis of the gaseous contents of the air-sac are given.

(13) The large and small nematocysts occurring on the tentacles are holotrichous isorhizas. Small isorhizas also occur in groups round the gastrozooid lip-region. Stenoteles occur in gastrozooids, palpons and float.

(14) Details are given concerning the fibrillar system found in cnidoblasts containing isorhizas.

(15) Cnidoblasts with developing nematocysts are found in the tentacular ampullae, and over other regions of the ectoderm. In the gastrozooids they tend to occur in small groups of even numbers,

pairs of them being interconnected by cytoplasmic bridges, some of which surround fibrillar strands thought to represent Hirschler's 'fusom'.

(16) Isorhizas discharge in an electrical field while *in situ*, but not when isolated. Discharge is inhibited by anaesthetics, either partially or completely depending on their strength. Cold acts in the same way.

(17) Endodermal glandular cells, presumed to produce proteolytic enzymes, occur in the distal part of the gastrozooids. Certain cells in the proximal region are also of a glandular character. The cells of the villi are phagocytic. The dark matter in them appears to derive from dermal pigments of captured fish. Nematocysts, apparently taken in with the food, also occur together with other matter undergoing digestion in the villus cells. The histology of the endoderm of palpons resembles that of gastrozooids except that the buccal gland cells are relatively less well developed.

(18) Non glandular absorptive cells occur throughout the endoderm in non-digestive regions, together with 'goblet' cells. All endoderm cells are flagellated but exceptionally long flagellae ( $25\ \mu$ ) are associated with the buccal endoderm of the gastrozooids.

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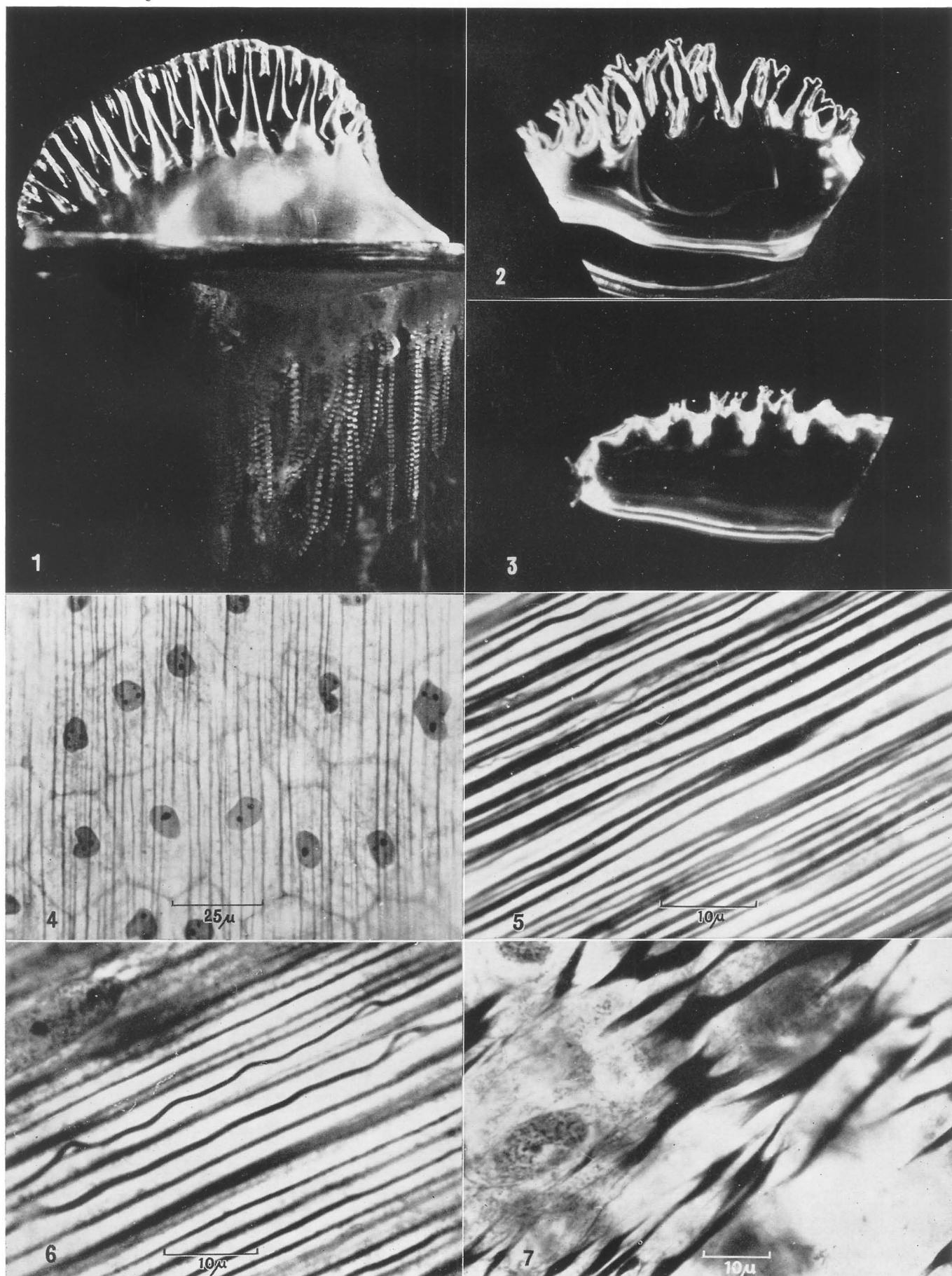
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## PLATE XXVI

- Fig. 1. A freshly caught specimen of *Physalia*, with the crest erect.
- Fig. 2. The saccus, removed from a living specimen, photographed from beneath.
- Fig. 3. The same saccus as in fig. 2, but with a 50 g. weight resting on it.
- Fig. 4. The endodermal muscle sheet of the saccus, showing cell outlines and muscle fibres (F.W.A., iron haematoxylin).
- Fig. 5. Detail of saccus muscle fibres, showing 'anastomoses' (F.W.A., iron haematoxylin).
- Fig. 6. Another region of the muscle sheet, where some fibres have buckled, revealing their double nature (F.W.A., iron haematoxylin).
- Fig. 7. Contracted muscle fibres from the dorsal outpushings of the saccus (Bouin, iron haematoxylin).



## PLATE XXVII

- Fig. 1. The diploid set of chromosomes from the saccus ectoderm.  
Late prophase. F.W.A., thionin. Scale 5  $\mu$
- Fig. 2. The codon ectoderm of *Physalia*, showing part of the nerve plexus.  
Formaldehyde-sublimate, Holmes's silver method. Scale 25  $\mu$
- Fig. 3. Frayed edge of mesogloea from the saccus, showing criss-cross  
laminae. Helly, iron haematoxylin. Scale 100  $\mu$
- Fig. 4. Gelatin section through the codon, near the base of some  
appendages. 'Bridge cells' cross the mesogloea from the endo-  
derm (bottom) to the ectoderm (top right). F.W.A., Mayer's  
haemalum. Scale 50  $\mu$
- Fig. 5. Surface view of the codon ectoderm, in a region where the  
cuticle is partly disintegrated. Strands of cuticular material are  
seen running obliquely down from the underside of the fragments  
of cuticle, their swollen bases being embedded in the ectoderm.  
Bouin, silver (see too, Text-fig. 3, gl.ec<sup>2</sup>). Scale 20  $\mu$
- Fig. 6. Paraffin section through the wall of a palpon (top) with a  
portion of a villus cut transversely (bottom). Zenker, P.A.S.  
Scale 50  $\mu$
- Fig. 7. Surface view of expanded buccal endoderm from a feeding  
gastrozooid, showing rows of short cilia, and a bunch of long ones.  
Formaldehyde-sublimate, silver. Scale 10  $\mu$



## PLATE XXVIII

- Fig. 1. Gas-gland ectoderm from a young *Physalia*. The nuclei only are stained (Helly, Feulgen).
- Fig. 2. Gas-gland ectoderm from another specimen, showing an 'islet' of six cells. The dark dots in the surrounding tissue are the nucleoli of the columnar cells. Some exceptionally large nucleoli to the right of the 'islet' belong to polyploid cells (F.W.A., iron haematoxylin).
- Fig. 3. A pair of young cnidoblasts from the ectoderm of a gastrozooid, showing Hirschler's 'fusom' (Ca-formaldehyde, silver).
- Fig. 4. A group of four prematurely discharged developing nematocysts from a gastrozooid. The cnidoblasts are not stained (formaldehyde-sубlimate, silver).
- Fig. 5. Part of the incompletely discharged filament of a large isorhiza. The undischarged part is seen coiled within the discharged part (phase contrast).
- Fig. 6. Group of large isorhizas from a tentacle. The arrow indicates part of a discharged filament showing the three spiral ridges beset with teeth (phase contrast).
- Fig. 7. Fibrillar 'basket' from a cnidoblast, isolated by peptic digestion. The nematocyst (a large isorhiza) has escaped from the 'basket' leaving it somewhat distorted. The reticular association of fine fibrils around the apical opening is shown (phase contrast).

