



**RESEARCH IN PHYSIOPATHOLOGY
AS BASIS OF
GUIDED CHEMOTHERAPY**

With Special Application to Cancer

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To My Wife

FOREWORD

THEORY AND FACTS

FEW OTHER PATHOLOGICAL CONDITIONS have aroused, as cancer has, the interest of so many scientific disciplines. Problems related to cancer have become of continuously increasing concern in virtually every field of medicine. In some, such as pathology, they are a major preoccupation. But in sciences other than medicine, cancer also has been receiving increased attention. One of the most urgent activities of synthetic chemistry today is the search for new compounds which might possibly be effective in the control of cancer. Physical chemistry is trying to provide new explanations about the variety of processes present in cancer. Even mathematical studies which recently have offered an interesting application of quantum theory to carcinogenesis, have found new applications in cancer.

With the rapid development of physical sciences, the medical research worker has hoped that from them might come some contribution that could help him ultimately in his difficult task. He also appears to have been anxious to take quick advantage of the progress of other disciplines for another reason, hoping that, through employing their findings and methodology, medicine in general and cancer research in particular, could be promptly changed from the empirical discipline it has been until now into a positive science. He has brought as many applications of other disciplines as possible into his study and this has led to a whole series of new methods of investigation through which interesting new information has been obtained. Yet, most of these applications have been tried chiefly because they have been at the immediate disposal of the scientist rather than because they have represented a missing link in the development of his own ideas.

The outcome has not been rewarding. Medical knowledge appears not to be sufficiently advanced to successfully utilize the avalanche of new, highly specialized information offered by the investigative methods derived

from other disciplines. Basic theoretical knowledge in medicine in general, and about cancer in particular, has not yet reached the level necessary to relate and assimilate the new data. To a large extent, basic concepts about pathogenic problems are not even formulated as yet. When the medical scientist has tried to transform the new data into effective therapeutic procedures, he has failed. And the failure has made more evident how much we need basic physiopathological knowledge before we are able to take advantage of detailed data.

Meanwhile, normal development of cancer research has been hindered, side-tracked from its logical course. While thousands of scientists with almost unlimited funds at their disposal are presently using the most advanced methods for the acquisition of details, almost no attempts are being made to resolve basic problems, although the cancer investigator is continuously obliged to realize the dearth of fundamental knowledge.

If we attempt to analyze this abnormal situation further, we can find indications that it may have its origin also in a distortion of the proper relationship between the two factors that, together, make for progress in research—ideas and experiment.

The experimental approach provides precise information about particular phenomena under defined conditions. The analytical method tries to investigate reality by recognizing the proper place of the various constituents of a whole, the parts being identified as such by the experimental findings. On the other hand, the conceptual method not only provides an inkling of what the completed whole will eventually look like, but also attempts to predict the properties and the relationship of the component parts.

In dealing with a highly refined and complicated subject, the analytical method by itself appears inadequate. For example, in atomic physics, the results of experiments are expressed by numbers giving the values of certain physical quantities that have been measured. In order to complete the analysis, we must simultaneously determine the numerical values of certain quantities defining the material bodies, the objects of the experiments. This is prohibitive so far as canonical coordinates by Heisenberg's uncertainty principles are concerned. With experimental knowledge somewhat curtailed, theory at present must attempt explanation.

In other areas as well, experiments present only limited numerical values pertaining to some physical quantities. Were we able to measure all quantities, we could analytically reconstruct the entire theme of the physical reality. However, when some quantities cannot be simultaneously deter-

mined, this direct reconstruction is not possible and experiments merely give an indirect approach to what we regard as "reality."

If the inadequacy of the analytical approach by itself is evident in the highly positive disciplines, such as in the physical sciences, it is even more so in biology. As Bohr and others have intimated, the conditions of uncertainty seem to be much more pronounced in biology than in physical science. The fact that experiments in biology give only fragmentary and unrelated results is not surprising; the need for a synthetic theoretical method in this field is clear.

In medicine, which is applied biology, the need for the conceptual approach is especially profound. It is true that this approach, as the sole approach, has shown its inherent weakness in the past. There was a time in the development of medicine when available data were so scarce and unreliable, and the need for ideas to provide some sort of guidance was so great, that the worker resorted to broad imagination, using it to replace almost entirely any other form of investigation.

Largely as a reaction to the high proportion of "speculations" prevalent in the early years, the experimental approach in medicine came to be emphasized. Claude Bernard, who almost single-handedly was responsible for this, tried to give experimentation its rightful role. However, in ensuing decades, the relationship between theory and experimentation has been progressively distorted. An unrestrained exaggeration of the role of the experiment, the erroneous view that pure facts represent the aim of research, has led to an entirely unbalanced approach. Not only have almost any data obtained by research been considered intrinsically interesting, but obtaining them has become the sole purpose of much research. In scientific papers today, experimental data must be reported as such; any allusion to theoretical meaning is considered undesirable. Generations of scientists have been schooled to believe in the intrinsic value of the experiment. As they have applied this belief to research in biology, and as they have made unlimited use of new methods taken from other disciplines with no ideological requirement for their use, we have had more and more data and fewer ideas. Today, with great astonishment, some scientists are at last beginning to recognize not only that data alone do not generate ideas, but that science cannot progress without theory.

Ideas and experiments are integral parts of all scientific research. A balance between them is needed to assure progress. It must be understood that the function of experimentation is to guide our thinking, to help build up new concepts, and to prove their accuracy in accordance with reality. Certainly, fundamental concepts must not be mere "speculations." They

should be accepted only after confirmation through experimentation. Experimentation is the necessary link between mental concept and reality. To the attempts to consider any unresolved fundamental problems in biology, one has to try to bring a rightful balance between conceptual views and experimentation.

The exaggerated importance attributed to experimentation in biological science, its use even as a substitute for ideas, has led recently to a massive attempt to solve the therapeutic problem of cancer by indiscriminate screening of chemical agents. Here, empiricism has been brought to its culmination. After tests of tens of thousands of agents, many workers are now beginning to realize that the results are almost worthless for cancer therapy in humans, that seemingly promising agents have an effectiveness limited to the conditions present in the actual animal experiments. By its impressive magnitude, the failure of indiscriminate screening, of empiricism epitomized, has begun to impel many workers to change their idea as to what must be done if the cancer problem is to be solved. A first result of this change has been a new and, this time, unbiased evaluation of just where we stand in our assault on the cancer problem. Every day more scientists are making the evaluation in their reports to the medical profession and to the public with a candidness which, only a few years ago, very few would have employed.

The Present State of the Cancer Problem

Surgery in cancer can be considered to have arrived now at or near its maximum efficiency. Thanks to progress in operative techniques, and to advances in pre- and post-operative care, ultraradical surgery is available today. The propensity of cancer to spread far from its original site has made such surgery obligatory in many cases if there is to be an effort to eliminate all malignant cells. Yet ultraradical surgery has not sufficiently increased the cure rate to justify horrifying mutilations, especially when the face is involved. With few exceptions, surgical procedures do not prevent the patient from dying of cancer sooner or later. The so-called five-year-cure-rate represents, to say the least, an unrealistic appraisal. Many authors consider that even the rate of five-year survival is not improved by surgical procedures, and the ultimate fate of these five-year survivors, with few exceptions, is still disastrous. Most of the "cured" cases still die from cancer.

Other recently discovered facts have increased skepticism about the value of surgery in cancer. The polycentric origin of cancer, especially in

cases where the lesions are far apart—considered by some workers to be true even in malignant melanoma, for instance—would greatly limit the value of surgery as a means of eliminating all cancerous cells. It is recognized that to operate on a lymphoma is useless. Furthermore, it is known today that cancer cells are present in the circulating blood. Surgical manipulation has been found to induce a flow of these cells into the blood even from relatively small primary tumors.

In view of all this, cancer cannot be considered to be a condition for which surgery is a major hope. Surgery represents only an expedient—to be tried so long as nothing better can be offered. It is probable that in the future it will be reserved, in cancer treatment, for the correction of mechanical complications, such as intestinal or other duct occlusion.

Unfortunately, radiation has not been much more successful in its long range results. In order to control cancer, it is necessary that radiation destroy all the cancer cells present in the organism while producing minimal damage to normal tissue. It appears that such high selectivity of action cannot be obtained. The lack of it may be implicit in the nature of the effects achieved by radiation. A study of the biological effects of radiation, which is to be presented later in this monograph, has shown that an important part of the action of radiation is to induce changes in certain constituents of the body, principally fatty acids. These changes are largely responsible for the favorable effects of radiation but they also are largely responsible for the undesired effects. It is the nature of these changes which limits qualitatively the capacity of radiation to influence cancerous processes, and makes it dubious that progress in technique can ever greatly improve the qualitatively insufficient effectiveness of radiation. Clinical results to date provide confirmation of this pessimistic view. The recent use of extremely high voltage radiation, of radioactive cobalt, and of other radioactive particles has not greatly improved results over those obtained with older forms of radiation twenty years ago, except for reducing some harmful immediate skin and systemic effects. Now, as earlier, with few exceptions, the benefits of radiation are no more than temporary. Long lasting good effects still are limited to only a few radio-sensitive tumors.

The resort to isotopes, in which the scientific world has put so much hope and millions of dollars, also has proved greatly disappointing. Of the thousands of cases of various kinds of cancer in which isotope therapy has been tried, only a very limited number of cancers of the thyroid have responded. Not only because of its continuing failures, but because of its inherent qualitative inadequacy, radiation does not appear, any more than surgery, to represent the solution for the problem of cancer.

With surgery and radiation therapy incapable of resolving the problem, more and more research workers have turned their efforts in other directions. The existence of some cases of spontaneous remission has led many investigators to believe that immunological procedures related to cancer would be able to resolve these problems. Unfortunately the existing knowledge in this specific field is too meager to permit more than some tentative investigations, usually only repetitions of similar researches made many years ago with limited success. Fruitful development of this approach would have to follow the normal pathway, starting with more knowledge of the complex immunological processes intervening in cancer.

An enormous amount of cancer research in recent years has been directed toward chemotherapy. It is a fact that many agents and groups of agents have shown the capacity to influence tumor evolution. However, each has had limited usefulness. Results of treatment have been characterized by inconsistency. Even in seemingly susceptible types of cancers, results have been good in one case, poor in another and have varied even for the same patient at different times. The inability to explain and remedy these variations has discouraged many workers. Although it appears evident that the source of discrepancies resides in the patients themselves, the general tendency among researchers has been to try to resolve the problem by finding agents able to act independently of any differences which exist between subjects.

In despair at the lack of progress in this approach, many workers today are using the screening enterprise mentioned above as a kind of last resort. For this project, they have renounced the scientific concept that pharmacodynamic activity must serve as the basis on which an agent is to be tried in therapy. They have fastened into a purely empiric approach. Now, all available chemical substances—and many others which will be synthesized especially for the purpose—are to be screened indiscriminately, for their effects on animal tumors with no reason for this test other than that the agents are, or can be made, available. We will not dwell here on the assumption that routine technique is more likely than imaginative brain power to resolve the problem of cancer. The results of this screening to date have shown it to be an invalid procedure, as expected by most critical workers. With tens of thousands of substances already tested, the busy screeners are obliged to recognize that the approach itself is fundamentally erroneous. Experience has proved that an agent can be wonderfully effective against one tumor and still be entirely inactive in others. Of tens of thousands of agents tested, less than a hundred have shown effects on

tumors in animals. None appears to have significant value when applied in humans.

These results have emphasized again the importance of factors other than the agent itself. One factor lies in the differences which exist between various tumors. Some of the other factors include variations between species, between individuals of the same species, between origins of tumors, between spontaneous and transplanted tumors, and even variations in any one individual at different times.

Faced with this situation, some workers have concluded that not one treatment but at least hundreds of different treatments must be found in order to cope with the huge variety of conditions.

Taking cognizance of these considerations, it has seemed to us that a more realistic and logical approach is to try to understand the nature of the existing differences and to attempt to make the treatment adequate on the basis of that understanding. It has been this approach which has been followed in our research.

We have studied the problem of cancer for the last thirty years from an entirely different vantage point than that used by other workers. Attention has been focused on the physiopathological aspect of cancer, on the basic changes that occur in the different patients, with the ultimate aim of understanding the part played by these changes in the response of cancer to therapeutic attempts. This emphasis on the physiopathological aspect of cancer has been made possible by applying a more general overall idea of the nature of the disease.

- This approach is based under various new concepts. They concern,
- 1) The role of the organization in the pathogenesis of the conditions.
 - 2) A dualistic systematization of the manifestations related to normal and abnormal physiology.
 - 3) The predominant intervention of certain constituents such as lipoids and chemical elements in the induction of the opposite manifestations.
 - 4) The possibility to integrate the occurring processes into a system of *defense mechanism* against the noxious influence exerted by the environment.

Many general and special problems of physiopathology, some of them concerning cancer and other conditions, have been analyzed in this framework.

The application of this approach to therapy has resulted from a logical development of that approach. The recognition of the intervention of a variety of pathogenic factors, not only differing from one subject to the other, but even changing in the same subject during the evolution of the

condition has emphasized the need for individualized therapy. As opposed to the tendency to overcome the differences existing between individual subjects through a standard therapy, the "guided therapy" utilizes the knowledge of the occurring different pathogenic particularities in order to correct them. A high degree of flexibility in the treatment has appeared necessary.

As part of this approach to therapy, has appeared the need for more complete knowledge of the existing differences and their interpretation in terms of the pathogenesis of the condition. The search for adequate analytical tests has thus represented the first task. The development of day-by-day analysis of the condition has been possible by choosing relatively simple but reliable procedures. The information they offered was used to determine the nature of the agents able to correct with a certain specificity, the encountered pathological conditions. These two parts, the recognition of the existing condition and the adequate agents, have concretized this approach.

These considerations explain also why the new developed "guided therapy" cannot be understood and correctly applied without a sufficient knowledge of its physiopathological and pharmacological basis. These same considerations have led us to present the research concerning this approach as a block, instead of fragmented communications. The form of a monograph has appeared consequently the best suited. In a further effort to achieve a cohesive presentation, we have separated from the text most of the technical and experimental data, and presented them as notes at the end of the text.

ACKNOWLEDGEMENT

Progress in our research has been made possible only through the day by day cooperation of different groups of co-workers who have contributed years of assiduous work. Many of them are mentioned in the following pages where the research in which they took part is presented. I am deeply indebted to them.

I wish to thank all those friends whose personal efforts or, who through their organizations, have given their material and moral support to the continuation of our research. Special thanks go to Mrs. Sherman Pratt for her tireless efforts on our behalf.

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CHAPTER 1

BASIC CONCEPTS OF ORGANIZATION

THE INTELLECTUAL MECHANISM used by man to acquire knowledge has led him to recognize the existence of relationships between the various manifestations encountered in nature. He has employed abstraction and integration to build up conceptual individualities which he identified as separate entities in nature. Structural characteristics and dynamic properties have appeared to be the most suitable criteria for defining these entities. However, curiosity has constantly impelled man to attempt to extend his knowledge by explaining and correlating these entities, and an important means has been analyses breaking them down into their component parts.

Out of these analyses has come recognition of the fundamental importance of *organization*. For, as entities have been analyzed one after the other, it has become clear that the seemingly infinite variety of them perceived by our senses is in reality the result of the arrangement of a relatively small number of basic units, the molecules. Moreover, analysis has shown that only a very small number of chemical elements make up even the most complex molecules; that combinations of less than one hundred elements, in different proportions and relationships, account for tens of thousands of compounds and many billions of entities. And further analysis has revealed that elements themselves represent different dynamic arrangements of only a few—according to some hypotheses, only two—fundamental corpuscles.

Upon close analysis, nature, which appears to be so greatly varied, turns out, in fact, to be based upon only a very few fundamental constituents and it is the manner in which these constituents are bound together, their organization into a multitude of combinations, which provides variety.

The study of organization obviously, then, could furnish the most valuable information about nature. And it would not appear to be too much to expect that, if nature's seemingly infinite variety stems from organization of only a few constituents, then organization itself might also be achieved through a few, relatively simple fundamental patterns. If so, seeking out such patterns—systematic analysis of organization comparable to the efforts to systematize constituents—could be of primary importance to better understanding of a host of problems.

Homotropy and Heterotropy in Nature

As man attempted to recognize order in the constant changes seen in nature, he noted certain patterns that appeared to indicate definite "laws of nature." Some of these laws have been observed to operate under such a wide variety of circumstances that they have come to be accepted as "fundamental laws."

In 1824, Sadi Carnot formulated one which is known as the Second Law of Thermodynamics. Carnot observed that, in a given system, work involving the transformation of thermal into mechanical energy is only accomplished as heat drops from high to low temperature. In more general terms, this means that work accomplished in an isolated system results in progressively eliminating differences in temperature. Clausius recognized this as a fundamental principle and postulated that the amount of energy available for work always tends toward a maximum. This condition, called "maximum entropy," corresponds to uniformization of temperature and also to homogeneous disorganization. At first, it appeared that the principle was in conflict with the First Law of Thermodynamics which expresses the rule of conservation of energy. However, Helmholtz soon was able to demonstrate its validity by showing that only the second law could reconcile the first with the impossibility of perpetual motion.

In a more philosophical vein, we considered, in our research, that this Second Law of Thermodynamics in its broadest sense could define a fundamental trend toward annihilation of any existing differences in nature, through the triumph of total uniformity. Since Clausius used the term "entropy" in applying Carnot's original observation to closed mechanical systems, it has seemed preferable to avoid confusion by utilizing another term for this general tendency toward uniformity in its broadest sense. Therefore, we have chosen the term "homotropy."

Despite the theoretically rapid trend in the direction of absolute uniformity, or homotropy, no such final state has yet been achieved. It must be concluded, therefore, that some other factor opposed to that trend exists.

We have chosen the term "heterotropy" for this other factor, which tends to maintain or produce inequality and thus to preserve the order that is evident in nature.

In order to understand the roles of these two opposing fundamental tendencies in the organization of nature in as logical a fashion and with as much ease as possible, it seemed advisable to try to study their operation first in one of the simplest and best known natural organizations, the atom, passing later on to higher and lower levels of organization.

The Atom

The role of the two opposing tendencies in atom organization becomes clear when we study the relationship between the forces that form this entity. Each atom consists of a positively charged nucleus surrounded by negatively charged electrons in adequate number to balance the nuclear charge.

The existence of the atom depends upon forces acting between nucleus and electrons. One group is of coulombian nature. These are the electrostatic forces that account for the attraction between oppositely charged electrons and nuclei, and for the repulsion between electrons bearing similar charges. If such forces did not exist, electrons would wander irregularly and would not be retained around the nucleus.

Yet, if electrostatic forces were unopposed, electrons would be drawn closer and closer to the nucleus and would finally fall into it, thereby bringing about complete annihilation of all charges. The fact that electrons are not absorbed by the nucleus indicates the existence of a second, opposing force.

This second force is defined by quantum mechanics and the quantum theory of fields. Quantum mechanics ascribes a series of discrete energy levels to electrons within atoms. Radiation is emitted or absorbed only when electrons pass from one stationary level to another. The energy levels are relatively stable, and a state of minimum energy exists when each of the electrons of the atom is as close as it can be to the nucleus on the ground level.

The energy levels correspond to the orbits described by Bohr's theory which, although not entirely accurate, affords a good basis for understanding atomic properties. Bohr envisaged the electrons revolving around the nucleus in definite orbits, each orbit moving continuously in these states, the atom not emitting radiation. This differs from the older theory according to which the electrons can revolve around the nucleus on any orbit. Such casual orbit motions would lead to loss of energy by radiation. The

electrons would come closer and closer to the nucleus and would, as already pointed out, finally be absorbed by it. The quantum theory of fields accounts for the absence of radiation and for electrons remaining in their particular orbits. However, the concept of stationary states fails to explain all the properties of the atom, particularly its chemical reactivity, by virtue of which different atoms combine to form molecules.

According to another tenet of the quantum theory, the Pauli Exclusion Principle, an orbit cannot be occupied by an indefinite number of electrons but, at most, by two electrons that spin in opposite directions. The orbits are arranged in shells, each shell having a definite level of energy. A shell is complete when it contains the maximum number of electrons compatible with the Pauli Principle. Complete shells consist of 2, 8, 18, etc., electrons. When an inner shell has its quota of electrons, additional electrons must occupy an outer shell. Consequently, instead of falling into the nucleus, the electrons in their lowest energy states will continue to revolve at a considerable distance from the nucleus.

As already indicated, if there were only electrostatic forces, the electrons would have long since fallen into their nuclei, neutralizing all electric charges. The universe would be in a state of maximum homotropy. No strong atomic forces would exist and no chemical reactions would take place. The intervention of quantum forces avoids this. It is apparent, then, that the organization of the atom results from the operation of two types of forces, electrostatic and quantum, the electrostatic serving to bring and keep nucleus and electrons together to constitute the atom, the quantum accounting for a motion of electrons which prevents their total annihilation and the neutralization of all electrical charges.

Homotropic and Heterotropic Forces in the Atom

We may now attempt to consider electrostatic and quantum forces in the atom in terms of homotropic and heterotropic trends. Let us hypothesize an atomic system in which only electrostatic forces are active and compare it with a real system which also has active quantum forces. Whereas the fictitious system will rapidly evolve towards a state of maximum homotropy, with annihilation of all charges, this will not occur in the real system. When the two systems have reached final states of equilibrium, the homotropy of the imaginary system will be greater than that of the real system. If the quantum forces that keep the electrons away from the nucleus in the real atom could be withdrawn, the electrostatic forces acting alone would bring about a state of complete annihilation, thus making available a certain amount of energy that had previously been preserved by the

quantum forces. In this sense, it is apparent that the electrostatic attraction between nuclei and electrons is of a homotropic character, while quantum forces are heterotropic.

*Fulfillment * of Quantum and Electrostatic Forces*

There are diverse consequences from the operation of quantum and electrostatic forces in the atom. The partial fulfillment of the electrostatic forces keeps the nucleus and electrons together in the atom, while quantum forces cease to exist with the establishment of complete electron shells, and have, therefore, been called "saturation forces."

In the atoms of the noble gases, quantum and electrostatic forces are simultaneously fulfilled. As a result, these atoms are inert. They have no physical or chemical activity and their entry into the formation of molecules is explained by the intervention of van der Waal's cohesion forces. In all other atoms, the electrostatic forces are fulfilled when the number of orbital electrons corresponds to the nuclear charge. However, when this occurs, the electron shells are incomplete and consequently unfulfilled quantum forces are present. When the quantum forces are fulfilled, other electrostatic forces appear.

Under these circumstances, in order to complete its outer electron shell, *i.e.*, to fulfill the quantum forces, an atom may borrow or lose one or more electrons. This is achieved with a second atom which, by the exchange, reduces or increases its orbital electrons to fulfill its quantum forces and is left with a number of electrons consistent with a complete outer shell. The fulfillment of quantum forces requires changes that involve a displacement of electrons outside the atom itself. This exchange of electrons, properly called "electron transfer," fulfills, to be sure, the quantum forces of the atom. However, as a result of the transfer, the relationship between each nucleus and its orbital electrons is changed, resulting in covalent ions. Those atoms that have gained by the transfer and have an excess of electrons now have a negative charge while those that have lost electrons have a positive charge. As a result, new electrostatic forces appear which, although confined to the atoms themselves, influence their external behavior, as evidenced by the interaction between atoms.

An antagonistic relationship can be conceived between electrostatic and quantum forces in the sense that the fulfillment of one usually leads to appearance of the other.

Electron transfer represents only one mechanism for fulfilling the quan-

* We have unwillingly resorted to this too anthropomorphic term, the use of which has to be excused as didactic license.

tum forces of the atom. Two atoms which do not have sufficient electrons in their external shells to complete the external shells of both can fulfill their quantum forces by sharing some of their electrons. By achieving a complete external shell for each atom, the sharing process satisfies the quantum forces of both atoms. This method of quantum fulfillment through the sharing of electrons also can lead to the appearance of electrostatic forces. If the two atoms are identical, the shared electrons have an intermediate position and, therefore, do not influence them. As a result, the atoms have their quantum forces fulfilled without inducing new electrostatic forces. This is the so-called "homopolar bond." If, however, two atoms are dissimilar energetically, their shared electrons will be located closer to one atom than to the other, the distance being determined by the competitive influence exerted by the atoms upon the shared electrons. At the same time, other electrons will be influenced by the bond, and, as a result, their orbits will be altered to some extent. Weaker electrostatic forces will result and the bond will be intermediary between the ionic and the homopolar. Both kinds of fulfillment of quantum forces—one achieved by transfer, the other by sharing—thus lead to the appearance of new electrostatic forces in the ions or ionoids.

We must repeat here for emphasis that the fulfillment of quantum forces can take place through various avenues, either by loss or gain of electrons, or by sharing which can range from ionic to homopolar. The plurality of possibilities for fulfillment of quantum forces is very important, making it necessary to consider the results of such fulfillment on a statistical basis.

The electrostatic forces act between charged ions of opposite signs, or between atoms bound by shared electrons. Through the balance of these electrostatic forces, bound atoms appear and correspond to neutral formations, having their electrostatic forces fulfilled. However, it is only with the intervention of suitable quantum forces that the bound atoms can form a new entity, the molecule.

Quantum and Electrostatic Forces in Molecules

Alternate operation of electrostatic and quantum forces leads to the organization of atoms into molecules. The quantum forces in the molecules intervene to permit organization of these entities so that the constituents are maintained at proper distances and positions. The result is electrostatic neutrality. The appearance of new quantum forces that maintain the constituents, through their organized movement, at certain distances and in certain positions, insures not only the establishment but also the stability of the new formations. Besides vibrational movements, other more definite

movements can be recognized in the new molecule. When two or more atoms become associated by shared electron bonds, the shared electrons no longer are confined to one atom but are displaced from their own orbits. Under certain conditions, electrons can travel between two or more atoms, or even surround the molecule as a whole. These movements which correspond to the intervention of quantum forces give stability to the molecule.

The fulfillment of intramolecular quantum forces will affect molecules in a similar way as fulfillment of atomic quantum forces affects atoms. By a process similar to that governing motion of electrons in atoms, motion of entities that enter into the structure of molecules is also controlled. The fulfillment of quantum forces is achieved in various ways. For example, there may be localization of the movement of electrons in the molecule. As the result of relative immobilization of these electrons, electrostatic forces appear in the molecule as a whole.

The relatively immobilized electrons can be considered as being related to the molecule as an entity, since they cannot definitely be attributed to any of the constituent atoms. As a result, the molecule becomes electrostatically active.

The positions of electrons and even of atoms in molecules can be understood easily by considering events at the molecular level in the same way we considered those at the atom level. The molecule whose electrostatic forces are balanced is neutral. However, it has active quantum forces which govern the position and mobility of the constituents and the relative positions of bound atoms or of certain electrons in the entity. Fulfillment of molecular quantum forces is realized through changes in movement of electrons which lead to loss or gain of one or more electrons, protons, ions or even groups of atoms. This leads to appearance of electrostatic forces and the molecule becomes an active entity. In the molecule, as in the atom, quantum forces can be fulfilled in more ways than one—although one may represent a preferred situation. For this reason, activation of molecules, through changes in mobility of molecular electrons, has to be considered on a statistical basis.

The electrostatic coulombian character of an activated molecule is the result of the changes in mobility of the electrons. Positive or negative areas in the molecule develop according to the abundance or dearth of electrons, at these positions. The new electronic arrangements in a molecule can be seen as representing a preparatory step for the molecule to become an active entity in the same way that atoms are activated and become ions. The molecule loses or gains one or more electrons, (or protons, ions or groups of atoms) and becomes electrostatically active, with positive or nega-

tive charge, depending upon the nature of the lost or gained entity, and this is the outcome of the fulfillment of the molecular quantum forces. This is illustrated by the following examples concerning the benzene molecule, and the carboxyl and hydroxonium radicals, in which we shall limit ourselves to changes produced by quantum and electrostatic forces.

In the benzene molecule, which is electrostatically neutral, the electrostatic positive and negative forces of the constituent atoms are balanced. However, all the electrons are not in fixed positions. The π electrons of the double bonds move around in the molecule. Because the molecule is closed, this movement is circular, thus accounting for the stability of the molecule, recognized in part by the equal reactivity of all its carbon atoms which is encountered under certain conditions and results in the Kekulian forms.

The fulfillment of the quantum forces accounts for a kind of relative fixation of these wandering π electrons which is responsible for the other structures of the benzene molecule different from the Kekulian ones. It is this localization of electrons with the capacity to enter into further reactions which results in the activation of the molecule as seen in the resulting Dewar structures which in turn accounts for active centers such as ortho, meta, and para positions. These excited molecules, electrostatically active, can readily take part in chemical reactions. Study of the mobile π electrons in many other molecules allows us to understand their role in providing molecular stability, while their relative localization favors the appearance of electrostatically active centers in the molecule and resulting reactivity. Here again, localization of electrons opens up many possible avenues to activation.

The carboxyl and hydroxonium ions represent typical examples of another kind of activation. Inactive carboxyl occurs when the quantum forces cause the electrons to wander continuously between the two oxygens of carboxyl. Because of this electronic condition, the H atom seems no longer to be bound to either of the O atoms, but is situated between both; this form corresponds to the electrostatically fulfilled condition. With fulfillment of quantum forces, the wandering electron takes a more fixed position at one or the other oxygen. When this occurs, the H^+ ion leaves the carboxyl group, and the carboxyl acquires a negative electrostatic equilibrium, leading to further combining activity. This fulfillment of the quantum forces is responsible not only for the appearance of an activated group of electrostatic character, but also for the existence of two structures, each one with another active oxygen.

A similar activation takes place when a molecule acquires an ion, as seen for the hydroxonium ion. Water can, under certain circumstances,

bind a proton resulting from a hydrogen atom which has lost its electron. This bond is achieved through a valency bridge, and can be regarded as the fulfillment of molecular quantum forces. Different structures can be considered as resulting from the fixation of the hydrogen bridge in different positions in relation to the tetrahedral constitution of the oxygen atom. They help to give this bridge bond its high resistance.

Bonding of Molecules

Electrostatic forces in radicals or activated molecules may be further balanced when new bonds are realized between entities with opposite electrostatic forces. Bonding of molecules having electrostatically excited centers may or may not be of chemical nature which is considered to correspond to changes in the structure of the molecules. More often, only a physical bond between molecules takes place, in which case there are no changes in molecular structure. Both types of bonding result in fulfillment of the electrostatic forces through a balanced neutralization, but bonding alone is not sufficient to establish a new entity. A new entity, with structural and functional individuality, apparently is realized only when quantum forces appear and establish definite relationships between the bonded constituents' molecules, placing them in certain positions and organizing their movements. The holistic concept emphasizes the difference between molecules or radicals bound only by the fulfillment of their electrostatic forces of general coulombian character, and the new entities resulting from the appearance of specific quantum forces proper to them. Here again, then, at the molecular as at the atomic level, progress in organization is achieved by alternate operation of electrostatic and quantum forces.

*Polymolecular Formations **

When an electrostatically active molecule or radical binds an electron, ion or even a small radical, the resulting entity is still considered a simple molecule. The group resulting from the bonding of several polyatomic radicals is a complex molecule. Like simple molecules, complex molecules also can group together and the bonding of several leads to still more complex formations, the macromolecules. In turn, macromolecules also can be grouped and the bonding of two or more produces polymolecular formation. Thus, organization progresses from simple molecules to polymolecular formations, first through the grouping together of similar entities. A

* All three terms—macromolecules, polymolecules and complex molecules—are chosen only for didactic convenience.

new entity appears when one of these groups binds a respective secondary part.

Micelles

A distinctive type of new entity results from the bonding of molecular formations with simpler constituents, such as ions or ionized molecules. To this type of entity we have applied the name of "micelle." * Polymolecules, macromolecules, complex molecules or even simple molecules can form the principal part of these micellar entities.

According to the above definition, micelles are entities formed by the binding of molecules, as principal units, to ionized molecules or ions, as secondary units. The latter originally were considered to be "impurities" until Duclay showed their important role in establishing specific entities. According to our concept, micelles are produced when grouped molecules and active impurities become bonded as the result of reciprocal balance of electrostatic forces and the alternate operation of electrostatic and quantum forces, seen for atoms and molecules, applies again. Fulfillment of electrostatic forces leads to appearance of quantum forces, this time proper to the micelles. The quantum forces maintain the micelle constituents in proper positions and govern their movements, described as vibratory for these entities. The operation of the quantum forces, together with fulfillment of the electrostatic forces, accounts for the stability of micelles.

The micellar quantum forces also can be fulfilled, leading to the appearance of unequal distributions of micelle constituents. The micelle then passes from relatively neutral to an electrostatically active form which can enter into further bonds, and it is primarily in further bondage that micelles appear in a reticular aspect.

In an overall view of the development of organization, from atoms to micelles, the relatively simple pattern of alternating operation of electrostatic and quantum forces can be recognized. The regularity of the pattern allows us to consider it as fundamental to the progress of organization. We have tried to go further and to recognize the existence of this same simple organizational system for formations below the atom and above the micelles. We tentatively conceived of subatomic formations being organized in the same manner, *i.e.*, by alternate operation of electrostatic and quantum forces. We will not go into this study here as it would lead us too far from

* It is to this type of structure that we apply the term micelle, as distinguished from various other meanings found in the literature.

the subject of this presentation. An outline of this subject is presented in Note 1.

Organization of Motion as Heterotropic Achievement

An interesting aspect of the concept presented above is the influence of homotropic and heterotropic forces upon the motion of particles within the organizational framework. At-random mobility must be considered to be an attribute of entities free of any constraint, and thus corresponding to a homotropic state. Any change toward constraint, leading to a degree of immobilization, must be considered a heterotropic effect. The systematized mobility produced by quantum forces, which appears to prevent annihilation of opposing charges, accounts for the relative immobilization. While mobility itself is an homotropic attribute, its systematization is heterotropic.

The correlation of mobility with homotropy, and of fixation with heterotropy, appears basic. The process of uniformization which corresponds to homotropy appears to be possible only in the presence of a maximum of free mobility. The heterotropic systematization of movement can be seen at various levels of organization. Electrons in movement in the atom differ from electrons in movement in the environment through a systematization of their mobility, as they are constrained to follow definite patterns. The relative fixation of certain electrons—for instance, shared electrons—following the fulfillment of quantum forces also marks a further heterotropic influence. This also applies to radicals such as the carboxyl.

In the formation of $\text{--C} \equiv \begin{matrix} = \text{O} \\ \text{= O} \end{matrix}$ the movement of electrons, in itself, is

homotropic, while the limitation of movement between the two oxygens is an heterotropic effect. With the electron fixed in one position, bound to only one oxygen, a further step in immobilization is achieved and represents a heterotropic factor. In more complex molecules, such as unsaturated fatty acids, for example, the tendency of the electrons to wander is related to homotropy, while their restriction to the molecule or even to certain areas of the molecule represents an heterotropic effect. This also applies to micelles, where the water molecules and impurities have a certain degree of mobility. This mobility must be considered to be a vestige of the movement of free water molecules, with a high mobility considered as a homopolar effect at this level. The retention of water or other molecules in the micelle may be considered as a heterotropic effect. And their further fixation, as in the activated micelle, is a further heterotropic effect.

Organization, which results from the alternate operation of electrostatic

coulombian homotropic and organizational heterotropic tendencies, leads to the realization not merely of stable configurations but, more significantly, to entities capable of reactivity, and consequently able to respond actively to the various changes of the environment. The fact that fulfillment of quantum forces causes appearance of new electrostatic forces, which will be further neutralized, leads to the progress of organization to ever-higher levels.

At each step of organization, however, another characteristic can be recognized. It results, in part, from the fact that progress toward higher entities is accomplished by an increase in complexity rather than only in size. The increase in the positive charge of nuclei in atoms, for instance, brings about a parallel increase in the number of surrounding electrons, but this goes on only up to a certain point. Actually, the size of the atoms is limited by the size of the nucleus which, in turn, is limited by the quantum forces able to insure stability for the nucleus. Nuclei become unstable when they contain too many protons.

Levels, Entities and Constituent Parts

As already noted, we defined an entity through its structural and functional individuality. We used the term "level" to indicate a conceptual grouping of entities having the same basic constitution, such as, respectively, nuclei, atoms, molecules, micelles, etc.

We have used the term "part" to define an entity when it contributes to the formation of another entity. Nuclei and electrons are parts that form an atom. Molecules are parts when they are bound through electrostatic and quantum forces to form micellar entities. In progressive organization, each new entity thus is composed of parts which are entities from the level immediately below, and the new entity itself serves as a part for the immediately superior entity. We call this relationship "hierarchic," one entity being inferior to that which it forms and superior to those which have formed it. So conceived, each new organizational entity can be identified not only through the nature of the parts forming it and the manner in which they are bound, but also through its level in the hierarchic succession.

We have seen that most of the entities are made up of dissimilar parts. Analysis of what happens when an entity is formed has shown that the process is complex. In order for an entity to act as a part in a higher level, it must first pass through an activated stage. Activation opens up many opportunities, a plurality of possible formations. So does another process, an immediate consequence of activation. Almost continuously, several similar entities are seen to join together in a kind of "common grouping,"

adding further to the multiple possibilities of new entities. The multiplicity of possibilities at each step in organization explains the exponential increase in the number and complexity of the entities, resulting from the hierarchic pattern of their formation.

According to the holistic approach, an entity exists only through its own qualities. It must have characteristics other than those of its constituents. It is the relationship between the constituents, in the new entity, largely resulting from the operation of quantum forces, that characterize the entity.

Principal and Secondary Parts

As noted, entities at progressive levels of organization are formed of dissimilar parts. These parts do not have equally important roles. There is a "principal" part which is characteristic for a given level. There are "secondary" parts that are nonspecific for the level, the same ones can serve at different levels. The secondary part for a hierarchic entity often is an entity of a far lower level. (*Fig. 1*)

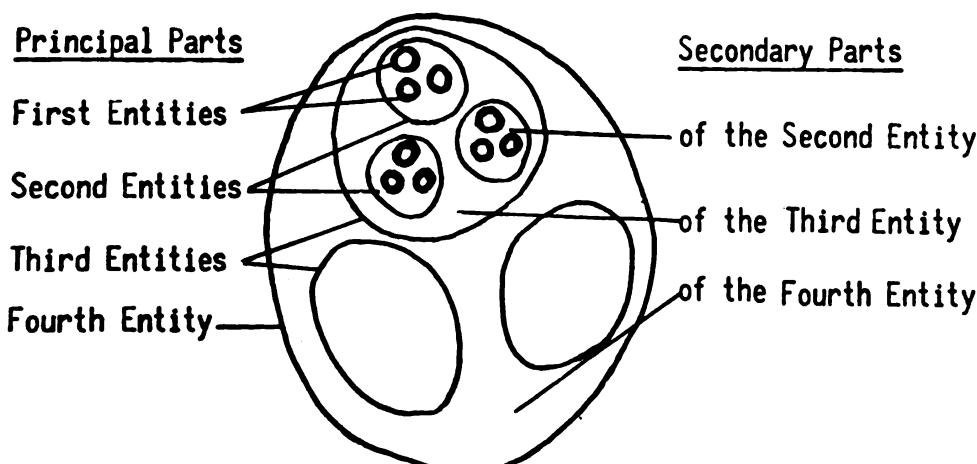


FIG. 1. *The hierarchic organization.* In the organization in general, the different entities appear interrelated according to a characteristic hierarchic pattern. Each entity is formed by a principal and a secondary part. An entity is hierarchically "superior" to the entities which form its principal part and "inferior" to those in the formation of which principal part it enters.

Secondary parts have important characteristics in common. These become especially evident when lower level entities are examined. At levels below the atom, secondary parts for all entities are electrons. It is of interest to observe that in higher entities as well, such as molecules or micelles, all secondary parts have a negative electrical charge.

Also characteristic of secondary parts is their derivation directly from the environment in which the entities of which they are components, appear. This is clear for many crystals, where water molecules represent the added secondary part. This water of crystallization is not free between the ions but bound to them. Water appears indispensable to crystal formation, since its loss results in disintegration. Water can be considered to play a secondary part. Similarly, in some crystals such as gold, some electrons wander between the atoms while others are concentrated in certain regions. (Brillouin) These electrons represent a secondary part in these crystals as do water molecules in others. Water and electrons can be related to the environment from which they derive.

Derivation of the secondary part from the environment appears to be even clearer at the micellar level. In the case of the gelatinous precipitate obtained by coagulating a colloidal solution, a part of the environment in which the gel is precipitated enters into the formation of the new micelle. For example, the micelle of colloidal copper ferrocyanate contains potassium ferrocyanate as a secondary part for each principal part of copper ferrocyanate. Micelles of ferric hydrate, obtained by the hydrolysis of a boiling iron perchloride solution, provide another example. Besides the Fe_2O_3 , molecules from the Fe_6Cl_6 solution used in the preparation enter into the formation of this hydrosol. The role of the negatively charged constituents becomes apparent when a part of the cation of this secondary part is removed from the intermicellar fluid and the hydrosol still persists. It is only when the chloride content becomes too low that coagulation results. The molecules of potassium ferrocyanate or iron perchloride, once considered to be impurities, must be looked upon as secondary parts of these micelle entities. Derived from the environment, they enter into the formation of the micelles, especially through their negative electrostatic character.

This concept becomes of even greater importance when entities are considered in relation to the constantly changing environment. The electrostatic balance between entity and environment realized at any given time cannot be considered to be permanent because of the changes which occur in the environment as it travels toward ultimate total homotropy. As a working hypothesis, it can be assumed that the relationship between entity and environment would change as the latter moves toward total homotropy. It can be assumed, too, that as hierarchic organization develops in time, secondary parts from the environment would differ for different entities. Changes in environment would provide evidence that these secondary parts are related more closely to the environment as it existed in the past when

these entities are assumed to have appeared. We will see below how important this is for the more complex entities of higher levels.

In the role played by secondary parts in progressive organization, the changes in their mobility are of special significance. We have already seen that in hierarchic entities the secondary parts are simpler units than the principal parts, a factor which facilitates their mobility. The mobility can be related to the fact that these secondary parts are derived from the environment where they are mobile, with their motion not systematized. The intervention of quantum and quantum-like forces, which help to create new entities, can be seen as a kind of organization of the relative mobility of the secondary parts. It is the systematization of their movement which prevents complete annihilation of electrostatic forces present. The relationship between secondary parts and environment thus explains the character of the mobility encountered throughout hierarchic organization.

It must be emphasized here that, because of the electrostatic nature of the bond between principal part and secondary parts, a principal part is capable of entering into the formation of more than one specific type of entity. Similarly, fulfillment of quantum forces can lead to more than one type of structure. However, of the many possible new entities, or structures, only a few will fulfill the requirements for developing a still higher organization, *i.e.* will be capable of acting as principal part in a new entity. Some remain at their original level without progressing, even after being bound to other entities. Even many of those which have some capability for higher organization can go only one or two steps. Only a very few will continue all the way up. In other terms, only a few will be able to utilize new quantum forces in order to realize new entities. While various bonds and structures offer a large variety of possible new entities, it is the entity with a capacity to adequately resist the effects of the changing environment which will take part in progressive organization.

In considering the forces which intervene in progressive hierarchic organization, one has to consider the free energy available in the environment. The immense amount of energy received from the sun represents a type of heterotropic energy which can intervene in organization. We will see that this is easily recognized for higher entities. The ability of certain entities to develop may be related to their peculiar ability to utilize heterotropic forces, most of them of solar origin. The less successful disappear or remain at lower levels.

The Organized Boundary

We have seen that an entity achieved through systematization of the movements of its components acquires a boundary between itself and the environment as a result of this restricted movement. The boundary does much more than delimit the entity and constitute a barrier between it and the environment. The electrons of the outermost shell form the boundary of the atom, for example. It is through them that the atom realizes its relationship with the environment. Chemical reaction is largely limited to this boundary. In the atom, where the nucleus is the principal part, and the electrons are the secondary part, it is evident that it is the organized movement of the electrons that provides the boundary. The form and organization of electronic shells, and specifically of the boundary shell of an atom, are results of quantum forces. The environmental nature of the secondary parts and their buffering role make them of great importance in complex boundary formation. Theoretically, hierarchic progress may be considered to depend upon the development of secondary parts which allow increasingly complex boundary formations. This explains the importance we attach to the study of boundary formation in higher entities.

To summarize the above concept of organization, the different entities can be integrated into a hierarchic organizational pattern which depends upon alternate operation of the two fundamental forces, electrostatic of coulombian nature, and quantum of organizational nature. Entities can be identified by the nature of the inferior entities that act as constituent parts and the relationship between constituents as principal and secondary parts. While the principal part is formed by an hierarchically developed entity, the secondary part is a second entity from the environment. The incorporation of a part of the environment into a new entity corresponds to a systematization of its motion. And it is through the organized motion that appears a boundary formation which marks the realization of a new hierarchic entity.

This concept of organization has made it possible to understand the relationship of the series of entities that compose the biological realm.

CHAPTER 2

BIOLOGICAL ENTITIES

WITH THE CONCEPT of hierarchic organization, it becomes possible to gain a new insight into, and understanding of, the biological realm.

In the classical view, just as simple substances in nature are conceived of as being formed by molecules and atoms, biologically complex organisms are considered to be composed of cells as fundamental entities. In arriving at complex organisms, however, it is granted that organization has followed a definite pattern. At first glance, it is apparent that cells are grouped in morphologically regular ways to form tissues. Similarly, tissues are grouped to form organs and these in turn compose the organism. In this classical systematization, a complex individual would appear to be the result of a grouping of cells, tissues and organs, bound together in what has been described as an harmonious morphological relationship.

In our study of organization of the biological realm, we have emphasized the individualization of both conceptual and material entities. In some cases, entities have been simple to identify because they are easily separable morphologically. Where morphological separation has not been immediately evident, other criteria—such as structural and functional properties—have been used for identification. Besides playing its part in organization, each entity has its own individuality, and consequently can be recognized holistically as a well-defined unity. Starting with chromomeres, the entity status is easily accepted because, in addition to clear morphological and functional properties, there is a degree of independent individuality. Following up, chromonemata, chromosomes, nuclei are other entities.

The study of the organization of biological entities has shown, however, that in all cases there is a specific pattern of interrelationship which is more complex than the classically accepted pattern. In the simplest micro-

scopically identified entities, it could be seen that a series of chromomeres are bound together through a special fibrillar formation, which stains differently from the chromomeres, to produce the chromonemata (1, 2), which can be considered holistically as a new entity. Two or four chromonematas (3), together with the chromosomal sap, form the chromosome as a new entity. Similarly, several chromosomes together with another part—this time represented by the nuclear sap and the proper nuclear membrane—form a nucleus. In turn, the nucleus, plus protoplasmic formations, cytoplasm and the cellular membrane, form the cell. Even superficial analysis indicates a common fundamental pattern in the organizational changes taking place for entities ranging from chromomeres to cells. Because of this pattern, these entities can be considered to be "hierarchically" interrelated. One entity is hierarchically "superior" to the entities which form it and "inferior" to those it will itself help form. Two immediately interrelated entities thus are hierarchically superior and inferior, respectively, just as in organization in other realms. (*Fig. 1*)

Analysis of the progression of organization from simple to more complex entities permits us to recognize other characteristics of the fundamental pattern. In order to form a hierarchically superior entity, several similar entities first join to form a group. It is the group which then will bind other constituents to bring into existence a new, hierarchically superior, biological entity. Thus, the chromomeres as a group join with a fibrillar formation to produce chromonemata; chromonemata join with chromosomal sap to form chromosomes. Groups of chromosomes plus nuclear sap form the nuclei. It has appeared evident that the parts which are bound to form each hierarchic entity do not play equal roles. In each case, the principal part is the one which is composed of similar entities acting as a group; the other part is the secondary. Figure 2 offers a graphic representation of hierarchic organization from chromomeres to cells.

In all entities, mentioned above, there is the same relationship between principal and secondary parts. The secondary surrounds the principal part. The morphological relationship has helped us to apply to those entities the hypothesis discussed previously concerning the mechanism through which hierarchic progression has taken place in nature. According to the hypothesis, several similar entities would first associate and form a group. In a second step, the group would tend to maintain around it a small portion of its immediate environment. From this portion of the environment would come the secondary part for the next superior hierarchic entity. With a boundary formation, separating this minute part from the rest of the environment, the new entity would be established. Such a process could oc-

cur, although rarely, with a single biological entity serving as principal part. Usually, several entities grouped together would be needed. This pattern explains why the secondary part can be conceived of as a part of the environment retained around the principal part, and why the establishment of a new and higher entity can be considered to occur only when this secondary part is detached from the rest of the environment and separated from it through the intervention of a boundary formation.

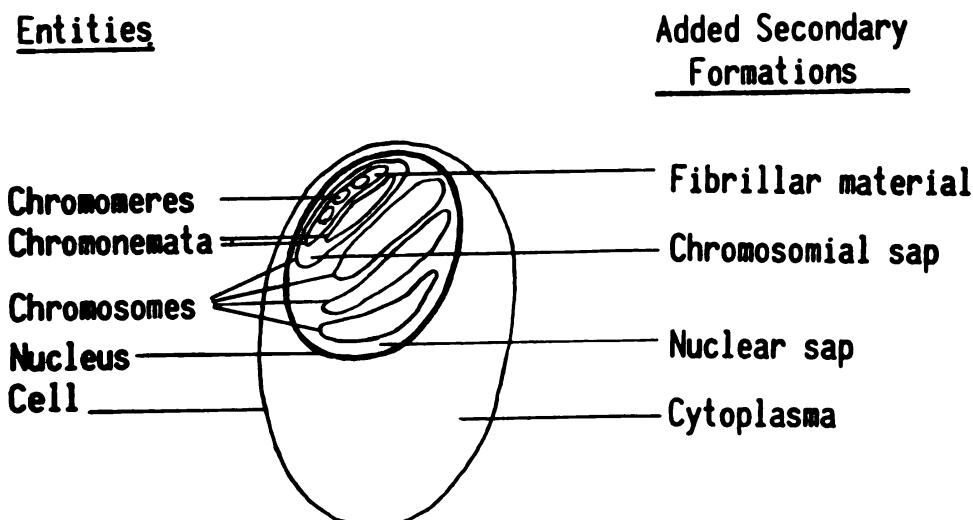


FIG. 2. *The hierarchic relationship* in the organization of morphological entities *below* cells. For each entity its principal part is recognized as being made by a grouping of entities hierarchically inferior to it. The secondary part which corresponds to a kind of environment for the principal part usually surrounds the principal part.

Having recognized this pattern of organization for lower entities, we went on to determine whether it remains the same for higher entities. It could be seen that groups of cells, along with interstitial formations and fluids around them serving as secondary part, create the tissue as a new hierarchically superior entity. Indeed, a proper boundary formation morphologically limits and conceptually defines the new entity. The interstitial fluids are separated by a continuous endothelium limiting the lymphatic spaces as a system closed toward the intercellular spaces. Under these circumstances, the lymphatic endothelium serves as the corresponding boundary formation that limits the tissue entity. Several tissues grouped together, playing the role of the principal part, bind the lymph, as secondary part, to form the organ, as a new hierachic entity. Lymphatic vessels and connective tissues represent the organ's boundary formations. Further-

more, organs grouped together, with blood as secondary part, form the entity called the organism. (*Fig. 3*)

Does the same pattern apply for entities hierarchically inferior to chromomeres? For these lower entities, morphological information to define the relationship between principal and secondary parts is unavailable for

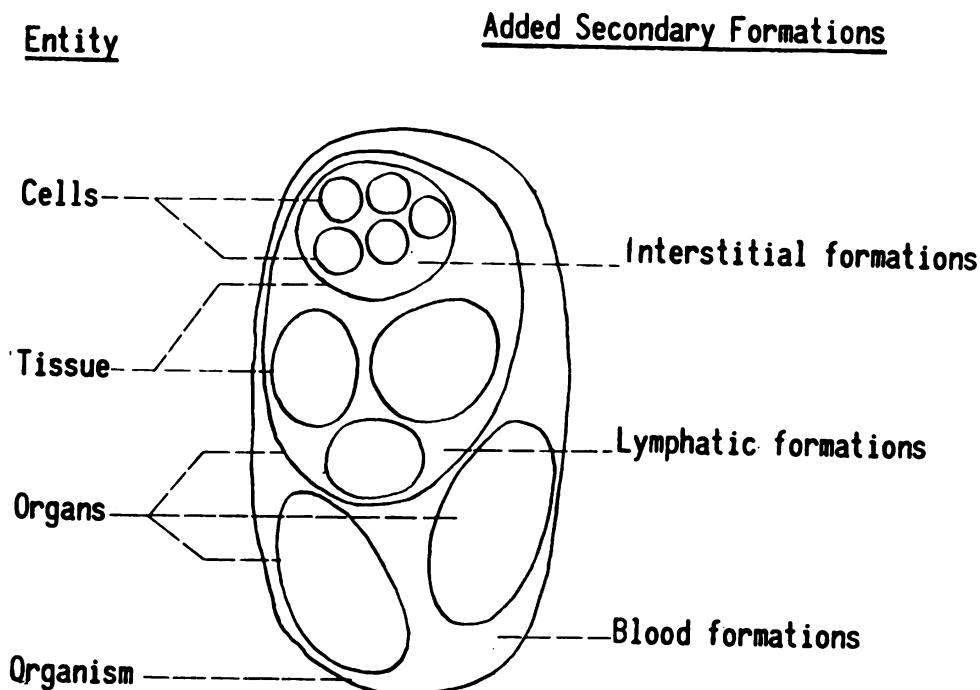


FIG. 3. The organization above the cells. The hierarchic pattern of the organization in general is recognized also in the organization above the cells. Tissues, organs, organism are seen to represent hierarchic entities, each one being made by a principal part formed by the grouping of entities hierarchically immediately inferior to it. The principal part is bound to a secondary part characteristic for each entity. This secondary part would correspond to the proper environment in which the entities forming the principal part, have evolved.

the moment. Until electron microscopy and other means provide such data, we are obliged to find other criteria to indicate which, in these hierarchic entities, is principal and which secondary part. We have considered electrical characteristics of the constituents as criteria for identifying their role in the hierarchic organization of biological entities below chromomeres.

Biological Realm

Before going further in trying to analyze submorphologic hierarchic entities, one problem was to establish the limits of the biological realm

itself. The question was, how far below the morphological formations could we go and still have entities which can be regarded as biological. With the progress of science, criteria previously used to define life have become outmoded. A separation line between the animate and inanimate no longer can be drawn. With the study of the properties of viruses, it appeared impossible to maintain the last vestige of the old vitalistic concept. According to most of the classical criteria, viruses would represent animate entities since they are able to multiply and conserve a strict identity. However, they also form salts, are crystallized, broken down, and then reconstructed in the same or another order. If the viruses are accepted as "borderline" entities, as proposed, then the concept of animate and inanimate can no longer be sustained.

Although the animate and inanimate cannot be distinguished in terms of a specific property, we cannot totally overlook the fact that an important group of entities appear quite different from others in nature. As animals and plants are different from stones, even without any vitalistic concept, we are obliged not only to recognize the difference, but also to try to establish where the difference lies. To conform to reality, we have applied the term "biological" to a group of entities, but we have given the term a new meaning.

Just as organic chemistry is considered—whether correctly or not—to comprise certain combinations of carbon, we consider the biological realm to comprise entities hierarchically developed from a specific chemical radical. This basic radical includes nitrogen and carbon atoms bound together to form the N-C-N-C group. According to this concept, starting from the basic nitrogen-carbon formation, an entire series of entities has been developed through hierarchic organization. Together they form the realm to which the term "biological" can be applied.

The N-C-N-C group, through combination with hydrogen, would result in radicals with a strong alkaline property; that is, with strong positive electrical character. Some of these N-C-N-C groups take part in the formation of nitrogenous bases, pyrimidines and purines, while some, by acting as principal part and binding various amino acid radicals would, with the necessary electrons, build up a new group of entities, arginine and histidine as alkaline amino acids. These can be considered, hierarchically, to be the immediate superiors of the N-C-N-C group and thus to represent the first biological molecules. The alkaline positive character of these molecules is noteworthy. Following the hierarchic pattern described above, several such alkaline amino acids linked together to a series of entities of the same level (simple amino acids) will form new groups that are still electrically positive:

the alkaline histones. In a new step, these histones will act as principal part to produce nuclear entities. (Fig. 4)

Principal parts, by binding different secondary parts, can form not one but many new and different entities. More than one hierarchic line can be identified. An especially important line results when histones bind one or more entities of the nucleic acid group, to form nucleo-proteins. Other histones can bind various other secondary parts such as carbohydrates or lipids and, in so doing, form different biological entities. Some of these

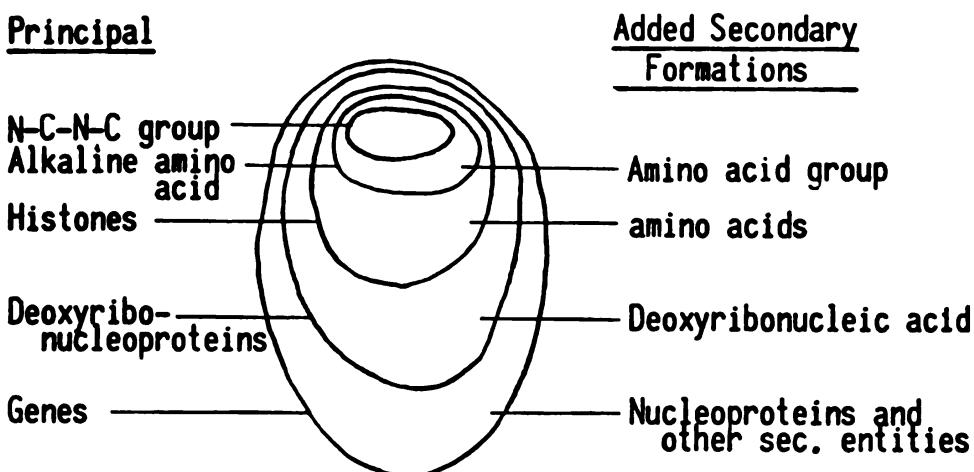


FIG. 4. *The organization of the submorphological entities of the biological realm.* The same hierarchic general pattern, with principal and secondary parts is recognized. Starting with the positive CNCN group, each entity has its principal part made by the grouping of immediately inferior entities bound to secondary parts, generally more electronegative.

entities can continue their hierarchic development. Nucleo-proteins can have ribose or deoxyribose and consequently form more complex nucleo-proteins. Through all hierarchic achievement, it can be seen that new lines are formed when groups of hierarchically lower entities, acting as principal organized part, bind different secondary parts, all more negative than the principal part. Theoretically, this would result in a series of entities all at the same level, *i.e.* entities with similar principal parts but having different secondary parts. Some would go on to develop superior hierarchic entities, others would evolve no further.

Different entities of the the same level can be grouped together in various ways to form a variety of principal parts. Always the group must be made up of entities of the same level. This requirement has been seen to be general at all hierarchic levels. Since differences can exist between the

constituents forming the principal part for a new entity, the predominance of one or another constituent will make entities at the same level differ. This mechanism of differentiation through the constitution of the principal part has been extremely important throughout the biological realm.

Nucleolus

The concept that plural groupings can enter into the principal part of the nucleus puts the role of the nucleolus in a new light. It was accepted for a long time that the nucleolus represents only the reserve material necessary for metabolism of the nucleus. The strong positive electrical character of the nucleus, as recognized through its rather alkaline reaction, would give it roles more important than that of the other constituents. According to a work hypothesis which we advance, successive nucleolar formations would represent the principal parts of hierarchic organization below the nucleus level. In chromonemata, chromosome and nucleus, the parts corresponding to the nucleolus can be recognized. These formations are grouped together with genes to form the principal part of the chromonemata. Similarly, chromatine formations representing entities of the same level as the respective nucleolar formations, will form together the groups characterizing the principal part of chromosomes. In the nucleus, the nucleolus is joining the other formations to form its principal part.

Protoplasmatic Formations

In the cell, a hierarchically superior entity, a similar condition also appears to persist. The protoplasmatic formations with ribo-nucleic acid can be conceived as representing entities of a nuclear level, that is, a level similar to that of the nucleus. Together with the nucleus they would form the group corresponding to the cell. This kind of evolution of entities in relatively separate parallel lines, with their further grouping together to form principal parts for new entities, is part of the typical pattern of organization especially evident in the biological realm.

Boundary Formations

We have mentioned that groupings of several entities would not be sufficient to form a new entity so long as the secondary environmental part is not isolated from the medium from which it originates. Consequently, the new entity appears only when a distinct boundary formation is formed. Progressive hierarchic development is dependent upon the appearance of such boundary formations. For the first biological entities, the radicals, the boundary seems to be more an energetic property than a morphologically

organized formation. For the molecules, it can be considered to consist of molecular surface forces, recognized as the van der Waals cohesion forces. A similar but more apparent boundary formation can be found in higher molecular complexes, especially the micelles. The molecular arrangement at the surface of micelles separates them from their environment and consequently insures their identity. In the case of morphologically identifiable entities, of course, boundary formations can be easily recognized. Chromomeres are well-defined and separated from the chromosomal sap. The chromosomes, in turn, show a real membrane just as the nucleus and the cell do. The next higher entity, the tissue, is bounded by the endothelial cellular layer, separating the interstitial formations from the lymphatic spaces. Usually the boundary of organs which have tissues as principal and lymph as secondary parts is represented by organized blood vessels. As far as the organism is concerned, the mucous membranes and skin are boundary formations. (*Fig. 5*)

Hierarchic Interrelationship

Viewed as a heterotropic effect, hierarchic organization can be considered to be a method of conserving existing entities as such, in spite of changes occurring in the environment. Teleologically speaking, by entering into the formation of a new and superior entity through the system of hierarchic organization, each entity, in fact, protects its own individuality. The hierarchic organization makes it possible for each entity to continue to live in a medium which corresponds to its own environment. The constituents of the secondary part in the new entity are chosen to correspond to the environment in which the principal part of the entity has existed. The successive secondary parts, added during the hierarchic development, act as multiple protective buffers for the first entities, thus insuring their unaltered conservation in spite of continuous changes in the environment brought about by increasing homotropy.

Phylogenetic Development

Hierarchic organization, when related to time, would appear to correspond to evolution. The concept of ontogenesis reproducing phylogenesis, appears in a new light when analyzed in accordance with hierarchic organization. The parallelism between actual hierarchic organization and hierarchic phylogenetic and ontogenetic development greatly helps to increase understanding of many principal problems of biology.

In accordance with the concept of hierarchic organization, when a new level is realized through the binding of entities from a lower level, as prin-

cipal part, to different secondary parts, several outcomes are possible. Some of the new entities are unable to continue to exist and disappear. Of the others, some are relatively well balanced entities and consequently can

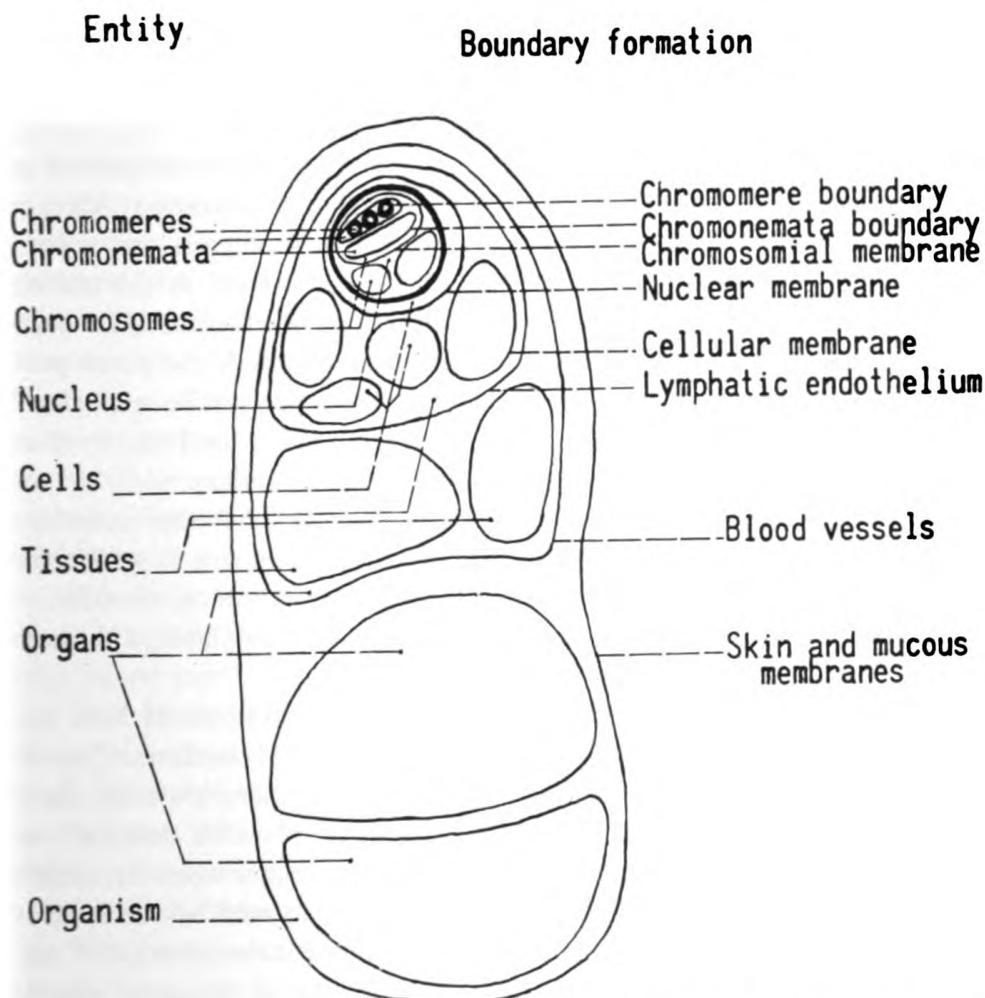


FIG. 5. The boundary formation in the hierarchic organization of the biological realm. A proper boundary formation delimits each entity, insuring thus its individuality. It is by separating each time the secondary part from the rest of the environment that the boundary formations have permitted the progressive development of the hierarchic organization. The boundary formation governs the relationship between the entity and its environment.

persist. But with further changes in the environment, some of these entities disappear. Even among those that remain, some do not represent fully satisfactory solutions and further hierarchic development is necessary to insure their persistence. They must evolve further to correspond to the

new unfavorable external conditions. This has led to the development of the complex entities present today in nature.

The multiple entity possibilities at each level have resulted in a wide variety not only of entities which, with further evolution, could produce higher complexes, but also of others which have ended their evolution at lower levels. So the existence of many varied entities appears to be the result of the existence of multiple solutions for the same problems which have been part of the mechanism of hierarchic evolution.

From this viewpoint, existing independent entities can be recognized as corresponding to the different levels of hierarchic organization. Viruses can be considered to be at the same level as genes or even the entities immediately below genes; the microbes at the same level as the nuclei; monocellular organisms at the level of cells, etc. Furthermore, in each independent organism, from simple to most complicated, the same progression in organization of successive hierarchic entities can be seen, starting with the simplest biological entities and continuing until the level at which the entity has actually stopped its hierarchic evolution.

In a further step, having arrived at the concept that the secondary part of each entity corresponds to the kind of environment in which the entity found itself at the time of its phylogenetic appearance, we tried to see what information about these environments could be obtained through study of the secondary parts.

The first biological entities, the alkaline amino acids, could have appeared in an atmosphere rich in ammonia, water and methane. Experimentally, electrical discharges through mixtures of these materials have induced the appearance of amino acids. It appears plausible that such an environment could have existed around volcanoes in times when the earth's atmosphere was formed by ammonia. With steam formed by the heat of the volcano, with methane resulting from the interactions of erupted metallic hydrocarbons with water, and with lightning so frequent around volcanoes in eruption, the necessary conditions for synthesis of amino acids may have been present. The simple amino acid molecules which would have resulted could have constituted a group needed for hierarchic development. Out of a series of such amino acids, some could have bound the group N-C-N-C which also could have been synthesized under the influence exerted by radioactive elements or radiation. (*Note 1*)

Additional information concerning the constituents of secondary parts which enter into the development of all the subnuclear entities is meager. Simple amino acids or urea are present in the chromosomal and nuclear sap which are practically free from K and Na. We could thus consider

ammonium as the predominant cation for all hierarchic entities up to the nucleus. This would accord with geological data concerning a primitive atmosphere in which ammonia was predominant at the time when the first biological entities would have appeared. We can then, tentatively, in view of this cation common to all, classify the hierarchic entities below the nucleus in what we will call "the nuclear compartment."

In the same way, we analyzed the composition of cytoplasm, with the thought that it could provide information about the constitution of a second environment in the evolution of biological entities. The principal cation of cytoplasm is potassium which also represents the principal cation of the earth's crust. Curiously enough, potassium and the other constituents could be found in the same relative proportions in the earth's crust as in cytoplasm, (*Note 2*) a fact which seems to confirm the hypothesis that mud, humid earth crust, represented the environment in which entities at nuclear levels lived. The cytoplasm conserved this constitution, with potassium as principal cation, when it was separated from the environment to become the secondary part which, with the nucleus, formed the cell as the next superior hierarchic entity. The cell by itself, represents a new compartment with potassium as principal cation.

It is only for the animal cell that the environment seems to have changed again. This time the new environment was the sea. When several cells joined together to organize tissue as a new entity, they had to maintain their environment, now represented by the sea. The hierarchic entities above the animal cell show sodium as principal cation in their secondary parts, thus indicating that when they were organized the sea was their environment. This characteristic allows us to group together animal hierarchic entities above cells to form a new compartment, the metazoic, with sodium as the principal cation.

With passage from marine to terrestrial life, air is found as the new environment. Not integrated as a new secondary part, without a separating boundary formation, the part of the environment kept in the respiratory apparatus does not enter however, into the formation of a new entity. Only the presence of air in the bones of birds can be seen as such integration.

A certain fundamental further development, in the same direction can be seen in animals as well as in humans, in the area of social life. (*Note 3*)

Division of complex hierarchic organization into compartments appears to be relatively simple. There are changes in the principal cation from compartment to compartment which correspond to similar fundamental changes in the environment through which the organism passed during its phylogenetic evolution—from volcano to mud, to sea, to the surface of

the earth. We tried similarly, to correlate other elements in the periodic chart with hierarchic compartments. The results will be presented in detail later. For the moment, it can be stated that elements correlated to different environments are also found in the different compartments corresponding to these environments. For example, Mg like Na represent an element of the metazoic compartment and of the sea where this compartment was phylogenetically organized. Fe, Cr, Ni, Zn, Ca, Mn, Co, As, and Se which fall into the cellular compartment, represent characteristic constituents of the earth's crust.

Constants

The concept that matter in general is the expression of the heterotropic trend permits us to explain further some of its characteristics which have been of great importance in biological development. Heterotropy can be seen working to maintain existing entities as long as possible, to conserve their characteristic properties in spite of changes in the environment. Heterotropy could result in unchanged values which would appear as constants of an entity and would indeed identify the entity. It is hierarchic organization which would tend to permit conservation of constants.

Each biological entity—just as any entity in nature—can ultimately be defined by the series of characteristic properties which it is able to conserve. Constancy is the criterion which permits us to judge the importance of a property to an entity. The longer a property is kept constant despite environmental changes, the greater its fundamental importance to the entity. The progressive addition of secondary parts through hierarchic organization represents an effective means of preserving constants of the lower entities. New properties, added with the formation of each new hierarchic entity, represented new constants. This explains why considering the constants, it can be seen that those incorporated in the lower entities are the best conserved. The higher a constant in the organizational hierarchy, the less well-preserved it will be.

The idea that constants correspond to the character of the environments through which the individual has phylogenetically passed, and that they are conserved through hierarchic organization, permits us to try to extend our understanding of conditions present in past environments. Cations and even anions would represent only one type (apparently the most important) of the constants maintained through hierarchical organization. Other constants, correctly interpreted, would indicate in what direction we must search for conditions which prevailed in the environ-

ment when the respective hierarchic entities that make up a given organism were established during phylogenetic development.

As examples, let us consider the conservation of salinity and temperature as constants. Values for salinity of the metazoic compartment and of the blood and values for temperature have been seen to be constants characterizing species. Differences between these constants in different species show a succession conforming with paleontological data. In the interpretation given by Rene Quinton, constants would indicate the times when various species originally appeared in nature.

According to our concept of hierarchic organization, these constants may be interpreted otherwise. They would not indicate the moment when the lowest entities of the respective species were formed, but rather the time in the development of these species when the hierarchic entities capable of conserving the respective constants appeared. In other words, they would not indicate the earliest moments of appearance of the first entities which later developed to form the respective species, but would indicate a relatively late moment in the creation of the metazoic entity which has appeared able to conserve, as its own constant, this specific attribute of the environment. In the case of salinity, this would correspond to the constitution of the metazoic entity itself which has retained the composition of the early sea in its intercellular fluids. As far as temperature is concerned, the entity which would appear able to conserve it has to be regarded as much more complex and even to be related to the appearance of systems of organs which are sufficiently sensitive to changes in temperature and which also possess the means of insuring constancy for temperature.

The conservation of different elements in different compartments appears to be characteristic. In order to maintain its constants for elements the entity has to oppose their uncontrolled circulation. The role of hierarchic entities in conserving ancestral conditions would explain why an entity would have to oppose particularly the penetration of the constituents which characterize the succeeding environments. This has appeared evident for the cations. The boundary formations which have to play the principal role in the creation of each entity must also insure its identity by barring uncontrolled penetration of elements characterizing the new environments. Invasion by such elements would correspond to an abnormal event which must be corrected. If the invasion progressed beyond a certain limit, it would create a condition incompatible with further existence of the entity.

Water Circulation

The passage of complex organized animals into the new environment of terrestrial life brings to the fore the problem of the place of water in hierarchic systematization. It appears to us an acceptable concept that water does not circulate freely in the organism. Its appearance in hierarchic entities can be understood if, as we did for the other constituents, we relate water to its place in the environment in which phylogenetic development has taken place. In the first near-volcano environments, which applied to the subnuclear entities, water was relatively scarce, which explains the high concentration of the constituents in the nucleus. The mud of the earth's crust is richer in water, which explains the difference between the nucleus and the cytoplasm, with the latter richer in water. The sea was the environment for the metazoic compartment, which explains the richness of water in this compartment. The so-called "internal sea" consequently can be seen only in the metazoic compartment. With the passage into the terrestrial environment with its air medium, the water again becomes scarce and has to be conserved. The circulation of water between compartments is governed by osmotic forces which are determined by the original richness in water of the respective environments. The importance of water circulation appears evident when an abnormality in its distribution occurs. Water arriving in a compartment—alone or with a cation—in an amount above that corresponding to the constant for that compartment, is separated from the constituents of the entity in order to reestablish the characteristic constant value. Such separation of abnormal amounts is accomplished according to the compartment, through the appearance of vacuoles, edema, exudates or diuresis.

Animals and Plants

The concept of hierarchic organization in which each entity can conserve its own environment allows us to consider in a new light various other problems of living organisms. One concerns the fundamental differences between animals and plants. Analysis of the constituents of the metazoic secondary part provides a new criterion for distinguishing between animals and plants and gives logical meaning to its distinction. Animals can be characterized as having sodium as the cation of their metazoic compartment; from the cell level on, they have had the sea as their temporary or even permanent environment. Plants, on the other hand, have potassium as the principal cation for their metazoic compartment, indicating that, from the cell level on, they have had the earth's crust as their environment,

passing thus directly from mud to air. By their actual attachment to the soil, plants continue this relationship to the mud. Their relative immobility is in accord with continuation of the terrestrial-air environment in their development. The mobility of animals, on the contrary, can be seen to have its origin in the fact that they have had the sea as their environment at least for a period of time, *i.e.*, from the cell period until the appearance of those animals which left the sea. We can interpret the appearance of cellulose and lignin as part of the plant-sustaining means which would bring to plants indispensable external protection against the hardness of the soil environment. Cellulose and lignin are not necessary for animals which experienced much of their evolution in the sea.

Multiplication

The hierarchic organization of organisms, with the conservation of successive entities, puts the problem of entity multiplication in a new light. According to the hierarchic organization concept, the multiplication of a complex entity means the reproduction of the entire series of hierarchic entities forming it. In this process, the intervention of each hierarchic entity appears highly individualized. And this applies not only for the morphologically identifiable entities, but even for the most primitive entities. The difference between the role played by the principal and secondary parts becomes capital for these processes. While for each entity the principal part has to be built as such, the parts corresponding to the secondary parts are taken from the immediate environment. The quantitative disproportion between some principal and secondary parts makes the role of the first difficult to be recognized. The complex entity, through changes that are the reverse of those of ontogenetic and phylogenetic hierarchic development, separates the successive principal parts which characterize it. With the replication which takes place the division occurs successively for these hierarchic entities. In scissiparity the division morphologically occurs at the cell level; in karyokinesis, it can be identified at the chromomere level and certainly takes place much lower in the hierarchic entities. In replication in general, different constituents available are adequately changed by the respective principal part to form the necessary secondary parts. Through these changes the same processes are reproduced which originally occurred when the entity had been phylogenetically organized.

With the individualization of the low hierarchic entities the problem of replication is simplified. Once replication occurs, the same process takes place successively for the progressively higher levels. Above chromomeres this appears very clear in karyokinesis.

After the chromomeres divide, two or four chromonemata appear. The process goes on within the chromosome, nucleus and cell. In order to protect its individuality each hierarchic entity is protected during its division. The chromosomal membrane, the cellular cytoplasm and the cellular membrane continue to protect the respective entities as they divide. The cell itself divides only when the two nuclei have had their protecting membranes rebuilt.

In the division and multiplication of a complex entity, the return to entities as low as subnucleic parts indicates the relative importance for the characterization of the complex entity and for the conservation of its particular properties of the parts added during the hierarchic development. The entity must, in fact, rid itself of these added parts which, although they have other importance, have a secondary role even in the processes of multiplication.

It is interesting to note that a similar return to more primitive component entities also occurs, although it is less pronounced, when an entity, tissue, organ or organism has to fight a noxious intervention. The defense is passed progressively from the organ to the tissues and from these to the cells. In effect, there is a renunciation of added parts during these moments of crisis. Even at the cell level, a similar process is seen. The added parts, represented by the protoplasmatic formations, disappear. The almost non-differentiated cell fights the noxious factor at the lowest levels of its organization.

Life and Death

We have seen that the term "biological realm" can be applied to hierarchic development starting from the N-C-N-C radicals. We have employed this term for didactic convenience although it is unrelated to the commonly accepted concept of life. The study of hierarchic organization also led us to consider a concept of life and death which, while retaining some of the common meaning, also accords with the phenomena of hierarchic organization.

In the complex entity, each lower level entity lives and dies with relative independence. An organ can be dead and yet have living cells in it for a time. There are always dead cells to be found in living tissues and organs. It is the relative independence of the different hierarchic entities making up a complex entity that explains these seeming peculiarities.

Our concept of life and death stems from consideration of the nature of hierarchic entities. We have seen that all matter in nature, from the simplest to the most complex entity, is a result of heterotropy. The per-

sistence of constants proper to each entity is distinctly opposed to homotropy. Life in its broadest sense, corresponds to the capacity of an entity to maintain heterotropy by conserving its characteristic constants. The life of any entity appears to be synonymous with conservation of its constants. An entity dies when it has permanently—that is, irremediably—lost its capacity to conserve the constants which characterize it. Death then represents exhaustion of heterotropy for the specific entity.

The fact that, in essence, life appears to be synonymous with the conservation of constants and is heterotropic, relates it, and especially its origin, to one of the important sources of heterotropic force, solar energy. A distinction has to be made between heterotropy as one of the fundamental laws of nature and the means by which it is exerted. Solar energy, with all of its quantas, would greatly increase the effects of heterotropic forces in nature. It would not create such forces but would simplify them and extend their applications. The origin of matter and, as we have seen above, of entities, biological or nonbiological, is in the final analysis the result of heterotropic forces. External conditions qualitatively and quantitatively influence operation of heterotropic forces.

The sun's heterotropic contributions have to be considered under this aspect. Through the quantas it disposes of, solar energy has not created life, as conceived above, but by permitting more and more entities to appear, has greatly facilitated their extension. Its effect, although certainly not limited to any group of entities, seems to be especially important to those forming the biological realm. Similarly, the effect, of a special type of energy, radiation, also must be considered. Radiation appears to be related to the elements, and will be discussed in a later chapter devoted to the elements.

Since life itself is related to changes directly aimed at conserving constants, in this broadest sense it is no longer limited to the specific group of entities found in the "biological" realm. Life has the same meaning for an atom, crystal or micelle, as for a cell, organ or organism. It is for this reason that knowledge of the mechanism through which constancy is achieved becomes of great importance in the study of all matter and especially of the biological realm.

Maintenance of Constants

To study the mechanism used to maintain constants, we must define exactly what constancy means. According to Cannon's principle of homeostasis, constants have been considered to correspond to a dynamic balance that results from the continuous operation of two opposing factors. And

it was conceived that by acting concomitantly as coupled antagonists, these factors or groups of factors insure constants. The intervention of these two opposite factors becomes apparent only if an exterior cause upsets their balance.

However, our study of the processes through which dynamic balance is maintained has permitted us to recognize a different mechanism than the one which is commonly accepted.

A value considered to be a constant for an entity is not fixed or static. It represents, rather, a statistical value, the result of a series of dynamic changes which must also be considered in terms of time. Consequently, a constant has to be seen not only as the average value of a series of organized changes, but also must be identified by the characteristics of the variations. An average value around which variations occur thus represents the first attribute of a constant. The second attribute is the existence of a rhythm in the variations, the third involves intensity of variations. For instance, when we say that human body temperature is constant, we mean that 37°C is average value for oral temperature, and also that body temperature presents characteristic variations having a 24-hour rhythm and also that the occurring changes consist of variations of a few tenths of a degree above and below the average value.

The two antagonistic intervening factors do not operate concomitantly to maintain a constant value, but rather act alternately, each being predominant for a period of time. The result is not a continuously steady value for the constant, but an oscillatory movement with successive passages from one side to the other of the average value. This oscillatory movement appears to be the general rule throughout nature, prevailing in everything from the waves in the smallest subatomic particles to the pulsation of the universe. The rhythm periods appear to correspond to environmental rhythms. A rhythm related to the day, for instance, is seen for temperature. In other constants we recognize a 12-hour rhythm which could correspond to that of the ocean tides. Other rhythms, with periods ranging from two hours to a few minutes are seen for several changes occurring in blood. There are also some in which the influence of the moon is evident; for example, the hypophysis-ovarian cycles; and for others, the influence of the seasons is apparent.

Teleologically speaking, balance represents a very effective method for maintaining constants. Any deviation in any direction as a result of an external intervention will be counteracted by the opposing phase of the oscillatory balance. This occurs because of the existence of two phases of the

oscillatory movement itself. Such would not be the case if there were fixed values for constants.

Related to the pattern of the organization of matter in general, this oscillatory movement can be considered to be another instance in which the two opposite fundamental forces of heterotropy and homotropy, which are basic to progressive hierarchic development itself, also operate. This oscillatory balance can be related ultimately to the alternate successive intervention of the heterotropic and homotropic trends in the organization and the manifestations of entities existing in nature.

Dualism

The concept of dynamic oscillatory balance is of great importance in the study of biological phenomena. Coupled factors with opposite properties characterize all constants and are involved both in the processes through which constants are maintained and in their manifestations as well. Recognition of this dualism in all biological phenomena has been of great value in the investigation of normal and abnormal physiology.

According to our concept, dualism results from the alternate, not concomitant, operation of two opposing factors. And, as we have seen, these factors ultimately can be related to the two fundamental forces in nature, homotropic and heterotropic. Thus, in a unified concept, in every phenomenon in which dualism appears, one force will be homotropic the other will be heterotropic. Homotropy is related to fulfillment of electrostatic forces, and has general coulombian electric character. Heterotropy is quantum-like and organizational. Homotropy would keep entities simple. Heterotropy would lead to more organized bonds and to more complex synthesis. In every phenomenon studied, these characteristics of the two fundamental forces have permitted dualism to be recognized and interpreted. The dualistic view has become our basic approach for all of the problems related to matter and, more specifically, to biological entities.

The dualistic concept of intervening forces brings an entirely new light in any analysis in which a graphical representation is different from a straight line. From the curves of spectral analysis of constituents to those of complex phenomena, the existence of oscillations reveals the intervention of opposed forces and offers a valuable mean to study them. This broad approach has a special field of application in biology.

Dualism can be further recognized easily in the manifestations of the biological entities, in their function and in the substances composing them. In the case of the elements, such a dualism can be related to atomic structure and the properties of the electronic shells, as will be seen below. In

complex molecules, a simple form of dualism can be seen in acidity and alkalinity, electrophily and nucleophilic or, furthermore, in positive and negative electrical characteristics. We will see later, how important dualism is for the different groups of constituents and how, without this dualistic concept, it would be difficult to understand the roles of most of these constituents in biology.

In part, as a consequence of the separation of the constituents into two groups, dualism can be observed easily in the hierarchic organization of higher entities. Dualism appears in the relationship between primary and secondary parts, the first having a more positive character than the second. The study of cancer manifestations under this dualistic aspect has been highly rewarding and is the subject of the following chapters.

In a more concrete step, the dualistic concept has provided new understanding of abnormality.

Normal and Abnormal

A normal entity can be conceived of as one which is able to maintain its constants with their characteristic values, rhythms and intensities by means of the alternate operation of homotropic and heterotropic forces. A normal entity, thus, can be defined as one having constants within the limits that statistically characterize this particular kind of entity. We can define the abnormal entity as one in which a constant's characteristics—average value, rhythm, intensity—are altered. It is alteration, without complete loss of the characteristics of constants, that differentiates abnormality from death. In death the constants themselves are irremediably lost. This definition also distinguishes abnormal from physiological manifestations. In the physiological manifestation, oscillatory movement persists and only its intensity is influenced, usually becoming exaggerated.

As expected from the dualistic concept, abnormal changes can take place in either of two opposite directions and this is a significant fact of abnormality. The two possibilities are inherent in the oscillatory balance characterizing the constant itself. It is the offbalance, resulting from the exaggerated predominance of one of the coupled factors over its antagonist, which leads to the abnormal. Persistent predominance of one factor abnormally affects, and even suppresses, normal oscillatory rhythm.

For each normal condition, then, two opposite abnormalities are possible. By relating the abnormal condition to one or more altered concepts, and the alteration in each constant to one of the dual changes possible, a new systematized analysis of the abnormal becomes feasible. The large number of constants which compose each entity and which can become

abnormal help not only to explain the great variety of abnormalities but also offer a means of obtaining analytical pictures of disease.

It is with this approach that we have tried to study pathological conditions, with special emphasis on cancer. This study is presented in the following pages.

CHAPTER 3

CANCER AS AN ORGANIZED CONDITION

IN THE CONCEPT now most widely accepted, cancer is considered to be the result of abnormal changes within cells. Although it is admitted that the disease may have different etiologies, it is the cell which is regarded as the pathogenic entity. A group of specific changes in the cells is believed to represent the fundamental abnormality.

In today's prevailing outlook, differences between tumors are attributed to the multiple secondary characteristics present in the diseased cells along with a primary specific anomaly. The complex clinical manifestations of cancer are further explained in terms of the relationship between cancerous cells, as pathogenic entities, and the whole organism. Clinical evolution, from local innocuous process to lethal disease is related to anatomical spread of cancerous cells from their original site. Abnormal metabolic changes seen in the organism are believed to result from the influence exerted by functional abnormalities of the cancerous cells. In a still narrower view, cancer is considered to be the result of abnormality of a single specific function of the cell—its growth. Qualitatively and quantitatively, abnormal growth has been considered to be the capital factor in the pathogenesis of the disease. (292)

In contrast to this classical view, our studies have led us to regard cancer as something other than an abnormality limited to the cell alone.

As we have seen, the organism is a complex hierarchic organization of different biological entities. We sought to determine where cancer fits in this complex organization. Can cancer with its manifestations and its evolution be better understood if systematized in accordance with the hierarchical organization of the organism? Can both manifestations and evolution be related not alone to a cellular abnormality but rather to a progressive par-

ticipation in the disease of the different hierarchic levels of the organism?

We have found that such participation cannot be analyzed readily in the advanced cancerous subject with so many and such varied manifestations of the disease already present. Similarly, incipient cases with a paucity of clinical manifestations are not ideal for the purpose. It was only by following the successive appearance of manifestations during the evolution of cancer that their relationship to the level of hierarchic organization involved could be clearly seen.

Identification of the level involved at each point in the development of cancer was greatly facilitated by conceptually separating the clinical evolution of the disease into a series of successive phases and identifying the changes which characterize the passage from one phase to the next.

We have chosen to call these phases precancerous, noninvasive, invasive, painful, preterminal and terminal. We will briefly identify them and their salient features here.

Precancerous Phase

In the precancerous phase, the disease is not clinically apparent. Yet this phase has been recognized as pathogenic in experimental carcinogenesis and its characteristic changes also have been identified in human subjects. Morphological changes—abnormalities in size and form—can be observed in the chromosomes. These changes are not identified as related to cancer in human subjects except where multiple centers of cancerization are found (as in the stomach, for instance). (*Note 1*) These chromosomal abnormalities can be considered to be precancerous lesions, since experimental carcinogenesis has indicated that these changes precede the appearance of cancerous cells. In terms of hierarchic organization, then, the precancerous phase can be considered to be limited to the subnuclear levels.

Noninvasive Phase

In the noninvasive phase, also known as "cancer-in-situ," abnormal intra-epithelial cells are present. The abnormality involves two changes. One, morphological, affects the nucleus; the other affects arrangement of the cells in the epithelium. The abnormal changes in the nucleus in this phase have been widely studied in exfoliative cytology. (*Note 2*)

Abnormality in this phase appears to be limited entirely to the nucleus. The cells continue to have an almost normally differentiated cytoplasm, a fact which originally led to the description of this phase as "cancer of differentiated cells." Besides the nuclear changes, cells in this phase show, histologically, an anarchic arrangement different from the regular disposi-

tion which is one of the basic characteristic of the epithelium. Since the regular relationship between the cells forming epithelium can be attributed to dipolarity, the anarchic disposition seen in this phase of cancer can be ascribed to loss of cellular dipolarity.

Cancer-in-situ, in terms of hierarchic organization, would appear to involve the level of the nuclei, and the noninvasiveness, characteristic of this phase, persists as long as the "cancerous" abnormality remains limited to this level, that is, as long as the cytoplasm of cells remains apparently unaffected.

Invasive Phase

This phase is characterized by irregular proliferation of cells and penetration into neighboring tissues. To the anarchic arrangements noted in the noninvasive phase, now has been added exaggerated growth. And the change of a noninvasive cancer into an invasive one can be considered to result solely from the addition of the new factor of abnormal growth. The invading cells will persist only if, concurrently, there is a loss of the defense mechanism of the invaded tissues, as will be seen later.

Studies of invading cells have revealed, in this phase, an anomaly no longer limited to the nucleus but now encompassing the cytoplasm as well. Exfoliative cytology has shown an abnormal and rapidly disintegrating cytoplasm and this has served as an important diagnostic criterion. From the point of view of organization, it can be said that, with the participation of the cytoplasm, the disease has progressed from the nuclear to the cellular level.

Painful Phase

Pain is the principal clinical manifestation characterizing the next phase of the disease. As we shall explain in greater detail later, pain arises from changes in the pH of the intercellular fluid that bathes sensorial nerve endings. For the moment, we can remark that biochemical changes now occur outside the cells, and, with the participation of interstitial formations, the disease has progressed to the tissular level.

Preterminal and Terminal Phases

In the next stage, the preterminal, biochemical changes affect the function of various organs which may or may not in themselves contain cancerous cells. While some changes in function may be seen even before this preterminal phase, now, there is manifest impairment. And, while the invasion of an organ by cancerous masses is a factor precipitating the func-

tional changes, invasion is not indispensable. Abnormal biochemical changes leading to serious functional impairments are seen in organs entirely exempt from tumor masses.

With further progress of cancer, metabolic functions that are systemically important become abnormal. Later, we will analyze in detail these changes which affect the whole organism profoundly. For the moment we want only to note that, with these changes cancer passes from the clinically preterminal to the *terminal phase*.

In the light of this systematization, cancer then appears to progress clinically in organized fashion as it passes from the relatively innocuous nuclear noninvasive cancer-in-situ to a lethal systemic disease, the progress being marked by the successive participation of different hierarchic levels of the organization. Table I sums this up.

TABLE I

<i>Organizational Level</i>	<i>Physiopathological Changes</i>	<i>Clinical Phase</i>
Subnuclear	Gene and chromosome anomalies	Precancerous
Nuclear	Nuclear anomalies and atypical cellular arrangements	Noninvasive cancer
Cellular	Atypical growth	Invasive cancer
Tissular	Local pH changes	Painful cancer
Organic	Organic metabolic changes	Preterminal cancer
Systemic	Systemic metabolic changes	Terminal cancer

By extrapolation, a similar progressive participation of hierarchic entities can be conceived of below morphologically recognizable levels. This would permit us, as a working hypothesis, to attribute the pathogenesis of cancer to abnormalities in nucleo-proteins or, even lower in the scale, to abnormalities in histones or alkaline amino acids. (*Note 3*)

This concept—of progressive participation of successive hierarchic levels in cancer—contrasts sharply with the view generally held today which places the entire burden of anomaly on the cancerous cell itself. The classical concept has led to the currently prevailing all-or-nothing approach in which therapeutic attempts are directed to the cancerous cells as the only avenue for controlling the disease at any moment of its evolution. Under our hierarchic concept, therapeutic possibilities can be extended beyond the cancerous cell.

These considerations raise the question of the relative importance of the multiple changes which occur in cancer. Subnuclear and nuclear changes are of relatively little importance as long as there is no progress of disease beyond these levels. Corroboration for this can be found in the great num-

ber of cases in which cancer-in-situ cells are noted in an organ, yet clinical cancer does not follow. In our concept, the changes which occur at levels above the nuclear are critical in the evolution of the disease and, as such, are the important pathogenic factors. On the other hand, as we shall see later, changes at higher levels similar to those encountered in cancer may occur independently of cancer, and without a sequence of changes at lower levels. It is only when changes at higher levels appear in proper sequence, affecting already abnormal entities of the lower levels, that clinical cancer results and the malignancy moves relentlessly from the noninvasive cancer-in-situ to the terminal phase.

This concept, then, focuses attention on all changes occurring at different hierarchic levels of the organization rather than on those in the cell alone. It emphasizes the importance of the relative independence which exists between the different hierarchic levels, an independence which governs their participation in the complex condition which is cancer.

From the therapeutic standpoint, then, it seems logical to suppose that, if the progressive participation of successive levels can be interrupted, many if not all of the noxious manifestations and the course of cancer can be favorably influenced. In view of this, it has been essential, first, to obtain more information about the cancer manifestations which are added as the disease takes its hierarchically progressive course and about the mechanisms that account for these manifestations.

CHAPTER 4

DUALISM

As we have observed above, dualism prevails in nature. The concept of an oscillatory dynamic balance—the result of alternate operation of opposed forces—has been of special value in the study of most of the physiological phenomena. Over the years we have also constantly observed that in most physiopathological manifestations, dualistic patterns can be recognized. This dualistic pathogenic concept has helped to guide our study of disease. In cancer, it has permitted better understanding of many processes and manifestations. It has also served as a basis for our attempts to influence cancer and other conditions therapeutically. It was in the study of pain that, initially, we found clear evidence of pathogenic dualism.

PAIN

Many years ago, during experiments with an alcoholic extract of human placenta as a therapeutic agent in terminal cancer cases, a curious effect was observed. In some patients with painful lesions, administration of the preparation resulted in a decrease in the intensity of pain and even in its disappearance within a few minutes, with relief usually lasting for hours. In other cases, however, there was an opposite effect; pain increased in intensity within a few minutes after an injection. In some subjects, the exacerbation was so great and pain became so unbearable that the experimental treatment had to be discontinued quickly. In several cases in which the preparation was used in progressively larger doses, another noteworthy effect was observed: after the first injections, pain decreased and even disappeared for several days, only to have a new pain arise as treatment continued. This new pain became more intense after each injection so that the therapy had to be discontinued. Patients clearly recognized the difference between pains. The new one frequently had a burning character.

Thus, it became apparent that one substance could increase pain in some subjects and alleviate it in others, and could even alter the nature of the pain in the same subject. Pain, as demonstrated by antagonistic responses to a single agent, thus appeared to have a dual nature. Our immediate problem was to investigate this and its significance.

Physiological and Pathological Pain

In discussing the sensation of pain, most authors have found it necessary to distinguish between different types of pain. Some have classified pain as: 1) spontaneous, or 2) provoked, according to its mode of induction. Others have defined pain according to its site of origin and quality as: 1) superficial or cutaneous, and 2) deep visceral or somatic. Superficial pain from skin and mucous membranes near body orifices has been described as bright or burning in quality, while deep visceral or somatic pain arising from mesenchymal structures, certain mucous membranes and viscera has been described as diffuse and aching in quality. In many respects, attempts to define and classify pain in these terms have served to confuse rather than to clarify the problem.

That different types of pains do exist is an observation based upon common experience. For example, when a stimulus of sufficient intensity is applied to the skin for an adequate period of time, a sensation of pain is induced. This pain disappears rapidly when the stimulus is removed. But if the stimulus has been of such intensity and duration as to produce tissue damage, an after-pain may recur spontaneously some time after the stimulus has ceased. The original pain serves as a warning that the tissues are endangered. The after-pain, however, cannot be considered as a direct effect of the application of an external stimulus, but is rather a manifestation of true tissue damage.

As a first step, two categories of pain—one induced in normal tissue by external intervention, the other appearing as a pathological manifestation of an existing lesion—were established. We called the first, which is a normal sensorial sensation, “physiological” or “sensorial” pain. The second, a symptom of an abnormal local condition, was called “pathological” or “symptomatic” pain. This separation helped to eliminate discrepancies otherwise encountered in the study of pain, discrepancies which result when two entirely different manifestations, one sensorial and the other symptomatic, are studied under the same heading and investigated by the same methods.

Pain may be induced in damaged tissues by various stimuli not of sufficient intensity to arouse pain sensations in normal tissues. This sensi-

tivity constitutes an abnormal response of tissues that have undergone pathological changes. Inflammatory, traumatic, circulatory, neoplastic or other pathological changes similarly may bring about either spontaneous pain or an abnormal degree of sensitivity of the involved tissues to external stimuli.

There are, therefore, two general types of pain which are biologically different. The first is a direct response of normal tissues to external stimuli which serves as a warning of danger. The organism reacts to this type of pain by seeking to run or to fight. The second type of pain arises as a consequence of tissue damage or disease to which the body responds by attempting to put the injured area at rest. This second type of pain, whether spontaneous or provoked, superficial or deep, is biologically different from the first pain experienced following the application of sufficiently intense external stimuli to normal tissues.

For purposes of further study, it would appear to be advantageous to distinguish between physiological or sensorial pain which is the response of normal tissues to noxious external stimuli, and pathological or symptomatic pain which is a manifestation of abnormal tissues. In the study of pain in all its aspects, it is necessary to keep this distinction in mind. While various investigative methods have furnished data concerning physiological pain, the information thus obtained is of very limited value when applied to the problem of pathological pain. However, it is pathological pain which constitutes the vital clinical problem, physiological pain being of concern in medicine primarily in the field of anaesthesia. (*Note 1*)

Dualism in Pathological Pain

It was only in pathological pain that a dual character was encountered. For one thing, it was noted that in some patients with chronic pain—associated with tumors, arthritis or other conditions—the pain intensity was not constant. In many of these patients, variations in pain intensity could be seen to follow a pattern. Although the variations usually are referred to as "spontaneous," we could show that they were related to the time of day. Furthermore, the variations were not the same for all patients. In one group, pain was severe in the morning and diminished toward evening, while in another group, little or no pain was felt in the morning and exacerbations occurred in the evening.

The intake of food also had a dual influence. In some patients with tumors far removed from the gastro-intestinal tract, pain was increased by eating while in others pain decreased. Patients themselves often recognized this relationship and many whose pain was increased with the intake of

food refused to eat for fear of aggravating their suffering, while those of the other group wanted to eat whenever pain was severe in order to reduce its intensity.

These observations on the influence of time of day and intake of food on pain led to the study of the acid-base balance of the body since this balance is known to be influenced by the same two factors—time of day and food intake.

In a preliminary study we considered a special aspect of the acid-base balance of the body, that is, the several mechanisms involved and their possible intervention in the change of pain intensity. A study was made of blood pH, titrimetric alkalinity, CO₂ combining power, relative chloride distribution between erythrocytes and plasma, as well as urinary pH for the indications they furnish concerning the acid-base balance. The blood is highly buffered in order to avoid damage, through abnormal pH values, to cells in general and especially to those of the nervous system. Consequently, the variations in the blood pH are as limited as possible. Alkaline reserve and the chlorides repartition represent only part systems in the general acid-base balance. Titrimetric alkalinity, corresponding to the sum of ionized and nonionized constituents, furnish information of the broadest scale of the acid-base balance. Consequently it appears to be a highly significant measurement. Through the non-ionized constituents, it can vary greatly without influencing blood pH. It reflects thus otherwise hidden changes in the acid-base balance. We could show that alone, among all the variable factors of the blood acid-base balance, total titrimetric alkalinity of blood varies in parallel with the urinary pH. (*Note 2*)

As an immediate result of this research, it was possible to utilize the changes in the urinary pH as an indication of the most important variations occurring in the systemic acid-base balance. This makes it possible to use changes in the urinary pH as an indicator of the relationship between acid-base balance and variations in pain intensity.

Pain and Acid-Base Balance Changes

When changes in pathological pain intensity were studied in relation to changes of the urinary pH, a correlation could be established in the majority of cases. Two opposite kinds of relationship were observed when curves of the variations in pain intensity were compared with those of the urinary pH.

Patients who had experienced pain associated with chronic pathological lesions over prolonged periods of time were instructed to record carefully the relative intensity of their pain at regular intervals, such as every hour.

No analgesics were administered for at least six hours before or during the test period which was continued as long as possible, even for twenty-four hours. Patients were instructed to concentrate on a single painful area and to estimate the degree of pain intensity. They were told to consider an average degree of pain during each hour rather than momentary peaks during the observation period or the pain at the moment of recording. The

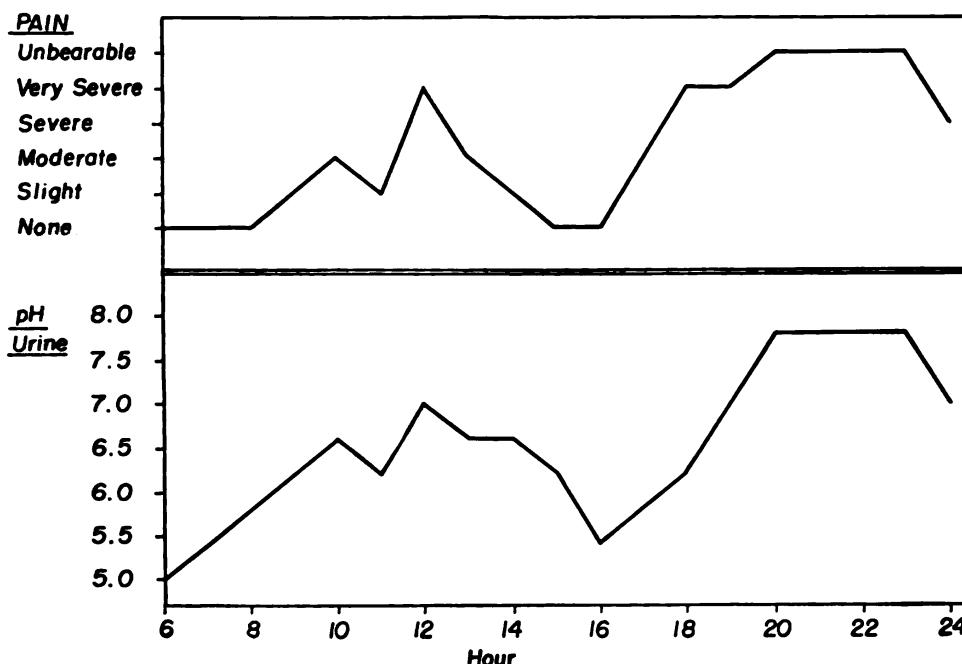


FIG. 6. A pain pattern is recognized by comparing the concomitant changes present in the curves of pain intensity with those of the urinary pH. The parallel variations of the two curves indicate an alkaline pattern with the pain more intensive when the urine is more alkaline, as seen in a case of carcinoma of the colon with painful abdominal mass.

degree was recorded in relative terms of no pain, slight, moderate, severe, very severe and unbearable, or as figures from 0 to 10.

Urine specimens were obtained each hour immediately after the pain intensity observations were recorded and the pH was determined electrometrically. Two curves—for the hourly variations in pain intensity and for urine pH—were then plotted.

Two distinct types of correlations were found. In the first, the two curves paralleled each other, the pain being more intense when the urine pH was higher, and less severe when the pH was lower. (Figs. 6 and 7) Because the maximal pain of this type of correlation is associated with a change toward alkalinity, this was called an alkaline pattern of pain.

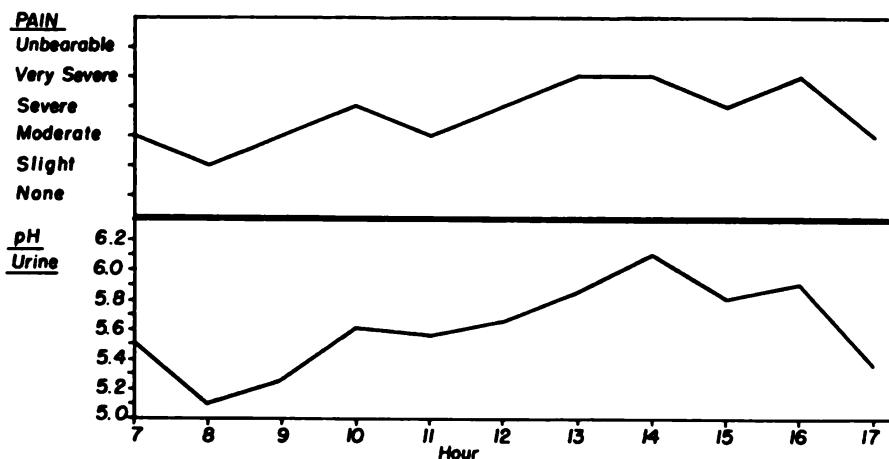


FIG. 7. *The alkaline pattern of pain* in which the concomitant variations in the curves of pain intensity and urinary pH are parallel, seen in a case of arthritis.

In the second type of correlation, the two curves varied inversely, pain being most marked when the urine was most acid, and least so when the urine was most alkaline. (*Figs. 8 and 9*) This second type was called acid pattern of pain because of its association with a change toward acidity. Considering the highly subjective nature of pain, the inconsistencies which occur are minor. Fig. 10 shows these curves followed during days.

The correlation between the pain intensity and urine pH curves are relative rather than absolute. The general level of urine pH apparently

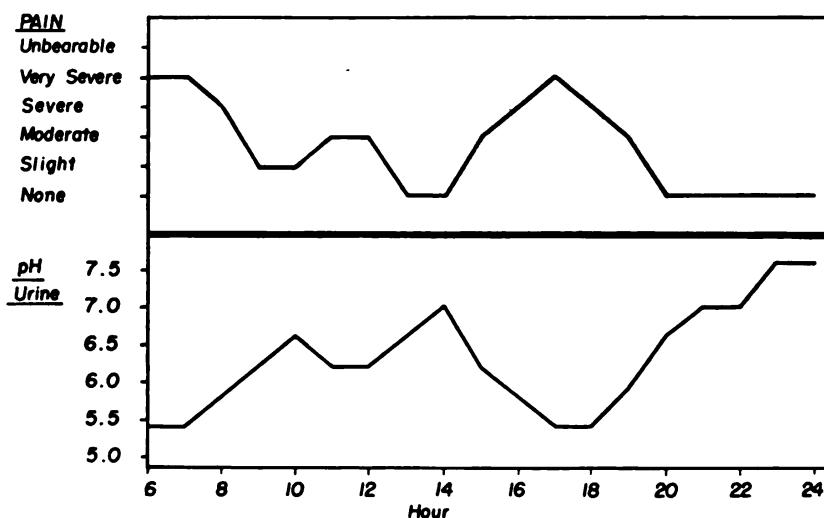
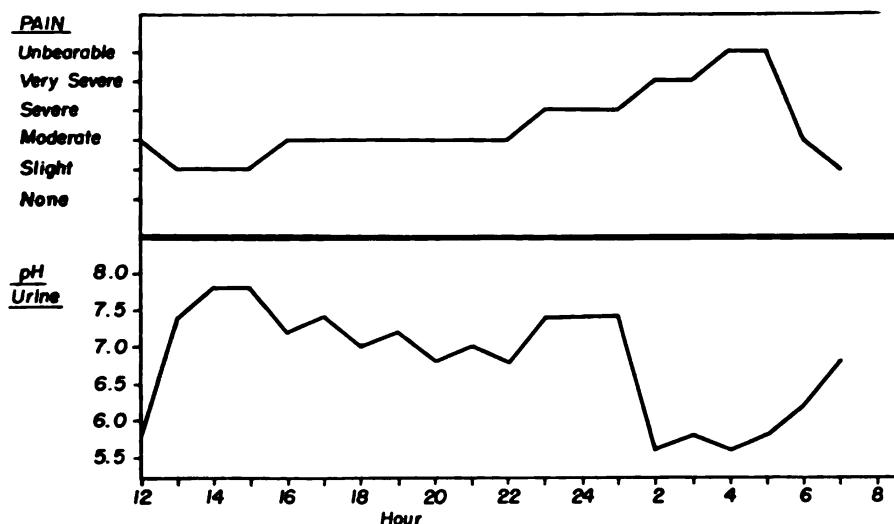


FIG. 8. *An acid pain pattern* is seen in a case of carcinoma of the prostate with metastatic bone lesions, in which the concomitant variations of the curves of pain intensity and urinary pH are divergent.

depends upon other factors. Consequently, only the fluctuations of the hydrogen ion concentration, rather than the absolute levels, are considered in this relationship to pain.

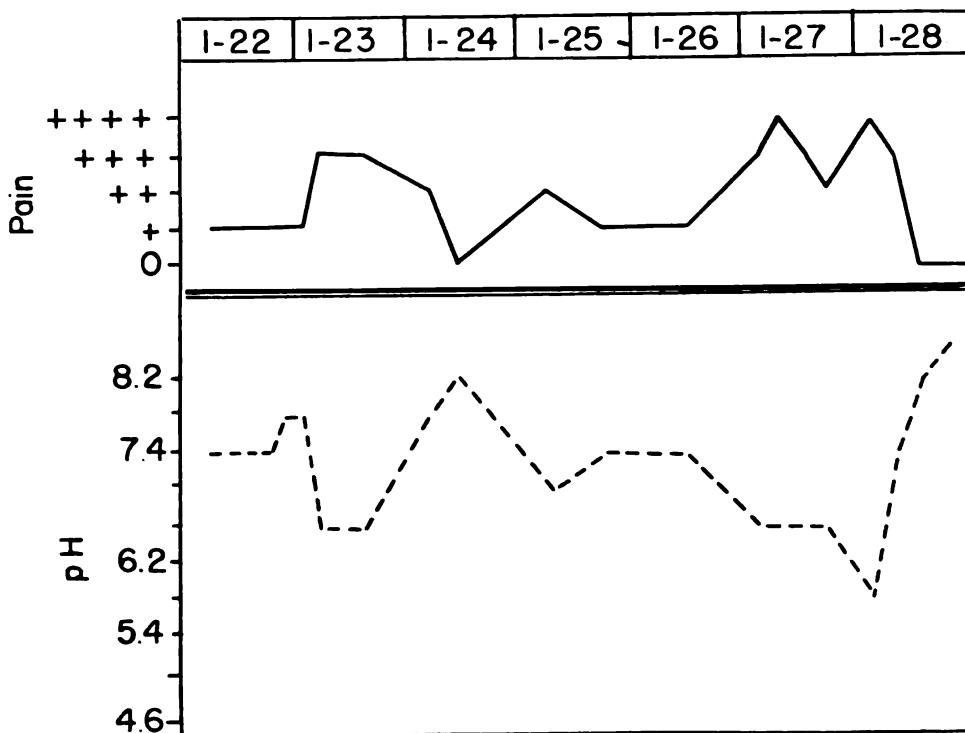
Changes in pain intensity were found to have a similar dualistic correlation with other factors as well as the acid-base balance—with potassium content of blood serum, for example. Studies were made to compare the concomitant changes in pain intensity and in potassium content of blood serum. In several cases the acid and alkaline patterns of these pains were determined through the relationship to urinary pH variations. At different



variations of pain intensity and body acid-base balance, from cancer cases, in which a frank dualism had been seen, to other painful conditions. It was interesting to note that, in many conditions, pain can have one or the other pattern but there are some conditions in which only one pattern is consistently found. Pain following trauma of any kind—the pain of post-

Carcinoma of Rectum

1943



Courtesy of Dr. Mario Rognoni

FIG. 10. The *pain pattern* can be recognized also through the characteristic opposite variations of the curves of pain intensity and urinary pH, followed during successive days—instead of hours—as seen in the above curves of a case of cancer of the rectum. (Courtesy of Dr. Rognoni)

operative and accidental wounds, burns and fractures, for example—always has an alkaline pattern. This is also true for the pain of gallbladder colic. In other conditions, either pattern may be present and must be determined by analysis. For instance, the pain of neuritis and simple headache has an acid pattern in some cases, alkaline in others.

In rheumatoid arthritis, an alkaline pain is almost constantly found. In osteoarthritis, the pain is of an acid type. In arthritic patients in whom

this relationship did not seem to hold, it was possible to recognize not only the existence of both rheumatoid and osteoarthritis but also to note that the pain pattern, as shown by test, was related to the more painful condition. We utilized the diagnosis of the type of pain present as an indication of the nature of painful processes. We will see later how this correlation has been confirmed by therapeutic trials.

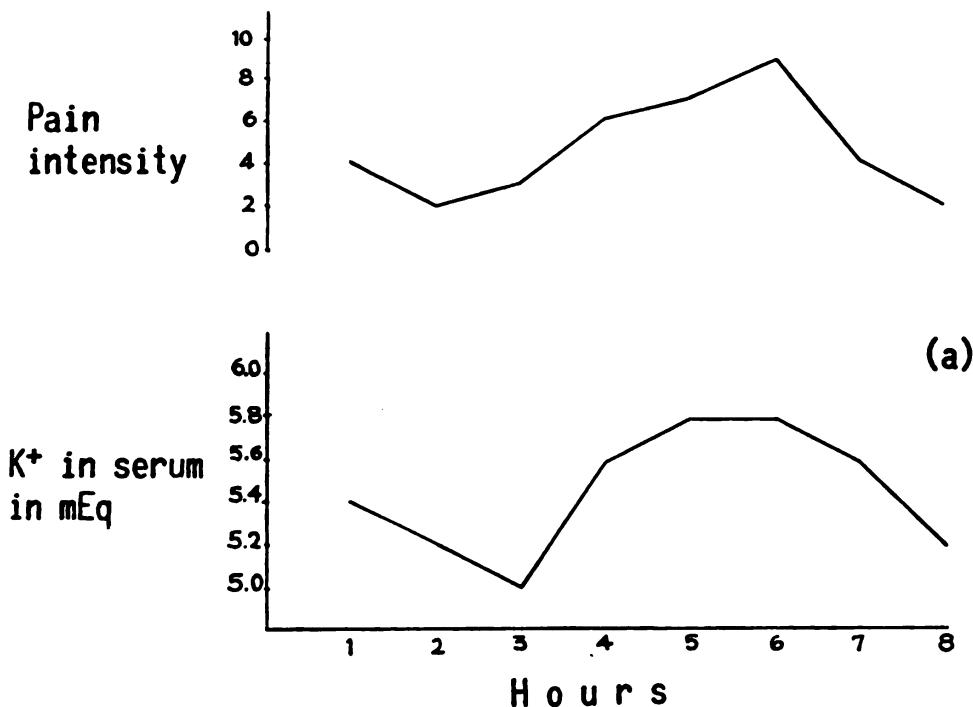


FIG. 11. *Pain pattern and potassium in blood serum.* The comparison of the concomitant changes in the curves of pain intensity and those of the amount of potassium in blood serum shows parallel variations in a case with an alkaline pattern.

Two types of pain associated with two different conditions present in the same individual have been found to occur more frequently than expected, although usually not simultaneously active.

In most patients with two or more anatomically separated painful foci, parallel variations occurred between the curves of the different pains. Only in occasional cases were the two pains found to vary simultaneously but in opposite fashion. Their opposite patterns were well described by patients who observed "the two pains act as if they were part of a balance; when one goes up, the other goes down, and the opposite." In Figure 13, the pain curve of lesion A is seen to vary inversely with the curve of urinary pH, while the pain curve of lesion B is parallel with the urinary pH curve. Thus,

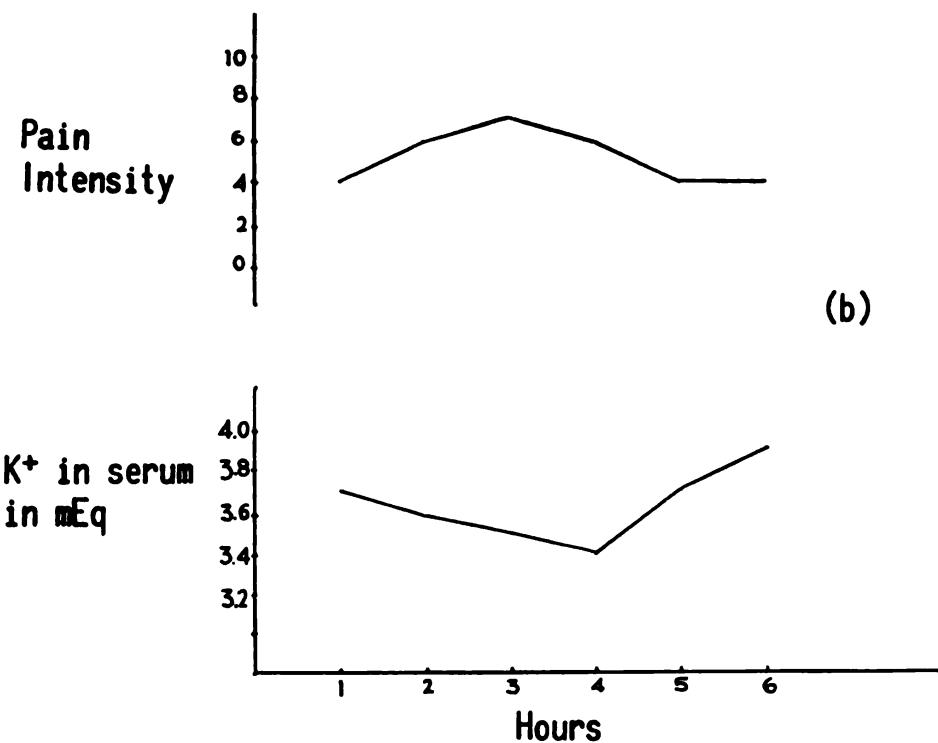


FIG. 12. Divergent variations between pain intensity changes and those of the blood serum potassium in a case of acid pain pattern.

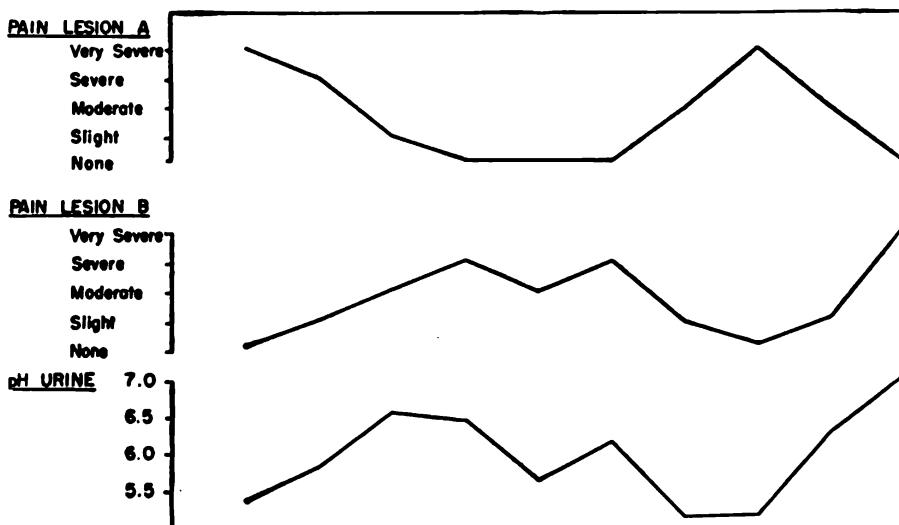


FIG. 13. Pains. Acid and alkaline pains can co-exist on different lesions, as seen in a patient with multiple osseous metastases from breast carcinoma. Lesion A, which corresponds to an acid pattern, shows divergent variations between the curve of urinary pH and that of its pain intensity, while for lesion B, with an alkaline pattern, the variations of the curves of pain intensity and of urinary pH are parallel.

the pain of lesion A is of an acid pattern while that of lesion B is of alkaline pattern.

Also interesting to note is the persistence of the same pattern for pains associated with chronic conditions. We have headache patients, for example, in whom, during the 20 years since we first determined the pattern of pain, there has been no changes of pattern. In others, on the contrary,

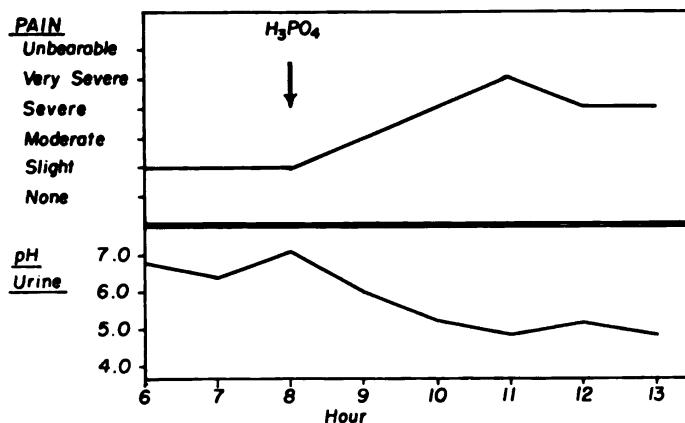


FIG. 14. The changes induced in the pain intensity by the administration of an acidifying agent indicate the pattern present. Pain with an *acid pattern* is intensified following oral administration of 1.5 cc. of a 50% sol. of phosphoric acid. Urinary pH changes reflect the induced systemic acidification.

changes occur rapidly. In a case of sciatica, we have seen rapid and frequent changes in pattern, especially in response to therapeutic measures.

Worthy of being noted is the correlation found between variations in pain intensity and changes in the acid-base balance of the body even in cases in which nerves are directly involved in lesions and in which a mechanical pathogenesis usually has been accepted. This would indicate that the chemical factor mentioned above has a role in the pathogenesis of pain even in these cases.

Acidifying and Alkalizing Agents

We next demonstrated the cause-effect correlation between acid-base changes and variations in the intensity of pain. Administration of acidifying or alkalizing substances could induce the same changes in pain intensity as those caused by spontaneous variations in the acid-base balance of the body.

The relationship between acid-base balance changes and pain intensity was thus investigated by administering strong acidifying and alkalizing

agents to patients after the type of pain-urinary pH correlation had been determined. Administered orally, strong acidifying substances, such as phosphoric acid, ammonium chloride or mono ammonium phosphate, increased the severity of pain with an acid pattern, (Fig. 14) and reduced the severity of one with an alkaline pattern. (Fig. 15) At the same time, it caused a lowering of the urine pH.

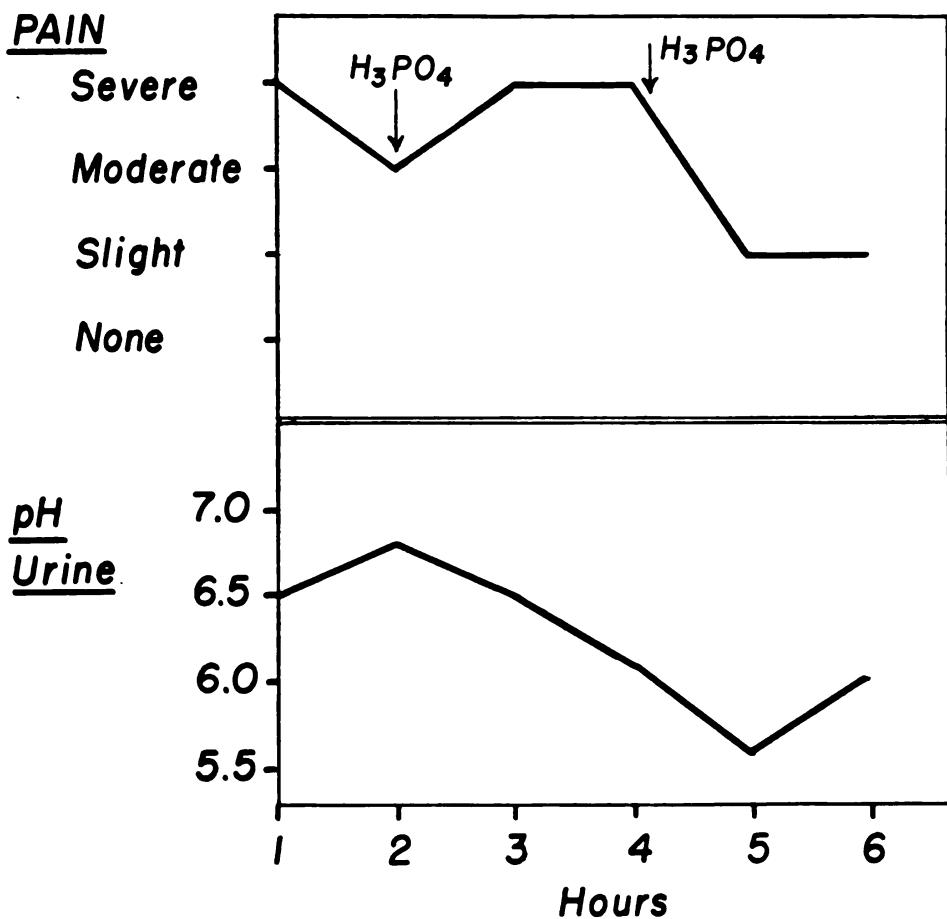


FIG. 15. Pain with an *alkaline pattern* is relieved by oral administration of two doses of 1.5 cc phosphoric acid (50% sol.). Urinary pH changes reflect the systemic acidification.

Sodium bicarbonate or ammonium acetate in quantities that alkalinized the urine increased intensity of pain with an alkaline (Fig. 16) and diminished intensity of pain with an acid pattern. (Fig. 17)

These changes of the systemic acid-base balance induced by administration of strong acidifying or alkalinizing agents explains how similar changes, when they occur spontaneously, affect pain intensity. The effect

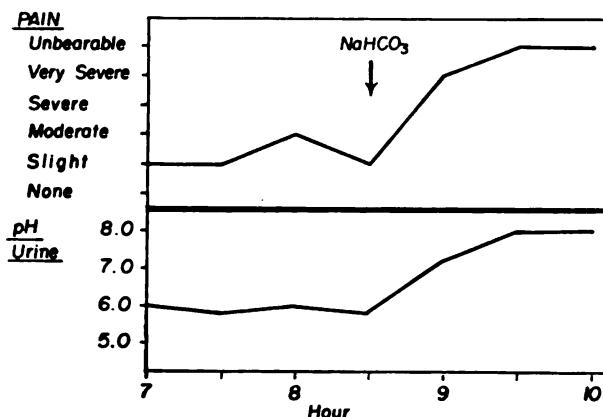


FIG. 16. Pain with an *alkaline pattern* is intensified by oral administration of 5 grams of sodium bicarbonate. Urinary pH changes reflect the systemic alkalinization.

may be to increase or decrease the intensity, depending upon the pattern of the existing pain. It is interesting to mention that similar changes of the systemic acid-base balance, spontaneous or induced by the administration of acidifying or alkalinizing agents, do not influence either the threshold or intensity of physiological pain. We have often utilized this response to alkalinizing or acidifying substances as a method of recognizing the acid or alkaline pattern of pain. (Fig. 18 bis)

Dualism in Local pH Measurements

Since it had been observed that a definite correlation exists between the variations in pain intensity of abnormal foci and changes in the gen-

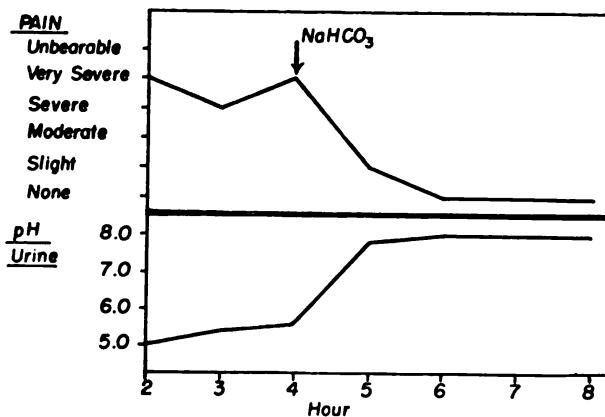


FIG. 17. Pain with an *acid pattern* is relieved by oral administration of 5 grams of sodium bicarbonate. Urinary pH changes reflect the systemic alkalinization.

eral reaction of the body, it was desirable to ascertain what changes were taking place within the abnormal foci themselves at the same time.

Patients with easily accessible superficial lesions, especially tumors, in which painful areas could be well localized, were employed in these experiments. The pattern of pain, acid or alkaline, was first determined in the manner previously described. Local pH determinations were then per-

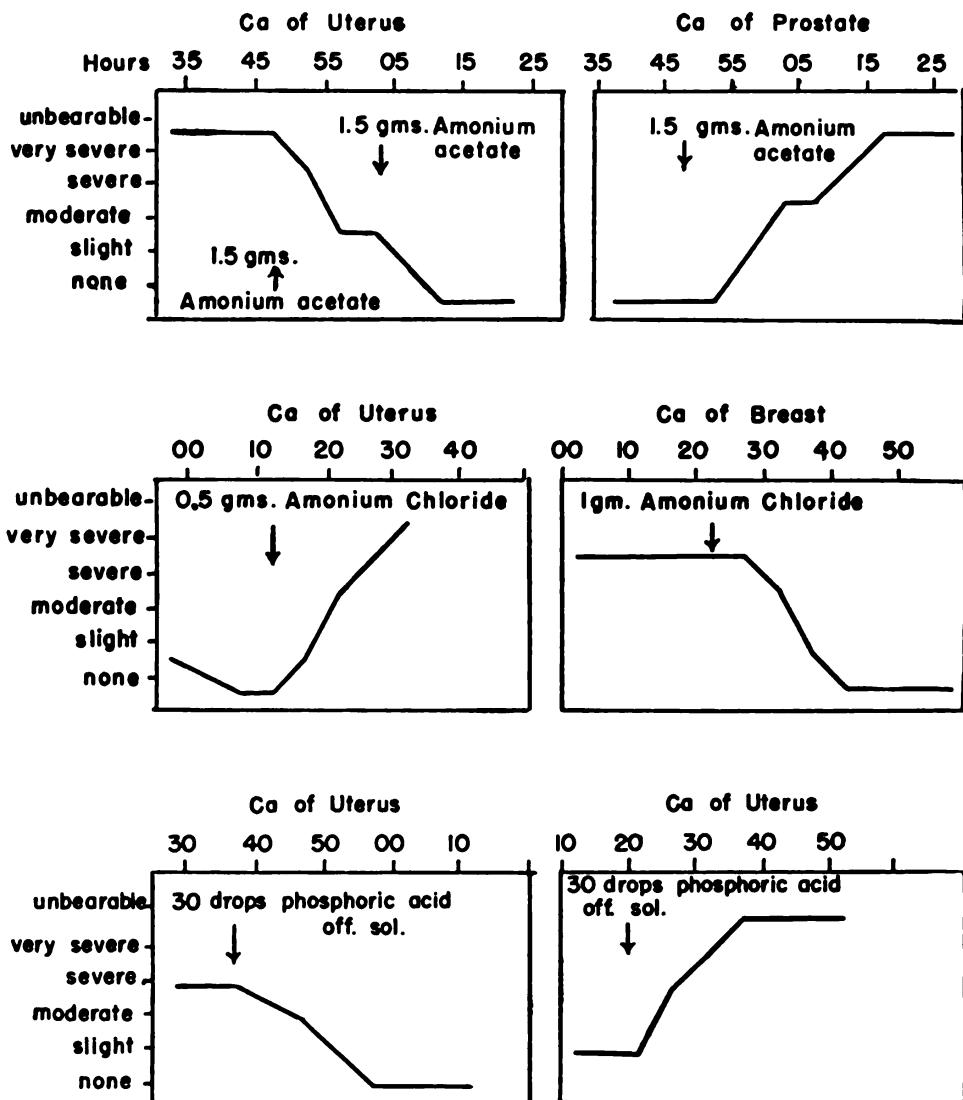


FIG. 18bis. The response of the pain of a lesion to an agent permits to identify the acid or alkaline pattern present. The effect of an acidifying and alkalinizing agent corresponds to an increase or a decrease in pain intensity, according to the pain pattern present. First row, left side—acid pattern, right side—alkaline; second row, left side—acid, right side—alkaline; third row, right side—alkaline, left side—acid pattern.

formed. Special glass electrodes* were used for this purpose and determinations were carried out employing a sensitive pH meter. The tip of the electrode was placed on the surface of the area to be tested if ulcerated, or was introduced into the tissue to be tested through a small incision. In reality this gives a measurement of the pH of the local interstitial fluid.

Urine pH and local pH determinations were then performed at different times corresponding with spontaneous variations in the pain intensity experienced by the patient. Simultaneously, the pH values of normal tissue areas and, when possible, of nonpainful tumor areas were determined. Similar studies were carried out after administration of strong acidifying and alkalizing agents.

Many difficulties were encountered both in the choice of suitable clinical subjects and in techniques. Neoplasms proved to be the simplest type of painful abnormal process to employ. The neoplasm had to be located in a readily accessible region so that the electrodes could be introduced into an ulcerated area or through small incisions. The patient had to be able to very accurately localize the area of pain since considerable differences in pH values were found to exist in different parts of the same lesion. The pain had to be superficially localized because accurate determinations in the depths were not possible. Finally, the complete cooperation of the patient was essential.

The data obtained for a patient with an ulcerated, profusely draining carcinoma of the breast is recorded in TABLE II. Pain was most intense

TABLE II
OBSERVATIONS IN A CASE WITH ALKALINE PAIN PATTERN

Treatment (oral)	Pain Intensity	pH Urine	pH Normal Tissues	pH Tumor
Phosphoric acid 50%, 2 cc.	None	5.4	7.3	7.6
	Moderate	6.2		8.1
	Very severe	7.1	7.4	8.5
Sodium bicarbonate 5 grams	Slight	5.5	7.3	7.9
	Unbearable	7.8	7.4	8.8

when the acid-base balance of the blood, as reflected in the urine pH changes, was relatively most alkaline, and was less intense when the balance was more acid. The pH values of the painful areas of the tumor in

* Supplied by Hartman and Braun, Paris.

this case showed considerable lability under the influence of spontaneous changes in the general acid-base balance of the body, reaching a high of 8.5. At this time, the pain was very severe. The pain became unbearable following oral administration of 5 grams of sodium bicarbonate, and the pH within the same tumor area reached 8.8. The pH of the normal tissues in this case, even after the administration of strong alkalizing agents, never exceeded 7.4, while the tumor pH was never below 7.6.

The findings in a case with acid pain are recorded in TABLE III. This

TABLE III
OBSERVATIONS IN A CASE WITH ACID PAIN PATTERN

Treatment (oral)	Pain Intensity	pH Urine	pH Normal Tissues	pH TUMOR	
				Painful area	Nonpainful area
Phosphoric acid 50%, 1.5 cc. Sodium bicarbonate 5 grams	None	7.0	7.4	6.8	7.2
	Moderate	5.8		6.3	7.1
	Severe	5.3	7.3	5.8	6.9
	Unbearable	5.0	7.3	5.5	6.8
	None	7.4		6.6	7.2

patient had an extensive sarcoma of the face which was not ulcerated but involved the skin. Pain was most intense when the blood titrimetric alkalinity was relatively low and less severe when the alkalinity was higher. The pH values in the tissues of a painful area of the tumor were always more acid than the values found in another nonpainful area. The pH of the nonpainful area was slightly below that within normal tissues. Spontaneous changes in the acid-base balance of the body brought about a reduction of the pH of the painful tumor area to 5.8, at which time the patient reported severe pain. The nonpainful tumor area had a pH of 6.9 at the same time. Following administration of 1.5 cc. of 50% phosphoric acid, pain became unbearable and the pH within the tumor tissues of the painful area fell at once to 5.5. At the same time, the nonpainful tumor area pH was 6.8 and the normal tissue pH was 7.3.

Similar results were obtained in other cases and led to the following conclusions: The pH values of the interstitial fluid of painful lesions studied *in vivo* differ from those of normal tissues, the hydrogen ion concentration being either higher or lower than that of normal tissues of the same individual. The pH of painful abnormal tissues is much more labile and

extremely sensitive to general body pH changes. Spontaneous or induced variations in the acid-base balance of the body produce slight changes or none at all in the pH of the interstitial fluid in normal tissues, but give rise to more pronounced changes of the pH within painful pathological tissues.

Through this research, we have thus been able to connect pathological pain to the pH of interstitial fluid of painful lesions. A further connection could be made with the richness of the interstitial fluids in potassium. In the alkaline pattern of pain, more potassium was found in the interstitial fluids. The presence in these fluids of potassium—the cation of the cytoplasm—in higher amounts than in normal conditions was seen to induce pain. The subcutaneous administration of potassium compounds was seen to be painful, while similar salts of sodium were well tolerated. As a local acidosis was found in lesions having an acid pain pattern, a local alkalosis and an increase in potassium content appeared in those with an alkaline pain pattern. The intensity of pain in this case was found to be proportionate to the degree of abnormal deviation of the local pH and to the abnormal amounts of potassium in the interstitial fluids. In addition to variations in the acid-base balance of the body, variations in the amount of serum potassium appear able to alter pain intensity in cases with an alkaline pattern. This correlation is further explained below by the place of potassium in the organization.

Oxido-reduction Potential

Differences indicating the same dualism were also found in other manifestations in painful lesions. The oxido-reduction potential was measured in tumors with pain. Patients chosen were those with easily accessible superficial tumors in which painful areas could be localized. Platinum needle electrodes were introduced in the painful areas through small incisions or, if the lesions were ulcerated in the lesions themselves. The measurements of the potential present were made using a Beckman pH meter. As had been the case for the pH, it appeared important that the patient be able to indicate clearly the painful areas. Because exact location of these areas in the depths was almost impossible, superficial lesions usually were chosen for these determinations. In general, in lesions with an alkaline pattern, the measurements showed high values (such as from +100 to +350 millivolts) while in lesions with pain of the acid pattern, the values were low (such as -2 to -15 millivolts).

Abnormal Substances

Changes in the local pH, like changes in acid and alkaline patterns, could be related to the appearance and subsequent accumulation of different substances in the lesion. Increased concentration of lactic acid was found in the interstitial fluids of acid pattern lesions, while increased concentrations of sodium and especially potassium ions were found in the interstitial fluid of lesions with an alkaline pattern.

Processes leading to a local acidosis are known and ascribed to the well-known anoxybiotic metabolism of carbohydrates. They result from lack of the "respiratory" oxybiotic phase of carbohydrate metabolism, with the consequent conversion of pyruvic acid into lactic acid. Only part of the lactic acid is changed into glycogen through the Pasteur-Myerhoff reaction, and an accumulation of lactic acid results. While the presence of lactic acid is well known, its presence has not previously been related to pain or other manifestations. We could show this correlation in some cases. (*Note 3*)

The scientific literature offered no information concerning the appearance of alkaline compounds. We were able to establish that the presence of sodium ions coincides with another anomaly found in these lesions—a high fixation of chlorides in the lesions themselves. Values of 1600 mgr. of Cl., or even higher, per 100 gms. of wet tissue were found in these lesions instead of about 400 mgr./100 gms. measured in normal tissues. The local alkalosis and the resulting alkaline pain pattern could thus be correlated with an abnormal sodium chloride metabolism at the tissular level in which with chloride ions fixed by the cells, sodium ions remain free to combine with carbonate anions and form alkaline compounds. If the abnormal NaCl metabolism occurs in the interstitial fluid, the subsequent alkalosis induces pain. We will see later how abnormal NaCl metabolism also takes place at other levels of the organization. In these cases of alkaline pattern of pain, the fact that abnormal amounts of sodium ions still enter the cells will result in a loss by these cells of potassium which will accumulate in the pericellular fluids, and form alkaline compounds.

An immediate conclusion that could be drawn from these studies was that there is a definite dualism in pathogenesis of pain originating in abnormal tissues and that the two pain patterns evidenced in lesions with acidosis or alkalosis of the interstitial fluids indicate that processes of two opposite natures go on at the tissular level.

OTHER ACID AND ALKALINE SYMPTOMS

Using the same method of investigation as in the study of pain, a relationship between variations in intensity of certain other symptoms and variations in urinary pH could be found. Acid and alkaline patterns of itching could be recognized. The same patterns could be found for vertigo and impaired hearing. Among psychiatric manifestations, manic-depressive states showed changes that could be related to acid-base variations.

The correlation between dyspnea and acid-base variations was contrary to what we expected. Classically, dyspnea is believed to be associated only with an increase of acidity of blood. However, both acid and alkaline patterns of dyspnea were observed, and could be related more directly to changes at the tissue level. We will review here the pathogenesis of itching, vertigo, dyspnea, and other conditions, under this dualistic aspect.

Itching

The problem of itching is interesting from other than the therapeutic point of view, since little is known regarding the nature and cause of this condition. Various hypotheses have been offered but have failed to explain its pathogenesis. Our interest in itching originated during the study of pain and led to an hypothesis which allows us to approach the problem from a new angle. By analogy with pain, we separated itching into two types: physiological, as the response of normal tissues to external stimuli; and pathological, as a sensation arising within diseased or damaged tissues. Tickling the normal skin or even stimulating it through heat, cold, etc., may cause a sensation of itching and lead to scratching. Certain mucous membranes, such as those of the nose, and the skin around natural orifices, are especially sensitive to such stimulation. This type of itching, as a response of normal tissues is *physiological or sensorial*.

Under pathological conditions however, the skin, mucous membranes and other formations may itch without external stimulation or in response to stimuli which ordinarily do not produce this sensation. The itching then can be considered as a manifestation of diseased or damaged tissues and, as such can be described as *pathological*. Just as does pathological pain, pathological itching represents a symptom related to abnormal changes already present. (*Note 4*)

In spite of their relative independence, the fact that similar fundamental mechanisms are involved in the production of itching and pain explains certain characteristics they have in common. Like pathological

pain, pathological itching varies in intensity with the time of day. Patients with chronic pruritus are aware of this. Some have more itching in the morning, others experience exacerbations at night. The same dualism is seen with intake of food. For these reasons, the relationship between changes in acid-base balance of the body, as reflected in the urinary pH, and variations in intensity of itching was investigated.

Patients with long standing pruritus associated with a variety of chronic skin conditions were studied. They were asked to note over periods of six to twelve hours the changes in itching intensity. Evaluation of the changes was made by the patients themselves, using a series of qualifications such as none, slight, moderate, severe, very severe and unbearable, or a scale ranging from 0 to 10. They were instructed to consider the average inten-

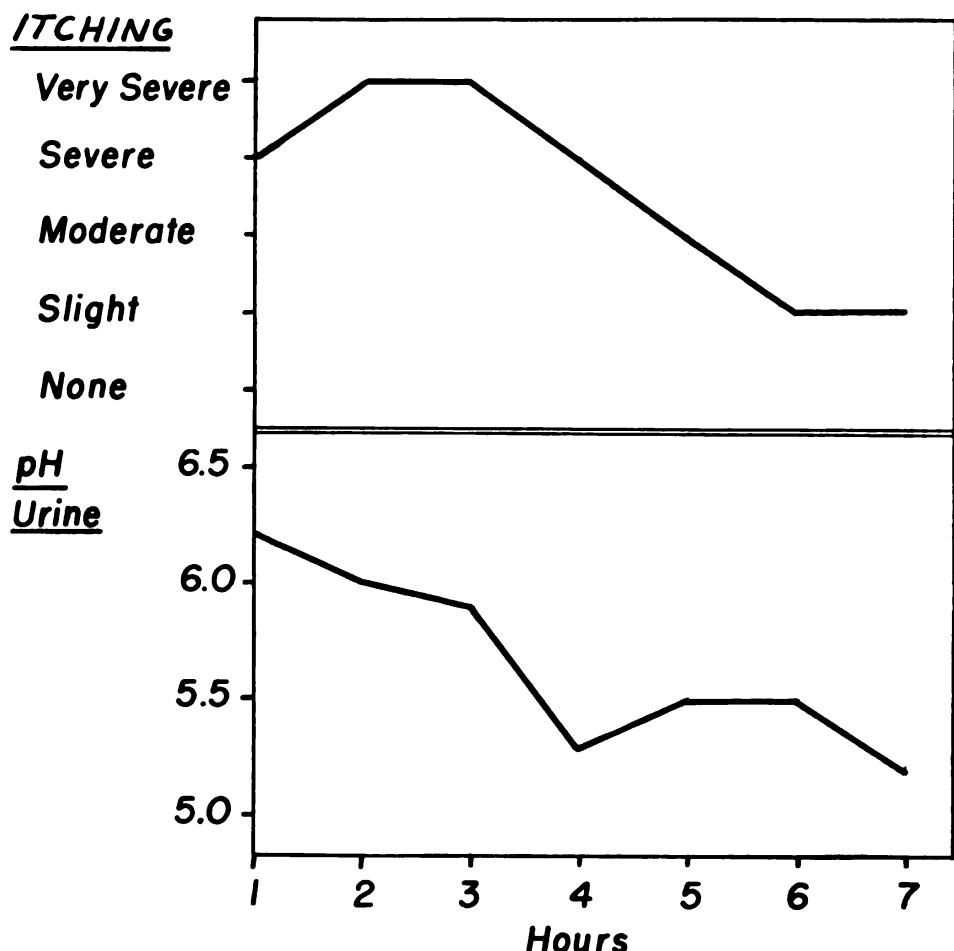


FIG. 20. *The alkaline pattern of itching is recognized in a case of senile vulvar pruritus, through parallel variations of the curves of the intensity of the itching and of the urinary pH.*

sity of their itching for each hour, rather than to indicate the maximum intensity at the exact time of recording. Voided urine specimens were obtained at the end of each hour. The pH of the urine specimens was determined electrometrically. A graph was plotted to compare the variations in the subjective data furnished by the patient with the concomitant hourly changes in the pH of the urine. It was usually necessary to repeat the test several times before the patient appeared able to satisfactorily evaluate the changes in the intensity of the symptom for hour-long periods rather than

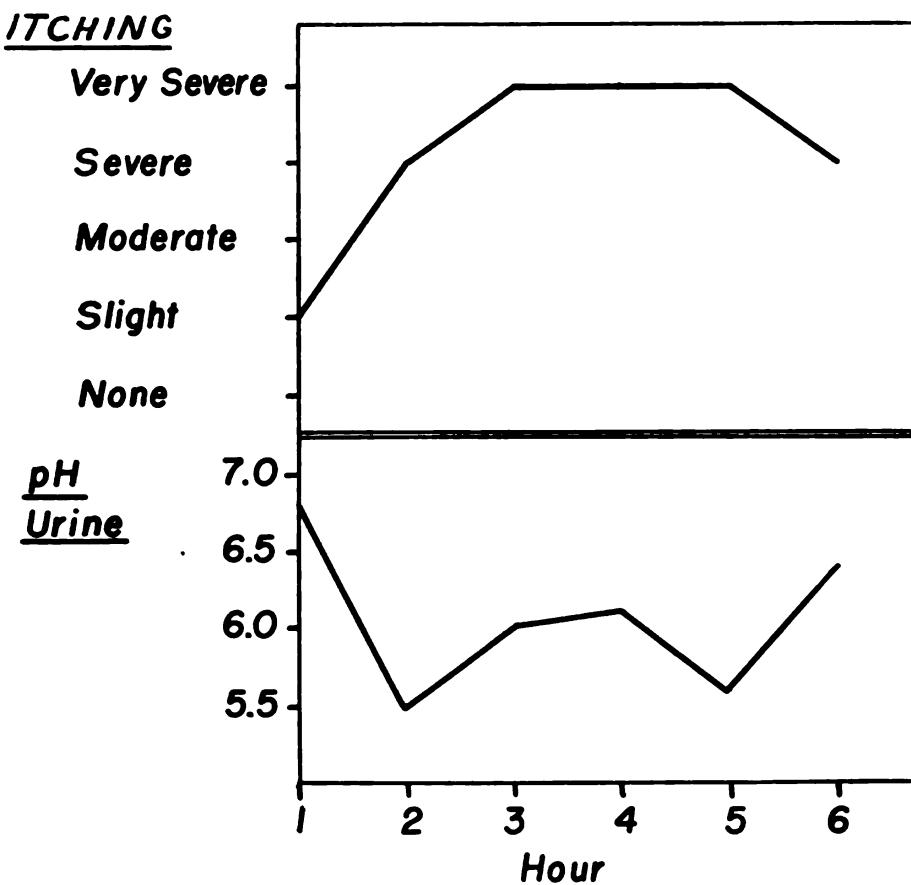


FIG. 21. *An acid pattern of itching* is recognized in a case of pruritus ani through the divergence in the concomitant variations of the curves of itching intensity and urinary pH.

for just the moment of recording. (We also tried to judge the intensity of itching through the frequency, intensity and duration of scratching, as noted by an observer, but without success.) Fifteen patients were studied and, because of the limited number, the results are presented as merely preliminary.

In four cases, the curves of itching and urinary pH did not show any definite correlation even after repeated tests. Of the remaining 11 cases, 7 showed a distinct parallelism of the two curves, and in the other 4, an inverse relationship between curves was apparent. The graphs obtained in two characteristic cases are presented here. (Figs. 20 and 21)

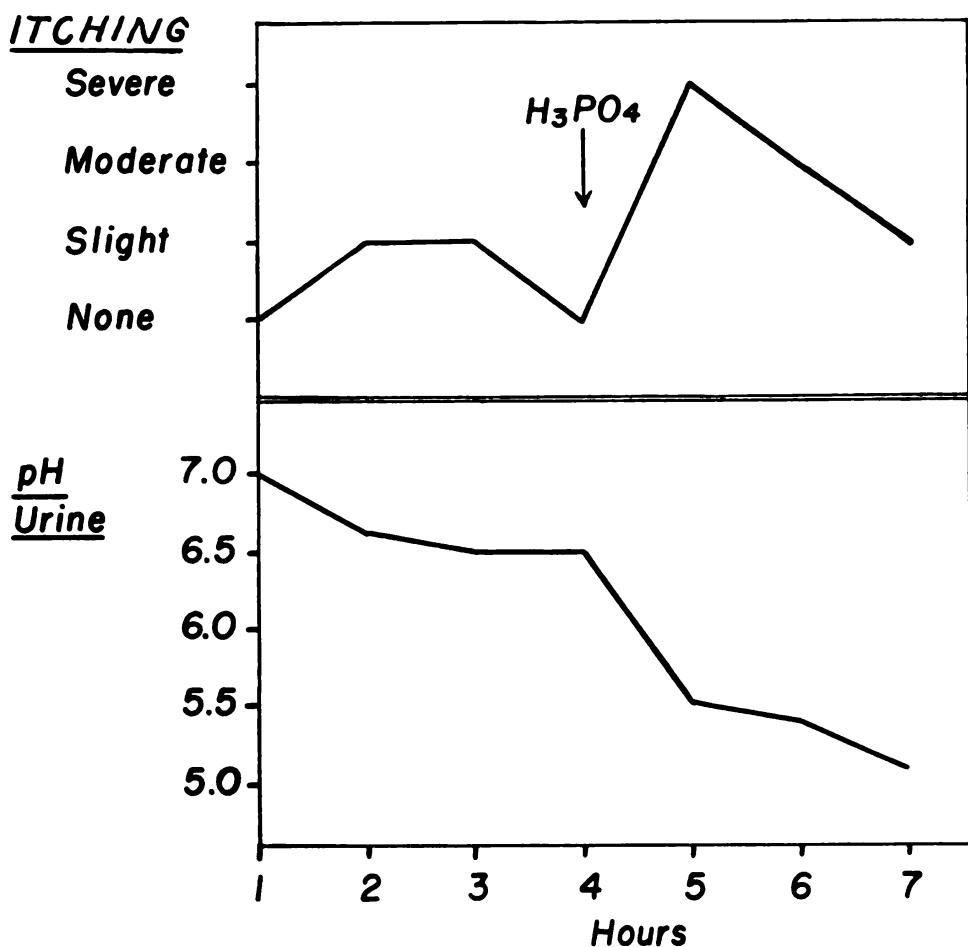


FIG. 22. Administration of phosphoric acid—1.5 cc phosphoric acid, sol. 50%—induces together with an acidification of the urine, an increase in the intensity of the itching with an *acid pattern*.

There is a distinct parallelism between the two curves in Figure 20, indicating that itching was more intense when the urine was relatively more alkaline, and slight or absent when the urine was more acid. We have considered this as an "alkaline pattern" of itching in accordance with the designation for pain. An inverse relationship between the two curves is seen in Figure 21. In this case, itching was more intense when the urine

was more acid, and less severe when the urine was more alkaline. This represents an acid pattern.

The effect of a strong acidifying agent, phosphoric acid, in cases with acid and alkaline itching, is illustrated in Figures 22 and 23. The intensity of the alkaline itching in the first case was reduced by the acidifying action of phosphoric acid, while the acid itching of the second case was intensified. In Figure 24, the response of a patient with alkaline itching to the administration of sodium bicarbonate is shown. The intensity of itching was greatly increased after the alkalinizing agent was given.

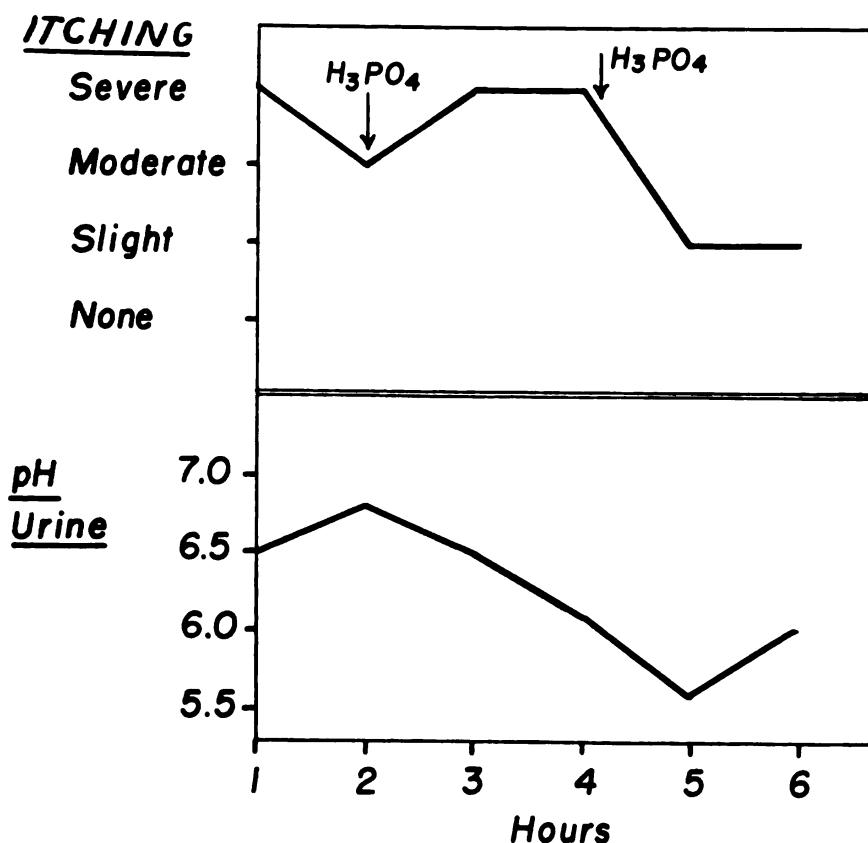


FIG. 23. Itching with an alkaline pattern is reduced in intensity by the administration of an acidifying agent. In the above example, two doses, each of 1.5 cc phosphoric acid (50%), were necessary in order to obtain this effect.

The fact that both pathological pain and pathological itching undergo the same changes in intensity related to the general acid-base balance would indicate that a similar mechanism may be involved in the pathogenesis of both symptoms. It can be conceived that a slight local pH change confined to the skin or mucous membrane could act on the itching end organs and

evoke the sensation of itching. More intense pH would result in pain. The fact that itching, one of the principal symptoms of dermatological conditions, can be related to local acid and alkaline changes within the skin would permit the integration of skin pathology in a more general physiopathological mechanism. The concept of the intervention of two different

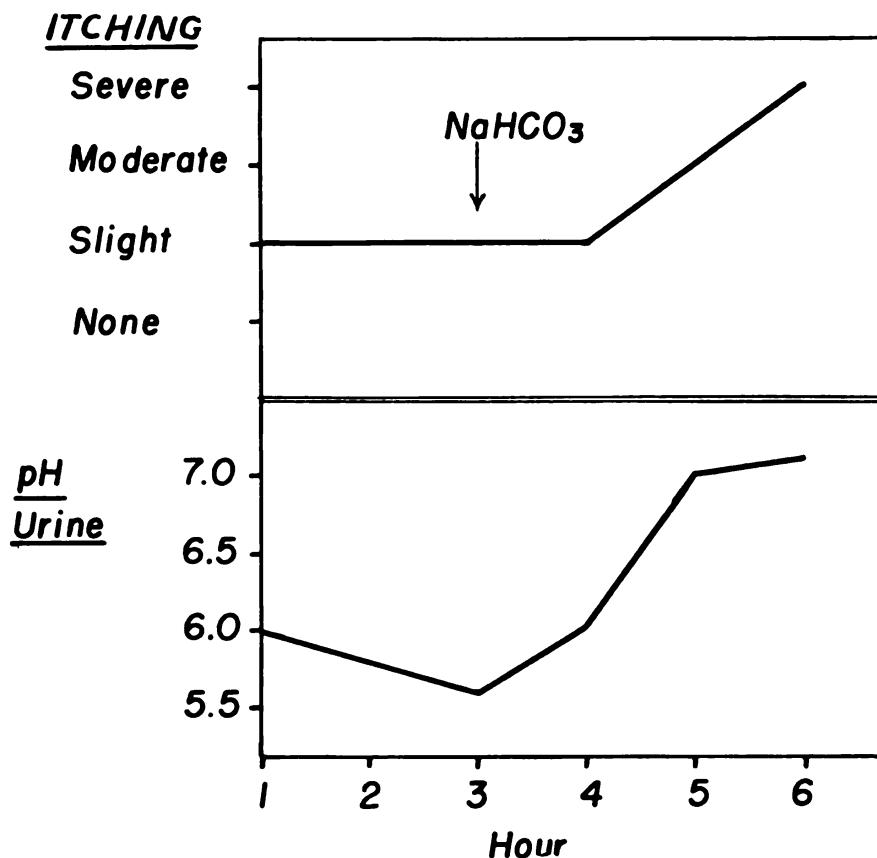


FIG. 24. The administration of 4 grams of sodium bicarbonate to a subject with an *alkaline pattern of itching* induces together with the alkalinization of the urine, exacerbation of the symptom.

abnormal processes, one resulting in acid substances and the other in alkaline compounds, represents a new approach to the study of many skin conditions. The therapeutic application of this concept has produced interesting results. (See Chapter XIV)

Vertigo

Vertigo has been of special interest in our research. While it can be induced by various etiological factors, a basic dualism in its pathogenesis is evident.

Many patients with vertigo have been found to experience wide variations in the intensity of the symptom. Some have exacerbations in the morning, others in the evening. This observation suggested a possible dual pattern and we investigated vertigo by the same method used for the study of pathological pain.

In patients with vertigo, the intensity was determined at hourly intervals by using either a scale ranging from 0 to 10, or a series of qualifica-

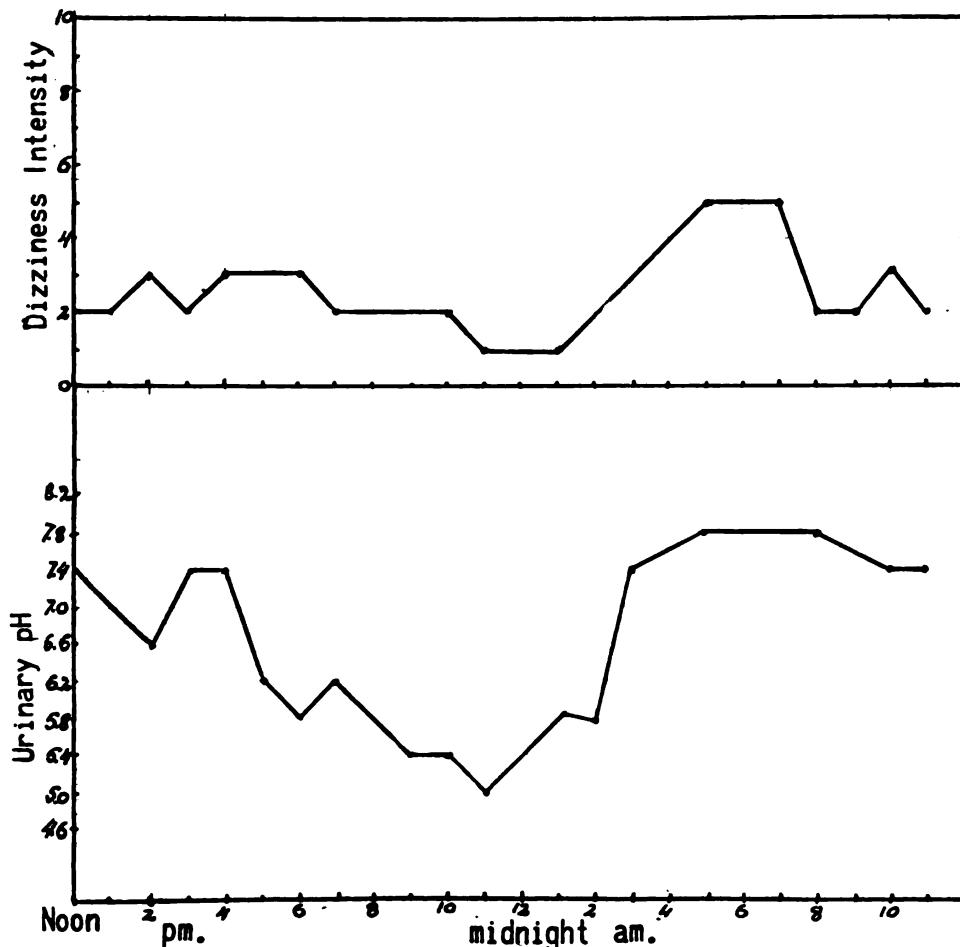


FIG. 25. Shows the curves of the intensity of *vertigo* and of the urinary pH in a case with an *alkaline pattern*, the variations of the two curves being parallel. (B. Welt, AMA Archives of Otolaryngology, 58:273-300, 1953.)

tions, such as none, slight, moderate, severe, very severe and unbearable. At the same intervals, urine samples were collected and their pH values measured. Data were plotted in curves having time as common abscissa. Here again, as for pain, two different correlations could be observed. In some cases, the two curves—one for intensity of vertigo, and the other for urinary pH level—showed parallel variations. (Fig. 25) The vertigo became more intense when the pH was high, and this was considered to be vertigo

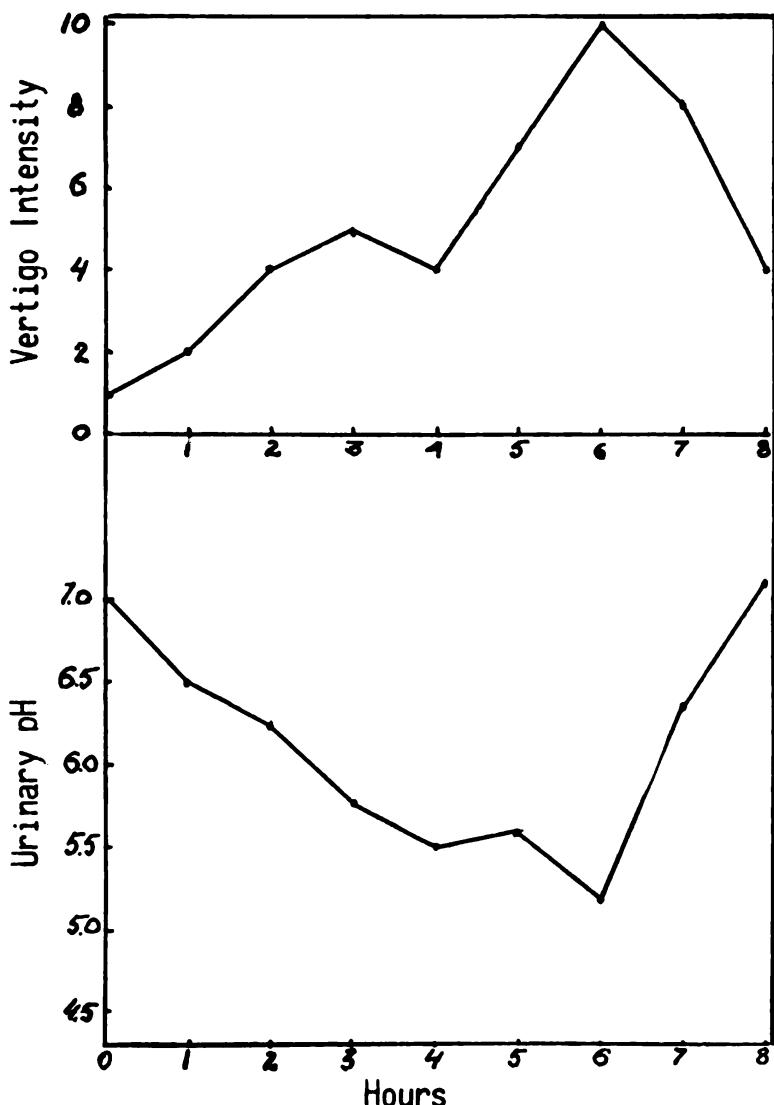


FIG. 26. *Vertigo with an acid pattern.* The symptom is more intensive when the urinary pH values are lower. (B. Welt AMA Archives of Otolaryngology, 58:273-300, 1953.)

of an alkaline pattern. In other cases—of an acid pattern—vertigo was more intense with lower values of urinary pH. (Fig. 26)

The acid-base pathogenesis of vertigo was further confirmed by the response to acidifying and alkalinizing agents. B. Welt has widely investigated vertigo by this method. He also has used the response to therapeutic agents as a criterion for the pattern present. The administration of butanol, heptanol or unsaturated fatty alcohols was seen to induce an increase in the intensity of vertigo of acid pattern and a decrease in the

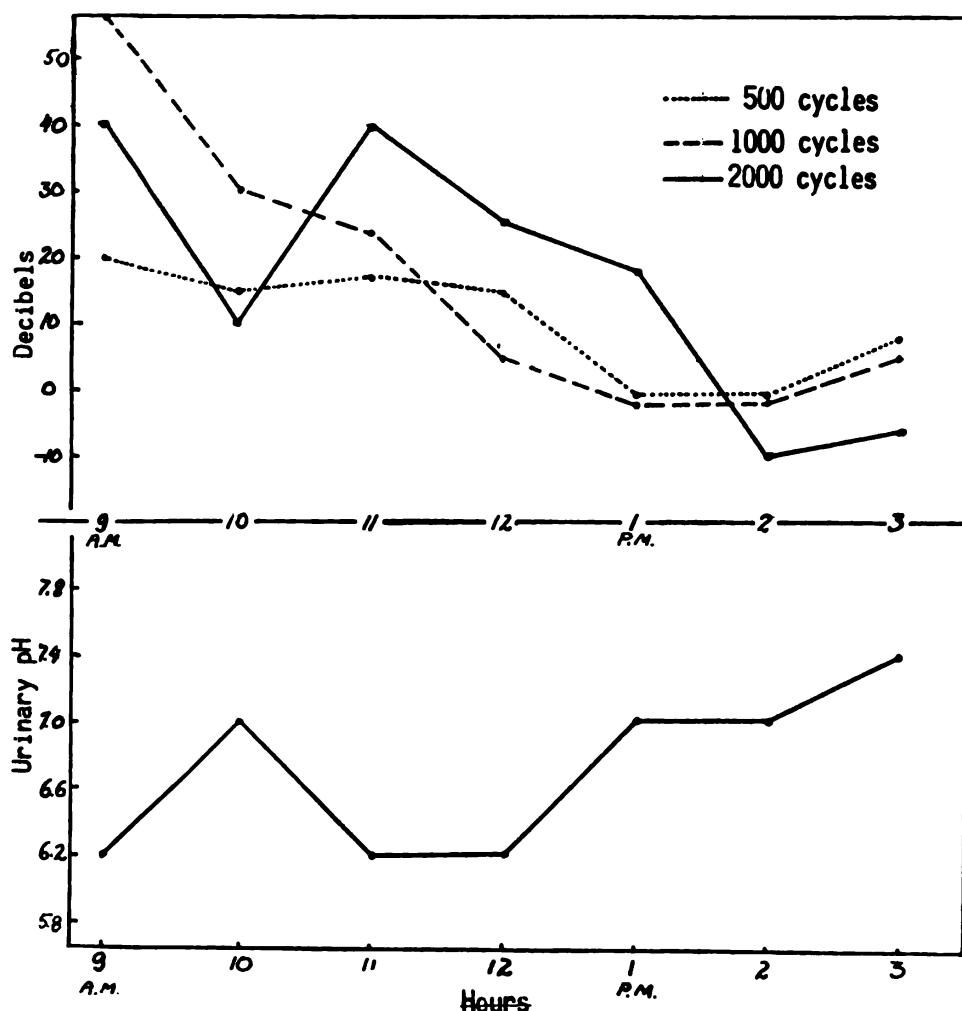


FIG. 27. The analysis of the concomitant changes in the curve of the intensity of the *auditive acuity* and of the urinary pH shows opposite variations in a subject with impaired hearing. The opposite variations are especially manifest for some values—2000 cycles in this case. This relationship corresponds to an *acid pattern*. (Courtesy of Dr. B. Welt.)

alkaline type. Agents such as sodium thiosulfate have an opposite effect.

The great similarity in the response of pain and vertigo to the same agents has established the role of acid-base changes in the pathogenesis of vertigo as well as the possibility of influencing these changes in order to relieve the symptom. These studies have shown that, in spite of the variety of etiological factors which can induce vertigo, the condition can be considered, from the point of view of therapy, in terms of its dualistic pathogenesis. This has simplified the therapeutic approach, limiting it to a choice between only two groups of agents. We will see later how successful this approach has been in Welt's hands.

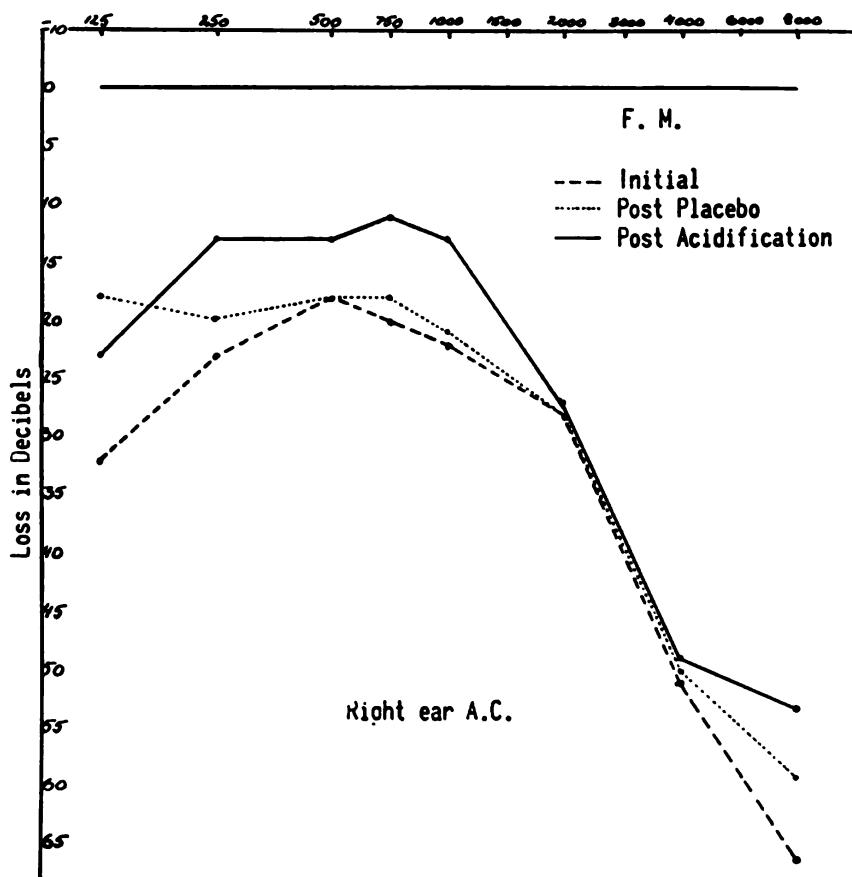


FIG. 28. The administration of an acidifying agent—2 grams of monoammonium phosphate—induces a marked increase of the *hearing acuity* if the abnormality present corresponds to an alkaline pattern. Only minimal or no changes are seen to occur if a few drops of acetic acid are used as placebo. (Courtesy of Dr. B. Welt.)

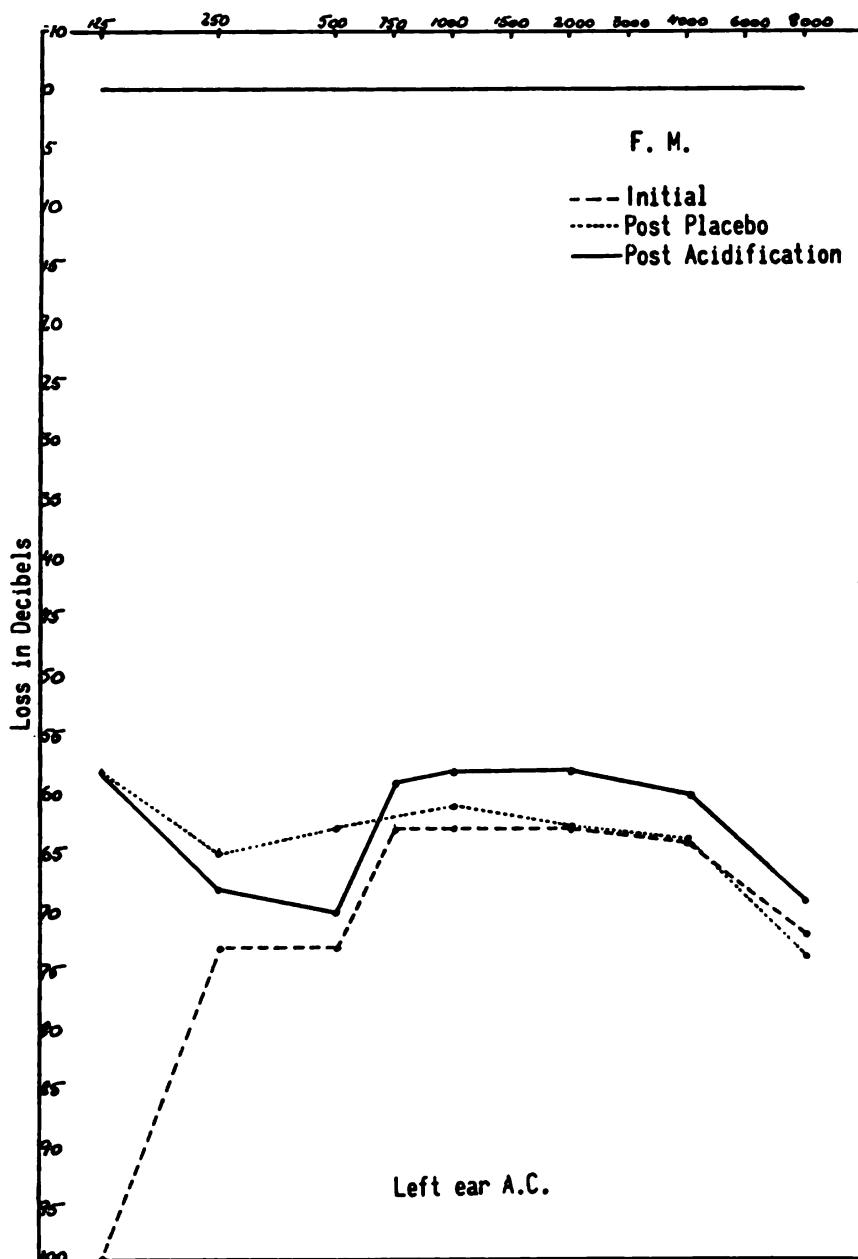


FIG. 29. Alkaline pattern of hearing impairment corresponds to an increased hearing acuity following the administration of 2 grams of ammonium phosphate.

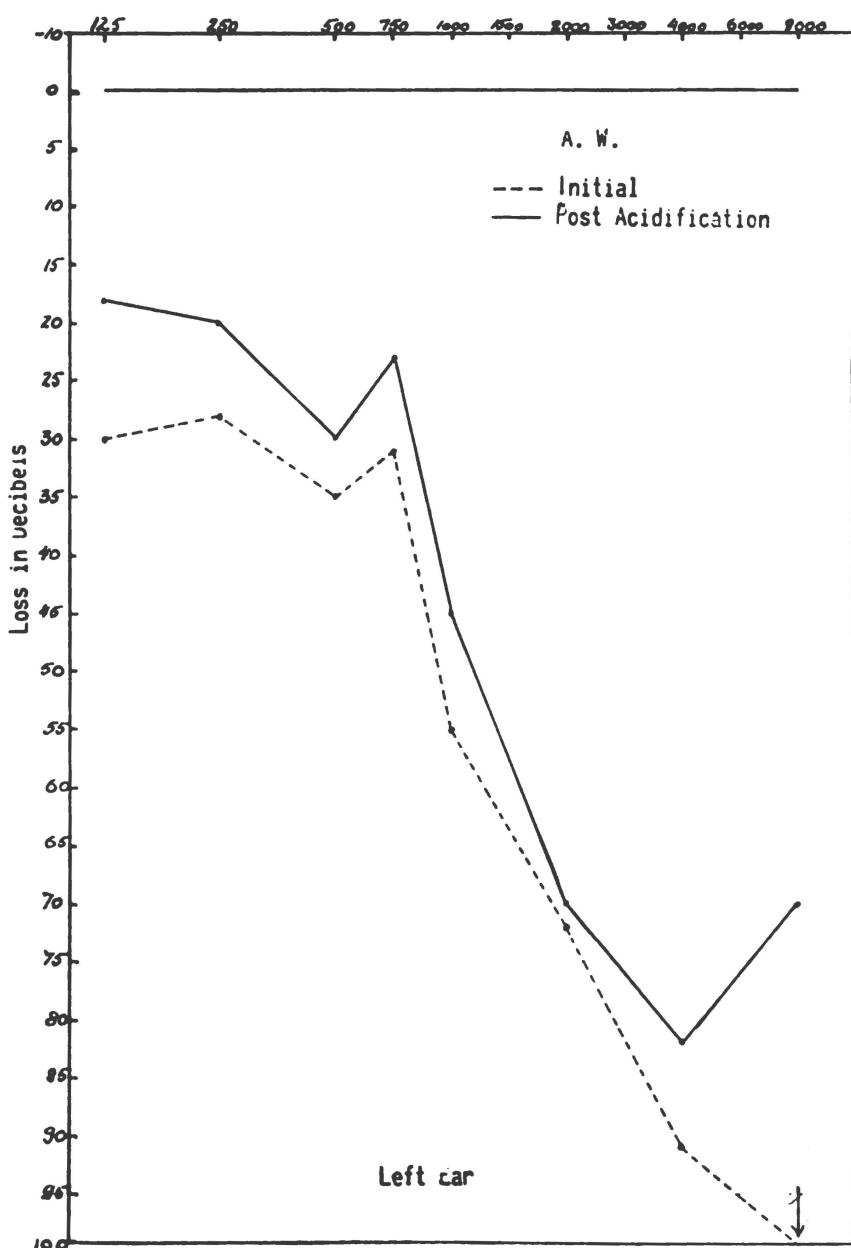


FIG. 30. Alkaline pattern of hearing impairment.

Impaired Hearing

In studying the various otological problems, B. Welt and I noted that many patients with impaired hearing experienced variations in auditive acuity at different times of the day, or even in conjunction with food intake. This led us to investigate impaired hearing by the same method used for pain. (Fig. 27) We studied the influence of acidifying and alkalizing agents upon auditive acuity in cases of impaired hearing.

Complete audiograms were obtained, employing differences of only two decibels between measurements and using all of the accepted frequencies for air and bone conduction. Audiograms were obtained in subjects before and after administration of acidifying agents, such as ammonium

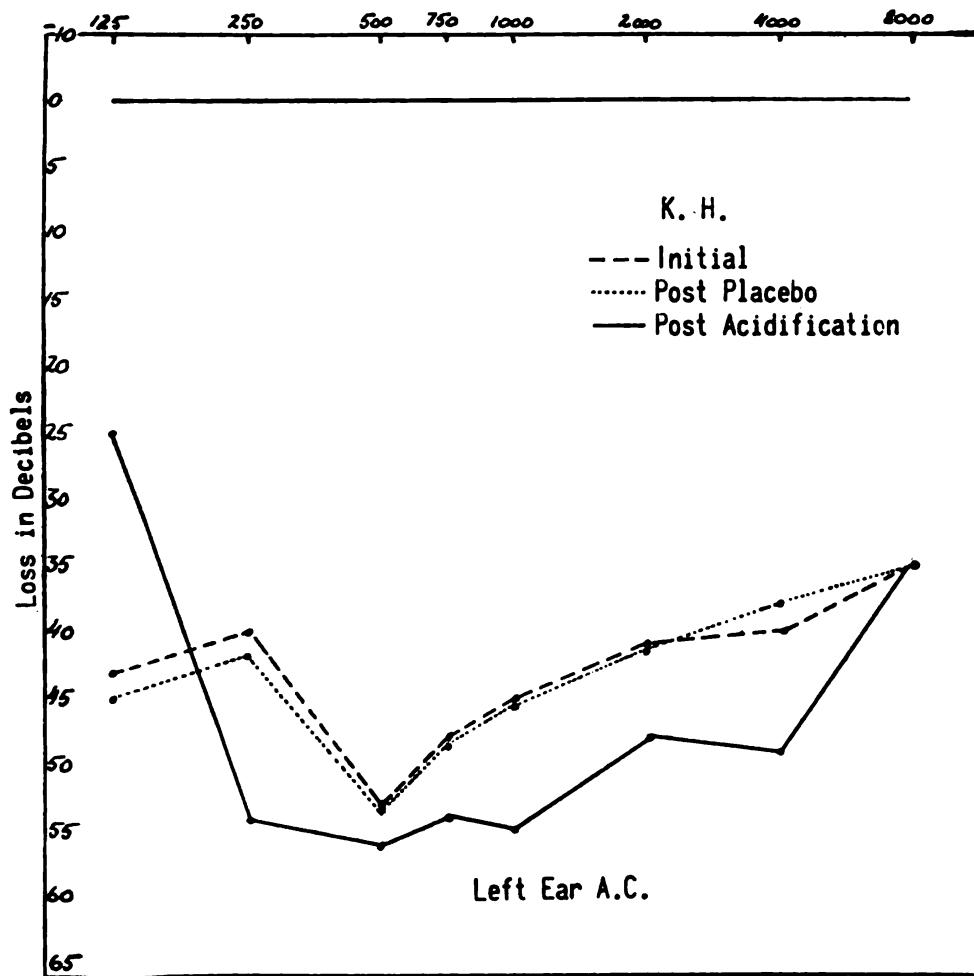


FIG. 31. The administration of 2 grams of monoammonium phosphate induces a decrease in hearing acuity if the abnormal pattern is acid.

chloride and ammonium monophosphate, and alkalinizing agents such as sodium bicarbonate. In normal subjects, the audiograms showed little or no change. In subjects with impaired hearing, three types of responses were noted for any one agent. The audiogram was either not changed at all or an increase or decrease in acuity was seen. If a manifest increase in acuity

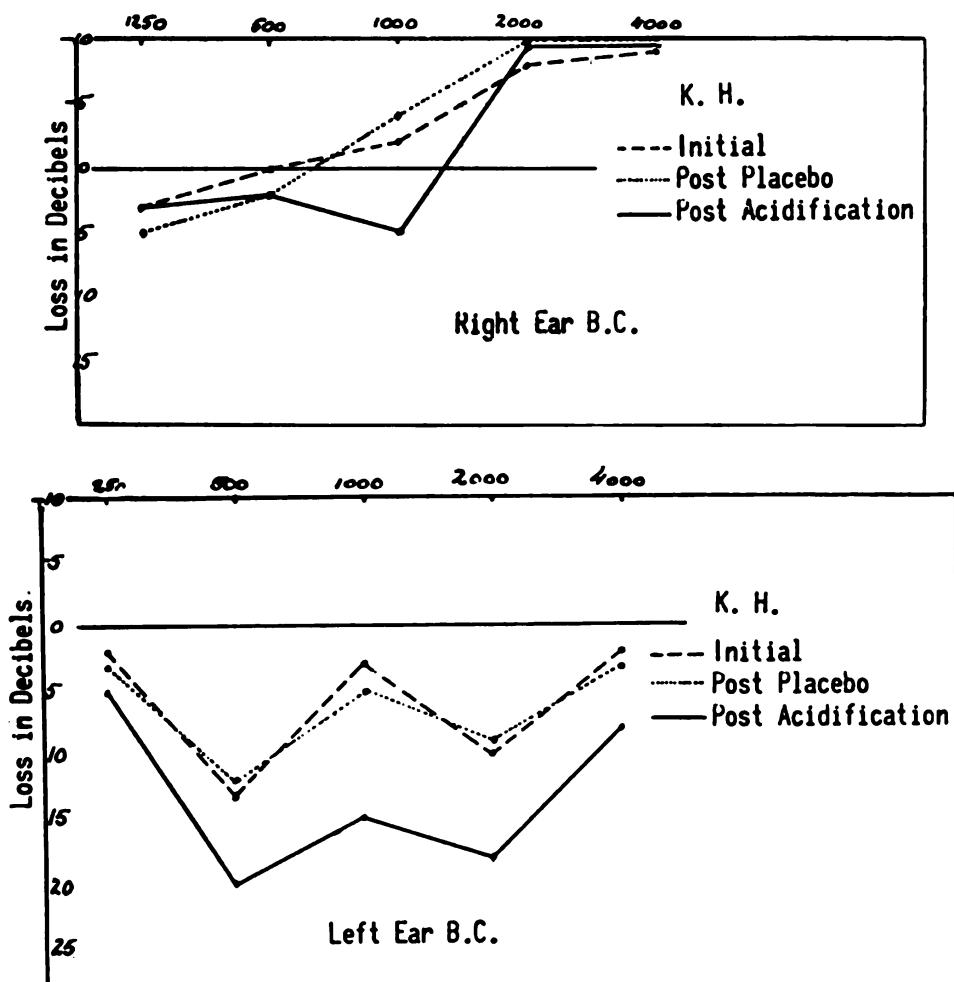


FIG. 32. Acid pattern of hearing impairment.

was obtained with one group of agents, an opposite effect was obtained when an agent of the opposite group was administered.

Changes of at least 6 decibels were required before they were considered to be induced by an agent. When changes of such intensity were obtained with an agent, it was invariably true that opposite changes would be obtained with an opposite agent. It was also true that the same responses

could be obtained in the same patient in repeated tests. This appears to be highly significant, indicating that the response was, in fact, correlated with changes induced by the administered agent, changes similar to those seen in the case of acid-base symptoms. It was thus possible to integrate hearing impairment in the group of acid-base symptoms and to recognize two well-defined types, one corresponding to an acid pattern, the other to an alkaline. Figures 28 through 32 illustrate several cases taken from Welt's observation. It must be noted that changes under the influence of the agents are seen at almost all frequencies in some cases but at only certain frequencies in others.

It must be noted also that not all cases of impaired hearing could be placed in one or the other category. While this could be done almost without exception for young subjects or for those with still evolving conditions, it could not be done for subjects with old, fully evolved impairment. It appears that once the pathological processes have arrived at a terminal point—and an inactive sclerotic scar is present—a response to acidifying or alkalinizing agents is no longer to be expected.

Manic-depressive Condition

We have studied the relationship between intensity of manifestations and systemic acid-base balance of the body in another group of subjects with various mental disorders. Of all the cases studied, the only condition in which a clear relationship could be shown was in manic-depressive subjects. From patients presenting changes during the day, passing from periods of high excitation to calm, from deep depression to calm, we obtained hourly urine specimens. At the same time, observations of their mental state were made. The evaluations of mental condition were made by trained observers or members of the family using a conventional scale which permitted translation into graph. The pH of the urine samples was measured electrometrically. Curves of the pH and of the mental conditions were plotted having the common time as abscissa. A striking correlation between the two curves was found in the first investigations in manic patients. With the pH of the urine at higher values, the patient was calm, while the more acid urine corresponded to periods of intensive agitation. Figure 33 shows an example.

This correlation also could be demonstrated by administering acidifying and alkalinizing substances to manic patients. Acidification through the administration of phosphoric acid was followed by a manifest increase in agitation while administration of an alkalinizing agent—sodium bicarbonate

—was followed by a period of calm. An opposite but less evident correlation was seen for several depressed patients. We must mention that the usual difficulty of judging accurately the degree of depression from one hour to another can explain this lesser correlation. Manic manifestations were much more readily evaluated.

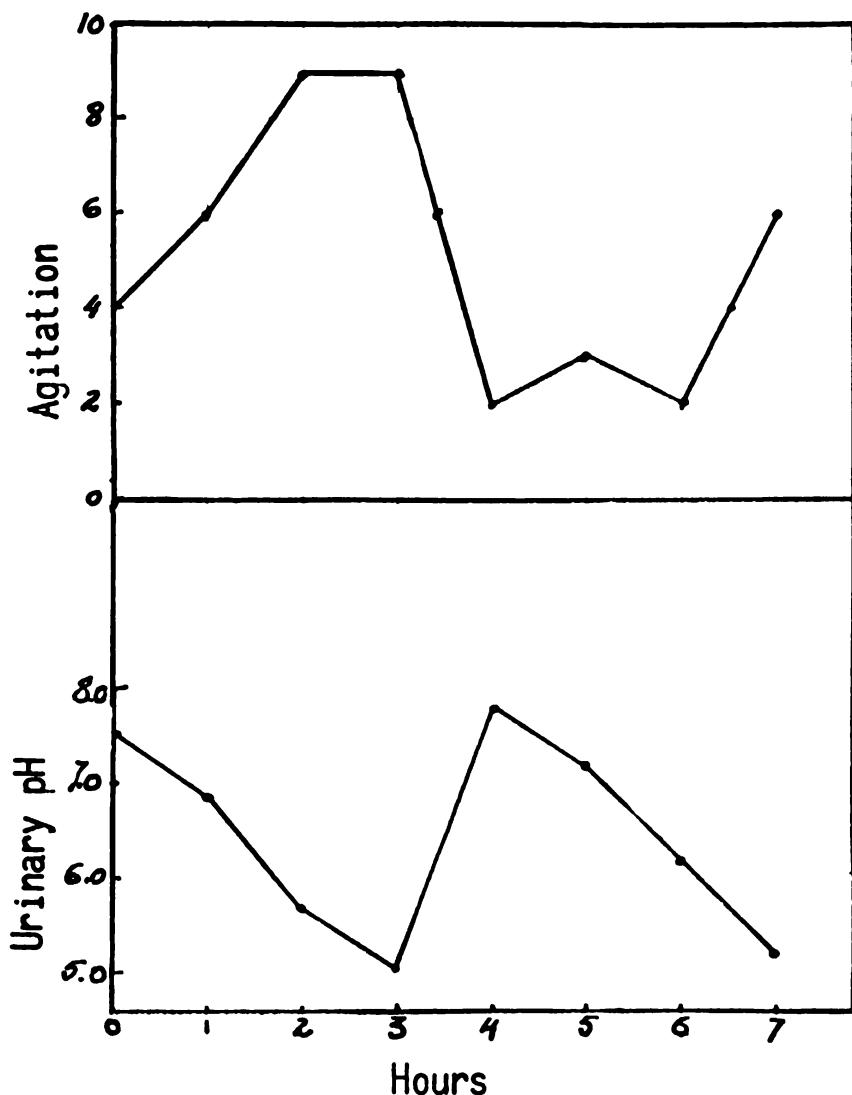


FIG. 33. The variations of the curve of the *agitation* of a 10-year-old boy are opposite to that of the urinary pH, indicating an *acid pattern*.

Dyspnea

Certain characteristics of dyspnea suggested that this symptom might be explored by the same means employed in studying pain associated with pathological tissue changes. A regular pattern is observed in certain types of dyspnea related to the time of day. Cardiac asthma or paroxysmal cardiac dyspnea occur mainly at night after patients have gone to sleep. However, this is not due to the recumbent position since patients may assume this position during the day without any ill effect. Harrison's "evening dyspnea" (20) is characteristically absent in the morning, but develops slowly during the course of the day, reaching a maximum in the evening.

Just as for pain, the degree of intensity of dyspnea was studied in relation to hourly acid-base balance changes as indicated by changes in the pH of the urine. Patients having dyspnea of prolonged duration as a result of various pathological conditions, with no treatment of any kind for at least six hours before or during the period of observation, were the subjects of this investigation. The degree of intensity of the dyspnea was estimated by trained observers who were in constant attendance. Dyspnea was recorded by the observers as absent, slight, moderate, severe, or very severe, estimations being based upon rate depth and evident difficulty in respiration.

At the time of these observations, hourly urine specimens were obtained from patients with as little disturbance as possible. No patient showed evidence of renal disease. The pH of the urine specimen was determined potentiometrically. The curves showing hourly fluctuations in the intensity of the dyspnea and the changes in the pH of the hourly urine specimens were then plotted and compared. Acidifying and alkalinizing substances were administered to patients during the course of some tests in order to observe the influence of induced changes in the acid-base balance upon the degree of dyspnea. Phosphoric acid and sodium bicarbonate were used for this purpose.

Fourteen patients with different pathological conditions were studied. In ten cases, a distinct correlation between the intensity of the symptom and acid-base variations was found.

Four patients had pulmonary edema associated with the symptom of dyspnea. One patient had edema due to congestive heart failure; another had pulmonary edema and lung metastases from a carcinoma of the pancreas. Two other patients with cancer of the breast metastatic to the bone and skin also had pulmonary edema. In one of these, the pulmonary edema appeared to be a result of the accidental introduction of a fatty acid in oil preparation into the blood stream following an intramuscular injection. In

all four of the cases with pulmonary edema, the intensity of dyspnea was found to be increased when the urinary pH showed changes toward more alkaline values and was diminished when the pH changes were opposite. In these cases, the intensity of the dyspnea was relieved following admin-

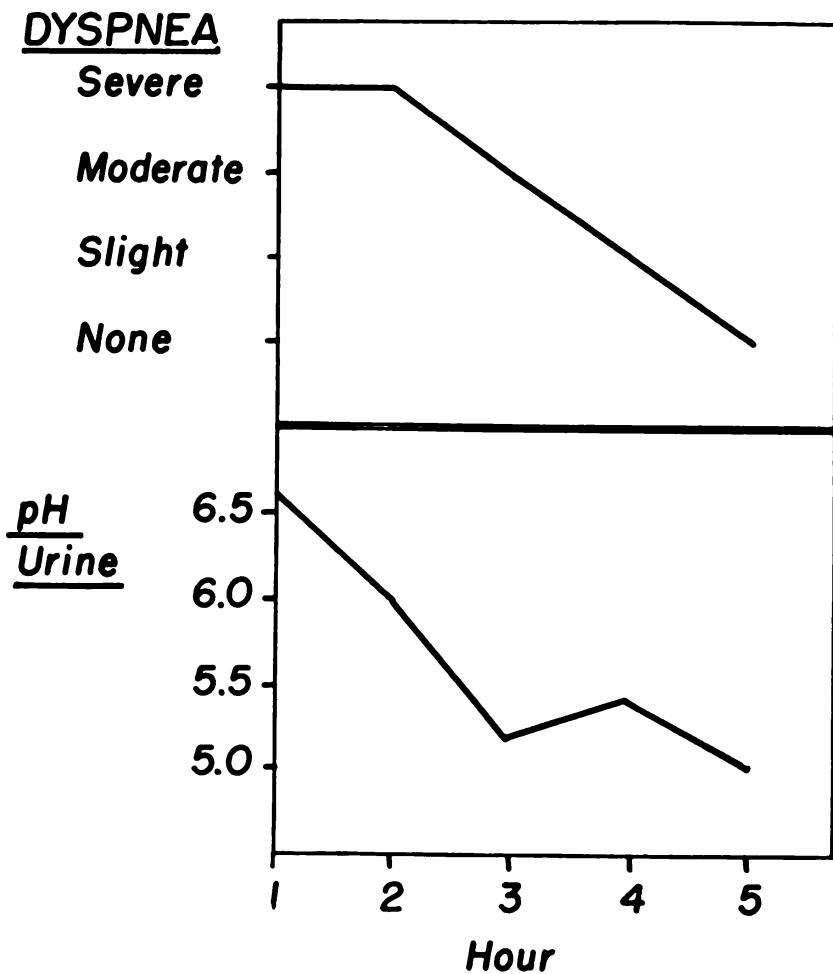


FIG. 34. The parallel variations between the curves of the intensity of *dyspnea* and of the value of the urinary pH in a case of pulmonary congestion following an accidental intravenous injection of a fatty acid preparation, indicates an *alkaline pattern*.

istration of phosphoric acid, while sodium bicarbonate increased the dyspnea. By analogy with pain, we called this correlation an alkaline pattern. (Fig. 34)

Six cases showed an opposite type of correlation between the intensity of dyspnea and the acid-base changes in the pH of the urine. All of these

cases had mediastinal or pulmonary masses and failed to show signs of pulmonary edema. In these cases, the maximum degree of dyspnea was associated with a relatively more acid urine, and the dyspnea was less intense when the urine was more alkaline. In these cases, phosphoric acid increased the degree of dyspnea, while conversely sodium bicarbonate decreased it. This would correspond to an acid pattern of dyspnea. (Figs. 35, 36)

These findings, although obtained in only a limited number of patients, strongly suggest a similarity between the fundamental origin of both pain and dyspnea. As in pain, the two patterns of dyspnea were associated with a relative alkalosis and a relative acidosis.

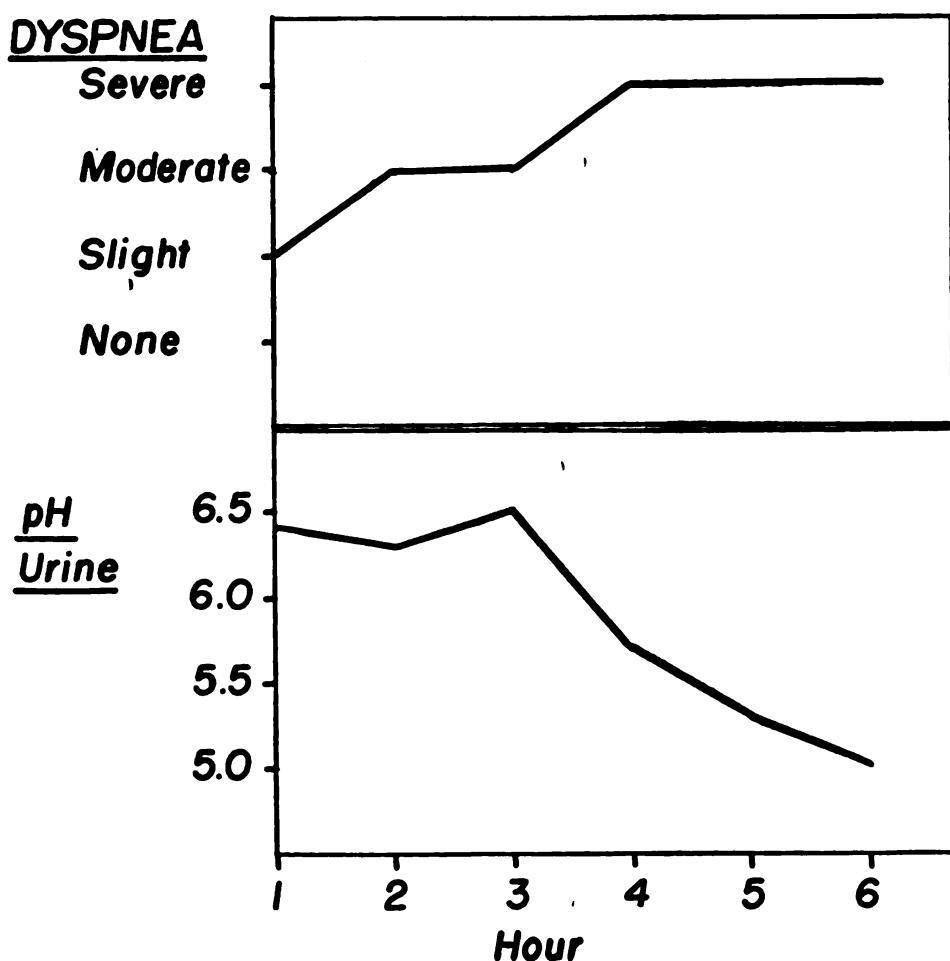


FIG. 35. The opposite concomitant variations between the intensity of *dyspnea* and urinary pH in a case of mediastinal metastases of a hypernephroma indicate an *acid pattern*.

Certain differences exist between the investigations of pain and dyspnea. In studying pain, it was necessary to depend entirely upon the observations of the patient as to the relative intensity of the pain experienced from hour to hour. In dyspnea, the patient's own observations were found

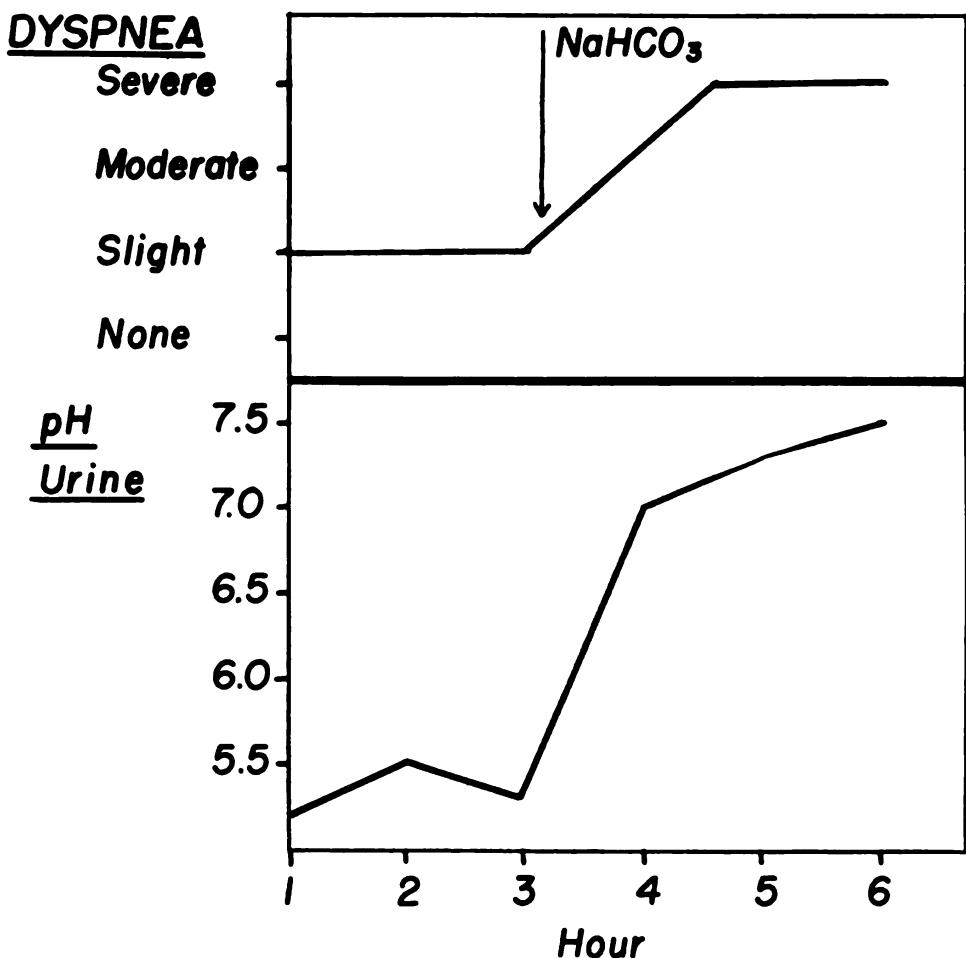


FIG. 36. The administration of sodium bicarbonate increases the intensity of the dyspnea in a case of pulmonary metastases of cancer of the gall-bladder, indicating an alkaline pattern.

to be less reliable due to the emotional factors associated with dyspnea. It was, however, possible to depend upon trained observers who could estimate accurately enough the degree of dyspnea on the basis of rate, depth and apparent difficulty of respiration. It was found that the greater the experience of the observer, the closer was the correlation between the curves of intensity of dyspnea and the changes of the urinary pH values.

Since it appeared evident that acid-base changes play a significant role in influencing the degree of dyspnea experienced by the patient in two opposite directions, it was important to consider this influence in relation to the physiology of respiration which is known not only to be affected by, but even dependent on acid-base changes.

Concerning the respiratory center, two different mechanisms of direct chemical stimulation have been suggested as being responsible for dyspnea. One is lack of oxygen, but considerable evidence exists to indicate that simple anoxemia is not a true cause of dyspnea. (21, 22) On the other hand, an acidosis of the respiratory center is known to cause dyspnea, but it has been established that only a very profound general acidosis, such as that connected with diabetes, acid poisoning or emphysema can create a change in blood pH sufficient to affect the respiratory center. This is also true for the chemoreceptor centers located in the carotid sinus, which are still less sensitive to the general lack of oxygen or acidosis than the respiratory center. While general acid-base changes seem to play an important role in dyspnea, these chemical changes would not appear to act directly upon the respiratory center since the actual blood pH is not sufficiently changed.

Other factors—involving direct local, rather than systemic influences—may be considered. One such direct action would be related to reflex stimulation which is generally accepted as having an important role in the control of respiration and even dyspnea. This reflex control of respiration is connected with nerve endings within the lung parenchyma which are stimulated when the walls of the alveoli are stretched in the respiratory phase. The stretch reflex induces impulses, carried by way of the vagus, which acting upon the respiratory center, stop the inspiratory phase and bring about expiration. It may be assumed that the nerve endings within the parenchyma can be stimulated not only by mechanical changes within the parenchyma but also by local chemical changes too. Under abnormal conditions such as pulmonary congestion, there may be a change in the tissue reaction, as has been recognized by a higher pH of the edema fluid itself. Through this reflex mechanism, these pulmonary tissue reaction changes may produce dyspnea. The general acid-base fluctuations influencing the local alkalosis would, as seen in pain, indirectly influence the degree of dyspnea. Such a mechanism may account for dyspnea with an alkaline pattern found in pulmonary congestion.

A similar local factor also could be seen for the acid pattern. It was observed that all patients with an acid pattern of dyspnea had tumors located within the mediastinum or in the lung parenchyma itself. From

the study of pain, we know that abnormal degrees of acidosis can occur in tumors. pH changes toward acidosis within a tumor tissue in the vicinity of chemoreceptors may produce impulse discharges in these centers especially sensitive to changes toward acidosis. They may alter the character of respiration and result in dyspnea. As in other conditions, these local changes occur even with reduced changes in the systemic acid-base balance. Through this mechanism, variations in the intensity of dyspnea can occur without the intensive change in the general acid-base balance which is considered necessary to affect directly the respiratory center itself.

The fact that relatively small acid-base balance changes affect the intensity of dyspnea in two opposite directions can thus be explained by this indirect influence exerted upon a local process, leading to alkalosis in abnormal conditions affecting the pulmonary parenchyma and to acidosis in lesions present in the neighborhood of the chemoreceptive centers. These two mechanisms proposed as part of this working hypothesis, appear able to explain the paradoxical experimental findings in clinical studies of this symptom, where opposite responses upon dyspnea are seen for the same acidifying and alkalinizing agents.

A more complete study of dyspnea under this aspect will be published separately.

Dualistic Patterns at Other Levels

Cellular Level

Cytological studies have revealed that some characteristics of the cell, other than those typifying the cancerous anomaly itself, exhibit dual patterns. For the cellular level, they could ultimately be related to changes in the aging processes. In tumoral foci having an acid pain pattern, cytological characteristics indicate a prolonged cellular youth. A round aspect of the nucleus, with a fine texture of chromatin and well-separated nucleolus, and a basophilic cytoplasm represent major characteristics of cellular youth. In lesions with an alkaline pain pattern, the cells show rapid early aging. The tendency to lobulation of the nucleus, to separation of chromatin and formation of clumps, to cytoplasmatic oxyphily and the appearance of azurophil granulae characterize such aging. Rapid aging was seen to lead to premature cellular death through piknosis and karyorrhexis. Opposite cellular aging processes could be further related to differences seen in evolution of tumors. Rapid aging of cells, associated with alkaline pain patterns, results in necrotic tumors and ulceration of superficial lesions. Frequently, it could be noted that a change from acid pattern to alkaline

occurs and is accompanied by a melting away of massive tumors and their replacement usually by ulceration.

Tissular Level

As seen above, pain, dyspnea, vertigo and itching can be considered tissue level manifestations which show dualism. Additionally, the nasal pH (*Note 5*) deviates either toward acid or alkaline values and these deviations can be correlated with similar acid-base changes at the tissular level. These measurements are useful as a diagnostic criterion for tissue level changes.

Organic Level

The same dualism observed at the cellular and tissue levels also was found in signs and symptoms involving the organ level. Dual patterns were found for dysfunctions of various organs. Insomnia and somnolence, diarrhea and constipation, oliguria and polyuria, tachycardia and bradycardia, all represent examples of dualism at the organic level.

The dualism evident for the length of persistence of a wheal (*Note 6*) induced by intracutaneous injection of a saline solution was also related to the organ level with the skin considered to be an organ. In normal subjects, the resorption of the wheal is completed in about 15-20 minutes. In one group the resorption time is short, even reduced to a few minutes. In other groups, on the contrary, the resorption time is greatly prolonged, the wheal sometimes being present even after more than 90 minutes.

Systemic Level

Studies of dualism at this level covered temperature variations and changes in various blood and urine values.

Temperature

Two patterns of temperature changes were found in cancer patients. For oral temperature, 37°C (98.6°F) was considered as the reference value. Temperatures measured several times during the day showed that, for many patients, the values were fixed either above or below this reference line. Normal individuals exhibit daily variations of temperature; the curve not only crosses the reference line but also shows broad changes. In contrast, variations usually are smaller in abnormal cases, and the curve remains on one or the other side of the reference line. Figures 37, 38 and 39 show examples of such curves. The two patterns have been found to be

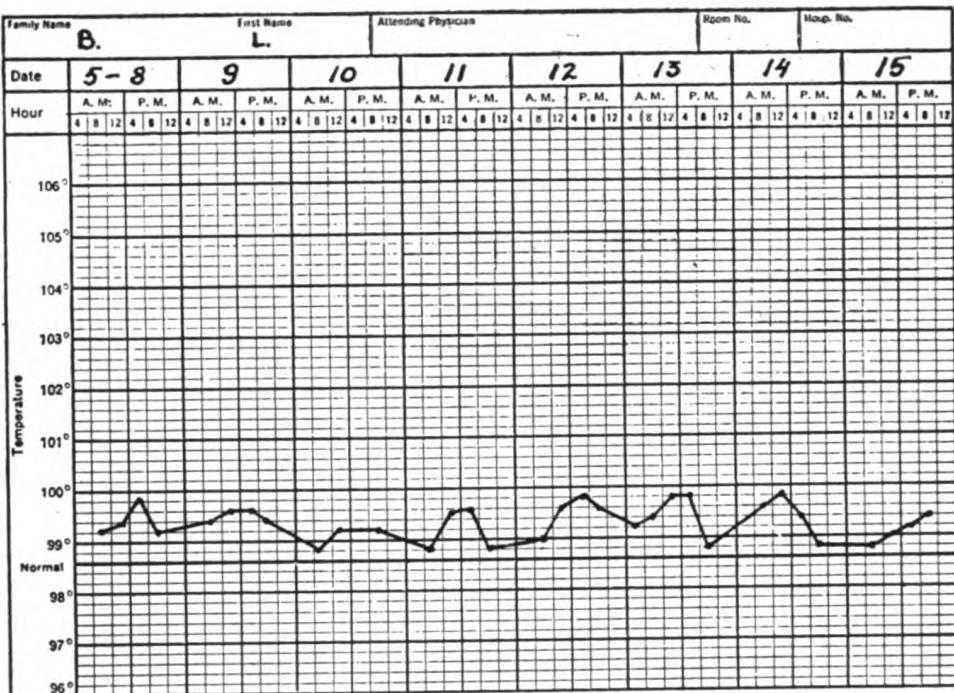


FIG. 37. The curve of oral *temperature* of a case with generalized metastatic melanoma is persistently above the 98.6°F (37°C) line, which corresponds to the average value of normal individuals.

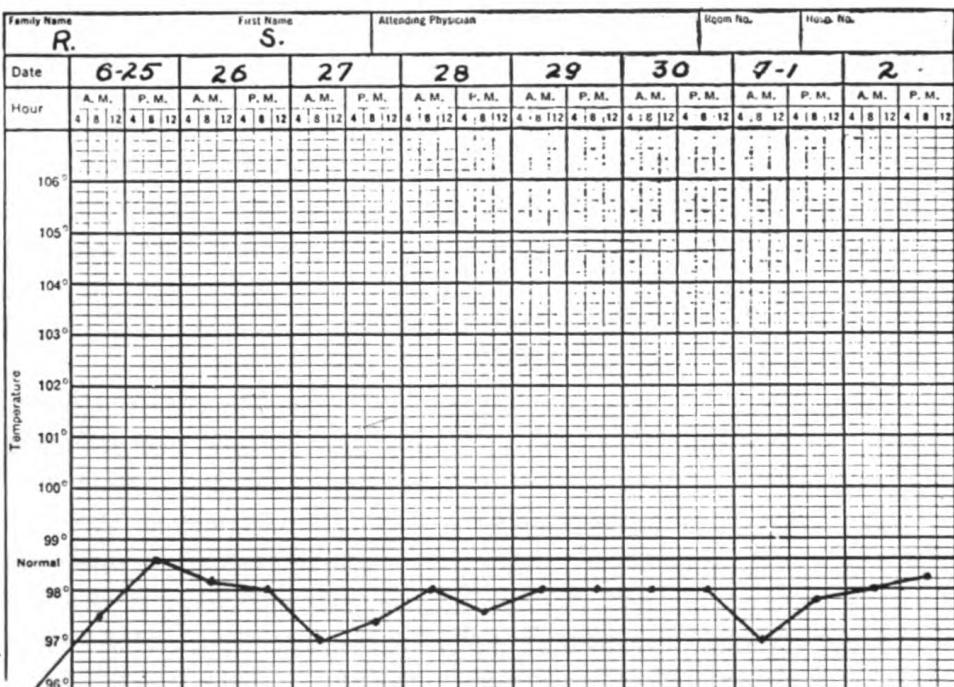


FIG. 38. The curve of the oral temperature of a patient with generalized metastases of adenocarcinoma of the breast shows values constantly *below* the 98.6°F average line.

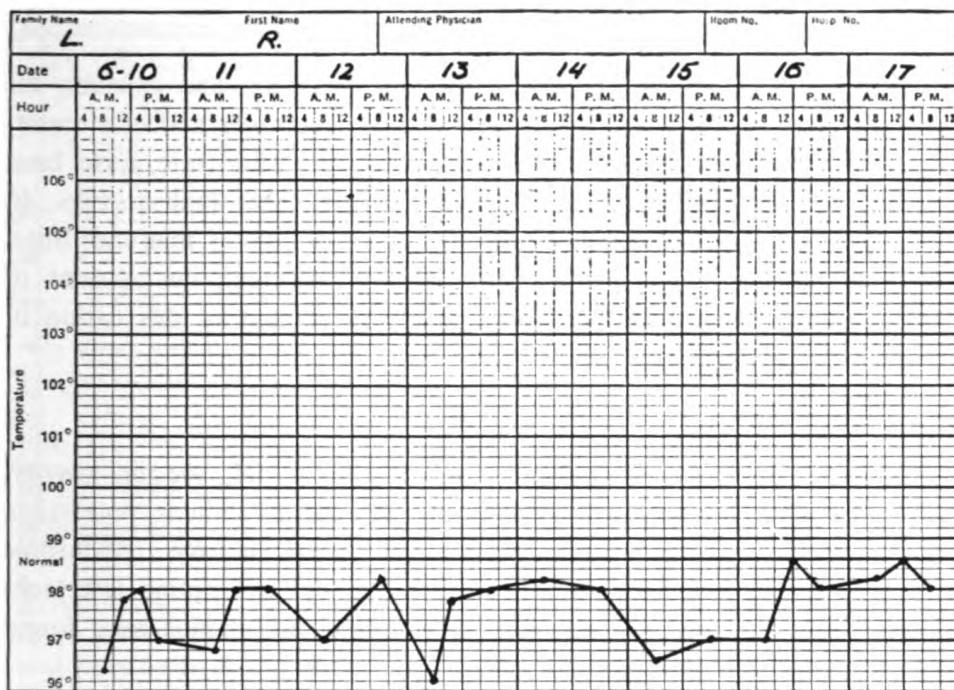


FIG. 39. Curve of the oral temperature of a patient with bronchogenic cancer with abdominal metastases shows values constantly *below* 98.6°F (37°C) average line.

independent of type or site of origin of tumors. We will discuss the significance of temperature patterns later.

Systemic Analyses:

Changes in urine or blood values were followed in hundreds of subjects for long periods, even years. It appeared essential to plot the data graphically. An average value, obtained from a significant number of normal subjects, was represented in graphs as a reference line. In normal subjects, as a general rule, relatively wide oscillations occur around the average value line. By contrast, in disease states, there is a fixation of the curve on one or the other side of the average value, the curves exhibiting only slight, or even no, variations. Two opposing patterns are thus evident in disease states for each type of analysis of blood and urine. The dualism indicates once again the existence in systemic metabolism of two kinds of abnormal changes with antagonistic characteristics. It is important to note that the advanced cancerous condition is characterized by a marked dualistic pattern at the systemic level.

Blood

In blood analyses, the concentrations of potassium and calcium, the presence of C reactive proteins, the number of leucocytes and of circulating eosinophiles, (*Note 7*) and the red cell sedimentation rate, have been studied, using classical methods. They all show the same dualism. Figs. 40 to 43 show some of these curves with the average values as reference lines. With the intention of obtaining information concerning the amount of potassium present in cells, we investigated the content of this cation of the red cells. (*Note 8*)

Urine

In urine studies, measurements were made for pH, specific gravity, surface tension, oxido-reduction potential, excretion of sodium, potassium, calcium, chlorides, phosphates, sulfates, sulphydryl, indoxylo, glucuronic acid, peroxides, etc. Most studies were carried out by routine test techniques. For some analyses, however, conventional techniques were found to be inadequate and new tests devised.

For the urinary excretion of sulphydryl, a new technique was devised by M. Bier and P. Teitelbaum in our laboratories. Using the Warburg micromanometer, the nitrogen liberated from sodium azide in a buffered solution in the presence of free iodine was found to be directly related to the amount of sulphydryl present. The amount of nitrogen freed at a determined moment—13 minutes—was most indicative. (*Note 9*)

For the information which we needed concerning the amount of calcium in urine a very simple method was devised. (*Note 10*)

For measurement of urinary surface tension, we devised a new technique. We used a capillary so calibrated as to give the surface tension in dyne/cm for a fluid with a specific gravity of 1.015. In this method, several arrests or slowdowns of the descending column are noted and make it possible to obtain information about an extremely important factor which is usually not considered in the measurement of surface tension with other methods. It is known that urine is formed of different constituents, some of them with the tendency to move toward the surface while others tend to move toward the bulk of the fluid. Changes in the distribution of these constituents take place. They induce changes in the value of the surface tension of the urine which occur even during the time measurements are taken. The descending column in a capillary will indicate these changes which are important for precise measurement of surface tension of urine. (*Note 11*)

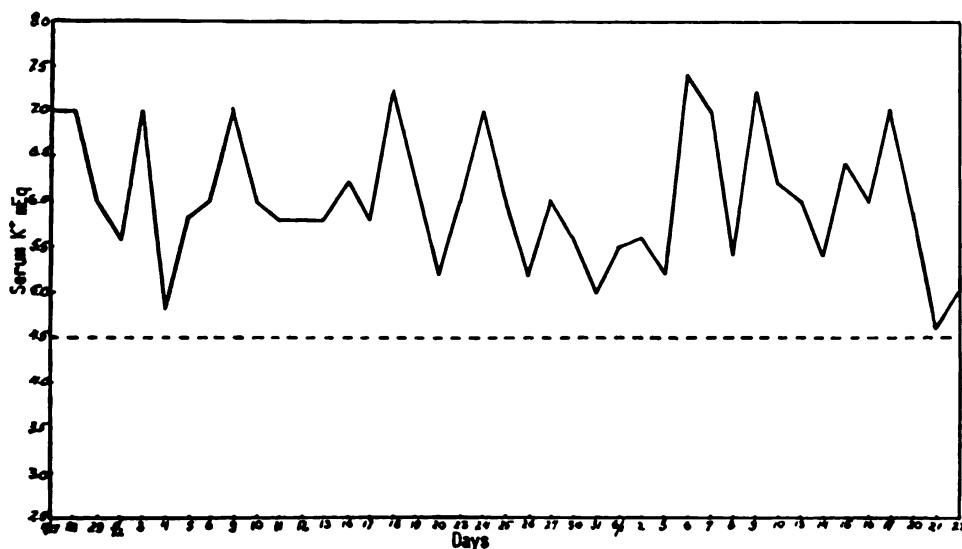


FIG. 40. Curve of the value of the K^+ in blood serum in a case with periarteritis nodosa. The values remain above 4.5 mEq, which represent the average value obtained from series of normals.

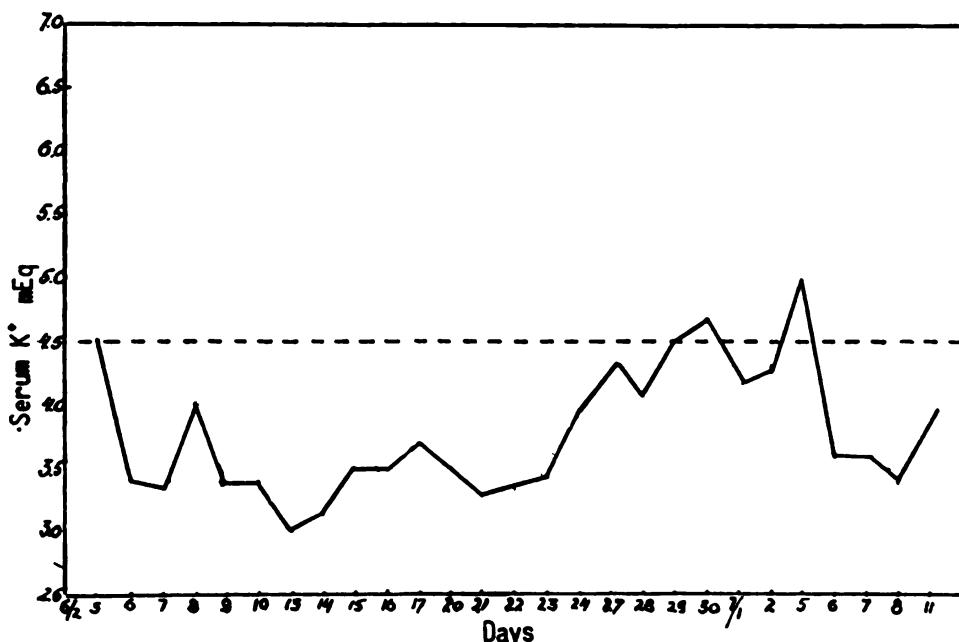


FIG. 41. Curve of the values of K^+ in the blood serum of a subject with carcinoma of the breast, liver metastases and jaundice. The values remain almost constantly below the 4.5 mEq line.

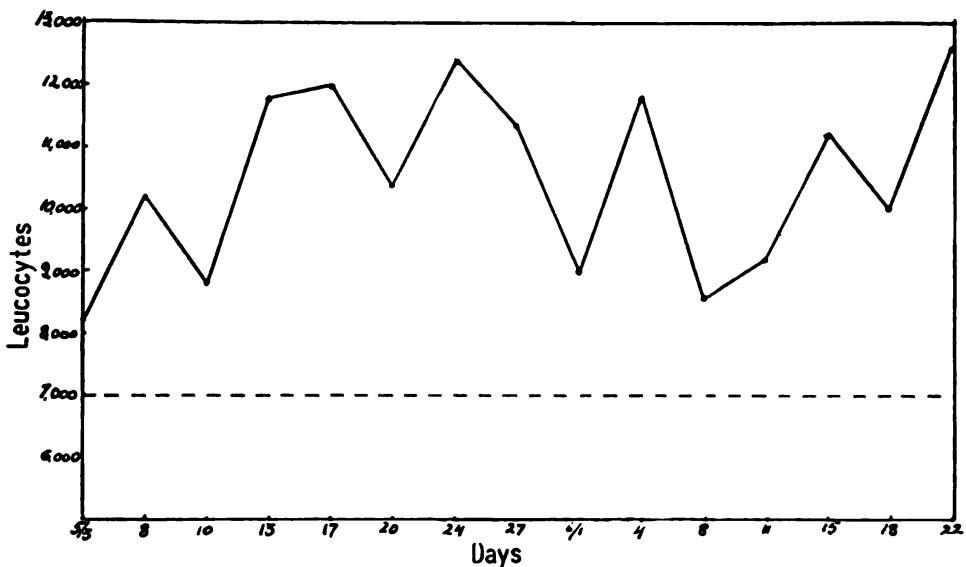


FIG. 42. The curve of the number of blood leucocytes of a case of breast adenocarcinoma shows constantly values above 7,000, considered as the average value for normals.

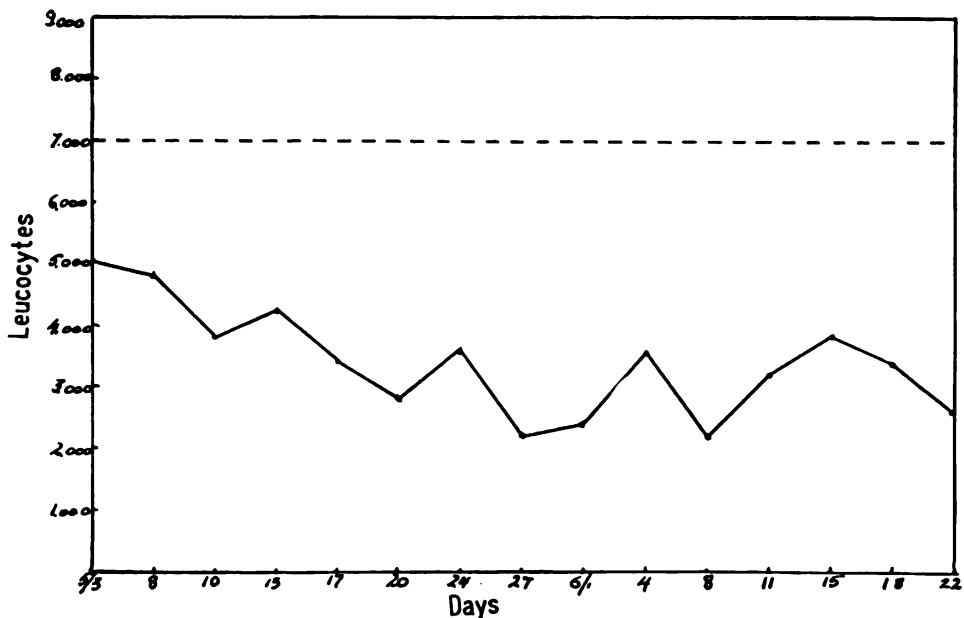


FIG. 43. The curve of the number of blood leucocytes of a case of breast carcinoma shows persistently values below the average line of 7,000.

Two methods were employed to measure the oxido-reduction potential of urine. In one, the measurement was made with a potentiometer using a platinum electrode, first at the pH of the urine and once again after bringing the pH to 7 by adding the necessary amount of NaOH or HCl. (*Note 12*) In the second technique, differences in potential were measured through the time necessary to discolor a solution of toluidine blue in an acid medium at 100°C. The value was determined in seconds, the concentration of the dye having been chosen so that discoloration in 100 seconds represented an average value for normal subjects. More rapid discoloration indicated higher potential, while a low potential corresponded to slow or even no discoloration. (*Note 13*)

The presence and relative amounts of oxydizing substances in the urine were also investigated by means of two reactions: in one, through the passage of indoxylo into indigotine and indigorubine under the influence of sulfuric acid (*Note 14*); in the other, through liberation of iodine from iodides in the acid medium.

It is interesting to mention at this moment, the variations encountered in the metabolism of nitrogen and the form under which it is excreted through the kidney under normal and pathological conditions. We could show that in general the form of excretion is determined by the amount of water also excreted by the organism. High amounts of water will thus induce the excretion of nitrogen principally as ammonia, low amounts as uric acid. This relationship was explained by data found in comparative physiology. The availability of water in the environment of various animals was seen to determine the form under which these animals excrete nitrogen. The occurrence of similar conditions concerning the excretion of water in pathological states, furnishes an interesting explanation for the form under which it is excreted in these pathological conditions. In Note 15 this problem is discussed in more details.

In all tests requiring quantitative measurements, an important problem arose. It appeared practically impossible to obtain 24 hour urines routinely for periods of months in large groups of individuals in order to note the continuous changes in excretion of the different substances studied. Measurements of various substances eliminated in urine could be made under these circumstances only by using isolated samples. The values so obtained express the concentrations of the substances in the sample and consequently were directly related not only to the amount eliminated by the kidney but also to the amount of water excreted at the same time. The values varied greatly from one specimen to the next, according to the amount of water excreted. Therefore these data could only be of relative usefulness. Since

specific gravity is also a direct function of the amount of water excreted in a urine sample, we related the concentration of a substance to the specific gravity of the same sample. This ratio provided a new value which is independent of the amount of water in the sample and is more closely related to the amount of the other substances which vary much less in amount than water. From the physiopathological point of view, the data obtained were seen to correspond to the degree of active reabsorption of a given substance by the kidney. While the ratio of the concentration over specific gravity would vary directly with the excretion of the substance, the inverse ratio would represent an index of retention, which increases with the retention of the substance in the body. Such indices were routinely applied for the different substances tested in urine to obtain more reliable values than could be obtained from the concentration data alone. (Note 15)

Urinary Patterns

Various urinalyses were performed during sufficient lengths of time on a larger number of subjects considered to be normal. For each test, an average value was thus obtained. This average value served as a reference line for the curves traced with the data obtained from the subjects. The

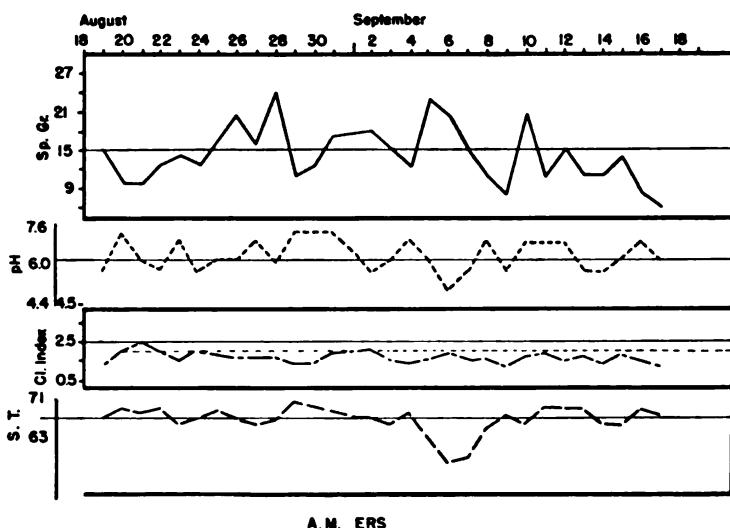


FIG. 44. Curves of *different analyses* of daily urine samples of a *normal* 40-year-old male. For each kind of analysis, the corresponding average value was calculated from the measurements obtained in more than one hundred normal subjects. The curves are seen to pass from one to the other side of the lines corresponding to the respective average values. A certain correspondence is seen for the changes which take place at the same time in these curves. Parallel variations are seen between specific gravity and chloride index, while opposite to those for the pH and surface tension.

study of these curves has permitted us to recognize several characteristic patterns. Under conditions considered normal, the curves were seen to pass from one side to the other on this average line with relatively wide variations. When an abnormality existed, the various urine analyses were characterized by a curve with only small variations, fixed on one or the other side of the average line. For each test, two such characteristic *abnormal patterns* are encountered. Fig. 44 shows the curves of different tests passing

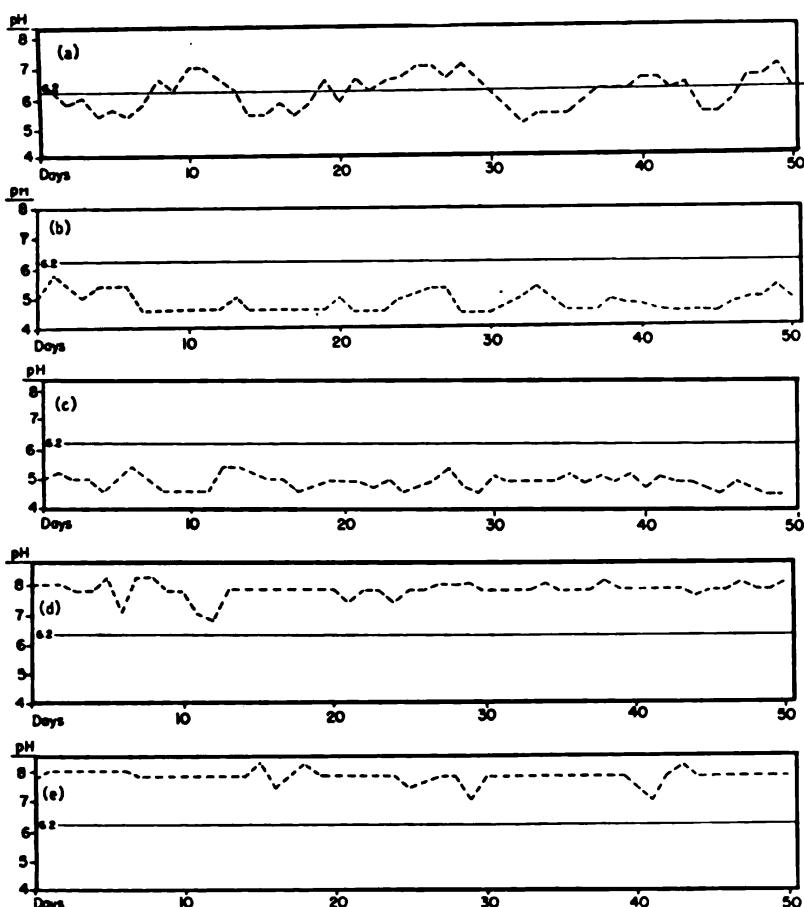


FIG. 45. Curves of the values of the *urinary pH* followed during 50 days in five subjects. The curve (a) corresponds to a normal individual while the other four (b, c, d, e) to subjects with different cancerous lesions. For the normal case, the values pass above and below the 6.2 value which is considered as the average computed values obtained from normals. For the abnormal cases, the curves which show little variations are fixed at one or the other side of the average line. For case (b), an adenocarcinoma of the ovary, and case (c), a bronchogenic cancer, the curves are fixed below the average line. For case (d), with a bronchogenic cancer, and case (e), a cancer of the breast with generalized metastases, the curves are above this average line.

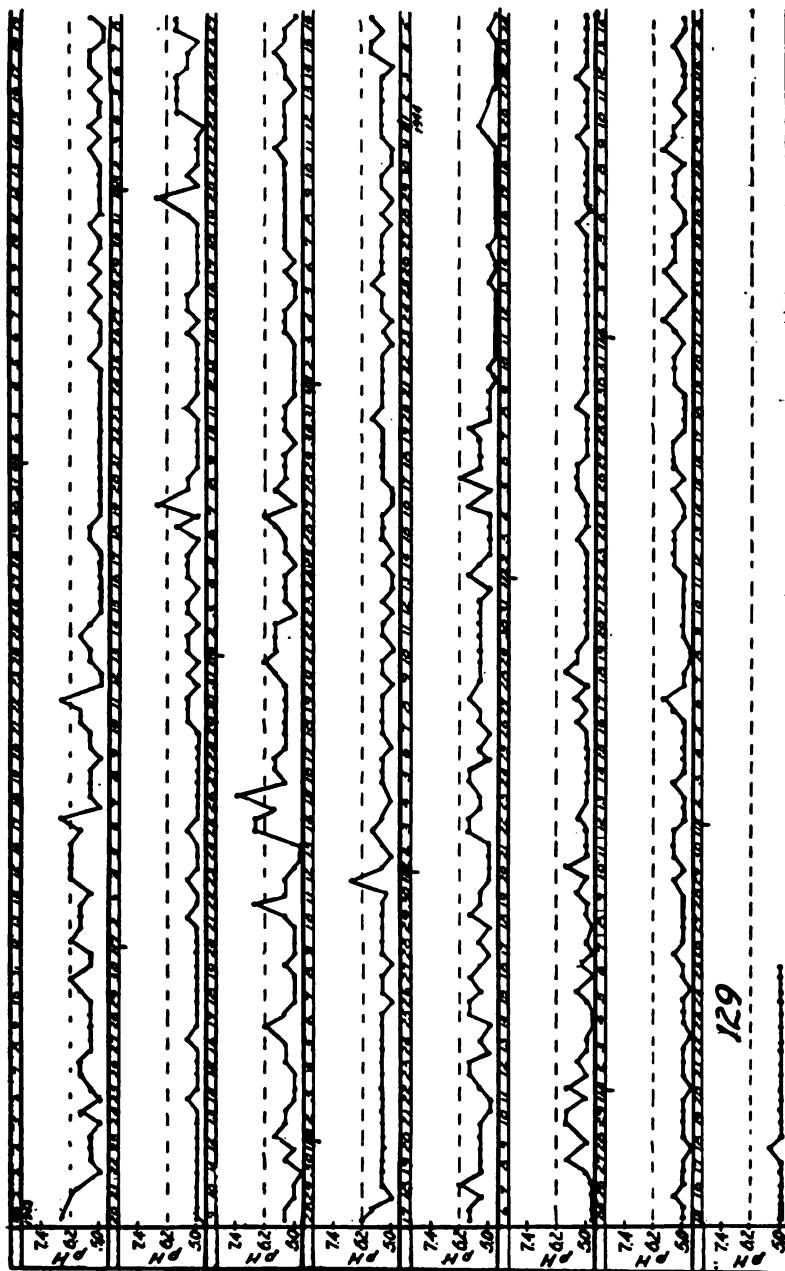


FIG. 46. Urinary pH values of a patient with a cancer of the prostate and multiple bone metastases measured in two daily urine samples for a period of more than 1½ years, remain fixed below the average value—6.2—despite of the changes in diet and of various therapeutic attempts. With the progression of the disease, the fixation of the pH at low values becomes more apparent.

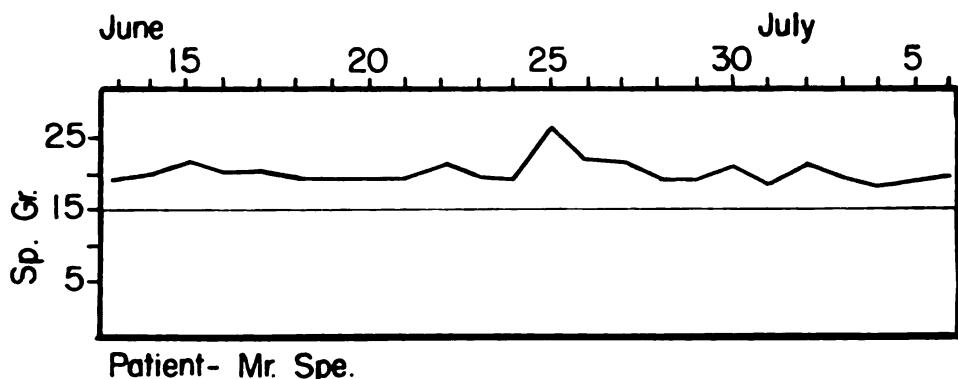


FIG. 47. Curve of the values of the urinary specific gravity in a case of cancer of the breast, with the values fixed above 1016 which is considered as an average value for the specific gravity as computed from a series of measurements in normals.

from one side to the other of the respective average lines as corresponding to a normal subject. Figures 45 to 51 show several examples of two opposite patterns for the various tests used such as urinary pH, specific gravity, surface tension, chloride index and sulfhydryl index.

Fundamental Offbalances

When the curves of various analyses obtained at the same time for the same subject were checked, an interesting relationship was found.

In patients with small localized tumors, only some of the analyses showed abnormal patterns. However, with the evolution of cancer toward the terminal stage, abnormal patterns became apparent for more and more analyses. In advanced cases, most of the analyses showed abnormal patterns.

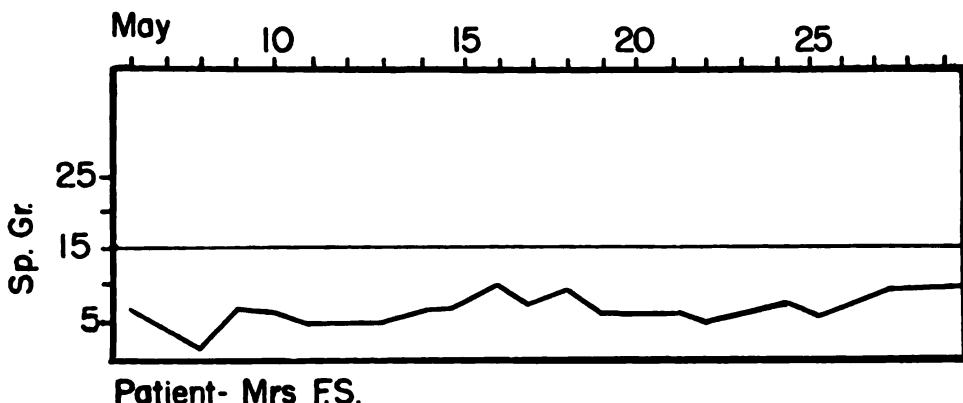


FIG. 48. The values of the urinary specific gravity in a case of cancer of the breast, are the whole time below the average value of 1016.

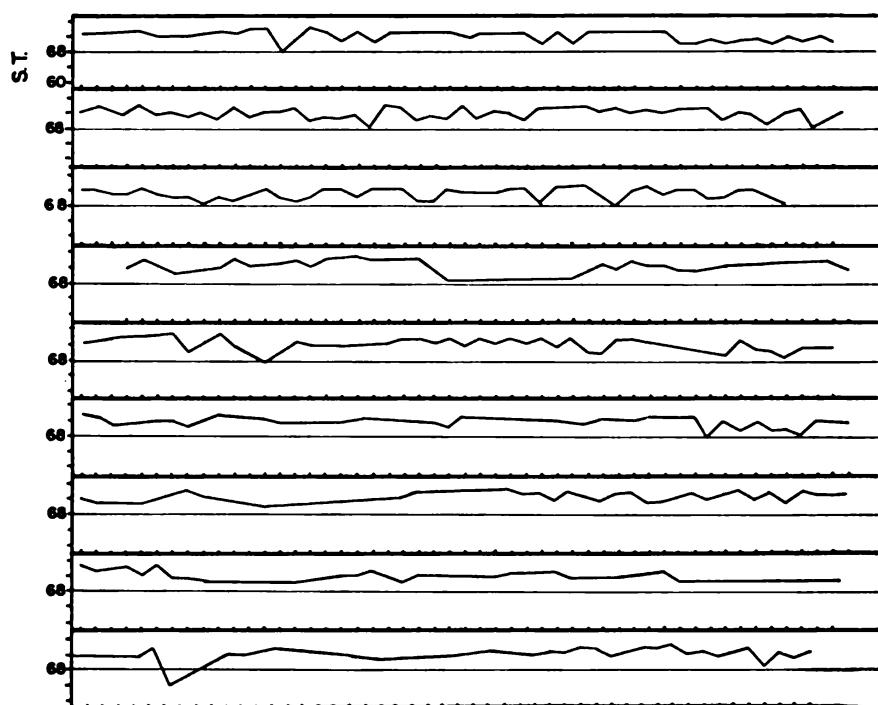


FIG. 49. The urinary *surface tension* remains constantly fixed *above* the average value of 68 for more than a year in a case of cancer of the urethra.

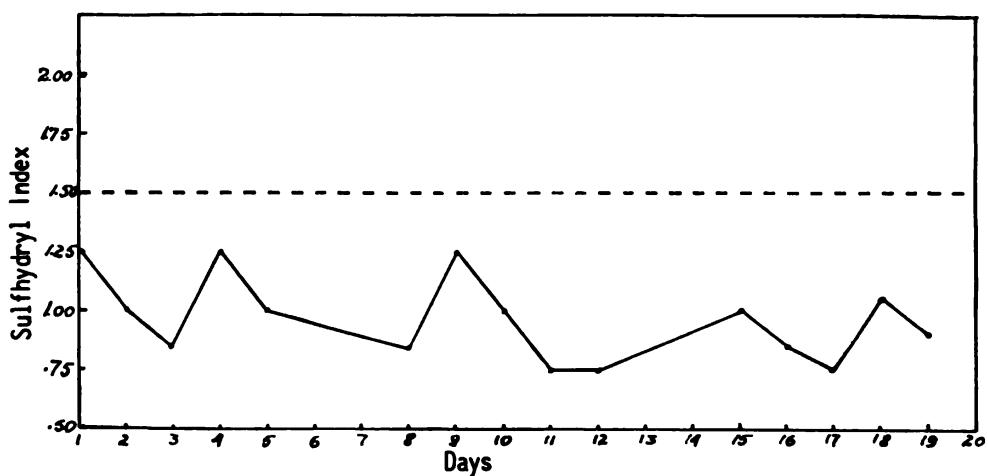


FIG. 50. The urinary sulfhydryl index remains below the average value of 1.5, in a case of cancer of the breast, with bone metastases.

It was especially in advanced stages that definite groupings of patterns with opposite characteristics could be recognized. They corresponded to two fundamental offbalances which we have called "Type A" and "Type D." ("A" for anoxybiosis, "D" for dysoxybiosis which represent the principal manifestations of oxygen metabolism in a phase of these offbalances.)

The correspondence between the different abnormal patterns defining the offbalance A or D, is seen in Figures 52 to 60. The abnormal pattern of

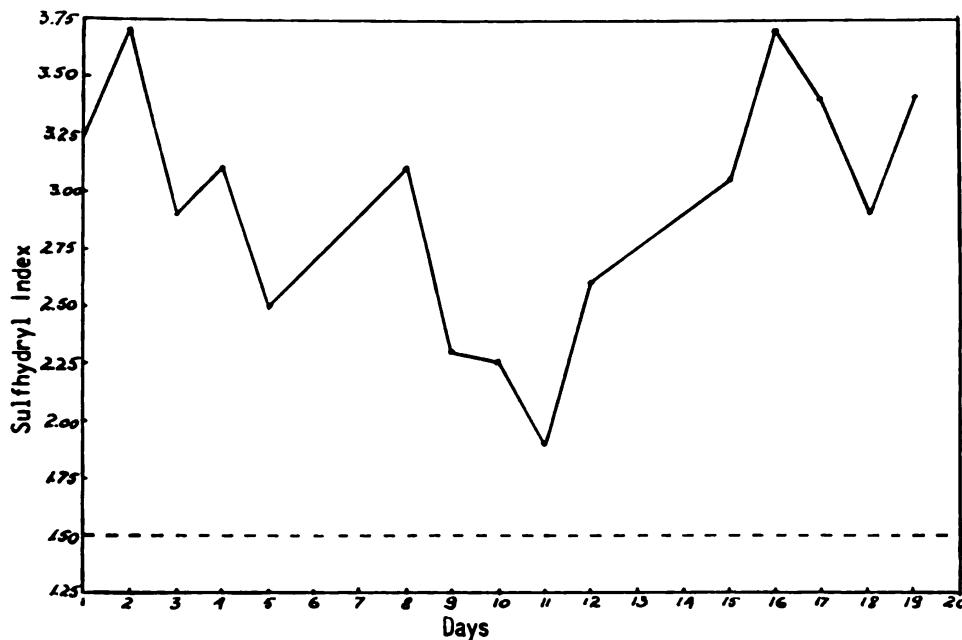
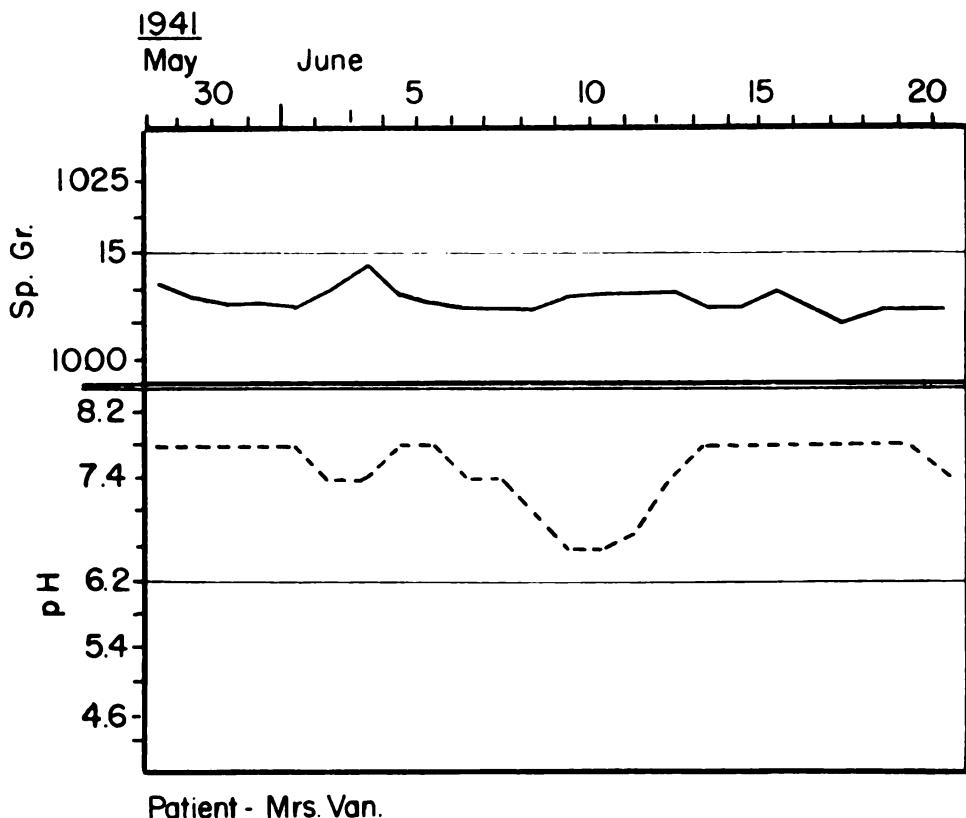


FIG. 51. The urinary sulfhydryl index remains above the average value of 1.5 in a case of hypernephroma with lung metastases.

the low urinary specific gravity appears together with a pattern of high values for the pH, both corresponding to the offbalance type A. (Fig. 52) A high urinary pH and low chloride index pattern correspond to the offbalance type A, as seen in Fig. 53. The analyses of a case with low specific gravity, high pH, low chloride index and high surface tension, as present in offbalance type A, is shown in Fig. 54. An opposite case, offbalance type D, with high specific gravity, low pH, high chloride index and low surface tension is shown in Fig. 55. A similar case of offbalance type D is shown in Fig. 56 with high urinary specific gravity, low pH, low surface tension and low blood leucocyte number. In Fig. 57, the low pH, high sulfhydryl index and low surface tension show an offbalance of the type D.

The independence of the levels may explain why, in the same subject,



Patient - Mrs. Van.

FIG. 52. A correspondence is seen between the patterns of *urinary specific gravity* and that of the *urinary pH* in a cancer of the colon corresponding to the type A offbalance. The changes still present in the curves are opposite.

not all the analytical patterns obligatory concord at all times in the same subject. Especially when defense reactions intervene, the offbalance at one level can be different from that at other levels. Fig. 58 shows such examples. Usually, as the disease progresses, many of these differences disappear, the manifestations—analytical and clinical at different levels—entering in the same type of offbalance. Fig. 59 shows such an example.

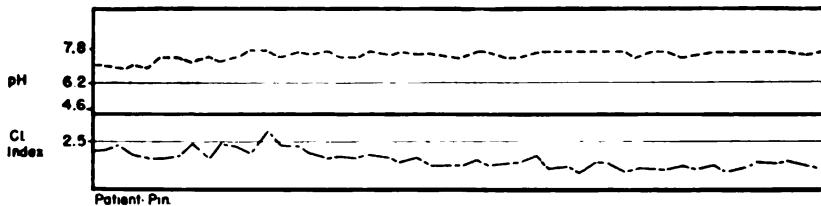


FIG. 53. In a case of sarcoma of the leg with lung metastases the curve of the *urinary pH* is fixed above the average line (6.2) while the curve of *chloride index* below the average line of 2.5. This relationship indicates a type A of the offbalance.

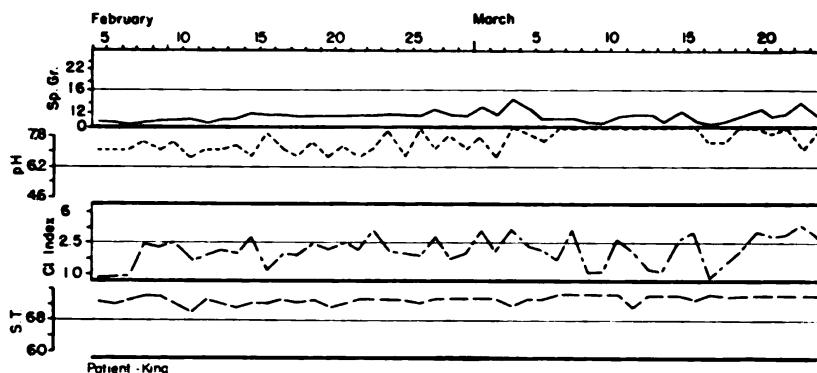


FIG. 54. The analysis of a terminal case of cancer of the breast, showing the fundamental offbalance, low specific gravity, high pH, low chloride index and high surface tension, corresponding thus to the offbalance A.

The passage of a subject from one offbalance into the opposite one is seen to occur during the evolution of the condition most often induced by therapeutic attempts. Fig. 60 shows such an example.

The two opposite offbalances, identified first through the urine analyses, could be recognized to exist for all the manifestations taking place at the different levels of the organization. The manifestations seen at these levels could thus be interpreted as corresponding to one of the two opposite fundamental offbalances, type A and D. Table IV shows this coordination of the different manifestations according to the two opposite offbalances.

On the basis of all these data, clinical manifestations and analyses, the dualistic pathogenic concept appeared to be well established and the dual-

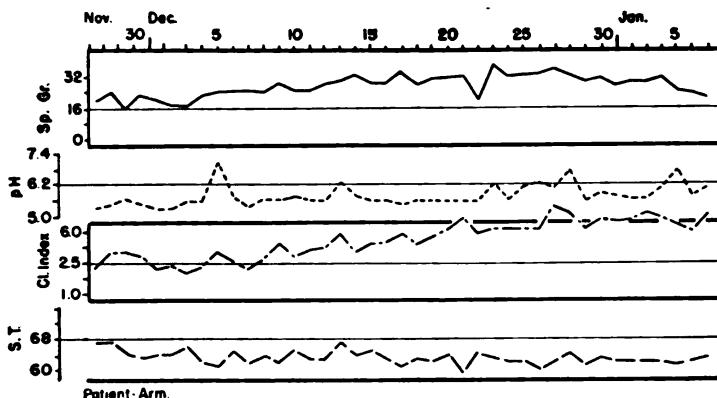


FIG. 55. A terminal case of cancer of the breast shows all the analyses fixed in opposite position to the case of Fig. 54, i.e., high specific gravity, low pH, high chloride index and low surface tension, corresponding to an offbalance of the type D.

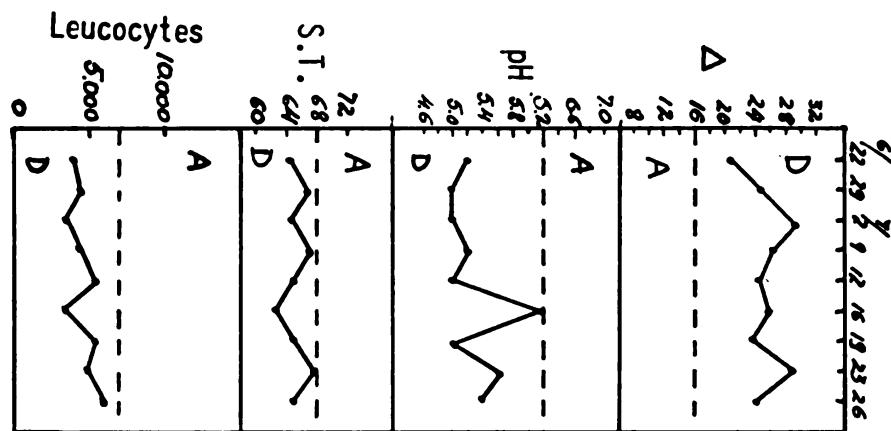


FIG. 56. Offbalance type D, shows high urine specific gravity, low pH, low surface tension and low blood leucocyte number.

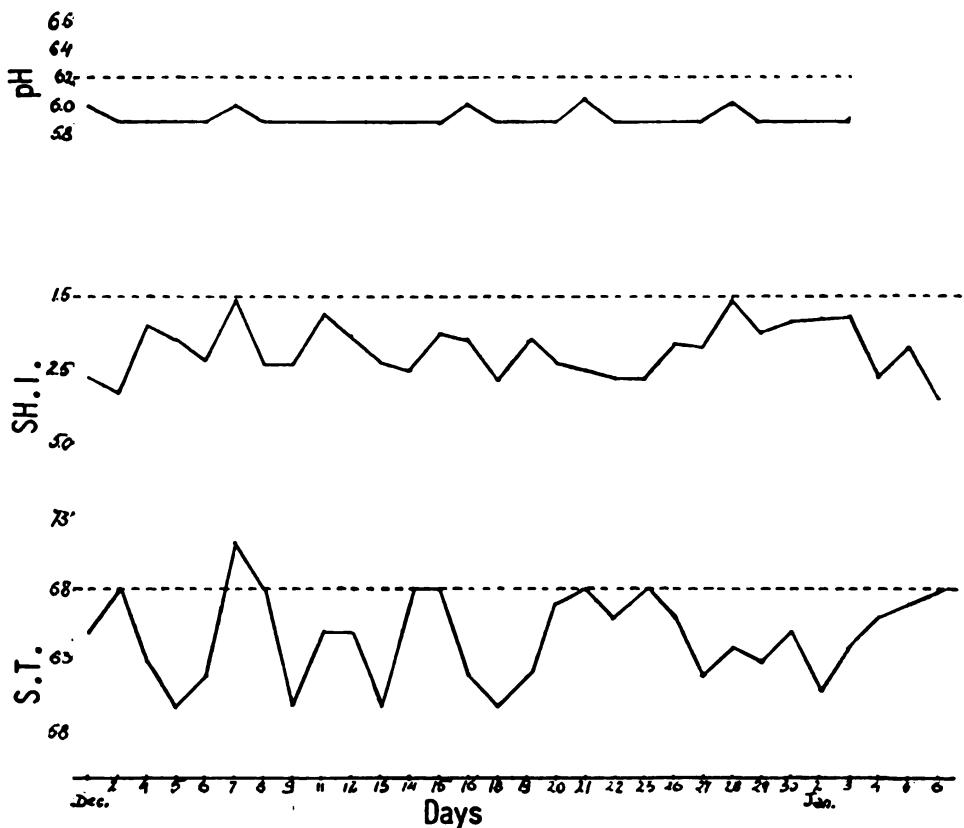


FIG. 57. The 3 curves, pH, sulphhydryl index and surface tension show an offbalance type D.

istic physiopathological mechanism studied in complex conditions in general and in cancer in particular.

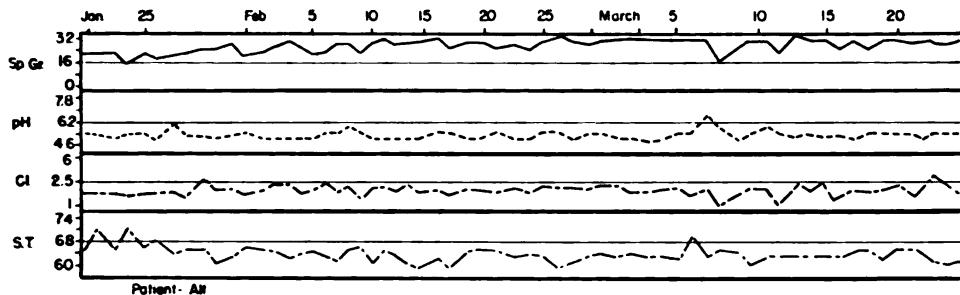


FIG. 58. The urine analyses of a patient with cancer of the ovary, showing a discordance between the patterns present. While all the analyses show patterns of the type D, the chloride index remains throughout the entire observation constantly of type A.

Place of Dualism in Cancer Physiopathology

After recognizing a dual pattern in most manifestations of cancer, the problem was to consider the relationship between this dualism and cancer itself.

One critical observation was that a pattern in a given patient may change. During the evolution of the condition, seldom is the change from abnormal to normal, but usually from one abnormal to the opposite abnormal pattern. This fact has a special importance as will be seen later. In some cases, even the immediate, direct cause of such changes could be established.

The following case is illustrative.

Mr. S. L., a patient with cancer of the prostate and metastatic destruction of half of the sacrum, was in very severe pain. Study of variations in pain intensity and in urinary pH, as well as the response to acidifying and alkalinizing substances, indicated that the pain was of a typical acid pattern. The patient started radiotherapy and pain decreased with almost every treatment, until after a few sessions it had disappeared completely. Out of bed and feeling well, he continued the treatment. However, at about the twelfth session, pain again appeared and thereafter increased with each treatment. After five more sessions, he was back in bed and suffering such unbearable pain that radiotherapy had to be discontinued. At that time, a new analysis of the pattern of pain showed that it had changed from the original acid to alkaline. It was hypothesized that this change might be the

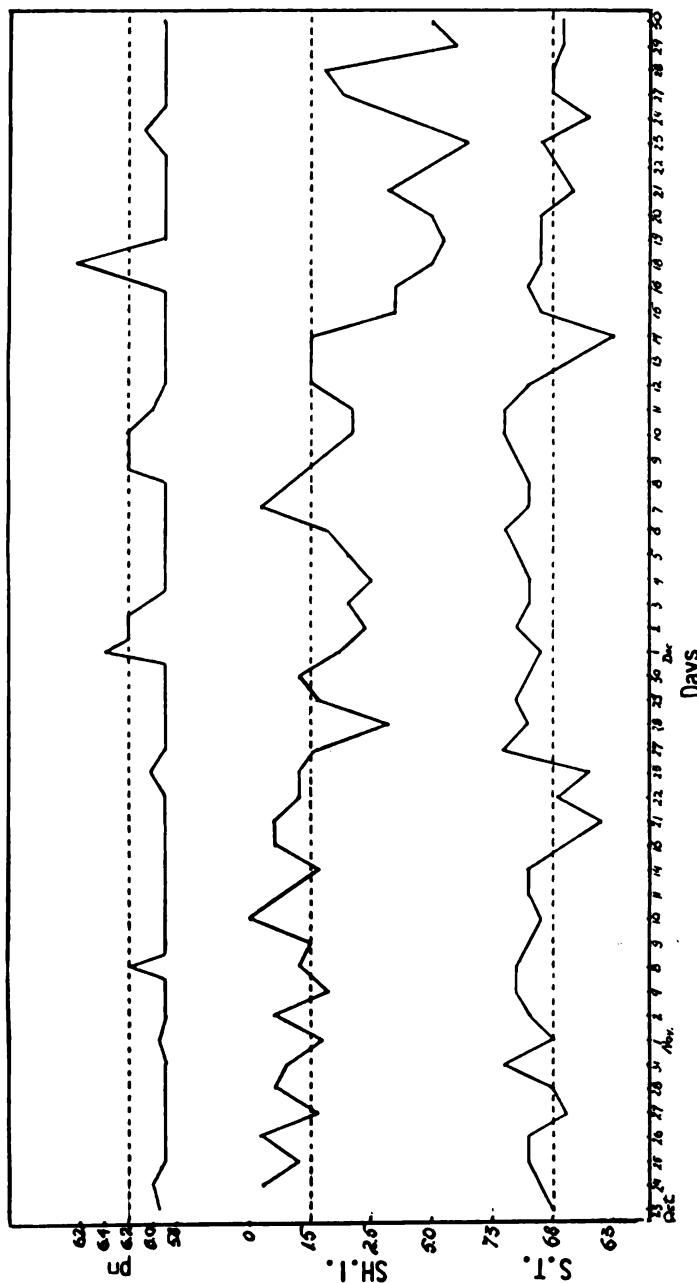


FIG. 59. The passage of discordant patterns into their opposite patterns, in order to realize a concordant offbalance as seen in a case of cancer of the stomach. At the beginning of the observation, low pH values show a pattern corresponding to type D, while two other analyses—sulphydryl index and surface tension—are of offbalance A. With the progress of the condition, sulphydryl index and surface tension values pass gradually from offbalance A also to offbalance D.

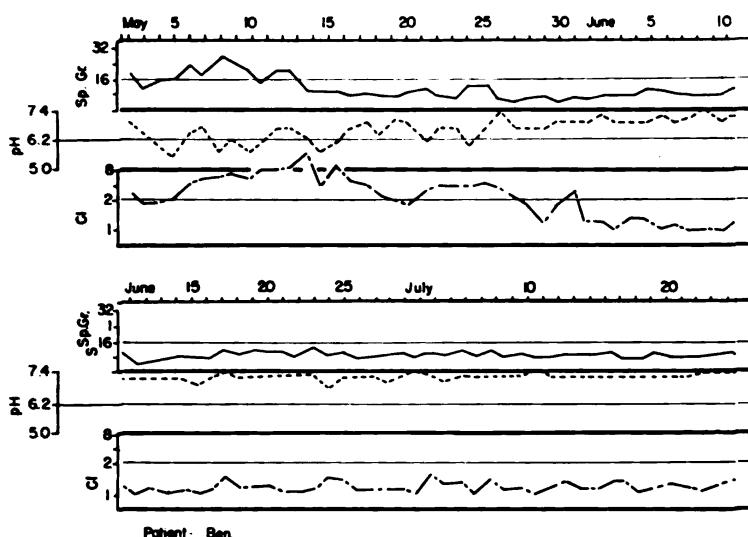


FIG. 60. A condition can pass from one offbalance to the opposite one. *The passage of the analyses from the offbalance type D into the offbalance type A is seen in a case of cancer of the lung. The analyses which first show high specific gravity, low pH and high chloride index, pass subsequently to the opposite side, with low specific gravity, high pH and low chloride index. This new offbalance remains unchanged for a long time as shown in the second part of the curve.*

result of irradiation. Study of other cases, after irradiation, has confirmed the hypothesis.

Changes of pain from an acid to an alkaline pattern also were observed in patients who had suffered shock or serious pyrogenic infections. Viral infection such as flu, or even smallpox vaccination, seemed to induce an opposite change—from alkaline to acid. Changes obtained under the influence of therapeutic agents were frequently observed and will be discussed later.

The dual pattern seen for most cancer manifestations was integrated into the concept of the disease as a complex organized condition that develops through progressive participation of hierarchically superior levels of the organization. From a clinical point of view, such participation corresponds to the successive addition of new manifestations to a previously less complex condition. According to their levels, these "Added Factors" correspond to prolonged youth or rapid cellular aging, to acid or alkaline pain, to blood and urinary analytical data fixed in one or another of two opposite patterns. Dualism is the principal characteristic shared in common by all these progressively added manifestations.

In trying to understand this dualism and its significance, we had to

consider again the place of dualism in the general organization of nature. The study of reactivity in nature has led to the recognition that a basic dualism exists—that the forces operating in nature can be separated into two opposite groups for almost all the intervening factors. Forces which would tend to lead toward annihilation of differences and produce a state of maximum entropy or homotropy or the antagonistic forces which tend

TABLE IV
MANIFESTATIONS

Level	Offbalance "A"	Offbalance "D"
Cellular	Prolonged cellular youth Less differentiated cells, especially of connective tissues Increased amount of connective tissue	Rapid cellular aging More differentiated cells, especially of connective tissues Decreased amount of connective tissue
Tissular	Increased lymphatic tissue Low oxido-reduction potential processes Low chloride content Local acidosis Acid pattern symptoms (Pain, dyspnea, itching, etc.)	Decreased lymphatic tissue High oxido-reduction potential processes High chloride content Local alkalosis Alkaline pattern symptoms (Pain, dyspnea, itching, etc.)
Organic	Somnolence Constipation Polyuria Exophthalmia Nasal pH below 6.5 Slow absorbtion of skin wheal	Insomnia Diarrhea Oliguria Enophthalmia Nasal pH above 6.5 Rapid absorbtion of skin wheal
Systemic	<i>Blood</i> Leucocytosis Eosinophilia High color index Low potassium content Low R.C. sed. rate No C reactive protein <i>Urine</i> Low specific gravity High pH High Cl, Na excretion Low SH, Ca, phosphate, sulfate excretion High surface tension Death in coma	<i>Blood</i> Leucopenia Eosinopenia Low color index High potassium content High R.C. sed. rate High C reactive protein <i>Urine</i> High specific gravity Low pH Low Cl, Na excretion High SH, Ca, phosphate, sulfate excretion Low surface tension Presence of oxidizing substances Death while conscious

to maintain and increase differences and lead to more complex organization—and are thus catalogued as negatively-entropic, ectropic or heterotropic—would thus appear with each new added factor when a higher hierarchic entity is realized. For this reason, they will appear especially manifest for the same added factors intervening under abnormal conditions. Dualism consequently concerns the manifestations progressively added. The result is seen in the alteration of the oscillatory movement with alternate predominance of one and then the other of the antagonistic forces for these added factors. This fact would explain the dualism seen especially in abnormalities. It also explains the intervention of the dual patterns mentioned above.

Dualism becomes even more important when it can be seen to play a capital role in the mechanism through which agents act upon abnormal conditions. Originally, the dualistic actions of agents could be ascertained through the changes induced in pain and various other patterns. It has consequently been possible to classify the effect of various therapeutic agents according to their influence upon these patterns and, through this more readily measurable influence, on the fundamental offbalances themselves.

At the same time, the study of the influence exerted by various agents upon these patterns, which is presented later, has posed the problem of precisely what effects upon these patterns are produced by the substances which are body constituents. And this led us to a third basic concept dealing with the important role played by body constituents and, in particular, by the lipids.

CHAPTER 5

THE CONSTITUENTS

HIERARCHIC ORGANIZATION and dualism have opened the way for a study of the body constituents in an attempt to systematize them and their functioning in accordance with these two concepts. In this research, we considered besides the constituents separated in groups as lipids, proteins, carbohydrates and electrolytes also the elements as a source of interesting information.

With most of the manifestations integrated in dualistic patterns, we planned to test the different constituents by noting their influence on these patterns. It was to be expected that some might have selective activity at certain levels of organization and the manifestations related to these levels. This selectivity did become evident but it also turned out that any agent, in sufficient amount, exerted an effect at any level. The problem was to select the manifestation which would respond most readily to the greatest number of agents. This would make comparisons between agents easier and serve as a practical criterion for the start of classification on this dual basis.

The measurement of the influence exerted by various agents upon the second day wound crust pH (s.d.c. pH) proved to be particularly rewarding and was employed in the first part of the investigation. The s.d.c. pH provided an indication not only of acidifying and alkalinizing effects but also comparative values for these effects. Later, the influence exerted upon many other manifestations was studied for corroboration. Details of the s.d.c. pH technique and the results obtained are in Note 1.

In studying the elements, we chose, first, simple combinations in which they appear. Each anion was investigated by studying it as it occurred in the respective acid and in compounds in combinations with different cations; each cation was studied in its combinations with different anions.

In this way, we obtained a series of data which enabled us to pinpoint the influence of each element.

The Elements

Using this method, we could determine that elements such as Li, K, Na, Fe, Ni, Zn, Hg, Bi, B, F, Cl, Br, I—in sufficient amount—produced in the s.d.c. pH an acidifying effect. The opposite effect—alkalization—was seen for Mg, Ca, Sr, Ba, Cu, Pb, S and Se. It must be emphasized that some elements—such as K, Fe, Zn, Hg, Cl in the acidifying group and Ca, Cu, S and Se in the other—produced an intense effect while others had a weak though still clear, action. We must add that Ni and Cr showed a relatively weak acidifying effect. This separation of elements on the basis of acidifying or alkalizing effect agreed with almost all data available about antagonism between elements—for example, the known antagonism between K and Ca, Mg and Cu, and between Mo, Zn and Cu.

As a second step, we related the elements, through their influence upon the s.d.c. pH, to one of the two fundamental offbalances. Those inducing acidification were thus classified as an “inducing offbalance type A,” or “anti offbalance type D” or “anti D,” while those producing alkalization were called “inducing D,” or “anti offbalance type A,” or “anti A.” Going another step, the acidifying elements were considered to have a tendency toward increasing heterotropy; the alkalinizing, a tendency toward increasing homotropy. This led us to attribute to the first, the acidifying group, the qualification “hetero” type, and to the second, the “homo” type. We did not give these designations any other meaning than that indicated above, using them for didactic facility.

The Series

After classifying elements into hetero and homo groups, we studied these groupings in terms of the place of the elements in the periodic chart. We could quickly see that when two or more elements are part of the same series, they also belong to the same group. For example, all elements in the I A and the VII A series are A inducing, or “hetero.” The members of the II A and VI A series are D inducing or homo. At this point, we tentatively extended these hetero or homo characters to an entire series after one or more elements in it had been recognized as such. The I A, II B, III A, V A, VII A series and the Fe subseries of VIII were classified as hetero or A inducing, while the II A, I B, VI A series and the Co subseries of VIII were labelled homo or D inducing.

This hetero or homo grouping of the various series permitted us to

make other correlations. We could see that among all the series designated as A in the periodic chart, those numbered oddly are hetero type, while those with even numbers are homo type. Among the B series, the opposite is true; those with odd numbers are homo type, while those with even are hetero type. Extrapolating, we could classify all the series according to this criterion. This view was confirmed by the hetero character seen in Cr and especially Mo, and the homo character for Mn.

We could go farther and correlate the above classification, made on the basis of biological properties, with the electronic configurations of the elements. For the members of the A series, those with an odd number of electrons in the valency shell were hetero type, while those with an even number were homo type. Among the B series, this criterion did not hold true. We found, however, that a similar correlation existed if consideration was given not to the valency shell alone but to the sum of the two external shells, the valency and the shell beneath it. This accords with the fact that in the B series elements, the two shells have insufficient electrons to fulfill the quantum numbers. We saw thus that those members of the B series with an odd number for the sum of electrons of the two shells have a homo character, while those with an even number have a hetero. This criterion applies to all members of the B group, including those in the I B and II B series, which have their full quantum quota of electrons in the shell beneath the valency shell. This same criterion was used to classify the three subseries of series VIII shown in the chart. The Fe subseries and Ni subseries were considered hetero type, the Co homo type. This antagonism was seen to be in accord with experimental findings.

These considerations also permitted us to classify the members of the Lanthanum and Actinium Series. Characteristically, all show nonfulfillment of three of their electron shells—the valency and the two shells beneath. We established for these elements a separate series designation, C. Using the sum of the electrons of the three shells, we separated the elements of the C series into hetero and homo categories. Here, the criterion was the opposite of that used for the B series. The members with odd numbers of electrons were considered hetero, those with even numbers homo.

We will discuss later the biological significance of this separation of elements into A, B and C series with their respective one, two and three unfulfilled shells. For the moment, we will only remark that if we consider an even number of electrons as corresponding to a kind of partial fulfillment of quantum forces, especially as compared to an odd number, such partial fulfillment is seen, among all the A series, in those with even num-

bers (II, IV, VI); among all the B series, in those with odd numbers (I B, III B, V B, VII B); and, among the C series, in those with alternate numbers which can be considered to correspond to even numbers. All these series—with partial fulfillment for the sum of their shells—have a D inducing or anti A, character, and thus a homotropic tendency.

Periods

Coming back to the influence exerted by the elements on test manifestations, we found that elements of the same series show some similar properties when acting at a certain level of organization, but some differences appear when they are acting at different levels. Some of these differences are important. Magnesium, calcium and strontium act similarly on the s.d.c. pH, and against convulsions as well. However, magnesium has been found to induce somnolence or even deep sleep in test animals, while calcium immediately wakes them from magnesium-induced somnolence. This type of antagonistic action among members of the same series sometimes appears especially pronounced between two consecutive members in the series—for example, between sodium and potassium, magnesium and calcium, oxygen and sulfur, and sulfur and selenium.

Study of this "antagonism" has permitted us to recognize a specific characteristic. When two elements of the same series act upon the same entity, one may substitute for the other. Sodium may replace potassium in cells. Magnesium and calcium, oxygen and sulfur, and sulfur and selenium can replace each other in this kind of reciprocal activity. There is no truly antagonistic action between them. This explains the fact that two elements of the same series, if in sufficient amount can have similar activity at a given level—that of the tissular, for instance, where the changes of the s.d.c. pH take place.

Further analysis of the activity of members of the same series has revealed another important characteristic which has permitted further classification. Differences in activity of members of the same series could be related to the organizational compartments involved. This became clear when activity of members of the I A series was analyzed according to whether these elements form constituents of the metazoic, nuclear or subcellular compartments. Sodium is the predominant cation of the metazoic compartment, which consists of the interstitial fluids, lymph and blood. Potassium is the principal cation of the cellular compartment. Ammonium, which corresponds in most of its properties to rubidium, represents the cation at the nuclear level. It could be seen that the development of hier-

archic organization has involved elements with progressively smaller atomic weights.

Study of constituents in compartments and in the environment has further permitted us—as seen above—to correlate the metazoic compartment with the sea, the cellular compartment with the crust of the earth, and the nuclear and subnuclear compartments with the formations in which their constituents were found in the vicinity of volcanoes.

This correlation of the metazoic compartment to the environment of the sea became especially interesting when we could recognize in its constituents not only sodium, but, curiously enough, the other members forming the same period in the periodic chart. Chlorine, magnesium and sulfur, predominant in the sea and also found in the metazoic compartment, are in the same period in the chart as sodium.

We have thus tried to extend the concept of a correlation between the periods of the chart and the different compartments of hierarchic organization. Tentatively we correlated the second period of the chart to the total organism as an entity. Oxygen, carbon and nitrogen—principal elements in air—enter into direct contact with the organism as such. The third period contains sodium, magnesium, sulfur and chlorine, which are found in the sea and can be correlated with the metazoic compartment. The fourth period contains potassium, calcium, iron, nickel, copper, selenium and bromine—all common to the earth's crust—and, according to our tentative systematization, correlated with the cellular compartment. Following the same plan, we could relate the fifth period—containing rubidium, molybdenum, silver, tellurium and iodine—to the nuclear compartment.

As a possible basis for a working hypothesis, we could consider the sixth period—with cesium, barium, gold, mercury, lead, bismuth—and the lanthanum series to belong to a subnuclear or, rather, submorphologic compartment. The seventh period includes the radium and actinium series, characterized by radioactivity. This period could be related to the lowest level of the biological organization, the primary one, probably even the submolecular level. This would relate the intervention of radioactivity—from cosmic rays and especially from the earth's radioactive elements—to the beginning of the biological realm. Such radioactive intervention could have brought together C and N to form N-C-N-C, which we considered in our hypothesis to be the first entity in the biological realm. This view represents, at least, a new basis for an interesting working hypothesis.

Thus we have the concept of hierarchic compartments related to changing environments. We also can correlate, further, the environments to the

periods in the periodic chart to which their principal constituents belong. It is difficult to accept as purely accidental the correlation of the changes in environments with the progressive displacement of their constituents toward periods in the chart with members each time having lower atomic weights. It is in this progression that we can see homotropy developing toward its maximum, complete value. This view, which will be discussed in more detail later, again relates evolution of the biological realm to progress of homotropy, with the environment representing the concrete realization of homotropic evolution.

The entire chart can be considered in terms of hetero and homo series and of periods that correspond to hierarchic compartments, as shown in TABLE V.

For the moment, the possibility of relating an element, through its membership in a series, to the hetero or homotropic trend and, through its place in a period, to an organizational hierarchic compartment, helps to explain many of the peculiarities seen in the biological distribution of the elements and especially in the role played by them at the "proper" levels to which they belong.

As a general rule, the presence of an element at the level to which it belongs is directly correlated to quantitative optimum values corresponding to the constants of the level and to the qualitative role which it performs. Its presence at levels other than its own must be interpreted in connection with its activity at its own proper level. Increase or decrease in the amount of an element has a different meaning according to the level at which the variation occurs. If it occurs at the specific proper level to which the element belongs, it would indicate a direct quantitative or qualitative change in the activity of the element. At other levels, this is not true. If the activity of an element is qualitatively impaired at its own level, the amount of the element at the immediate superior level will increase. The increase at the superior level can be interpreted as taking place in order to keep at the disposal of the impaired level a sufficient amount of the element for possible later use. On the other hand, an abnormally intensive activity of an element at its own level will reduce the amount of it present at the level immediately superior. The decrease at the upper level can be interpreted as a defensive attempt to reduce the abnormal activity by limiting the supply.

The general rule, which appears to govern the variations in distribution of an element within the organism, makes it important to know the proper level of an element. Some examples will illustrate what we mean. An increase of copper is seen in the blood serum of cancer patients, although

PERIODIC CHART OF THE ELEMENTS

* Lanthanum Series

Actinium Series

a manifest reduction in catalase as well as in copper content is seen in the tumor cells themselves and in the liver cells. According to the view presented above, these findings can be interpreted to reflect a primary insufficiency of copper at its specific level, that of the cell. Copper is quantitatively deficient at the level of the cell not because of its low availability, but because it cannot be utilized well enough qualitatively to form catalase. The qualitative impairment in copper's use at its proper level would lead to an increased amount of copper in the immediately superior compartment, that of the blood serum.

The knowledge that copper belongs to the cellular level, because of the period to which its belongs, could explain this peculiarity. The organism does not have *too much* copper although the amount of it in the blood is increased. Neither does it have too little copper at the proper level. The abnormality resides in a qualitatively impaired capacity of abnormal cells to utilize copper. In tentative therapeutic application, we have to try neither to increase nor decrease the amount of copper but to obtain its proper utilization by the abnormal cells.

This view applies also to potassium. An increase of potassium in blood serum is seen in subjects with type D offbalance. With potassium belonging to the cellular level, the primary abnormality has to be sought at this level. In fact such a primary anomaly of potassium metabolism is seen in the cells for, in offbalance D, the cells are poor in potassium, possibly because the cation moves out of the cells as the result of being displaced by sodium. The increase in the amount of potassium found in the circulating blood thus can be interpreted as secondary, designed to offer the cells a sufficient amount of potassium to be utilized in attempts to overcome this offbalance. On the other hand, in abnormal offbalance A, when quantities of potassium are found present in proliferating cells, an abnormally low amount of potassium is found in blood (as low as 3.0 m Eq or less). As potassium is still excreted through the kidney, this low blood potassium is not to be interpreted as a quantitative deficiency but rather as a teleological response to the abnormally high utilization at the cellular level.

A study of potassium, presented under this aspect, is the subject of Note 2.

The relationship between elements, periods and levels of the organization explains a curious distribution of elements as seen in the following experiment. 1/10 molar solutions with pH, of dibasic phosphates of lithium, sodium, potassium, ammonium and rubidium were prepared. Each solution was injected intravenously into mice, 1/4 cc. per minute, until the animal died. The organs, and especially the brains, were immediately

fixed in Bouin solution and studied histologically. For ammonium and rubidium, vacuoles were seen present in cells and especially in nuclei; for potassium, the vacuoles were present in the cytoplasm, while for sodium, only a pericellular edema was noted. No such changes were observed for lithium. Considering the dimension of the atoms, an opposite occurrence would have been expected with lithium penetrating most into the cells and rubidium the least. The fact that the heavier elements correspond to the lower levels of the organization, according to the concept presented above, explains the occurrence.

This could also be seen for the distribution of selenium and tellurium. We could show that while selenium accumulates in the cytoplasm, tellurium—which is the next heavier element of the VI series—is fixed preferentially in the nuclei.

It must be recognized that many problems result from exaggerated or reduced amounts of elements at compartments where they do not belong as characteristic constituents. The therapeutic effort, until now, has been to try to eliminate an excess or make up for a deficiency at any level. According to the view presented above, the main effort should be to try to correct the anomaly in the metabolism of the element at its proper level for this will lead to correction at other levels. We will consider, later, some examples of such effort.

The concept of dualism and of the place of elements in hierarchic organization has opened a new way to study the influence exerted by these elements in abnormal conditions as will be presented below.

The same type of analysis used for elements can be applied to the other body constituents. We will start with those which we believe to be the most important for the problem of the imbalances, the lipids.

CHAPTER 6

LIPIDS AND LIPOIDS

TWO DIFFERENT PATHWAYS, one theoretical and the other experimental, have led us to consider lipids as possibly the most important constituents involved in the dualistic patterns of physiopathological manifestations. The study of all the constituents of the organism—electrolytes, proteins, carbohydrates and lipids—has shown that for each of them, a rough division into two classes with antagonistic reactivity can be made according to the positive or negative electrostatic character of their polar groups—nucleophilic or electrophilic for some, anionic or cationic for others. However, these fundamental differences which can explain their intervention in processes in which dualism is apparent, do not represent the reason for their role in the induction of patterns.

The reactions in which some of these constituents take part are carried out as rapid changes while others are completed only slowly. It is these slow reactions, once accomplished, which tend to be stable for long periods of time. Since such stability is characteristic of clinical and analytical manifestations which have dual patterns, it appeared logical to consider the constituents with slow reactivity which are related to these manifestations. Because of their hydrosolubility, and the rapidity of the reactions in which they take part, most electrolytes, proteins and even carbohydrates probably play a lesser role in these long lasting processes.

The lipids, on the contrary, seem to be especially suited for this role. Many of the reactions in which the lipids participate are slow. As we will see later, this is primarily because of their insolubility in water. They form in the organism a group "apart" from all the water soluble constituents, a fact which permits them to function through proper reactions largely without continuous interference from the other constituents. For these reasons, the lipids appeared to be the most likely of all constituents to be

of major importance in physiopathological manifestations with long-lasting patterns. The study of the lipids has substantiated this.

However, before discussing these substances and their properties, a nosological problem must be considered: What are lipids? How can they be defined?

DEFINITION OF LIPIDS

The literature fails to furnish an adequate definition for the group of substances that show those properties which biochemistry and experimental biology attribute to the lipids. A definition on a chemical basis, such as one which considers lipids to be fatty acids and fatty acid derivatives, appears to be insufficient. It excludes substances such as those forming insaponifiable fractions which not only have properties attributed to lipids, but continuously intervene in the processes related to them.

Physical characteristics such as "greasiness" and solubility come nearer to the real situation without providing a satisfactory definition. Bloor's definition (*Note 1*), widely accepted today in spite of having been found inadequate, has introduced—in addition to the important solubility characteristics—certain less acceptable criteria such as the origin of these substances and the direct relationship to fatty acids. Without these criteria, lipids would have to include the group of hydrocarbons which have the same solubility property but usually are not encountered in organisms. However, with these criteria, Bloor's definition, besides limiting the field too much through the requirement for a relationship to fatty acids, excludes the entire important group of synthetic agents with similar properties. To be complete, a definition would have to include these artificial substances.

Thus confronted by the need for a satisfactory general definition, we proposed one in 1940 (23) which has since been of great help to us in all our research: *a lipoid is a polar-nonpolar substance in which the nonpolar part is predominant. It is thus formed by one or more polar groups bound to one or more nonpolar groups, the last being energetically predominant.* In terms of intervening forces, this definition considers the cohesion forces of the nonpolar part, and especially those related to its surface and known as the constant "b" of van der Waals forces, which in lipoids are predominant upon the electrostatic forces of the polar part. The definition has provided the key for the study of the multiple problems in which these substances appear to be involved. This definition appears acceptable since it explains all the known properties of the lipids. Further-

more, the study of the specific relationship between the forces involved could even predict new properties, as will be shown later.

The distinction between natural and synthetic substances as a basis for a definition has been obsolete in biochemistry for a long time. In our study however, it appeared to be didactically useful to indicate whether or not a substance is encountered naturally in the organism. Therefore, while adhering to our general definition, we have employed the term "lipoids" for the entire group of polar-nonpolar substances with a predominance of the nonpolar part, and have conserved the term "lipids" to designate the naturally occurring members. With this separation, we have also avoided

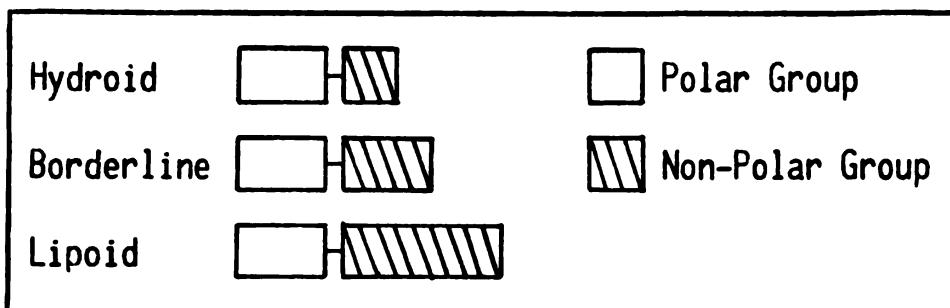


FIG. 61bis. Schematic representation of the predominant relationship of the polar and nonpolar parts in hydroids, borderline substances and lipoids.

a certain apprehension felt by many workers about incorporating indistinctly, in the same group of agents, substances with vastly different chemical constitutions, which until now have not been associated with lipids. The fact that, beyond physicochemical constitution, biological properties characterizing the lipids are common to the entire group of lipoids, will in time, we hope, help to reduce the importance of this separation between lipoids and lipids.

The structure of the lipoids—with a large variety of polar and nonpolar groups but always with the same characteristic energetic relationship between them—has led to a logical systematization of these substances, using the nature of the polar and nonpolar groups as criteria.

CLASSIFICATION OF LIPOIDS

Lipoids may be subdivided according to different criteria.

I. *According to the polar group.*

A. *Lipoids classified according to the nature of their polar group.*

1. Lipo-carboxylic acids $(-\text{COOH})$
2. Lipo-thiols $(-\text{SH})$
3. Lipo-sulfonic acids $(-\text{SO}_3\text{H})$

4. Lipo-amines	(—NH ₂)
5. Lipo-amides	(—CONH ₂)
6. Lipo-alcohols	(—OH)
7. Lipo-aldehydes	(—CHO)
8. Lipo-ketones	(=CO)
9. Lipo-halogens	(—Cl, etc.)
10. Lipo-metals	(—Na, etc.)

etc.

B. *Lipoids classified according to the predominant element of the polar group.*

1. Lipo-sulfur compounds

a. Lipo-thiols	(—SH)
b. Lipo-sulfonic acids	(—SO ₃ H)
c. Lipo-sulfides	(=S)
d. Lipo-sulfoxides	(—SO)
e. Lipo-sulfones	(=SO ₂)
f. Lipo-sulfites	(=SO ₃)

etc.

2. Lipo-nitrogen derivatives

a. Lipo-amines	(—NH ₂)
b. Lipo-amides	(—CONH ₂)
c. Lipo-nitriles	(—CN)
d. Lipo-isocyanides	(—NC)
e. Lipo-nitro derivatives	(—NO ₂)

etc.

C. *Lipoids classified according to the energetic character of their polar group.*

A. Lipoids with negative polar groups

1. Lipoacids
 - a. Lipo-carboxylic acids
 - b. Lipo-thiols
 - c. Lipo-sulfonic acids, etc.
2. Lipo-aldehydes

B. Lipoids with positive polar groups

3. Lipobases
 - a. Lipo-amines
 - b. Lipo-guanidines
 - c. Lipo-imines, etc.
4. Lipo-alcohols

II. *According to the nonpolar group.*

A. *Lipoids classified according to the structure of their hydrocarbon chain.*

1. Aliphatic
2. Alicyclic
3. Aromatic
4. Heterocyclic, etc.

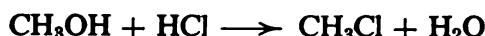
B. Lipoids classified according to their carbon bonds.

1. Saturated
2. Unsaturated
 - a. Ethenic (mono-, di-, poly-)
 - b. Ethynic

Some aspects of this classification require discussion.

The generic term lipoacid has been employed to describe simple lipoids having polar groups with acid functions. While the principal lipoacids are the fatty acids, other members have other acid polar groups, such as SO₂, SH, NO₂, etc. The significance of this grouping together of lipoids with negative polar groups has become evident especially in studying the similarities in the biological effects of these substances. In certain aspects of our research, this correlation has permitted us to substitute one category of lipoids (lipo-thiols or lipo-aldehydes) for another (lipo-carboxylic acids), thereby avoiding certain undesirable effects of the latter group of substances.

Lipoids having polar groups energetically opposite to those of the acids have been grouped together. Of these, the members with a polar group with alkaline functions have been classified as lipobases. The term base is generally applied to ionizable compounds which influence the pH of solutions and combine readily with acids by losing an OH⁻ and gaining a proton. Another group is formed by the lipoidic alcohols. Recent evidence indicates that, in many circumstances, the differences in the reactions of alcohols and common bases are quantitative rather than qualitative. Quite often the reaction of an alcohol with an acid is analogous to the reaction between an acid and sodium hydroxide, the H⁺ of the acid combining with the OH⁻ of the alcohol. The differences between reactions are considered to be matters of time-rate. Whereas the reaction of the base is almost instantaneous, that of alcohol is a slow reaction and is less complete. This behavior of alcoholic substances is particularly clear when the hydroxyl group of an alcohol is replaced by a halogen to prepare the alkyl halides. For instance, according to Karrer (24): "This can be done by the action of the concentrated halogen acids on the alcohol:

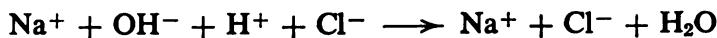


The reaction corresponds superficially to the formation of a salt from an acid and a base:



There is, however, a difference between the two processes. Bases and acids are largely dissociated; when they come together, hydrogen ions and hy-

droxyl ions combine almost at once to give the very little electrostatically dissociated water, so that the reaction which really occurs is:



Ionic reactions always occur instantaneously. Reaction between alcohol and hydrogen halides is governed by other laws. Alcohol is only very slightly ionized. For the removal of the hydroxyl group a certain time is required. The reaction between alcohol and acid with elimination of water, known as esterification, is therefore a time reaction." No essential difference exists between the reaction of lipo-alcohols or lipo-amines, for instance, with organic acids.

These considerations would have been sufficient to allow lipobases and lipo-alcohols to be grouped together. There are other considerations as well. Their common biological activity and mutual interchangeability also appear to justify grouping them together. Furthermore, the recognition of the existence of a general mutual antagonism between lipoacids on the one hand and lipobases and lipo-alcohols on the other hand, chemically, physically and biologically, has proven of considerable value in explaining a variety of experimentally observed facts in many aspects of our research.

Following this through, we have found it advantageous to define the two groups of lipoids by a more general character, the electrical aspect of the polar part, negative for the lipoacids and lipo-aldehydes and positive for the group of lipo-alcohols and lipobases. The terms, "positive and negative lipoids," serve also to emphasize the nature of their antagonism.

The structure of the nonpolar group as it confers physical, chemical and biological properties on the lipoids permits further subdivisions. Lipoids may be classified on the basis of the aliphatic, alicyclic, aromatic or heterocyclic character of the nonpolar group. While the negative lipids are principally formed by fatty acids, the positive are made up principally of sterols. The presence or absence of double bonds defining saturated and unsaturated carbon chains has been one subject of our study and considerable biological importance has been found to be related to this character as well as to the positional relationship of the double bond to the polar group and the polarity induced by the double bond.

The study of lipoids has shown that, besides properties contributed by the elements and groups which compose them, they have additional physico-chemical and even biological properties which are characteristic. We have termed these "lipoidic properties" to indicate that they are considered to result directly from the particular constitution of the lipoids.

Physical Lipoidic Properties

Solubility

Solubility represents the first and most important of these lipoidic properties. Characteristically, a lipoid has a greater solubility in neutral solvents than in water. This is explained by the fact that the two constituent groups, polar and nonpolar, induce different solubility properties.

As is well known, solubility corresponds to a free movement between molecules of the solvent and the solute. (25) Solubility is greater when the physical properties of the groups forming the solvent and those forming the solute are similar; it is impaired when they are different. Consequently, polar groups in a solute will tend to favor solubility in solvents with polar groups, such as water. At the same time, they will oppose solubility in neutral solvents formed by nonpolar groups. On the other hand, nonpolar groups in a substance will favor solubility in nonpolar neutral solvents but will oppose it in polar solvents such as water. Polar groups thus are hydrophilic and lipophobic, while nonpolar are lipophilic and hydrophobic. (26)

While the solubility characteristics of substances composed only of polar or nonpolar groups are readily apparent, the problem is more complex when a substance contains both polar and nonpolar groups. Since such a compound possesses groups with antagonistic solubility tendencies, its solubility "in toto" will depend upon the relationship between the opposing forces. For a borderline group of polar-nonpolar substances with approximately equal forces, there will be equal solubility in polar and nonpolar solvents. For other substances, the predominance of one or the other group will determine solubility characteristics. If the electrical forces of the polar group predominate, the substance will be hydrosoluble but insoluble or only partly soluble in nonpolar solvents. If, on the contrary, the cohesion—*i.e.*, the van der Waals forces—of the nonpolar group predominate, the substance will be soluble in nonpolar solvents and less, or even not at all, soluble in water. (TABLE VI)

Polar and nonpolar forces can be calculated and their study can indicate the place of a substance in this systematization. The importance of solubility for defining and systematizing lipoids became apparent in a physicomathematical study of these substances carried out by Jean Mariani in our laboratories. (*Note 2*) We have defined as "hydroids" those substances with predominant polar groups which are more soluble in polar solvents such as water. The "borderline substances," with no predominance of either group, show the same solubility in polar and neutral solvents.

TABLE VI
CLASSIFICATION OF CHEMICAL COMPOUNDS

Composition	Predominance	Name	Example
Polar groups only			Water
Polar-nonpolar groups	Polar group predominant	Hydrides	Glycerin
	No predominance	Borderline substances	n-Propyl alcohol
	Nonpolar group predominant	Lipoids	Oleic acid n-Butyl alcohol
Nonpolar groups only			Paraffin

The "lipoids," in which the nonpolar groups predominate, are more soluble in neutral solvents than in water.

As we have mentioned above, from a practical point of view, a substance could be judged to be a hydride, borderline substance, or lipoid by considering the differences in its solubility in water and in a nonpolar solvent, such as petroleum ether, which corresponds to a mixture of the first aliphatic saturated hydrocarbons liquid at normal temperature and pressure. *A polar-nonpolar substance more soluble in water than in neutral solvent is considered a hydride; one equally soluble in both solvents is classified as a borderline substance; while a substance more soluble in the neutral solvent than in water is a lipoid.*

Different polar groups such as COOH, OH, NH₂, CO, SO₂, SH, etc., enter into the constitution of various lipoids. They differ considerably in their electrostatic forces. As a result, the forces of the nonpolar groups required for predominance, if a lipoid is to be formed, also will differ. A different nonpolar group thus is necessary for each different polar group. For aliphatic molecules, it is principally the length of the chain which determines cohesion forces and a different number of carbons in the nonpolar group appears to be necessary, depending upon the polar group, in order to form a lipoid. The study of homologous series from this point of view is interesting.

Since the value of the electrostatic forces varies greatly from one polar group to another, the first members of the various homologous series, which are also lipoids, will differ from series to series, depending upon

the nature of the polar group. The length of the carbon chain of the non-polar group will thus indicate in what member of a series the lipoidic character appears. By comparing mathematically the value of the electrostatic forces of each polar group and the cohesion forces of the nonpolar group in the respective series, it is possible to determine which member of each homologous series of substances will first show the properties of the lipoids. This also can be determined experimentally, as seen above, using the solubility characteristics of the lipoids. For the different members of the series, degrees of solubility in a polar solvent such as water, and in a nonpolar solvent such as petroleum ether, were determined. The first member of an homologous series to be considered a lipoid was the one found to be more soluble in the nonpolar than in the polar solvent. All members with a large number of carbon atoms show lipoidic properties; those with fewer carbon atoms lack those properties.

Thus, lipoidic properties first become manifest, among the carboxylic acid series, in valeric acid, *i.e.*, the five-carbon member. The shorter carbon chain members are soluble to an equal or greater degree in water than in petroleum ether, while those having a carbon chain longer than four show a higher degree of solubility in the nonpolar solvents than in water. (TABLE VII)

TABLE VII
SOLUBILITIES OF CARBOXYLIC ACID HOMOLOGUES

Substance *	Common Name	No. of Carbon Atoms	% of solubility in	
			Polar Solvent (Water at 20°)	Nonpolar Solvent (Petroleum Ether)
Methanoic acid	Formic acid	1	∞	insol.
Ethanoic acid	Acetic acid	2	∞	∞
Propanoic acid	Propanoic acid	3	∞	∞
Butanoic acid	Butyric acid	4	∞	∞
Pentanoic acid	Valeric acid	5	3.7 (at 16°)	∞
Hexanoic acid	Caproic acid	6	0.4	∞
Heptanoic acid	Enanthic acid	7	0.24	∞
Octanoic acid	Caprylic acid	8	0.25 (at 100°)	∞

* Names approved by International Union of Chemistry.

The same is true for the alkyl alcohols. n-Propyl alcohol and the members below it are either miscible with both water and petroleum ether or more soluble in water, indicating that the nonpolar forces do not predomi-

nate in their molecules. Therefore, they are not lipoids. n-Butyl alcohol, more soluble in neutral solvent than in water, thus is the first lipoidic member of this homologous series. However, this is not true for all its isomers. The primary, secondary and iso butanol are the first in their respective series to possess the solubility properties characteristic of lipoids. In the tertiary alcohol series, however, the four-carbon member, the tert.-butanol, does not show the same solubility properties. Tert.-butanol is miscible with water and neutral solvent and as such, is not a lipoid. For this tertiary alcohol series, it is the five-carbon member, the tert.-amyl alcohol, which first shows the solubility properties of a lipoid, being only 12.5% soluble in water and infinitely soluble in petroleum ether. Thus, of the four isomers of butyl alcohol, three are lipoids, while one, tert.-butyl alcohol, is not. (TABLE VIII)

TABLE VIII
SOLUBILITIES OF THE ALKYL ALCOHOLS

Substance *	Common Name	No. of Carbon Atoms	% of solubility in	
			Polar Solvent (Water at 20°)	Non-polar Solvent (Petroleum Ether)
Methanol	Methyl alcohol	1	∞	∞
Ethanol	Ethyl alcohol	2	∞	∞
1-Propanol	Propyl alcohol	3	∞	∞
2-Propanol	Isopropyl alcohol	3	∞	∞
1-Butanol	n-Butyl alcohol	4	7.9	∞
2-Butanol	sec.-Butyl alcohol	4	12.5	∞
2-Methyl, 2-propanol	tert.-Butyl alcohol	4	∞	∞
2-Methyl, 1-propanol	Isobutyl alcohol	4	9.5	∞
1-Pentanol	n-Amyl alcohol	5	2.7	∞
2-Pentanol	sec. act. Amyl alcohol	5	5.3	∞
3-Pentanol	Diethyl carbinol	5	insol.	∞
2-Methyl, 2-butanol	tert.-Amyl alcohol	5	12.5	∞
2-Methyl, 1-butanol	n-act. Amyl alcohol	5	insol.	∞
3-Methyl, 2-butanol	Isoamyl sec. alcohol	5	sl. sol.	∞
1-Hexanol	n-Hexyl alcohol	6	very sl. sol.	∞
2-Hexanol	sec.-Hexyl alcohol	6	very sl. sol.	∞
3-Hexanol	Ethyl propyl alcohol	6	0.9	∞
1-Heptanol	n-Heptyl alcohol	7	insol.	∞
1-Octanol	n-Octyl alcohol	8	insol.	∞

* Names approved by International Union of Chemistry.

The same methods were used to recognize the first lipoidic members of various alkane derivatives studied. TABLE IX shows the first lipoid members of several homologous series.

TABLE IX
**FIRST LIPOIDIC MEMBERS IN VARIOUS ALKANE DERIVATIVE
HOMOLOGOUS SERIES**

Substance *	Common Name	Polar Group	No. of Carbon Atoms
Methanethiol	Methyl mercaptan	-SH	1
Propanal	Propionaldehyde	-CHO	3
Propylcarbylamine	Propyl isocyanide	-NC	3
1-Butanol	n-Butyl alcohol	-OH	4
2-Butanone	Butyl ketone	=CO	4
Butanamide	Butylamide	-CONH ₂	4
2-Methyl, 2-butanol	tert.-Amyl alcohol	-OH	5
Pentanoic acid (n)	Valeric acid	-COOH	5
Hexylamine (n)	Hexylamine	-NH ₂	6
1, 8 Octadiol	1, 8 Octadiol	-OH	8

* Names approved by International Union of Chemistry.

Molecular Layer Formation

Other fundamental characteristics result from the different solubility properties of the two parts, polar and nonpolar, forming a lipoid. Introduced in a diphasic medium in which one phase is water and the other oil or even air, the polar group, with the tendency to be soluble in water, will penetrate the water. Since the nonpolar group, which is hydrophobic, is predominant, not only will it not enter the water but it will also prevent the entire molecule from moving freely in water. Consequently, the lipoid molecule will remain at the surface of the water with only its polar group penetrating. Because of this, the molecule will assume an oriented position toward the surface of water. If the second phase is a neutral solvent, the lipoid molecules, which will accumulate at the interphase with the polar group in water, will have the nonpolar group penetrating the neutral solvent.

In both cases, the molecules form oriented molecular layers which, if present at the limit between two phases, would appear as organized formations. This property which appears as a direct consequence of the characteristic constitution of the lipoids has further consequences. In such a layer, the polar groups penetrating in water will influence the properties of its surface, and thus reduce its surface tension.

Through the coulombian character of their electrostatic forces, the polar groups will thus confer, according to their nature, a positive or negative electrical character to the layer.

In a mixture of water and oil, the presence of a lipoid layer will lower the intersurface tension and will favor the breaking down of the phases, facilitating the formation of an emulsion. The presence of the same electrical charge at the surface of these resulting emulsion droplets will act as a repellent force between them and increase the stability of the emulsion. (Fig. 62) This is another important characteristic of lipoids which results from their peculiar constitution.

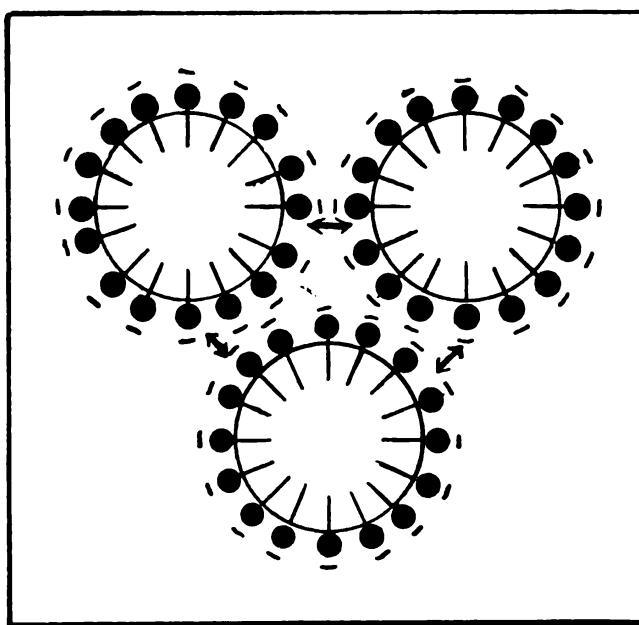


FIG. 62. The presence of the same electrical charge at the surface of droplets of an emulsion insures, through the repellent forces, the stability of the emulsion.

Chemical Properties

Lipoids have two groups of chemical properties which can be related to their two principal parts, polar and nonpolar. The polar groups with their electrostatic forces give to the lipoids one group of characteristic reactivities. A carboxylic lipoid will act like any other organic acid, while the lipo-alcohols will act like other alcohols, a thio-lipoid like a mercaptan, and so on. A characteristic of chemical reactions induced by the polar groups is that, while they are occurring in a water medium, they are largely limited to the site where the lipoid is localized due to the insolubility of

the entire molecule in water. Through this localization, the polar reactivity of the lipoids becomes largely a "surface reactivity." It is interesting that even minute amounts of lipids are able, through this localization at separating surfaces, to induce important changes.

A second group of reactions take place at the nonpolar group and especially at the different formations present in it, such as double bonds, cycles, etc. The hydrophobic and lipophilic character of the nonpolar groups confers a special character on these reactions. Most of them take place in nonionic nonpolar media. Many occur at the semipolar double bonds with nucleophilic or electrophilic carbons which appear to be especially suitable for this reactivity. This would explain the fact that nondissociated molecules may take part in these reactions. Most of these reactions are relatively slow. This double reactivity, ionic through the polar group and rather nonionic through the nonpolar group, makes the study of these lipoids one of great interest and it will be discussed below in more detail.

Biological Properties

The biological properties of lipoids in general also can be related directly to their physiochemical characteristics and, thus, to their peculiar constitution.

Lipidic System

The relative insolubility of the lipids in water and their solubility in neutral solvents has permitted us to separate these substances as a group from the other constituents of organisms. For more than just didactic purposes, we consider lipids to constitute a separate system in the organism. The part played by lipids in the organization and the functioning of various entities supports this concept. For example, when a lipoid is introduced into the organism, it will be selectively dissolved in, circulated through, retained by and metabolized as part of the lipidic system. Overton's "Index of Repartition" of anesthetics in the organism can be seen to be a direct corollary of the existence of such a system although the anesthetic agent can be a lipoid or a nonpolar substance.

A great degree of independence of this system is morphologically evident as in adipous cells, when fats circulate as chylomicrons or when they form oriented layers. We have seen above how the orientation of lipoids at the surface of water results from the relationship between the solubilities of the two constituent groups, polar and nonpolar. Along with

their insolubility in water, the orientation of lipoids has allowed them to play a very important role in biology.

The very existence of biological entities appears to depend upon the ability of lipids to build up boundary formations separating and thus assuring the individuality of biological entities.

Through peculiar, reciprocally opposed orientations, two or more layers of lipids can form a membrane with two polar faces which has the ability to separate two aqueous media. In its simplest form, such a membrane appears in mitochondria. (*Note 3*) Similar boundary formations identify nuclei and cells and appear in higher entities, as in the membranes and intercellular cements of lymphatic and blood vessel endothelia. It is this peculiar orientation which allows lipids to establish the necessary boundary formations resulting in complex hierarchic organisms. The existence of biological entities, at least from the chromosome level up (and probably even below that level), can be seen to result directly from the intervention of lipids as a separate system, particularly in the formation of the dipolar lipidic boundaries.

However, boundary formations which separate the biological entities would not have been efficient if they did not fulfill another capital role: that of allowing selective passage of metabolites. A totally impermeable membrane would isolate the respective entities and result in their death. On the other hand, a totally permeable membrane would have no usefulness. The boundary formation has to act selectively, permitting the passage of some, but not all, substances. But even this does not seem to be sufficient to insure an efficient boundary. Most important, such a membrane must be able to alter its permeability, quantitatively and qualitatively, according to variations in circumstances. Such capacity for altering permeability can be related to the presence of the two groups of lipids, fatty acids and sterols, with their antagonistic properties relating to permeability.

The fatty acids appear to induce permeability in the membrane they form, especially permeability for anions. The perpendicular position to the surface of water assumed by the nonpolar aliphatic groups when the fatty acids form this boundary membrane appears to be favorable for the passage of a substance through the membrane. The fatty acid molecules thus can be separated, permitting other molecules to pass between them; that is, to pass through the membrane formed by the fatty acids. The negative electrical character of the polar groups of these fatty acids explains why they represent a kind of barrier to the free passage of cations. These cations are attracted and retained by the acid polar group. This would explain the manifest changes in permeability under the influence of calcium ion. The

removal of calcium from cellular membranes, through treatment with oxalates, increases permeability, while treatment with calcium salts reduces permeability. The bivalent calcium ion, when it binds the polar groups of two adjacent fatty acid molecules in the membrane, prevents the passage of other molecules between these parts of the membrane, a fact which explains the manifest decrease in permeability induced by this cation.

The other group of lipids, the sterols, have an effect on permeability opposite to that of fatty acids. This can be related in part to the bond which these sterols make with the fatty acids. Consequently, they block any passage through the part of the membrane formed by fatty acids. The impermeability is due, to some extent, to a peculiarity of the layers formed by these sterols themselves. The polycyclic molecules of sterols do not take the same perpendicular position toward the surface of water as fatty acids do. (27) (Fig. 63) Since sterol molecules assume a position almost parallel

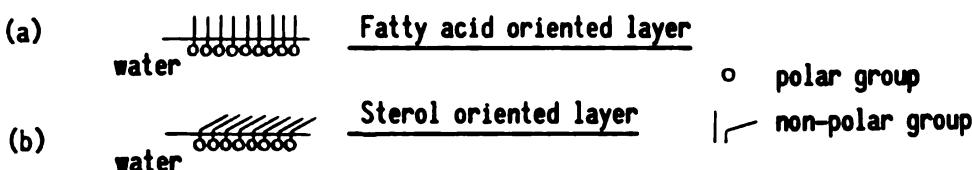


FIG. 63. *Schematic aspect of oriented interface layers.* The perpendicular position to the surface of water of fatty acid molecules (a) favors the passage of other molecules through the separating membranes they form. Oppositely, the almost parallel position to the surface of water of the polycyclic molecules of sterols (b) prevents the passage of other molecules through the membrane they form.

to this surface, the layer which they form exhibits no permeability properties. It even opposes any passage through it. It seems that fatty acids and sterols make separate "spots" in the cellular membranes so that, through their quantitative relationships, they confer different degrees of permeability to different regions of the membrane. The changes in permeability which result from the antagonistic intervention of the two groups of lipids seem to play an important role in normal and abnormal physiology.

With fatty acids inducing permeability, and sterols opposing it, the fundamental character of their biological relationship can be recognized. It would seem that part of the function of sterols is to oppose the activity of fatty acids. Conceptually, sterols would appear, in this specific activity, to be "anti-fatty" acid agents, with a capacity to control the activity of fatty acids rather than be active by themselves. Partly for this reason as well as for greater general understanding, it is necessary first to investigate fatty acid activity.

FATTY ACIDS

Besides their constructive role in establishing boundary formations, fatty acids appear to serve various other purposes in the organism. They can be used as caloric metabolites, and they play an active functional role in a biological change. While all fatty acids may exhibit these three activities—caloric, constructive and functional—there are important individual differences. With the carboxyl as common polar group, the differences between the various fatty acids can be related to the nonpolar groups. We will discuss this aspect of fatty acids, emphasizing only what can be considered to be new contributions to understanding the biological role of the substances.

Rancidity

The study of changes which take place in vitro, on lipids and especially on fatty acids after they have been separated from the organisms, led us to consider a possible parallelism between them and the changes which take place in the organism. We tried thus to utilize especially the knowledge furnished by the study of the chemical deterioration of natural fats generally known as rancidity (28), to better understand and also to systematize many of the processes occurring in vivo.

Three types of rancidity are described. In one—hydrolytic rancidity—fats are separated in free fatty acids and glycerol (or mono- or di-glycerides), through the intervention of lipolytic enzymes. These are often produced by molds (*Penicillium*, *Aspergillus*, etc.) or by microbes rich in such lipolytic enzymes or even the lipase present in the tissues from which the lipids are obtained. The characteristic of this type of rancidity is the intervention of enzymes and the appearance of free fatty acids as a result.

In a second type of rancidity, also occurring under the intervention of enzymes, an oxidative process is involved. The characteristic of this type of rancidity is that it affects almost, if not exclusively, saturated fatty acids, converting them into methyl-ketones by a beta oxidation process. This "perfume rancidity" called so because of the odor of the methyl-ketones with seven, nine or eleven carbons which result—takes place apparently through the intervention of a peroxidase present in certain molds (such as *penicillium glaucum*). One of its characteristics is that it occurs especially on saturated fatty acids with a low number of carbons (8 to 12).

The third type of rancidity groups together the oxidative changes which take place at the unsaturated nonpolar group of the lipids. As they result from the intervention of double bonds, the reactions differ according to

the energetic center present. In one which occurs at room temperature only for the conjugated fatty acids, such as eleostearic acid, and at 100°C only to some extent for oleic, linoleic and linolenic acid, the oxidation leads to the appearance of peroxides. (29) In another form of this oxidation, taking place for oleic, linoleic and linolenic fatty acids at room temperature or below 50°C, hydroperoxides result, as it has been shown by Farmer and coworkers, first for rubber (30) and after for fats (31). Another important fact seen in rancidity changes is that the atmospheric oxidation of polyethenoid fatty acids can result in a displacement of the double bonds with the appearance of conjugated isomers. (32)

The study of natural rancidity has represented the basic guide for our study and systematization of the processes encountered in normal and abnormal physiology. We searched and found this similarity not only in general outlines, but also for most of their details. By referring to the processes found in rancidity, we were able to identify, besides enzymatic lipolysis and enzymatic Knoop beta oxidation, known to occur in the organism, also the intervention of hydroperoxides, peroxides and the conjugation of double bonds. Not only the processes themselves but also the conditions under which they take place and their inter-relationship have been found to parallel *in vivo* those which can be seen *in vitro*.

We will see all along in the study of fatty acids how far the biological intervention of this parallelism goes.

Caloric Metabolism

Although all the fatty acids are ultimately used by different organisms as caloric metabolites, the saturated and monoethenic members are most important from this point of view. Among the saturated and monoethenic fatty acids, the members with long chains appear to be those which are kept in reserve for caloric purposes. We could show that the principal form of caloric desmolysis, the Knoop beta oxidation, takes place directly, almost exclusively, on members with relatively short chains, that is, with a maximum of 10 or 12 carbons.

While fatty acids with short chains take part directly in these caloric metabolic changes, those with longer carbon chains must undergo preliminary changes before entering into caloric metabolism. A desaturation, changing a saturated fatty acid into a monoethenic, appears to be a first step in caloric metabolism of the long chain members. The monoethenoids thus can be seen to be intermediary forms between the saturated reserve and the short-chain, easily metabolized fatty acids.

The double bond in these monoethenic acids would thus appear to

have two uses: one, to reduce the melting point below body temperature and thus permit easy mobilization, and two, to induce changes which lead to the breaking up of the long molecule into two shorter ones which can be metabolized through the Knoop oxidation.

All the data indicate that this fission would not take place at the double bond but through a more complex process. A first change consists of oxygen fixation at the carbon near the double bond. This leads to the appearance of a hydroperoxide group. It is only in a subsequent step that the molecule breaks at a place between this carbon near the double bond and the double bond itself, resulting in the appearance of short chains which have an even number of carbons capable of being directly metabolized through the beta oxidation. (*Note 4*)

The position of the double bond in the naturally occurring monoethenic fatty acids, separating almost always a group of nine carbons toward the carboxyl or the methyl end, (*Note 5*) acquires a special significance for the breaking down of the molecules for caloric purposes.

The desaturation of the saturated fatty acids, which would represent a first step toward allowing them to participate in metabolic caloric changes, would usually take place in the liver, apparently through the same processes by which polyunsaturated fatty acids are partially saturated. (*Note 6*)

An interesting part of the caloric metabolism of the saturated and monoethenic fatty acids, which will be shown below, is their combination with glycerol to form triglycerides.

Constitutional Role

Although saturated and monoethenoid fatty acids enter into the formation of boundary membranes, the di-, tri- and tetraenic members seem to have a particularly important role in the constructive function of fatty acids. Some of them enter directly into the formation of the membrane; some form complex lipoids such as lecithine with the glycerophosphoric radical and nitrogen containing bases. As a rule, these last represent a lipoidic substrate which would act as a neutral natural solvent present in membranes, and as such, intervene in the realization of a diphasic medium at the level of the boundary formation. This medium would largely insure the orientation of the fatty acids at the separation surface and the formation of permeable lipidic layers.

Functional Role

The third role of fatty acids is as functional agents taking part in certain reactions. This activity appears to be strongly related to two factors:

the presence of an uncombined carboxyl group and the energetic intervention of the double bonds of the nonpolar part of the polyunsaturated members.

Free fatty acids appear to be functionally active while the combined ones usually are inactive. The activity is related only partially to the direct capacity of the carboxyl to realize new combinations. It results from the induction exerted by the carboxyl upon the nonpolar group. The so-called free fatty acids of the organism are probably bound in a labile form to proteins, but this bond will not influence the induction effect exerted upon the nonpolar group. The intensive positive carbon of the carboxyl, together with the zig-zag disposition of the fatty acid molecule, causes the inductive effect to charge the successive carbons of the chain. They will thus show alternative signs. The even carbons show a negative character, while the odd ones are positive. The fact that oxygen combines with positive carbons explains not only why, as in Knoop oxidation, this bond occurs at C₃, which is strongly positive, but also explains the so-called alternate oxidation (33) where the other following odd carbons are binding oxygens. Through the influence exerted by the carboxyl, the double bond shows a special activity which has been worth studying.

Double Bonds

There has been some tendency to regard the double bond as a weak, easily broken point of the molecule. Actually, it emerges as an important center of activity. With its capacity to become a semipolar center, and consequently, to bind or lose radicals, the double bond is an energetic center in the molecule. Its important characteristic is the ability to effect such changes without altering the chain of the molecule itself. Since this type of reaction is reversible and can be repeated for the same molecule, the double bond appears to represent a functional entity. Because the reaction principally involves nonmetallic elements, the unsaturated fatty acid takes an active part in the metabolism in which these elements appear.

The study of rancidity has helped us, by analogy, to systematize oxidation processes as they take place *in vivo*. In addition to Knoop beta, several other types of oxidation could be recognized in which double bonds intervene more directly. The double bond, with its semipolar character, influences nearby carbons, rendering them highly reactive. In one form of oxidation, a molecular oxygen is bound to a nearby carbon to produce a hydroperoxide formation, as was shown to occur *in vitro* by Farmer. (31) When, under certain circumstances, this oxygen fixation becomes reversible, the fatty acid will liberate the oxygen. It appears highly probable that in

such a process, the oxygen is liberated as a free radical, the entire process thus corresponding to an activation of oxygen. The change of a molecular oxygen into a free radical would represent the physiological role of unsaturated fatty acids in oxidation processes.

The presence of two double bonds in non-parallel position, common to most of the naturally occurring polyunsaturated fatty acids, is even more important; the two double bonds exert a particularly strong influence on the special carbon which is in the intermediary position between them. Because of the alternate induction produced by the strongly positive carbon of the carboxyl, the carbons of the chain have alternate characters, positive and negative. When an intermediary carbon also has a strong positive character, it appears to be especially able to fix oxygen. This strongly positive intermediary carbon, occurring in natural polyunsaturated fatty acids with more than two double bonds, may be the reason for the important role played by these acids when they act as essential fatty acids in the organism. (*Note 7*)

The study of rancidity has further shown that, while the *in vitro* oxidation of an unsaturated fatty acid under mild conditions such as room temperature leads to the appearance of hydroperoxides, oxidation at a higher temperature will result in another fixation of oxygen, this time at the double bond itself. Epoxides or peroxides will appear according to the ionic or molecular character of the oxygen. This extremely important process also occurs in rancidity under the influence of an enzyme. It is highly probable that a similar process takes place *in vivo* in those pathological conditions in which peroxides appear in the urine. Radiation, certain inflammations (especially those due to streptococci), administration of selenium preparations or of highly polyunsaturated fatty acids are followed by the appearance of these oxidizing substances in urine. As mentioned above, when these substances appear, there also are increases in indoxyl and glucuronic acid, which can be considered, up to a certain point, to result from abnormally intensive oxidation taking place on tryptophane and glucose. (See below.) While activation of oxygen is a physiological process, peroxides appear under abnormal conditions.

ABNORMAL FATTY ACIDS

The study of the relationship between abnormal conditions and lipids has progressively led us to consider the existence of qualitative changes in these lipids, besides the quantitative ones. The existence of abnormal metabolic processes, and especially the fact that such abnormalities are often of long

duration, could hardly be attributed to variations in the quantity of the intervening lipids alone. More probably they would result from changes in the nature of the intervening lipids themselves. We have investigated this aspect of the fatty acids present under abnormal conditions.

As a guide for the direction to be followed in these investigations, we used the information furnished by the study of rancidity. We believed rancidity would be able to indicate broadly the nature of the qualitative changes which the lipids may undergo under abnormal conditions. Conceptually, the abnormal can be considered to result from a loss of the capacity of the organism to sufficiently control occurring processes and keep them in the frame of the constants which characterize the entity. Due to this lack of effective control, the *in vivo* occurring changes under abnormal conditions would closely approach those which take place *in vitro* where such a control does not exist. These considerations led us to search for changes similar to those seen in rancidity, or occurring *in vivo* in lipids, under abnormal conditions.

As mentioned above, in rancidity a first group of changes concerns the polar group. Some of them result in the appearance of free fatty acids, others correspond to changes in the carboxyls themselves, while still others are represented by processes of oxidation which occur in the chain near the polar group. A second group of rancidity changes concerns the nonpolar group and especially the energetic centers present in it, the double bonds.

The study of this last group of changes led us to consider, the changes appearing *in vitro* under the direct influence of heat and oxygen. As part of these changes, we considered of special importance the conjugation of the double bonds seen to occur *in vitro* as a step in the oxidation of polyunsaturated fatty acids. This conjugation corresponds to a characteristic displacement in the molecule of two or more of the double bonds present, so as to result in parallel reciprocal positions.

While in the simple bond two tetrahedral carbons are bound through their peaks, in the double bond they are bound by one edge, and in the triple bond by a surface. In the conjugated formation, the common edges of two double bonds, being separated by one simple bond, are consequently parallel. The planes in which the electrons of these double bonds are moving for each double bond and which are perpendicular to that of the bond itself, become parallel. Through the resulting reciprocal induction their energetic value is enhanced.

We have studied systematically the different qualitative abnormalities concerning the fatty acids, guided mainly by the information obtained through the study of rancidity.

Methods of Investigation Used

Following this line, we first investigated the forms under which the lipids in general are present in the organism. We utilized the differences in solubility between these different forms, separating them into free lipids, lipids kept in a labile bond with other constituents, as in cenapse, lipids bound through their polar group as in fats, or in the still stronger form as lipids in combinations so firm that they cannot be separated except through saponification. The method devised for this study and some examples are in Note 8A. This research showed that under abnormal conditions, very important variations occur in the amounts of the different forms. This study pointed out that the free lipids are greatly responsible for the important manifestations in which lipids appear as active agents.

In the study concerning the abnormal metabolism of the carboxyl and nearby carbons, we investigated the appearance of fatty aldehydes or ketones in blood, urine and in the cells.

One of the major problems encountered was the appearance *in vivo* of conjugated fatty acids, as abnormal fatty acids. In order to ascertain their presence and to measure their amounts, we had utilized three different methods of investigation: spectral analysis in ultra-violet and in the first portion of the visible spectrum (*Note 8B*); the study of the place of the double bond in the fatty acids molecule through the fission of these molecules and the analyses of the resulting fractions. (*See Note 1, Chapter 10*) More recently we have tried the vapor fractionation method (gas chromatography) (*Note 8C*).

The first, and especially the second method, gave us valuable data permitting us to recognize the intervention of conjugated fatty acids in abnormal conditions. These studies revealed the appearance of conjugated fatty acids, especially as trienes, the increase of their amount with the progress of the conditions and especially the fact that death occurs when their concentration in the bodies has reached a critical value. This has marked the importance of these substances in physiopathology. The fact that gas chromatography did not reveal the presence of conjugated fatty acids appears due to the conditions under which the method actually works.

Later, we will frequently return to the various problems related to intervention of conjugated fatty acids. We could thus directly correlate the intervention of these abnormal fatty acids with the pathogenesis of the manifestations of many conditions such as trauma, shock, adrenalectomy, and especially with the noxious manifestations following irradiation. (*Chapter 10*)

In abnormal metabolic changes, an important factor is the intervention of abnormal fatty acids in the metabolism of chloride ions, producing an especially strong fixation of the chloride ion to the carbons at the double bonds. The conjugated double bonds in a fatty acid molecule appear to be especially suitable for this since an abnormal, irreversible fixation of chlorides occurs in two steps. First, the halogen is fixed at the extreme carbons of conjugated formations with a displacement of the double bond in the intermediary position. In the second phase, the fixation takes place in the intermediary carbons, too. (*Note 8D*)

Functionally, fatty acids induce activation of oxygen as a normal process, but the appearance of peroxides or irreversible fixation of chloride ions is an abnormal event.

It is the abnormal fixation of chlorides by the conjugated fatty acids which leads to a more complex group of processes involving sodium chloride metabolism. With the chloride ion fixed, the sodium ion of sodium chloride remains free to enter into other combinations, especially with a carbonate ion, producing strongly alkaline compounds. This process explains the appearance of local alkalosis as a result of the intervention of conjugated abnormal fatty acids, corresponding to the chloride phase of "D."

The division of fatty acids into four groups—1) saturated and mono-unsaturated, 2) di-, tri- and possibly also tetra-unsaturated, 3) tetra- and higher polyunsaturated, and 4) conjugated—corresponds schematically to the four principal roles—caloric, organizational, functional and pathogenic—which fatty acids play in the organism. These roles are seen to be dictated both by the different structures of the fatty acids and the different substances to which they are preferentially bound. The fate of a fatty acid in the organism seems to be greatly influenced by its bond to other substances. As already noted, we have called these other substances "anti-fatty acids."

THE ANTI-FATTY ACIDS

Glycerol and Glycerophosphoric Acid

It is classically accepted that the intestinal absorption and circulation of fatty acids is made through bonding to various substances. The analysis of this absorption shows, however, that different fatty acids have preferential bonds. For saturated and monoethenic fatty acids, the bond is principally with glycerol. Although mono- and di-glycerides can be identified in the cells of the intestinal mucosa, these fatty acids leave the intestine as triglycerides, forming the largest part of the chylomicrons. They are

also found in reserve in adipose cells as triglycerides. The di-, tri- and even tetraenoic fatty acids usually enter the circulation as phospholipids, that is, in direct combination with glycerophosphoric ions. The polyunsaturated acids are bound to sterols when they enter the blood, circulate and are stored. While the structures of the various fatty acids determine their different roles in the organism, it is the anti-fatty-acid constituents to which they are bound which enhance these roles. The study of the anti-fatty acids has shown that these substances can even dictate, by themselves, different fates for the various fatty acids they bind.

The combination of glycerol with any fatty acid seems to establish a caloric metabolic character. This is true for the very different fatty acids found in plants and animals as triglycerides. Even the ricinoleic triglyceride, if fresh, is used as comestible oil, *castor oil*. The same is true of the oil of triglycerides of polyunsaturated fatty acids found in marine animal oils. In the seeds, all the triglycerides of fatty acids, even the conjugated ones such as eleostearic and parinaric, represent energetic sources. It seems that it is their combination with glycerol which has given all these fatty acids value as caloric metabolites. The same is true for the bond to glycerophosphoric ion.

Combination with glycerophosphoric acid endows various fatty acids with the ability to participate in the construction of membranes. The bond to sterols, on the contrary, induces an ultimate functional activity provided the fatty acid itself is so constituted as to be able to fulfill this function.

The influence exerted by anti-fatty acids can be understood in terms of the changes they induce in the activity of the fatty acids. Since the activity of the last is largely related to their presence as free substances, it is principally through their combination with fatty acids that the anti-fatty acids intervene. By inactivating those free fatty acids which form a membrane and insure its permeability, an anti-fatty-acid agent can cause the membrane to change its permeability and even to become completely impermeable. Similarly, an anti-fatty acid, by combining with a polyunsaturated fatty acid, can reduce or even suppress its functional activity. It is to be noted that, by both changing permeability and suppressing functional activity, the anti-fatty acids exert their influence ultimately by altering oxygen metabolism. From this point of view, metabolism becomes predominantly anoxybiotic in contrast to normal oxybiotic metabolism. For glucose, for instance, suppression of the oxidative phase arrests metabolism at pyruvic acid which passes into lactic acid. The appearance of acid substances as a biological effect of the action of anti-fatty acids results, in fact, from the

reduction of the fatty acid's activity, affecting oxidative processes directly, or indirectly through reduction of membrane permeability.

While one group of anti-fatty acids can be directly related to hydrooids, and especially to glycerol or to glycerol bound to phosphoric acid as in the glycerophosphoric ion, a second group is represented by lipoids, principally formed by derivatives of a characteristic ring system, the cyclopentanophenanthrene. As anti-fatty acid lipoids, these compounds, the steroids, were of special interest in lipid research. Only some aspects of the biological activity of steroids—mainly, those which represent new views in the study of these substances—will be discussed here.

Steroids

A fundamental role of these substances in biology is determined by the fact that they are polycyclic. This leads us to consider the role of the ring itself in reactivity, as shown by a study of the steroids in opposition to the fatty acids. In the fatty acids, the bonds between carbons as present in the aliphatic chain, insure a high reciprocal mobility between these carbons. As a result, the entire aliphatic chain is highly flexible. On the other hand, rigidity is characteristic for all the rings, and is increased by the polycycling of the molecules. The constituents of the molecules are kept in fixed reciprocal positions. While, in the fatty acids, the flexibility of the chain permits the energetic centers to take different relative positions among themselves toward other molecules, the rigidity of the polycyclic molecules maintains the energetic centers of the cycle, or those attached to it, in the same relative position. This fundamental characteristic of the cyclic molecules appears to be an important factor in determining the biological role of the various agents which have such cycles in their molecules.

In the case of steroids, this attribute acquires special importance. An understanding of the different biological activities of steroids can be obtained by an analysis of the forces resulting from this characteristic composition. Besides the energetic centers or formations attached to it, two energetic centers appear as part of the steroid nucleus itself. One is at C₃ and the other center is represented by the cyclopentanic group. The fact that these centers are maintained in fixed relative position through the rigidity of this polycyclic nucleus has resulted in an important property of the nucleus itself which becomes translated into a dipolarity of the molecule. The study of these two energetic centers has advanced our knowledge of the role of steroids.

The study of the polar groups bound to C₃ of the polycycle skeleton of

steroids has permitted us to recognize the conditions which induce stronger activity for these polar groups, conditions which are usually fulfilled in the naturally occurring members. It could thus be seen that the reactivity of an oxygen bound to C₃ is increased if another double bond present in the cycle is parallel to the double bond through which the oxygen is bound to C₃. A double bond between C₄ and C₅, as shown in Figure 64 (a), fulfills

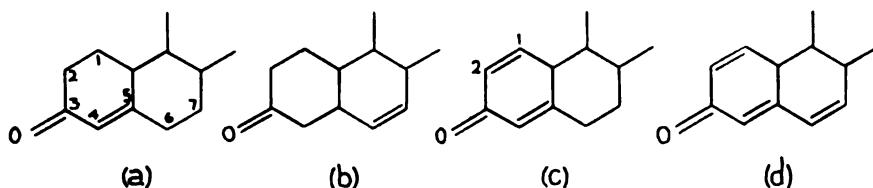


FIG. 64. Influence exerted upon the oxygen bond at C₃ by the position of the *double bond* in the cycles 1 and 2 of the cyclopentanophenanthrene molecule. A parallelism between the double bond of oxygen and that present between C₄ and C₅ increases the energetic character of the carbonyl (a). A similar influence but less active, is exerted by the double bond between C₆ and C₇. A double bond added between C₁ and C₂ (c) increases the activity. Still more activity would result from a third double bond added between C₆ and C₇ (d).

such a condition. A similar influence is exerted indirectly by a double bond between C₆ and C₇ (b) which, through induction, will influence the parallel C₄ and C₅ bond and further the double bond of the oxygen. This explains the influence exerted by the double bond present between C₁ and C₂ (c), as in the synthetic, prednisolone. Further enhancement of reactivity would be obtained with a third double bond added between C₆ and C₇. The parallelism between three double bonds (d) would produce an increased reactivity.

For the hydroxyl, a similar enhanced reactivity is induced by double bonding of the carbon to which the hydroxyl is attached with a double bond for the C₃ — C₄ or C₂ — C₃, as shown in Figure 65 (a and b). A similar condition is fulfilled if a double bond is present in the molecule parallel to any of these bonds, as seen in Figure 65 (c) where the double

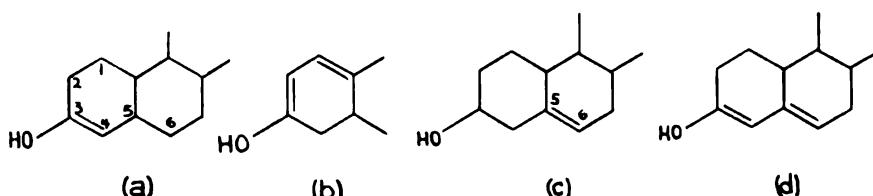


FIG. 65. The influence exerted upon the hydroxyl bond at C₃ by a double bond in the cycle 1 and 2 of the phenanthrene is increased if the double bond is adjacent or parallel to the bonds of C₃, bearing the hydroxyl.

bond is between C₅ and C₆. An enhanced reactivity of these compounds would be obtained with one double bond between C₃ and C₄ and another between C₅ and C₆ (d).

We will come back to this important intervention of the double bonds in cyclic molecules.

The energetic property of the cyclopentane group appears to be correlated with its odd number of carbons. The alternate succession of carbons with positive and negative characters resulting from the induction effect causes two carbons of this cycle to have the same sign. This "twin formation" induces a special molecular reactivity related to the pentanic cycle of the steroid molecule. (*Note 9*)

The special reactivity seen for C₃ of the cyclopentanophenanthrene molecule can be explained through a hypothesis covering the origin of these substances. Although the origin of a cholesterol molecule through a cyclization of squalene (35) appears plausible, this seems less probable for the corticoids. We have tried to connect their origin to arachidonic acid.

Several considerations such as the high levels of arachidonic acid and corticoids in the adrenals, and the reduction of the former when an important amount of the latter is excreted (*Note 10*), seem to establish a correlation between these substances. According to our hypothesis, the steroids with a two carbon chain at C₁₇, as seen present in the corticoids and luteoids, would result from a cyclization of the arachidonic molecule. (*Note 11*) This would explain the special reactivity of C₃, which would correspond to C₉ of the arachidonic acid bound in this molecule by a double bond.

A study of the different steroids under this energetic aspect has permitted us to understand their physiologic properties.

With the C₃ having a hydroxyl or an oxygen as polar group in almost all the steroids, the variety of the biological properties would be related to the different conditions at the other extremity of the molecule, principally at C₁₇, which result from the special energetic conditions prevalent at this region of the molecule. The simplest steroids are those having a polar group represented by an OH or O fixed at C₁₇. Such naturally occurring steroids have properties related to secondary sex characteristics. We will discuss them briefly here.

Sex Hormones

This group of steroids has two polar groups, one at C₃ and one at C₁₇. The energetic center at C₃ can have negative or positive polar characters, according to the presence of oxygen or hydroxyl. The energetic center at

C_{17} also can have an oxygen or hydroxyl group and thus be negative or positive. An important factor for the properties of the substance is the relationship between the two polar groups in the same molecule. It is apparent that the polarity of the molecule will vary according to what polar groups are present at C_3 and C_{17} . In a very simplified concept, which we consider only partially accurate, we have tried to associate female and male hormonal characteristics with this polarity.

In the simple steroid molecules, a folliculinoid or estrogenic biological property seems to be conferred if the two polar groups—at C_3 and C_{17} —are formed by hydroxyls. The molecule appears to have a dipositive polarity. It seems to be important that the two hydroxyls be kept in the relatively fixed reciprocal positions—corresponding to C_3 and C_{17} —as part of the solid skeleton of the steroids. Estrogenic properties are present in various steroids that fulfill this condition. Furthermore, substances far removed from the steroids have folliculinoid properties if they have this relationship between the two hydroxyls. As shown in Figure 66 diethylstilbestrol, which has its two hydroxyls maintained in a fixed relative position similar to that of steroid estrogens, also shows potent estrogenic activity.

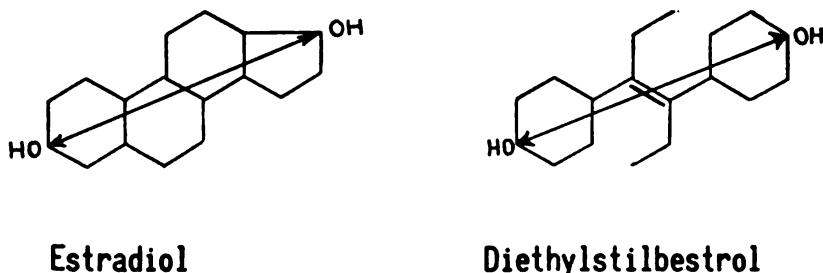
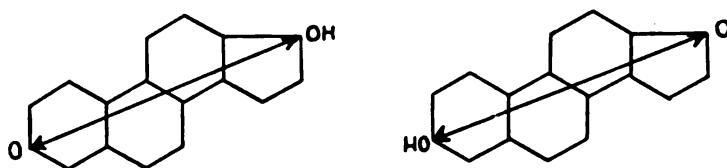


FIG. 66. *The folliculinoid* activity appears to be related to the existence of two hydroxyls kept at the same relative position, as it appears in estradiol and in diethylstilbestrol.

In the same way, we tried to correlate testoid activity with the presence of positive-negative polarity; that is, with two polar centers energetically different, one corresponding to an oxygen and the other to a hydroxyl, maintained in the same fixed relative position. The importance of this relative steric position of the two polar groups for testoid activity becomes evident when it is found in substances other than testosterone, the principal male hormone, with an oxygen at C_3 and a hydroxyl at C_{17} . Testoid activity is present in androsterone, which has an oxygen and an hydroxyl maintained in the same reciprocal positions, although here the oxygen is at C_{17} .

and the hydroxyl at C₃, the reverse of testosterone. In both substances, testosterone and androsterone, the two conditions for testoid activity, positive-negative polarity and the same relative position between the polar groups, are fulfilled. (Fig. 67) The differences which exist between these substances in their specific hormonal activity can be explained through the different influence exerted upon the two polar groups in these substances by the rest of the molecules.



Testosterone

Androsterone

FIG. 67. The *testoid* activity seems to be related to the presence of a hydroxyl and a carbonyl in the same fixed relative position which is insured by the rigidity of the steroid molecule. The same positional reciprocal relationship is seen to exist between these two polar groups in testosterone and androsterone.

The testoid activity seen for cortisone, hydrocortisone and other hormones also can be explained by the presence in these molecules of oxygen and hydroxyl at C₃ and C₁₇, and maintenance of the fixed position between these two polar groups.

Conceptually, the antagonism between estrogenic and testoid biological activities can be considered to be ultimately related to the differences in polarity, which in one form or another can be found in other factors differing for the sexes. We will mention here only that a similar difference between male and female character is seen in the sexual chromosomes, where the female character is related to the XX chromosome, and the male to an X and a Y chromosome. As we will see below, a relationship exists between lipids in general and sex.

Besides the sex hormones, fatty acids appear to be connected with male sex characteristics, while female characteristics are related to another group of steroids, the sterols.

Sterols

Characteristic of the structure of this group of steroids is the presence of a hydroxyl at C₃ and a long chain bond at C₁₇. Through the hydroxyl,

the center at C₃ has a nucleophilic character. This is reinforced by the presence of a double bond between C₅ and C₆ which, by paralleling the bond between C₃ and C₄, increases its ionic character and consequently the reactivity of the hydroxyl bond to C₃. Through this hydroxyl, sterols combine in general with substances having a negative polar group to form esters.

Besides the capacity to combine with fatty acids in general, one of the most important characteristics of the principal sterol of animals, cholesterol, is its selective affinity for certain fatty acid members, the polyunsaturated. We tried to explain the specificity of this bond through an interesting process which could be called "steric coupling."

In this process, two molecules, usually lipoids, are kept together not only by the combination of their polar groups but also through a bond between their nonpolar parts. The two molecules are reciprocally attracted through the multiple forces present in the nonpolar groups. Some are related to attached centers, while some, such as those corresponding to cohesion forces, are related to the rings themselves. An important factor is the rigidity of the sterol molecule which permits another molecule, if it is flexible, to make the steric coupling. The rigid skeleton not only keeps the energetic centers of one molecule in a fixed position but permits the flexible aliphatic chain to cover over the polycyclic molecule and thus bring the energetic centers of one molecule in contact with those of the other. Through this, steric coupling completes the bonding of the polar groups. The greater the concordance between energetic centers in both molecules, the more perfect the coupling is, for the more complete is the reciprocal neutralization of the energetic centers of the two molecules. Steric coupling explains why, of all the fatty acids present in the organism, cholesterol seems to prefer to bind those with polyunsaturated chains. It is these fatty acids which have several energetic centers in the nonpolar group as represented by double bonds. The long chains of these fatty acid molecules, having a certain degree of flexibility, will then complete the steric coupling.
(Note 12)

Steric coupling, in addition to its general importance in biology, where it represents a kind of molecular reactivity, seems to explain the antagonistic influence exercised by different constituents, especially the sterols and polyunsaturated fatty acids. Through steric coupling, cholesterol could influence the activity of these fatty acids more directly related to the nonpolar group. It has to be emphasized, however, that the neutralization resulting from steric coupling is not irreversible. On the contrary, through the intervention of various factors, such as the breaking down of the bond

between the polar groups, the two coupled molecules can regain their independence. This would explain the relative lability of the combinations between fatty acids and sterols. The antagonism between fatty acids and sterols is an important aspect of biological dualism which will be discussed in more detail later when these substances are studied in terms of their influence at the different levels of organization.

Steroids with a Two-Carbon Chain

Among the most important steroids are those having a two-carbon chain fixed at C₁₇.

Two groups, the luteoids and corticoids, appear directly related to allopregnane hydrocarbon, the steroid polycycle with a two-carbon lateral chain fixed at C₁₇. As we have already seen in the hypothesis concerning the origin of the steroids (*Note 11*), this hydrocarbon could have been directly derived from arachidonic acid, the two-carbon lateral chain corresponding to the tail chain of this acid, a tail which remains after cyclization.

The Luteoids

The prototype of the luteoids is progesterone. Two polar groups C = O are present, one at C₃ of the polycycle and the other at C₂₀ of the tail chain. A parallel double bond between C₄ and C₅ completes the formula. Energetically, progesterone presents a first center at C₃ which appears strongly nucleophilic for two reasons: first, because it corresponds to the potent electronegative C₃ and second, because it is reinforced by a double bond present between C₄ and C₅, and which is hence parallel with the double bond of the carbonyl. The second = O is attached to the C₂₀ of the tail chain. This also appears reinforced, the double bond of this carbonyl being parallel to the bond between the C₁₃ and C₁₇, which in the cyclopentane, according to the hypothesis of twin carbons, binds two negative charged carbons. Through its constitution progesterone is also a lipoid, the complex hydrocarbon group being predominant over the polar groups. With its polar nucleophilic centers, progesterone has the fundamental character of acid lipoids. Progesterone's luteoid activity corresponds to the presence of two relatively strong neutrophilic centers kept in the characteristic positions, one at C₃ and the other at C₂₀.

We can see that any disturbance in this energetic picture, any change from the dinucleophilic at any center, decreases the luteoid properties of the substance. With more profound changes, the luteoid activity is even suppressed. (*Note 13*)

Corticoids

The corticoids represent the group of hormones upon which the attention of scientists recently has been intensively focused because of their new therapeutic applications.

Chemically, they appear to be the same as luteoids, derivatives of the same parent hydrocarbon, allopregnane. Structurally, all these adrenocorticoid hormones have: a) a C₃ binding an O group; b) a double bond between C₄ and C₅ in the first cycle; c) a two-carbon tail chain with an O attached in ketone form to C₂₀; d) an OH as primary alcohol present at C₂₁. This structure, common to all corticoids, seems to be responsible for the principal properties of these substances. Corticoids have been separated into subgroups based upon the presence of attached groups OH or = O at C₁₁ or OH at C₁₇. The presence or absence of attached radicals at C₁₁ appears to be most important. Corticoids without attached radicals at the C₁₁ have a major influence on the metabolism of electrolytes. The second group of corticoids, having the radical, are known as neoglucogenic corticoids, the name indicating their principal biological characteristics.

Energetically, the corticoids present a nucleophilic center at C₃, reinforced by the presence of the double bond in the cycle between C₄ and C₅. The double bond is parallel to the double bond of the carboxyl, and thus inductively increases the ionic character of the latter.

A second energetic group of the tail chain appears in toto as a strong tripolar center with a nucleophilic center at C₂₀ of this chain and an electrophilic center at C₂₁. (*Note 14*) To this basic pattern is added, in the neoglucogenic corticoid, a separate energetic center at C₁₁, which can be either electrophilic, formed by a hydroxyl, or nucleophilic, formed by an oxygen.

Corticoids appear, in general, to act as positive lipoids. (*Note 15*)

Because of their importance in relation to anti-fatty acid activity, we will discuss first the neoglucogenic corticoids, the members with a polar group also at C₁₁. According to our hypothesis, these steroids have a special biological activity, a role in the process of synthesis in the organism. The part of the molecule between C₁₁ and C₂₁ constitutes an energetic formation with a peculiar property. It represents a kind of energetic mold or template, in which each carbon has its specific energetic character. Different radicals would be attracted by the energetic centers of this template formation according to their own energetic nature. Kept in their respective positions, they would be induced to bind together in order to form new substances. In this manner this template formation would promote new

syntheses. In different corticoids the constitution of the $C_{11} = C_{21}$ formation will differ and this will determine which substance is to be synthesized by the respective mold or template formation. (*Note 16*)

Using the template hypothesis, we studied an entire series of body constituents forming the "gluco group." Glucose, galactose, glucosamine and galactosamine, with their respective acids, as well as ascorbic acid, are among these substances. According to our hypothesis, these neoglucogenic corticoids would have the important role of producing, possibly along with other mechanisms, the entire series of "gluco" constituents. The existence of different template formations would result in a variety of synthesized constituents.

The intervention of the template formation in synthesis can occur again and again without affecting the molecule of the corticoid as such. It is interesting to note here a structural curiosity which could be interpreted as being related to template activity. In this template, the group of successive C_{11} , C_{12} , C_{13} and C_{17} are part of the rigid skeleton of the cyclic molecule, while C_{20} and C_{21} are forming the lateral chain attached to C_{17} . This can be regarded as conferring a certain proper mobility to this lateral chain as related to the polycycle. It is conceivable that this lateral chain would become a closed formation when synthesis takes place. A movement of the chain at C_{17} would permit the mold to open and thus liberate the synthesized molecule. It is interesting to note here the importance of the structure of the template for the constitution of the substances synthesized. Besides the polar group at C_{17} , that at C_{11} is also important for neoglucogenic activity since it insures a six-carbon chain in the synthesized molecules. A hydroxyl or carboxyl at the C_8 of the synthesized substance will appear, according to the nature of the polar group at C_{11} of the steroid. The respective characters and positions of C_{21} and C_{12} will permit the appearance of a cycle formed by five carbons and an oxygen, characteristic for the pyranic form of newly synthesized substances.

An interesting confirmation of the template hypothesis was obtained when glucosamine which, according to the hypothesis, is synthesized by the cortisone molecule, was found to induce in patients many of the clinical changes which are obtained by treatment with cortisone. We will consider these results later in our discussion of therapy. The capital role played by glucosamine, galactosamine and the respective uronic acids in the constitution of the connective tissue represents the "missing link" for the explanation of the relationship between cortisone, the other neoglucogenic corticoids, and this tissue. Some part of the therapeutic effect obtained

with these neoglucogenic corticoids in diseases of the connective tissue has to be attributed to the intervention of the amino sugars.

In the study of anti-fatty acid activity, glycerol and glycerophosphoric ion were found to control the absorption and circulation of saturated mono-, di-, or tri-unsaturated free fatty acids. The sterols appear to counterbalance the normal polyunsaturated members while adrenal corticoids, and especially the neoglucogenic corticoids, counteract the toxicity of fatty acids in general and of the abnormal conjugated members in particular. Research done in our laboratories by E. F. Taskier indicates that the adrenals intervene in the defense mechanism against fatty acids, and especially against the conjugated members which appear to be related to abnormal conditions. (*Note 17*)

The part of our research concerned with the role of lipoids in normal and abnormal physiology has been almost entirely guided by the concept of an antagonism between the two groups, one with a positive and the other with a negative polar character. This specific aspect has led us to study, together with the fatty acids and the anti-fatty acids, other substances related to this antagonism. In the group of lipoacids or acidic lipoids, as obtained from tissues, organs or organisms, we recognized the group of porphyrinic acids, related to various hemes present in the organisms. In the group of anti-fatty acids obtained from the same sources, different constituents form the insaponifiable fractions.

As related to this dualistic aspect we have studied another group of substances, which appear to act in the organism against the anti-fatty acids themselves. These other substances would represent a kind of biological brake to counteract an exaggerated intervention of anti-fatty acid constituents.

We have made a special investigation of two substances of this group, glucuronic and sulfuric acid anions, which characteristically seem to oppose certain anti-fatty acid substances. These substances appear as a result of an exaggerated oxidation of normal metabolites. Under abnormal conditions, the oxygen resulting from the intervention of peroxide may be fixed to carbohydrates even before they have undergone the preliminary fermentative transformations seen in normal metabolism. With the aldehyde group bound to phosphoric acid, the oxidation takes place at C₆, the second most reactive carbon in the molecule. This direct oxidation would represent, according to our view, one of the sources of glucuronic acid. Similarly the sulfuric anion would result from the oxidation of sulfur present in the organism. They correspond to the oxygen phase of offbalance D.

Glucuronic and Sulfuric Anions

Urine specimens that contain abnormal oxidizing substances show significant amounts of glucuronic and sulfuric acid compounds. (*Note 18*)

The analysis of the conditions under which these two substances exert anti-toxic activity permits a better understanding of their role in general biology. A certain parallelism exists, and has always been emphasized, between a detoxifying and an eliminating function exerted by these two radicals. Not only do sulfo- and glucurono-derivatives appear in the urine, but it often has been noted that glucuronic acid intervenes when large amounts of certain substances, such as menthol and phenol, are present and there are insufficient sulfuric acid radicals to insure detoxification and elimination. When mineral sulfates are administered, the proportion of sulfo-derivatives increases.

This parallelism appears especially interesting when we recognize that sulfuric acid represents the end result of the oxidation of sulfur introduced into the organism in combinations in which it is a bivalent negative element. Only a smaller amount of sulfur is introduced as a hexavalent positive element: that is, as sulfate. Sulfur is introduced mostly in bivalent negative form, as in methionine, cystine, etc. Both sulfuric and glucuronic acid result from oxidative processes, acting in one case upon the thiol group and in the other upon glucose.

The relationship between sulfuric and glucuronic acid goes still further. It has been noted that glucuronic acid appears when enough sulfuric radicals necessary for detoxifying action are not available. However, this is not entirely true since one process does not duplicate the other. Qualitative differences intervene. (*Note 19*)

The significance of glucuronic acid in the defense mechanism seems clearer when we recognize that, with but few exceptions such as benzoic acid, all the substances with which glucuronic acid combines are lipids or lipoids having one or more *positive* polar groups. The combination with glucuronic acid takes place through these positive polar groups. (*Note 20*)

In our view, glucuronic acid like sulfuric acid, has a specific role in the defense of the organism and this seems to be directed especially against lipids or lipoids with a positive polar group. Bound by glucuronic acids, the latter are eliminated as excremental substances. Glucuronic acid thus would act against many anti-fatty acid agents. We can conceive of sulfuric and glucuronic acids as means by which organisms are protected against an exaggerated activity of anti-fatty acid agents. Along the same lines, when lipoids with positive polar groups are predominant and able to act

in an exaggerated manner to oppose the fatty acids physically and chemically, the same means can be utilized to reduce this exaggeration. Thus, the intervention of glucuronic acid as a result of an abnormal oxidation of glucose induced by fatty acids appears to be biologically sound. This is also true for the sulfuric radical.

The importance of these substances does not reside only in the fact that the organism can easily produce them in larger quantities than fatty acids. The fact that they combine to form excremental substances is important too, for in this way, they help in materially eliminating the anti-fatty acid substances from the organism. This would not take place if only a combination with fatty acids were possible, since the esters of fatty acids are usually retained in the organism and, under certain circumstances, can again liberate their constituents. The intervention of glucuronic acid and sulfuric acid appears to be more effective than that of the fatty acids which have their own activity and are more toxic in exaggerated amounts. This appears to be especially true in the case of glucuronic acid because the amount of glucose available is practically unlimited as compared with other metabolites. Glucuronic and sulfuric acid would thus intervene in the biological antagonism between fatty acids and anti-fatty acid substances, inactivating and eliminating agents from the last group, especially when in excess. Teleologically speaking, their intervention appears to be still more interesting since the body has, as part of its defense mechanism, a tendency to manufacture anti-fatty acids in excess. The intervention of agents other than fatty acids would prevent a vicious circle and permit an excess of anti-fatty acids to be removed by excretion.

FATTY ACIDS VS ANTI-FATTY ACIDS

This study of the relationship between fatty acids and anti-fatty acids has been guided by the dualistic concept. It must be recognized, however, that the direct activity of these substances could be largely reduced to that of one group, the fatty acids. The action of anti-fatty acids is largely indirect. They control and thus limit the activity of the fatty acids. It is within this framework that the different anti-fatty acids selectively influence different specific functions of the free fatty acids.

The lipids and associated constituents with their multiple activities create for each entity a balance responsible for many of the manifestations of the entity. Variations in manifestations can be attributed in large part to qualitative and quantitative variations in the intervening lipids. A sys-

tematization of these variations would help us understand many of the processes encountered in normal and abnormal physiology.

As we have mentioned before, the balance between two antagonistic forces, especially for normal conditions, is not static. Instead, there is alternating predominance of the forces, which results in an oscillatory movement. Several groups of such coupled forces, each group with its proper rhythm, are at work. Operating simultaneously, they make for a series of very complicated variations. Yet analysis is possible since each of the variations follows a dualistic pattern. The variations, as they occur at different levels and with different intensities, have been identified through various tests. In a second step they have been tentatively correlated with changes in lipids. And next, didactically, lipid changes have been related to various etiologic factors, some intrinsic and some extrinsic.

Sex

The influence exerted by the sex of the organism upon lipidic balance was brought to our attention by a curious effect seen when cholesterol was administered in an ether-oil solution to rats. Only the females showed paraplegia and ulcerations of the hind legs. While castration or administration of sex hormones did not alter this response, it was influenced by the administration of two groups of lipids. The insaponifiable fraction of human placenta, for instance, was seen to induce a high sensitivity to this preparation of cholesterol even for males, while the acid lipidic fraction of placenta prevented paraplegia in females. (*Note 21*)

Similarly, the fact that in females alone, adipous cells appeared quickly in the skin of the ear after the application of sulfur mustard could be related to the intervention of the insaponifiable fractions. (*Note 22*)

Starting with these observations, it could be seen that, in general, a higher proportion of positive lipids exists in females than in males. This could be shown by direct analyses and by analyses of manifestations related to such lipids. While many differences are to be seen in various manifestations between females and males, only some could be related to the direct or indirect intervention of sex hormones. In such instances, castration with or without the administration of sex hormones was able to change, and even to reverse, the differences in manifestations seen between sexes. However, in instances in which these measures were without effect, the differences could be related to the intervention of lipids.

Age

The changes in lipidic balance related to age have been made the object of an extensive study which also sought to determine the role of lipids in aging processes. A general predominance of positive lipids, more manifest in the cellular and tissue levels than in the blood, was seen in youth. This would be expected in view of the special metabolic influence exerted by this group of lipids. The anoxybiotic character of metabolism induced by sterols results in the intervention of dehydrogenases which lead to an abundance of hydrogen ions. This, in turn, leads to a predominance of the kind of syntheses which favor anabolism. Growth thus could be related to the predominance of lipids with positive polar groups, especially sterols.

Aging processes, on the contrary, could be related to a predominance of lipids with negative polar groups, especially fatty acids. This predominance could be found especially at the cellular level, as seen in cultures of tetrahymena. (*Note 23*) In complex organisms or in rats (*Note 24*) in which an increase in the proportion of fatty acids at the cellular level is present, an opposite change occurs at the systemic and even at the organic level. There is an excess of cholesterol, this time limited to the higher levels, as revealed through analyses of the blood, for instance. Changes in the blood vessels are related in part to this excess of sterols at the systemic level. Many manifestations have confirmed such an offbalance with sterol predominance at higher levels. For example, we found the urine surface tension abnormally high in old age. (*Note 25*) Similarly, skin wheal absorption in old people requires more than 90 minutes for completion as against approximately 20 minutes in middle-aged adults. A predominance of fatty acids at lower levels and of sterols at higher levels would thus characterize the changes in lipidic balance related to old age. (*Fig. 68*)

Other Physiological Factors

The study of the role of lipids in various physiological functions was made indirectly for the most part, using the tests previously mentioned which were interpreted in terms of dualistic patterns. These were related to the general offbalances A and D and, through them, attributed ultimately to a predominance of sterols or fatty acids.

Sleep in itself, without relation to night or day, was found to induce a marked change, comparable to a type A offbalance with predominance of sterols. Subjects with pain of an acid pattern often correlate the appearance of pain with sleep, the pain occurring uniformly at the moment they wake up. In these cases, the urine shows a low specific gravity with

a high pH and a high surface tension, corresponding to an A type offbalance. As we will see, in subjects with an intensive A type offbalance, nocturnal polyuria and pollakiuria occur.

Sexual intercourse in males was seen to induce, in analyses, transitory changes similar to an offbalance of type D, corresponding to a predominance of fatty acids. In females the change corresponds to a transitory offbalance of type A, manifested by changes at the systemic level. *Muscular exercise* was seen to induce, in a first phase during the exercise itself,

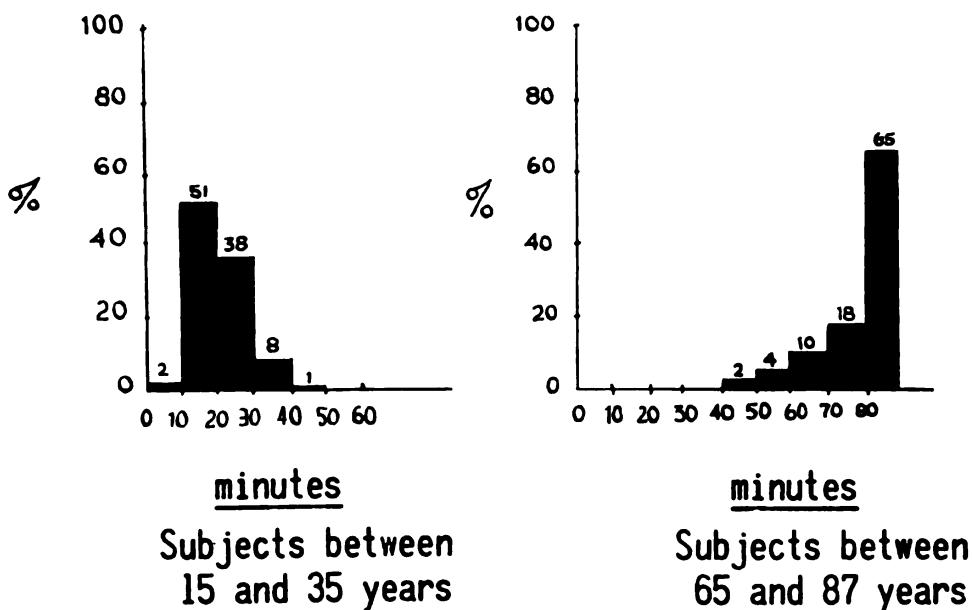


FIG. 68. The disappearance time for the wheal induced by the intradermic injection of 0.2 cc saline, varies with the age. In old age, the wheal often persists for more than 90 minutes.

changes comparable to an offbalance type D. This phase is followed by a much longer phase of type A, indicating sterol predominance. *Intensive mental exercise* produces a marked change similar to offbalance A, with all urinary tests showing the patterns found with predominance of sterols.

The responses attributed to influences exerted by *external factors* could be integrated in the same dualistic mechanism. All the data indicate the manifest influence exerted by the time of day. Two marked changes are seen, one around four o'clock in the morning and the other usually around eight or nine o'clock in the evening. The morning change corresponds to a predominance of sterols, the evening to predominance of fatty acids. These changes together with the clinical manifestations related to time of day

appear in a new light when interpreted not as being the direct results of time changes but rather of patterns of diurnal activity and nocturnal rest. This explains why in rats and mice, which are nocturnal animals, most of the analyses show variations related to the time of day opposite to those in humans. Other variations could be recognized more strongly related to time of day. Variations with a 24-hour rhythm could be seen, for instance, for urinary surface tension in mice. But, when rats and mice were maintained for a length of time under artificial conditions, with light during the night and dark during the day, the animals changed their habits, becoming active during the day and sleeping during the night. After a certain time, most of their analytical patterns such as urinary pH, blood leucocytes, eosinophiles etc. changed, acquiring the type of variation seen in humans. Urinary surface tension remained unchanged for a long time. (*Note 8 Chapter IV*) Even more interesting were other changes which could be related to changes in external temperature. The urinary surface tension measured in rats in the morning for long periods of time showed variations related to changes in the temperature of the environment. (*Note 26*) (*Fig. 69*)

The importance of temperature led to its more detailed investigation. Variations in lipidic balance have been found to parallel variations in body temperature. The blood of normal individuals is richer in sterols than the blood of those with hypothermia. Furthermore, in moments of high temperature, more sterols are found than in moments of low or normal temperatures. An increase of fatty acids occurs in conditions with hypothermia. These changes were confirmed also by the correlation between blood content in lipids and temperature in different abnormal conditions. In shock with hypothermia the blood is rich in fatty acids, while in infections with fever, it is rich in sterols.

The role of *temperature* was also investigated by studying the influence upon the lipidic balance by externally applied heat or cold. Characteristic variations could be seen in human analyses under the influence of hot and cold days, and of local applications of heat and cold. Manifestations corresponding to predominance of lipids with positive character were induced by heat, while others corresponding to predominance of lipids with negative character were induced by cold. Variations were seen in animals kept in an incubator or in a refrigerator. (*Note 27*) We will see below, by studying their influence at different levels, the importance of these variations produced by temperature.

The influence exerted by *barometric changes* could be seen in changes in total blood potassium, the two curves being parallel. Similar changes

could be observed related to the atmospheric humidity. Other tests as well, such as urinary pH, calcium excretion, etc., show a similar relationship but to a much lesser degree. (*Note 28*) The influence exerted by the environment could explain the changes seen from one day to the other in various analyses. (*Note 28*)

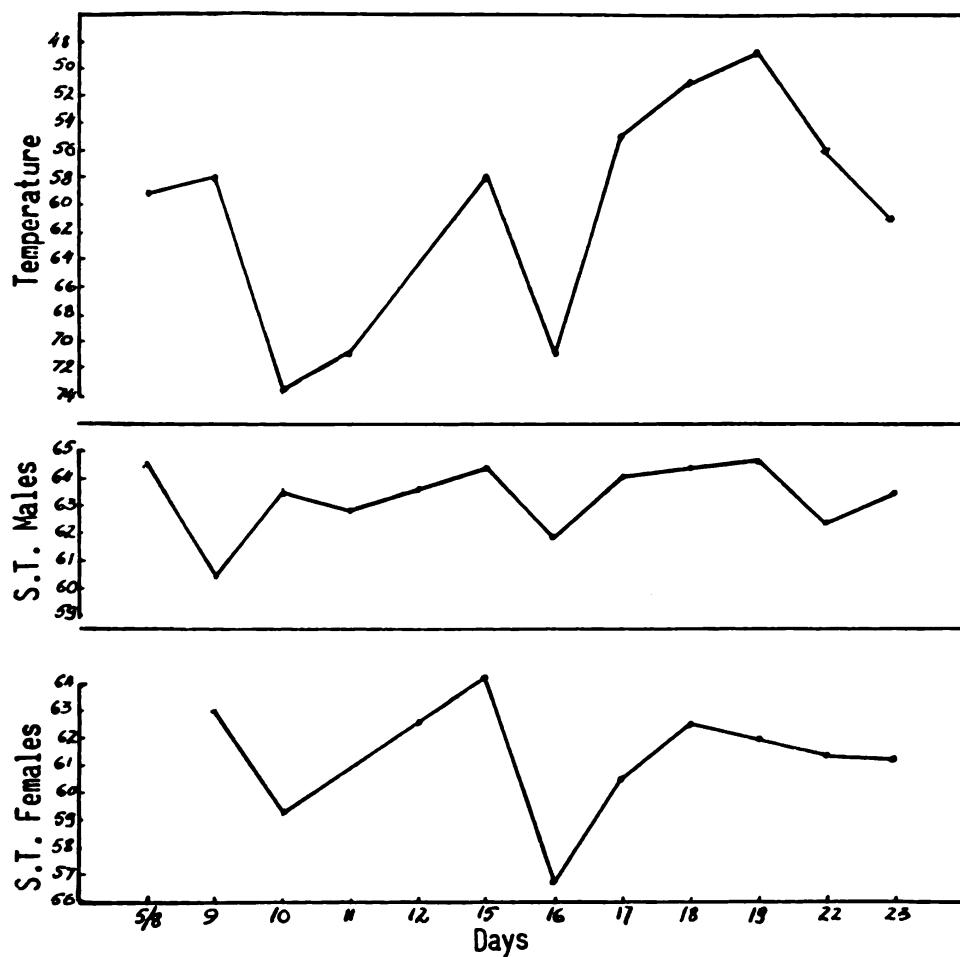


FIG. 69. The curves of the average values of surface tension of two groups of 20 male rats each, and two groups of female rats each, show parallel changes with the inverse curve of the temperature of the environment.

The influence exerted by changes in seasons was studied. An increase in fatty acids in winter and of sterols in summer could be noted. These increases also could be largely related to the seasonal variations in temperature. Very hot days were marked by analyses indicating intervention of lipids with a positive character. The influence exerted by the seasons was seen even in the responses of organisms to pathological conditions such as

tumors; variations in character and growth of experimental cancer could be noted. (*Note 29*) The relationship of many viral infectious diseases to seasonal changes which has been noted in many epidemiological studies could be related to the changes in lipids. (*Note 30*)

Effect of Antagonistic Lipids at Different Levels

A more complete study of lipids under the dualistic concept was made by considering their activity at different levels of hierarchic organization. This research was greatly facilitated by the degree of individuality which different biological levels exhibit when they are part of the hierarchic organization of complex organisms. It was also aided by the availability of lower organisms in nature which correspond to various hierarchic levels. Through this double approach, the information obtained showed the importance of the relationship between the levels of the complex organism and the influence exerted by lipoids. If high doses of the agents are applied, the influence is exerted upon all the levels. A preferential influence is exerted upon a single level if reduced doses are used. When medium size doses are administered, to the preferential effect upon one level a reactional response at other levels is added. This results often in concomitant opposite effect at these levels.

Effects of Lipoids on Viruses

The antagonistic effects of positive and negative lipids were evident in the study of their action upon viruses. Generally, agents with a positive polar group appeared to create favorable conditions for the development of viruses, while those with a negative polar group had an opposite effect. This influence, which was first seen in phages in vitro, became still more evident in viral infections.

Subcutaneous administration of positive lipids, such as sterols or in-saponifiable fractions of organs, induced greater local receptivity to viruses. In experiments with smallpox virus in rabbits, for instance, virus inoculation of the skin induced an exaggerated response in those areas where positive lipids previously had been injected subcutaneously compared with the response in other previously untreated areas. In less sensitive species such as mice and rats, positive lipid injections induced abnormally high local receptivity to virus inoculation. Intracerebral injection of sterols followed by subcutaneous inoculation with smallpox virus invariably produced nervous system localization of the virus. Intraperitoneal administration of sterols in very high doses in mice prior to smallpox inoculation produced a great degree of central nervous system localization. Intra-

cerebral virus inoculation, after subcutaneous administration of high doses of anti-fatty acids, brought death earlier in test animals than in controls given intracerebral virus alone.

A striking opposite effect was noted for lipids with a negative polar character. In rabbits, subcutaneous injection of a polyunsaturated fatty acid set up a local skin area refractory to smallpox virus inoculation, although inoculation was positive in other areas of the body. Death also occurred later, following intracerebral inoculation with a neurotropic virus in test animals given subcutaneous or intraperitoneal injections of fatty acids, than in controls. This partially protective effect was opposite to the increased receptivity seen in animals injected subcutaneously or intraperitoneally with insaponifiable fractions and intracerebrally with the same virus, where death appeared earlier than in controls.

The antagonistic effects of the two groups of lipids for viral infection appeared interesting from several points of view. The effects were local, at the cellular level, where viruses themselves act. Subcutaneous injection of lipids induced manifest changes in response toward the virus in the skin at the site of injection, and little or no change at all elsewhere. We have utilized this fact, as we will see below, to obtain information regarding the level at which various agents act. A change induced in receptivity to viruses, limited to the skin at the site of injection, would indicate activity of the agent at the cellular level. Tests based upon the skin response to smallpox virus infection have shown that, among the lipids with a negative polar character, a maximum of influence is exerted by the insaponifiable fraction of organs of exodermic origin from species sensitive to the virus. The insaponifiable fractions of rabbit skin and rabbit brain were the most active of the lipids tested. Among the fatty acids, the preventive effect was seen to increase with the degree of desaturation. It was almost entirely absent in saturated fatty acids, notably present in polyunsaturated fatty acids.

The increase and decrease in receptivity of the skin to smallpox virus following injection of lipids also furnished information about the roles of the polar and nonpolar parts of lipids in this specific activity. An opposite effect was seen between two groups of substances having the same nonpolar group but differing in their polar groups. While the polyunsaturated fatty acids of safflower oil, for instance, greatly reduced receptivity, the same polyunsaturated members having alcohols as polar groups increased receptivity. The polar group—negative or positive—appears to be the factor inducing the opposite effect.

The role of the nonpolar group was studied by comparing saturated

and unsaturated acids and alcohols. Almost no activity was seen for the saturated. The unsaturated members were active in general, with activity in any direction increasing with the degree of desaturation of the nonpolar group. Thus, it appears that the nonpolar group determines whether a substance is active or inactive, but the nature of the activity—that is, increasing or decreasing receptivity—is determined by the polar group.

The influence exerted by agents with a positive character upon viral infection would explain the seasonal changes in clinical manifestations which are especially interesting for the paralytic form of poliomyelitis.

We could show experimentally that when mice, after being inoculated subcutaneously with smallpox vaccine virus, are kept in an incubator at 37°C, all develop cerebral involvement, while such involvement appears in only a small proportion of other animals kept at room temperature, and does not appear at all in those kept in a cool place. As we could also show that one of the effects of exposure of an animal to a higher temperature is an increase in the body of the amount of free lipids with positive character, this could explain the increase in the virus sensitivity of cells in the central nervous system which are especially sensitive to these lipids. This relationship would also explain the increased incidence of paralytic polio cases during hot weather.

The presence of greater amount of lipids with positive character in youth helps also to explain the frequency and intensity of viral infections in children. (*Note 31*)

The study of the effects of temperature and lipids upon viruses has shown that those effects are not limited to the host but also are exerted upon the viruses. (*Note 32*) The influence of heat and cold upon virus activity was studied in bacteriophages, where effects for virus and host could be separated. The direct influence upon the virus appeared relatively small and secondary to the changes which appear in the host itself. Bacteriophage, separated from microbes by filtration and kept in an incubator at temperatures 2-3 degrees C higher or lower than controls, showed no change in virulence. This was true as long as microbes were not present. Microbes kept at higher temperature were more sensitive to phages; when kept at lower temperature, they were less sensitive. This influence went so far as to change a sensitive strain to a refractory one, and vice-versa.

The fact that microbes grown at higher temperatures favor the development of bacteriophage while those grown at lower temperature hinder it could be correlated with the change in the richness of lipids in the microbes themselves. Similar results were obtained when microbes were grown for a time in media containing fatty acids or insaponifiable fractions and were

then removed and exposed to phages. These experiments (*Note 33*) indicate the direct role played by the lipids of the hosts in the activity of phages, and would explain the influence exerted by temperature. Through the change in the lipids of the host, the virus changes too, becoming more active if grown in microbes at a higher temperature and less aggressive if passed through microbes kept at lower temperature.

Effects of Lipids on Microbes

The antagonistic effects of the two groups of lipids upon microbes were investigated. As an example, we will mention here the characteristic changes in *Bac. anthracis* treated with polyunsaturated fatty acids and insaponifiable fraction preparations. (*Fig. 70*) We investigated the microbes for their morphological, tinctorial, cultural and virulence characteristics. With the fatty acids added to media, changes which can be considered to be mutational were induced, leading to tiny Gram negative microbes growing on agar as transparent small colonies. The changes, however, were reversible. Usually several passages in normal media were sufficient to produce reversal. First small and separate, then larger and more confluent Gram positive granules were seen to appear in the microbes which, themselves, also became progressively plumper. Ultimately, all the characteristics,—morphological, tinctorial and cultural—of the normal microbes reappeared. (*Fig. 71*)

Microbes showed opposite changes when treated with insaponifiable fractions, (*Fig. 70*) losing their bacillus form. Abnormally intensive Gram

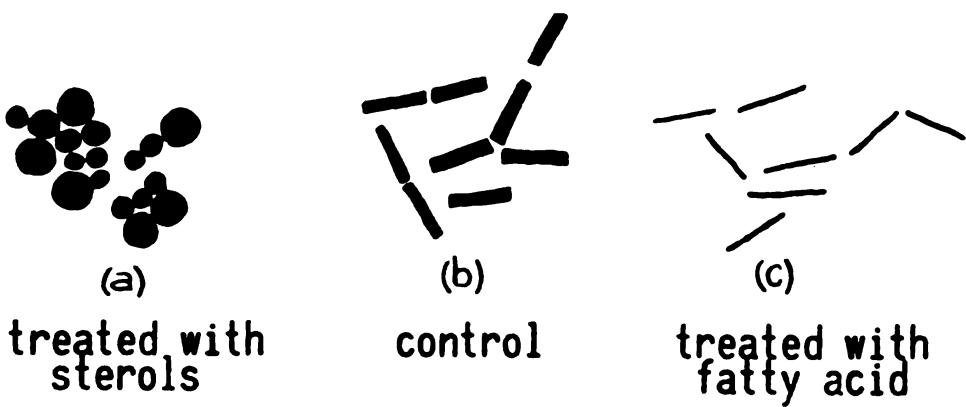


FIG. 70. *Influence of lipids upon microbes*. Schematic drawing of the changes induced in *Bacillus Anthracis* by the influence exerted by the two groups of lipids. Treated with sterols (a) as in the unsaponifiable fraction of placenta, the microbes change into cocci irregularly shaped and intensely retaining the gram stain. Treated with fatty acids (c) from cod liver oil, the bacilli change into very tiny gram negative microbes. (b) shows untreated microbes.

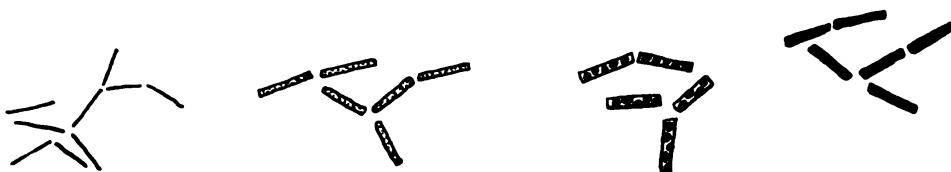


FIG. 71. *Lipids and microbes.* Drawing of the progressive passage toward normal bacilli of the tiny gram negative microbes obtained through the treatment of *Bac. Anthracis* with fatty acids. The passage takes place usually in successive steps. The gram positive formations appear first as fine granules; they later become clumps and finally give the microbes their normal aspect.

positive cocci appeared. They grew on agar as very thick creamy white colonies. These changes were seen to persist for a long time and seldom were spontaneously reversed. Treatment with fatty acids induced reversal although inconsistently. We attempted to correlate the differences in changes induced by different lipids to the different levels of the microbe at which they work. The change to cocci can be regarded as corresponding to an influence exerted upon the membrane and the change to Gram positive to an influence upon differentiated formations present in the body. (313)

Effects of Lipids on Protozoa

The effects of lipids upon monocellular organisms, especially *Tetrahymena pyriformis*, were studied and an effort made to relate the nature of the main changes induced in these protozoa to changes observed at the cellular level of complex organisms. An initial effect was noted on the polarity in protozoa which seemed to be oppositely influenced by long chain polyunsaturated fatty acids and sterols. Lipids with a positive character were seen to induce a change in the form of protozoa causing them to become almost round, a change considered to correspond to reduced polarity. Lipids with a negative character had an opposite effect; the *Tetrahymena* became abnormally elongated.

The administration of higher amounts of polyunsaturated fatty acids was seen to induce immediate changes localized at the anterior pole of the organism, changes which ultimately lead to the breakdown of the membrane particularly at this point. This effect parallels in intensity the degree of desaturation of the fatty acids. Other changes were seen in growth rate and survival time and, thus, in the aging process. (Note 34) (Fig. 74)

At the same time, resistance to heat was seen to increase as the result of treatment with negative lipids, while it decreased after treatment with

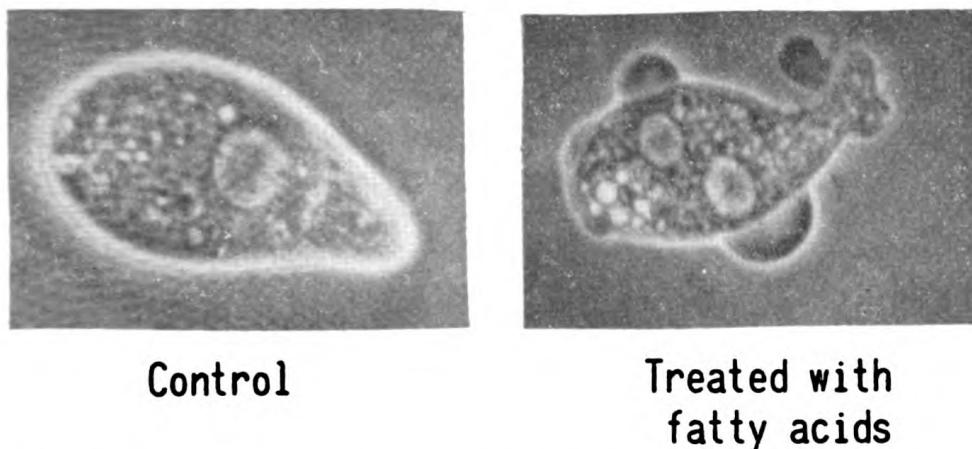


FIG. 74. In a direct action of fatty acids on *Tetrahymena*, a passage of fluid occurs at the surface with a break of the membrane especially manifest at the anterior pole (a), control untreated (b), (1200x).

the positive sterols. (*Note 35*) The same influence upon the aging processes, as manifested in a prolongation of the life-span, was noted for polyunsaturated fatty acids with a long chain and even for some members of the saturated series but with a shorter chain.

Effects of Lipids on Complex Organisms

Morphological Changes—The same level separation was used in the study of the effects of lipids on complex organisms. Acting at chromosomal levels, lipids led to the appearance of monstrosities. Various lipids, especially insaponifiable fractions of organs, were injected into larvae of flies. While an immediate change in the cells of the larvae could be traced to the subnuclear level as seen in chromosomes, monstrosities were seen to be induced in the resulting flies. A similar effect became evident when lipids were injected into hens' eggs before or during incubation. Especially with cholesterol but also with insaponifiable fractions of organs, a high proportion of chickens were hatched with spastic paraplegia.

The same problem is being studied, in collaboration with P. Fluss, in *Drosophila melanogaster*, grown for many generations in media to which an entire series of different lipids from one or the other group are added. This study is in progress and the results will be published later.

We have seen that the antagonistic effects induced by the two groups of lipids could be related ultimately to opposite changes in the fundamental biological process of aging. This appeared clear for lower morphological levels of organization and was especially evident for cells. While anti-fatty

acids induce changes which can be regarded as corresponding to prolonged youth, the polyunsaturated fatty acids induce rapid aging with pyknosis and karyorrhexis and death of the cells as old entities. This could be seen clearly in tumors, in which cells with youthful character lead to non-necrotic tumoral masses, while cells that age rapidly produce necrosis in the tumors followed by ulceration if the tumor is superficial.

The effect of conjugated fatty acids was somewhat more complex, indicating an abnormality in the induced processes. Their administration was followed by the appearance of cytoplasmic and even nuclear vacuoles, corresponding ultimately to an anomaly of water metabolism.

The effect of lipids upon adipous cells appeared to be of special interest. The anti-fatty acids, especially the sterols, when injected subcutaneously in animals, induced a characteristic process in the adipous cells near the injection site. These cells became very enlarged and highly irregular, with their content changed into an emulsion only slightly stained with sudan. The fatty acids, on the contrary, imparted to adipous cells an abnormal resistance to destruction. They remained persistently unchanged even in the midst of very active processes which usually cause them to disappear. Unchanged adipous cells were found encircled by the invading cancerous cells, deep within tumors in animals treated with fatty acids.

On Pain—From the start of this research, the opposing effects of the two groups of lipids upon pain has been most impressive. For the fatty acids the degree of saturation is important. The saturated members of the fatty acid series and even oleic acid are entirely without effect. Linoleic and linolenic acids show a slight influence, while the polyunsaturated members show a marked effect. Administration of highly unsaturated fatty acids and of acid lipidic fractions of certain organs, such as placenta, liver, spleen or blood, uniformly decreased pain of an acid pattern and increased pain of an alkaline pattern. These opposite effects have, from the beginning of our study, contradicted the idea that this influence upon pain was the result of the direct action of these agents upon the nervous system. Furthermore, the opposite effects exerted upon the same pain by the other group of lipids have confirmed the hypothesis that the action takes place at the level of the painful lesion, where the differences between the two pains was found to correspond to two opposite acid-base offbalances.

In the study of the effects of lipids on the pH of the second day wound crust, made in collaboration with Carlos Huesca, we have demonstrated that lipids influence pain through changes induced on the acid-base balance present at the tissue level. The positive lipids constantly lowered this pH while the negative lipids elevated it. (*Note 1 Chapter V*) Even more im-

portant than this temporary pH effect in establishing the mechanism of lipid action in pain was the change in the actual pattern of an existing pain after administration of these agents. Polyunsaturated fatty acids in sufficient amount were found to convert an acid-pain pattern to an alkaline pattern, while sterols changed an alkaline pattern into an acid one. We will return to this important fact later.

A pathogenic role for lipids becomes evident too, when pain can be induced through the administration of lipids in previously painless lesions. Such lesions treated with large amounts of lipidic preparations became painful. An alkaline pattern of pain was seen to appear after fatty acid administration, while an acid pattern followed use of the insaponifiable fraction. (*Note 36*)

At the tissue level, lipids also affect such acid-base symptoms as vertigo, itching, dyspnea, tremor, and even mental diseases. In these conditions the same antagonism between the two groups of lipids—and the same opposite effects upon the acid and the alkaline pattern—can be noted along with the same possibility for changing the pattern to the opposite type if big doses of lipids are administered.

Wound Healing—The same manifest antagonism between the two groups of lipids was also noted in their influence upon the evolution of wounds. Changes in the sloughing and healing process were followed by measuring the size of wounds (*Note 37*) as well as by serial histological examinations. The lipids with a negative polar group were seen to retard the evolution of the processes by prolonging the first catabolic phase. Positive lipids generally had an opposite effect. However, here too it could be observed that sterols have relatively little effect on the healing of connective tissue, but manifestly favor proliferation of the epithelia. This was especially evident in the changes in scar formation of the skin of treated animals. In rabbits treated with cholesterol, the epithelial scars were found to have 8-10 layers instead of the 2-3 characteristic for the rest of the skin and for the scars in control animals.

Regeneration—In collaboration with E. F. Taskier we studied the effect of lipids upon the regeneration of liver in rats, after the resection of almost $\frac{3}{4}$ of this organ. The rate of regeneration could be estimated by correlating it to the time of appearance of fatty droplets filling up almost all the cells, as a first step in the regenerative process. In very young animals, this change in fatty liver cells was seen to take place even within the first 24 hours after resection. The change was progressively delayed as the age of operated animals increased. In old animals the change in fatty liver cells appeared only after the fourth day.

The administration of lipids had a marked effect on appearance time of fatty cells. Sterols induced precocious appearance in old animals. From this point of view, sterol-treated animals appeared to react as young individuals, with fatty cells evident even on the second day. The fatty acids and acid-lipidic fractions of organs showed an opposite effect, delaying the time of appearance of the fatty cells. Young animals treated with polyunsaturated or conjugated fatty acids showed no fatty droplets in the liver cells for as long as three to four days. With higher doses of the same agents, the fatty infiltration did not occur at all.

It is interesting to note a parallelism between fatty infiltration of liver cells and the richness of adrenals in sudanophil substances. An almost complete lipid depletion of the adrenals was seen after high doses of fatty acids and coincided with a total lack of fatty cells in liver regeneration.

(Note 38)

Organic Level—Effects of the two antagonistic groups of lipids at the organic level have been studied in terms of manifestations clearly associated with various organs. We will review these effects briefly here, with more details to come when the therapeutic use of lipoids is discussed.

Intestines—The influence of lipids upon intestinal function is marked by the same antagonism between the two groups of agents. Oral administration of large amounts of fatty acids, especially higher unsaturated such as obtained from cod liver oil, was usually followed by diarrhea. Diarrhea also occurred after parenteral administration of these substances in large amounts. It was interesting to note that parenteral administration of the acid lipidic fraction of placenta, blood or even organs had a marked influence upon the colon and rectum in particular. High doses produced tenesmus with a mucous or even sanguinolent secretion. This localization of the effects of the lipidic fraction appeared to be especially interesting from a therapeutic point of view, as will be seen later. The oral or parenteral administration of the opposite group of lipids, sterols and insaponifiable fractions, has an opposite effect, a constipating one, which we will discuss later together with its therapeutic aspects.

Kidney—The manifest opposite effects exerted by the two groups of antagonistic lipids upon diuresis raise the question of where these effects take place. While a systemic effect can be recognized, a more direct intervention upon the kidney also must be considered. The addition of the acid lipidic fraction of organs, and especially those obtained from pork kidney, to the perfusion fluid in a dog kidney preparation produces a manifest decrease of excreted urine. The administration of insaponifiable fraction

has a marked diuretic effect which we will discuss below with its therapeutic aspect.

Nervous System—Interesting effects by the two groups of antagonistic lipids upon many manifestations of the central nervous system have been noted.

Convulsions—Administration of sterols and insaponifiable fractions of many organs such as placenta, liver, butter, eggs, etc., in large amounts induces convulsions in rats. Convulsions also were noted in humans when huge doses of these agents were administered. But even in relatively small amounts, these lipid agents sensitized animals to the administration of other convulsant agents. In rats or mice receiving such lipids, thiamine chloride induced convulsions in doses without effect in controls. (*Note 39*)

An opposite effect was observed for lipids with negative character. Saturated fatty acids showed no influence on thiamine-induced convulsions. Such convulsions were prevented by the administration of nonsaturated members. The effect was related to the degree of desaturation of the fatty acids. With increases of the iodine number, the necessary effective doses of these fatty acids became progressively smaller. While hundreds of milligrams of mono- and diethenic acids were necessary for each 100 gram of body weight, the anti-convulsant effect was obtained with only a few milligrams of clupanodonic acids, and with still less of the nonenic acid, bixine.

The study of the pathogenesis of convulsions also covered the influence exerted by these lipids of the adrenal corticoids. The administration of mineralocorticoids, especially desoxycorticosterol, even in small doses, to subjects who had received any one of the lipids with a positive polar group, such as cholesterol or insaponifiable fraction of placenta, liver or kidney, was followed almost invariably by convulsions. We will present more details on this effect later in the discussion of synthetic substances. For the moment we want only to note the relationship between mineralocorticoids and lipids with positive character in the pathogenesis of convulsions. The concomitant intervention of the two factors—an offbalance induced by lipids with positive character, and action of mineralcorticoids—seems to provide new light on the pathogenic problem of epilepsy and convulsions in general.

Coma—The role of cortical hormones in the pathogenesis of convulsions was confirmed by the opposite effect produced by neoglucogenic corticoids. We will see later that the administration of cortisone to subjects receiving higher alcohols such as heptanol, octanol or octandiol in large doses, induced a subcomatose condition at first which progressively changed into coma. (*Note 40*) Opposite properties of the mineral and neoglucogenic

corticoids, which made Seyle separate them according to their "phlogistic" and "antiphlogistic" activity, would explain the two opposite manifestations inducing convulsions and coma, produced in individuals previously treated with the same anti-fatty acid agents.

On Cardiac Rhythm—The influence exerted by the two groups of lipoids upon the cardiac rhythm was studied under the same dualistic aspect. The effects observed can easily be interpreted considering the role of the differentiation of the cardiac cells for their part in the cardiac physiology. The role of a cell in cardiac physiology is a direct function of its own automatism which can ultimately be related to its degree of differentiation. The fact that the two groups of lipids act antagonistically upon this cellular differentiation, the acid lipids exaggerating it and the insaponifiable fraction of sterols reducing it, has explained some of the effects induced by these agents upon normal and abnormal cardiac rhythm. (*Note 41*)

On Oestral Cycles—The action of the two groups of lipids at the organic level was also studied in the rat ovarian cycle. Daily, and even twice a day, vaginal smears were made in animals treated with these agents. When large amounts were administered, both groups suppressed the cycle. With smaller doses, only the lipids with positive polar groups, especially sterols, produced this effect.

Systemic Level—Blood has appeared especially suitable for *in vitro* and *in vivo* studies of the effects of the two groups of lipids at the systemic level. The effects on different blood constituents were analyzed and led to very conclusive results. We will outline here the principal points of this study.

Under the influence of anti-fatty acids, the erythrocytes become more turgescent, increase in volume, show a strong refringency of their crown in dark field examination, and remain isolated from one another. The sedimentation rate, if previously high, is reduced by treating blood *in vitro* with insaponifiable fractions of organs. Oxygen appears to be retained longer in treated red cells than in controls.

The fatty acids have an opposite effect. Under their influence, the red cells become crenelated and develop a tendency to form sludges. The sedimentation rate is increased. The color of the treated blood is dark and, even after oxidation, rapidly darkens again. *In vivo*, lipids with a positive character induce leucocytosis, those with a negative character leucopenia. This last effect is seen even *in vitro*. In Note 42, the influence exerted by lipids upon the blood is presented with more details. (36)

On Temperature—The administration of sufficient amounts of positive lipids induces a frank elevation of temperature, while hypothermia follows

the administration of negative lipids. The relationship between temperature and lipids, however, is not so simple since changes in external temperature influence the balance between the antagonistic lipids. For example, animals kept in incubators at a temperature of 35°C show an increase in lipids with a positive character. Animals kept in a cool place, such as a refrigerator, show an increase in lipids with a negative polar group. The organisms are able to combat the increase of lipids with negative character by means of the normal defense mechanism, but are less capable of dealing with an increase of lipids of positive character. Therefore, while a high proportion of animals kept in refrigerators adapt themselves to the new conditions, those in incubators die in a few days.

On Systemic Patterns—The influence exerted by lipids upon various other systemic manifestations which are reflected in abnormal patterns in urine analyses has been studied. In general, the fatty acids induce patterns corresponding to the offbalance of type D, while the sterols induce patterns of the type A offbalance. Here again we must emphasize that any lipid, if administered in large quantity, influences all analytical values. A certain specificity, however, is noted since, in relatively small doses, lipids induce changes only in certain values. Because of the inherent technical problems concerning the patterns, only a few analyses could be followed accurately over the period of time necessary for a clear recognition of changes in small laboratory animals. It is for this reason that most of our studies in this area were made on humans where pattern changes could be easily identified and followed over long periods.

It is to be emphasized that, under these conditions, the influence of lipids is exerted especially upon already existing abnormal patterns, increasing or decreasing their deviations from the normal, or changing the patterns entirely. Abnormal patterns were induced through huge amounts of lipids, which very seldom were administered to patients. TABLE X shows schematically the analytical changes induced by the two groups of lipids upon various urine and blood analyses, expressed as patterns corresponding to abnormal conditions, as well as upon the manifestations present at other levels.

We will discuss these effects in more detail when describing the pharmacodynamic properties of lipids and lipoids.

Mechanism of the Lipidic Biological Activity

The analysis of the changes induced by lipids has emphasized certain characters which appear of capital importance for the understanding of the biological intervention of these substances. In one kind of activity a lipid

acts through its *lipoidic properties*. From the data concerning its distribution in the organism it can be seen that, due to its solubility characters, a lipid introduced in an organism will be selectively retained by the existing lipidic system. When such intervention through its lipoidic properties takes place, the nonspecific character of the activity of the lipid is prevalent. A second kind of activity results from the *bond realized through the charge of the polar groups*. The positive or negative character of these polar groups deter-

TABLE X

LEVEL	EFFECTS OF STEROLS	EFFECTS OF FATTY ACIDS
<i>Cells</i>	Prolongs youth character Increases potassium content Decreases sodium content Reduces membrane permeability Reduces cellular oxidation Reduces chloride content	Induces rapid aging Decreases potassium content Increases sodium content Increases membrane permeability Increases cellular oxidation Increases chloride content
<i>Tissues</i>	Lowers pH of lesions Lowers chloride content of lesions Lowers water content of lesions	Raises pH of lesions Raises chloride content of lesions Raises water content of lesions
<i>Organs</i>	Induces somnolence Induces diuresis Induces constipation Induces tachycardia	Induces insomnia Induces oliguria Induces diarrhea Induces bradycardia
<i>Systemic</i>	Induces hyperthermia Induces hypertension	Induces hypothermia Induces hypotension
<i>Blood</i>	Increases RC volume Decreases RC sed. rate Increases persistence of oxygen fixation Determines persistence of RC isolation Determines hyperleucocytosis Determines eosinophilia Decreases kalemia	Decreases RC volume Increases RC sed. rate Decreases persistence of oxygen fixation Determines formation of sludge Determines leucopenia Determines eosinopenia Increases kalemia
<i>Urine</i>	Induces water excretion Induces sulphydryl retention Induces calcium excretion Induces chloride excretion Induces sodium excretion Induces phosphate retention Induces retention of surface active substances	Induces water retention Induces sulphydryl excretion Induces calcium retention Induces chloride retention Induces sodium retention Induces phosphate excretion Induces excretion of surface active substances

mines thus the nature of this second kind of activity. A third kind of activity results from the *chemical constitution* of the polar group, which will induce selective combinations and consequently will have a more specific influence. A fourth group of changes are induced by the activity which takes place at the nonpolar group of the lipid and more specifically at the *energetic formations* present in it. They will have a still higher character of specificity.

With this systematization of the activity of the lipids, a further systematic analysis of the influence exerted by the lipids appears possible.

Through its selective distribution, the administration of a substance having lipoidic properties will influence those entities which have lipids in an active form in their constitution. The influence exerted will thus be proportional to the richness of the entity in these active lipids. This fact explains why the administration of a lipid or lipoid affects selectively the abnormal entities rich in free lipids and to a much lesser degree, the normal ones. It is this selective distribution which will further limit the activity of the lipoid to the lipidic system and most manifestly to the abnormal entities. In the frame of this limitation, this activity results from the charge of the polar group. Similar effects are thus obtained for all the different lipoids which have the same electric positive or negative character of their polar group. This explains why one can use different agents from the same group and still obtain similar results. Agents chemically so different as fatty acids, mercaptans, persulfides, aldehydes or epichlorohydrine, have similar activity because they all have negative polar groups. The characteristic of the effects resulting from the electrical character of the polar groups, is that they are common for the groups having the same sign and diametrically opposite for the agents with a positive or a negative polar group.

This effect was clearly seen in fatty acids in which the negative carboxylic polar group was changed into the positive primary alcohol. The biological effects of the new substance were opposite.

It is in the third kind of activity that the chemical nature of the lipoids intervene. Certain effects resulting from the bond of an amino polar group will thus be different from that of the alcohols, although both act as positive energetic centers and as such have exerted other common effects. The same is true for the carboxyl and thiol groups.

Still more specific appear the effects resulting from the intervention of the energetic factors present in the nonpolar group, such as the double bonds, and the energetic formations they realize such as conjugated, or two double bonds separated by a methylenic carbon.

The various mechanisms involved have explained further the different kinds of biological effects which result. The action of the lipid by means of

the lipoidic effect will thus influence general, nonspecific manifestations, such as those concerning the permeability of membranes. Only secondarily, will these changes in membrane permeability influence the different metabolic processes which the membrane governs.

In the second group of changes, related to the intervention of the polar groups, the antagonistic effects induced were seen to concern processes resulting from membrane permeability. It is only in a third change that a more specific action upon the different metabolic processes has to be considered. These are concerned with an intervention upon metabolites or the agents governing them. The character of this last lipidic intervention is its specific influence exerted upon a definite metabolic system.

We tried to interpret the influence exerted by a lipid or lipoid according to the above systematization. The recent development of the biochemical methods of investigation has put into limelight many biochemical processes by considering them as isolated metabolic entities. Most of them were seen to result from the intervention of enzymes upon more or less specific substrata. One of the principal objectives of the actual pharmacodynamic studies is to correlate as directly as possible, biological effects of different agents to specific metabolic processes, most of them corresponding to a change in an enzymatic process. This approach, while very interesting, would not take into consideration the important role played by the non-specific activity of lipids and lipoids. These nonspecific influences through changes in the lipidic system induce different changes in different metabolic processes. A nonspecific change in membrane permeability will affect many enzymatic processes. It explains the existence of similar influences exerted upon these processes common to agents which have nothing more in common than their lipoidic properties and the presence of a positive or a negative character of their polar group. It is this character which binds an effect to the nonspecific intervention. This so-systematized analysis has thus permitted to separate the biological activity of the lipids and lipoids, the more specific from the lesser influences, and correlate each one to a proper or common character of the agent. This view has amply simplified the study of the pharmacodynamic intervention of these substances.

OTHER CONSTITUENTS

In addition to the chemical elements and lipids, other constituents have been studied from the dualistic point of view. Although the other constituents have received less emphasis, interesting information has been obtained.

Amino Acids

Amino acids have been separated into groups based upon their effects at different levels. The first group includes the simple amino acids. In these members the portions of the molecule which are added to the amphoteric amino acid group, are usually electrically neutral. The amino acids polymerized through the amphoteric group serve as building materials for the bigger protein molecules. They have appeared to be inert without effects upon the different levels. Beyond these simple amino acids, are two groups, energetically active, which have a second energetic center with a negative or positive character in their molecules. While the amino acid group serves to make these substances parts of higher proteins through the same bonds of amino acid groups as the simple members, it is the other energetic center, with acid or alkaline character, which confers upon these amino acids a positive or negative character.

We studied effects, at different levels, of arginine, lysine and histidine, which are members of the group with alkaline centers; of glutamic and aspartic acids which have acid centers; and of methionine with a thiolic center. Like for the lipids, the last two groups have shown similar properties, but opposite to those of the members with alkaline centers. The nature of their intervention appeared evident through the interesting opposite effects exerted upon microbes. Cultures of *B. subtilis* in broth containing members of one or the other of the antagonistic groups show characteristic changes. Unlike controls in which the long chains of microbes remain isolated, the microbes were seen to be kept together in media with alkaline amino acids, forming a consistent gelatinous mass separated from the medium. In broth with acidic or thiolic amino acids, the microbes remained separated or formed very small aggregates. This appeared interesting when we considered the positive character present in alkaline amino acids, as related to the heterotropic, constructive trend, while the negative, as in the acid and thiolic members, is related to the opposite trend. We saw further the same antagonism between the influence exerted by histones and nucleic acids, the first paralleling the alkaline amino acid groups and the second the opposite group. The more manifest effect of the ribonucleic acids could be seen to take place at higher levels of the organization and possibly explains the more direct action upon the genes.

We investigated the effect of the two groups of amino acids at the tissue level upon pain. Arginine, lysine and histidine displayed an analgesic effect upon alkaline pain, while glutamic acid and methionine had this effect upon acid pain. The effect could be related more to the basic tend-

ency of these substances to act through metabolic changes, than to a direct influence upon the acid-base systemic balance. The first group acts as heterotropic agents and the second as homotropic, as mentioned above.

Abnormal Amino Acids

Our research led us to several tentatives to define abnormal amino acids and the proteins they form. One concerned their rotatory capacity. The naturally occurring amino acids are all levorotatory. However, the organism constantly has enzymes able to attack dextrorotatory amino acid members as if it would have to be prepared to encounter and destroy them. Such dextrorotatory members can be conceived to appear on a statistical basis as the result of the resonance process seen to occur in all the synthesis in nature. The intervention of specific enzymes against them would have the aim to control their existence and especially to prevent their intervention in further evolution. In a work hypothesis concerning the cancerous process, we considered that their persistence and especially their participation in forming hierarchic entities would correspond to the specific abnormality characterizing this condition.

In another work hypothesis which concerns also cancerous processes and which we will discuss later, abnormal proteins are thought to appear as a result of the bond of a carbamic radical (295) to the amino acid group. The resulting cyclic formation having the characteristic NCNC group in it, would correspond to abnormal amino acids which would represent the primary characteristic formation of the cancerous condition. (*See Chapter 11, Note 1*)

Carbohydrates—Glucose acts as an anti-fatty acid agent, possibly because of the glyceryl compounds resulting from its metabolism. We have studied it in opposition to the respective acids—gluconic, glucuronic and saccharic. Glucose has an analgesic effect, although limited, upon pain of an alkaline pattern, and an opposite effect upon pain of an acid pattern.

The acid group has an opposite effect upon pain. This could be correlated with the changes toward acidosis seen in the local pH of the lesions. A manifest change toward acidosis was seen under the influence of glucose in the second day wound crust pH. We have noted previously the role played by glucuronic acid as an agent with anti-positive-lipid activity. We believe that it is largely through this mechanism that it favorably influences acid pain, having an indirect action similar to that of fatty acids.

CHAPTER 7

DEFENSE

THE RECOGNITION THAT multiple factors are responsible for abnormal conditions, and that these factors can be systematized according to the concepts presented above, throws new light on a specific aspect of the relationship between the different entities and the environment when this tends to alter their characteristic organization. This response is concretized as the defense against the noxious. The analysis of this defense has been facilitated by emphasizing the relative independence of the entities forming the complex hierarchic organism, the dualistic patterns of response, and the critical role of the lipids as well as of proteins. Abnormal processes in an organism's defense system may be better understood when they are compared to those corresponding to normal physiological processes. For this reason, we start with this last aspect.

The direct intervention of a noxious agent upon a biological entity can be characterized by its tendency to induce heterogenization, through an alteration of the entity's constituents or the relationship between them. This, in turn, affects one or more of the constants that characterize the entity. The ensuing defense response is directed ultimately at restoring the altered constants to their normal values.

Involved in a first stage of defense are those very factors which normally maintain the constants, the factors which induce the oscillatory dynamic balance. As a first response, they become exaggerated. Such exaggeration, which takes place successively for the opposite phases, resolves many slight noxious interventions without clinical manifestations. Through a damping movement, the exaggerated oscillations soon return to normal. If the normal constants are reestablished, the phenomenon can be considered to be a physiological response.

But if the alterations induced by the noxious agent persist, an abnormal

condition results. Indeed, in this case the exaggeration of oscillatory movement can be so great that an abnormality may result even from this exaggerated attempt of the entity to reestablish normalcy. In fact, offbalances are induced by just such changes which often represent, by themselves as will be seen later, one of the major immediate factors inducing the abnormalcy. As long as an abnormal condition is not resolved, the biological entity will try to utilize new means in order to reestablish the normal balance. If the constants disturbed by the noxious intervention are fundamental, or if the changes resulting from the defense mechanism itself are too great, death of the entity will result.

As expected, responses will differ according to the level to which an affected entity belongs. However, despite the many differences related to levels, a common and relatively simple pattern can be recognized when manifestations occurring at different levels as the result of the noxious intervention are compared and referred to the basic pathological concepts already noted.

Most of the information about this simple pattern was originally obtained by studying responses at the systemic level. Blood was particularly suitable because of its availability, its multiple constants and manifest capacity to conserve them, and particularly because of the facility with which noxious agents could be induced to act upon it.

The intervention of a noxious agent able to change the energetic balance of blood sets in motion immediately a group of successive processes which may or may not be clinically apparent, depending upon their intensity. They have been described as hemoclasia by Widal and hemo-shock by many authors. Although widely investigated, the mechanism did not appear clear. From our studies, we have arrived at certain conclusions which we will briefly present here.

Diphasic Phenomenon

As a noxious factor, we used an intravenous injection of killed microbes or of a colloidal suspension of a metal. Within a few minutes, a group of changes occurred. They were revealed through a series of analyses made at very short intervals. (*Note 1*) The changes were found to affect most of the blood constituents. The most characteristic change in our opinion is a leucopenia which especially affects the granulocytes. With it, there is a lowering of serum antitryptic power; a decrease of serum albumin; appearance of degraded proteins, esterase and amylase; increase of free fatty acids; and a lowering of coagulability with reduced clot retraction. Clinically, these changes are accompanied by hypothermia and hypotension.

Together they represent what we will call the "negative phase" of the immediate response.

This group of changes represents, in fact, only the first part of a diphasic phenomenon. The negative phase is usually followed by a second and opposite one which we call the "positive phase" of this immediate response. It results from the tendency of the body to correct, and even over-correct, the changes occurring in the first phase. After hypothermia and hypotension, hyperthermia and slight hypertension follow. At the same time, the number of granulocytes increases, as does the antitryptic power of serum and its albumin content. The serum appears richer in free sterols. Blood coagulability and clot retraction also increase. After moving rapidly to a peak, all these values return slowly to normal. The existence

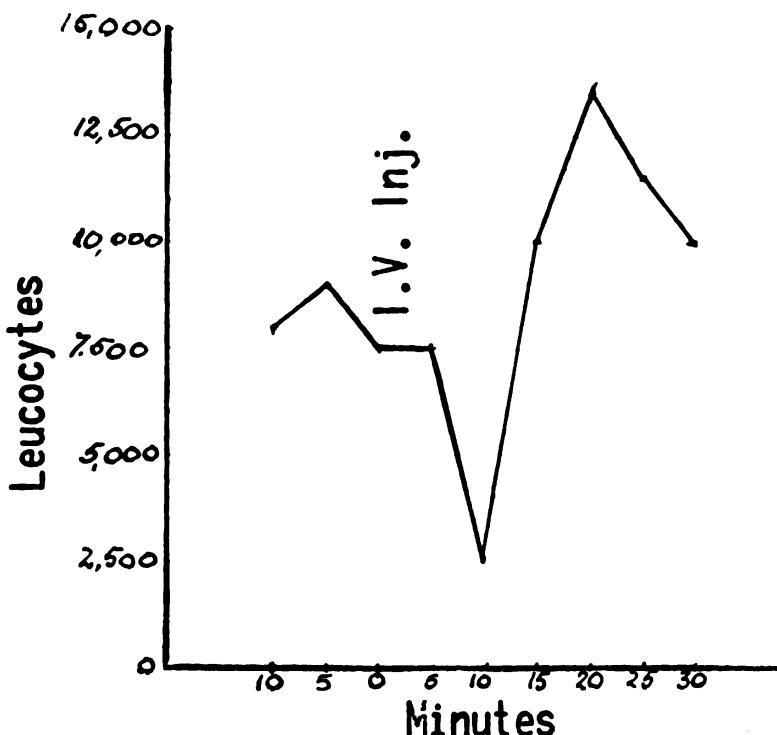


FIG. 75. *Diphasic response in the defense.* The intravenous injections to a normal individual of a foreign material such as of a suspension of killed microbes or of a colloidal metal induces a typical response which corresponds to the hemoshock. A *diphasic* curve seen in most of the analyses characterizes the occurring variations. The curve presented corresponds to the total number of the blood leucocytes. A parallel diphasic curve is seen for other blood analyses such as clot retraction, albumin content of the serum, and antitryptic values of the serum. Similar diphasic curves, but opposite in sense, are seen for blood coagulation time, amount of amylase and esterase in the serum, amount of K⁺ in the serum, and for the amounts of proteoses and peptones

of two phases can be recognized in all the changes occurring in hemo-shock. (*Fig. 75*)

In trying to correlate the multiple changes taking place, it is the lysis of leucocytes, especially granulocytes, which can be considered of primary importance in the development of hemo-shock. This is evident from the relationship between granulocytopenia and the intensity of the diphasic phenomenon. The administration of morphine or other opium derivatives to an individual, prior to the application of the noxious factor, will reduce or suppress the granulocytopenia together with all the manifestations. (*Note 2*) Intensive physical exercise concomitant with the application of the noxious factor will increase the granulocytopenia parallel with all the manifestations of hemo-shock. (*Note 3*)

According to our hypothesis, lysis leads to liberation of proteolytic enzymes which may be present as such or may be present in precursor form in the leucocytes. And it is the intervention of these enzymes which reduces the antitryptic power of the blood and, by digesting blood constituents, lowers the amount of albumin present in the serum, and induces a parallel increase of products of protein hydrolysis. The increase in amylase as well as in esterase present in blood is related to the other hydrolytic enzymes liberated in this phase, and is also probably correlated with leucolysis. The esterase acts hydrolytically upon the neutral fats present and this would explain, at least in part, the liberation of free fatty acids seen in this phase. In the changes corresponding to the first phase, digestive effect of these enzymes upon the blood constituents can be recognized as being one of the most important intervening factors.

We confirmed the correlation between these changes and leucolysis not only through their parallel variations, as mentioned above, but also through in-vitro experiments. Lysis of leucocytes resulted in liberation of hydrolytic enzymes. An exudate rich in granulocytes was obtained by injecting sterile broth, or an aleuron suspension, into the pleura of rabbits. To this exudate, removed through pleural puncture, a small amount of a colloidal silver-protein preparation (Collargol 0.1%) was added and the preparation maintained at 38°C. This was seen to induce the appearance of vacuoles in the leucocytes, following the phagocytosis of silver grains. The vacuoles were observed to grow rapidly to huge dimensions followed by bursting of the leucocytes. (*Fig. 76*)

Analysis of the pleural fluid treated in this manner has shown the same change as those seen in the first phase of hemo-shock: lowering of anti-tryptic power with a decrease in albumin content, increase in products formed by partial digestion of proteins, appearance of amylase and ester-

ase, and an increase of free fatty acids. There were also the same nuclear "shadows" as encountered in large amounts in the circulating blood at this phase. The increase of the potassium content of serum seen in this phase, and the increase found also in the supernatant part following centrifugation of the exudate to which Collargol had been added, represents a further confirmation of the role of leucolysis in this first phase. These data enabled us to consider that the mechanism through which the blood tries to combat the intervention of a noxious agent corresponds, in the first phase, primarily to a lysis of granulocytes followed by hydrolytic digestion.

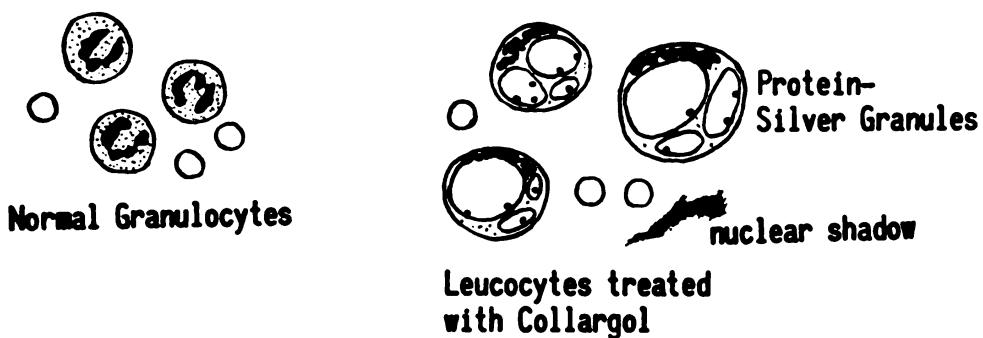


FIG. 76. Drawing of the changes induced by a *colloidal suspension of silver proteinate upon leucocytes*. The leucocytes were obtained by injecting broth intrapleurally to rabbits. Silver proteinate was added to the suspension of leucocytes and the changes observed in a microscope heated chamber maintained at 38°C. The phagocytosis of the silver proteinate leads first to the appearance of this substance as intracellular granules, followed by the formation of vacuoles. As these grow to a huge size the cells burst. The nucleus remains as nuclear shadow.

The second phase, which would correspond to efforts to correct the exaggerated effects of the first digestive phase, involves largely a mobilization of reserves of those blood constituents which were altered during the first digestive phase. The spleen pours a part of its stored blood into the circulation. The richness of spleen in reticuloendothelial cells explains the liberation of sterols which is seen during this second phase. This is recognized by the fact that, at this time, the spleen efferent blood is richer in sterols than the afferent blood. Other constituents come from intercellular and lymphatic spaces. This mobilization, characterizing the second phase of hemo-shock, appears to be achieved in large part mechanically, through a direct intervention of the vegetative nervous system inducing the contraction of the smooth muscular fibers, as seen during chill, which marks the beginning of this second phase. Fever which follows, is in part, due to the sterols liberated largely by the reticuloendothelial system.

If this hemo-shock, in spite of its frequently violent clinical manifestations, resolves the effects of the noxious intervention upon the blood, it can be considered to be, to a certain degree, a physiological response. It amounts to an exaggeration of the oscillatory mechanism through which the characteristic constants of the blood are maintained. By employing the hydrolytic enzymes stored in the leucocytes, the blood tries to resolve the influence exercised by the noxious factor, digesting and thus breaking down either the factor itself or the results of its direct intervention. Acting upon blood constituents, the noxious agent often induces the appearance of micelles bigger than those normally circulating. The fatty acids liberated by hydrolytic enzymes would insure, in the first place, a higher boundary permeability, thus permitting the passage through the capillaries of substances otherwise barred. At the same time the fatty acids bind the antigen in a lipidic complex.

In the second phase, the organism tries to repair damages caused by the exaggerated digestive process or by the intervention of fatty acids. If the organism is able to resolve through a successful diphasic reaction the changes induced by the noxious agent, it returns to normal.

Prolonged Hemoshock

Inability of the organism to resolve the noxious intervention through the mechanism involved in the diphasic phenomenon leads to abnormal prolongation of one phase or another. If it is unable to destroy the noxious factor in the first phase or to mobilize the repair process in the second and thus correct the damage induced by the first phase, the organism remains in a prolonged first phase of hemo-shock. If the second phase is quantitatively or especially qualitatively inadequate, the organism remains in a prolonged second phase, continuing to try to resolve the offbalance by a quantitatively greater mobilization of the otherwise qualitatively inadequate weapons which are at its disposal. It is the predominant intervention of the lipids which characterizes these extended phases. We wish to note again that the fatty acids intervene in the prolonged first phase while anti-fatty acid agents, especially sterols, are active in the second.

The adrenals play a particularly important role in the immediate and prolonged defense process. In the first phase, the increased amount of fatty acids with four or more double bonds found in blood and in the organism in general appears to come from the adrenals, which are usually extremely rich in these substances. In an exaggeratedly intensive prolonged first phase, we found small reddish adrenals practically devoid of fatty acids. This occurrence, together with the concurrent increase in fatty acids

in the blood, relates these changes in fatty acid content of the blood largely to a liberation of the adrenal fatty acids into the circulation. Another important factor for the prolonged first phase appears in the intervention of lymphocytes able to induce a lysis of compounds of very high fatty acids such as present even in waxes. (*Note 4*) A lymphopenia corresponds to the prolonged first phase. In the elevation of the amount of anti-fatty acid agents in blood, characteristic of the prolonged second phase of the diphasic phenomenon, the adrenals seem to intervene again providing a portion of the increased circulating sterols. The exaggerated manufacture of sterols can be attributed to the reticuloendothelial system in general. Granulocytosis and lymphocytosis occur in this prolonged second phase. The intervention of sterols, which are relatively simple steroids, can explain the clinical manifestations such as fever, which characterize the prolonged second phase, since fever can be induced by the administration of large amounts of sterols.

We can separate, from the point of view of its manifestations, the immediate diphasic hemo-shock phenomenon with a short evolution, from the more prolonged forms. While the former, if not too exaggerated, would correspond to a physiological phenomenon, the latter is always abnormal. In the former, the principal intervention is that of hydrolytic enzymes; in the latter, lipids play the most predominant role. Pathogenically, each phase of the diphasic phenomenon, if unable by itself to resolve the immediate problem, will be followed by a corresponding lipidic predominance. The result may be either one of the two phases, with fatty acids or sterols predominant. We call this entire response "the antiheterogeneous reaction" of the defense, separating its diphasic manifestations into immediate hydrolytic and prolonged lipidic stages.

Antiheterogeneous Reaction

Although, in the prolonged lipidic stage, a certain specificity for particular antigens can be recognized, the antiheterogeneous response in general represents rather a nonspecific effort of the organism to resolve the problems caused by the presence of any heterogeneous factors as such.

Before going further, we want to emphasize some important characteristics of this antiheterogeneous response related to organization. The catabolic processes present in the first phase appear to result in part from the direct hydrolytic process and in part from the biological intervention of the products of hydrolysis, especially fatty acids. The hydrolytic enzymatic process is homotropic in nature by definition, as it breaks down different constituents, liberating groups with anionic and cationic character.

Enhancing the catabolic character of the first phase processes are the anionic groups which appear to have a predominant role. The second phase, a reparative one, is anabolic and therefore, heterotropic in character.

The study of the antiheterogeneous response emphasizes another fundamental characteristic of the processes. A basic difference exists between the direct effects induced by the intervening agent and those resulting from the defense processes themselves. A direct effect of a noxious intervention corresponds to heterogenization of the constituents. Some changes will appear through this heterogenization itself, others through the defense processes which represent the response of the organism to the heterogenization. While the first corresponds to a direct action, the last is catalogued as antigenic, its manifestations being grouped as defense processes. The same substance can have a direct action and an antigenic one revealed principally through the manifestations which it induces. A direct action can be noted instantaneously if the changes induced are sufficiently intensive. The antigen effects always require a certain time before manifestations appear. This time can range from a few minutes for the first enzymatic response to hours or days for the prolonged response.

An important feature of the prolonged stage is that it persists as long as the noxious agent is present. This is evident in cases in which the noxious agent can be suppressed through external intervention. For example, with suppression of microbial activity by antibiotics, the corresponding clinical condition disappears. Of more interest is the effect of antimicrobial and antitoxic immune sera. Administration of a specific serum, if it can neutralize the noxious agent, produces a curative effect at this stage. In a short time, symptoms disappear and the organism reverts to normal. Although nonspecific, the prolonged antiheterogeneous reaction shows such a straight correlation with the presence of the antigen as to make us designate this stage as primary or toxic. In this stage, the organism reacts with clinical manifestations of disease if the antigen is capable of inducing sufficient noxious changes; if not, there are no clinical manifestations. Persistence in the organism of an antigen beyond the rapid diphasic phenomenon indicates, in general, an incapacity of the organism to achieve its disposal successfully. The need for more complex means to combat the antigen becomes imperative.

With or without clinical manifestations—that is, even without a primary toxic stage of the disease—as long as the antigen has not been fully neutralized, the organism will still try to resolve its intervention and return to normal, resorting to other means. It will produce antibodies with a cer-

tain degree of specificity toward the antigen. Two kinds of antibodies will be manufactured and will differ in their fundamental characteristics, the time of their appearance, and their role in the defense processes.

Coagulant Antibodies

The first group of antibodies have a characteristic property. Together with the antigen, on which they fix with a degree of specificity, they form highly energetic complexes. This is manifest in a marked tendency to bind together such complexes as well as constituents of the blood and form huge aggregates. When such antibodies are produced for, and act against, a specific microbe, agglutination results. Conglutination, precipitation and flocculation occur when similar antibodies act against other antigens. Due to their tendency to establish antigen antibody complexes resulting in huge formations, these antibodies are generally grouped as coagulant antibodies. Although the coagulation characteristic is not demonstrable *in vitro* for all antibodies in this group, we use the term "coagulant antibodies" for didactic purposes.

The huge complex formation resulting from the binding of coagulant antibodies with an antigen can appear as a precipitate, agglutinated microbe or conglutinated red cells. Once established, this formation represents a new heterogeneous entity of much larger dimensions than the antigen alone. As such, it becomes by itself a new noxious agent for the organism which consequently reacts against it. The organism utilizes the same processes against this noxious antigen-coagulant antibody complex as it uses for any heterogeneous agent, with the same immediate diphasic or prolonged mechanism.

Teleologically, the formation of coagulant antibodies can be interpreted as an attempt of the organism to defend itself anew against the antigen. The antigen, this time fixed through these antibodies in a new and more noxious formation, will once again incite the nonspecific defense mechanism. First there will be the antiheterogeneous response with its diphasic phenomenon and, if once more this is not effective, a prolonged new lipidic intervention will follow. If the quantity of heterogeneous formations is great, the first phase of the diphasic phenomenon can be so severe as to cause death in a few minutes. If less severe, this first phase is followed by the second, with chills and high temperature. As in all the antiheterogeneous reactions, the organism tries to combat the presence of the noxious factor—in this case, the flocculate produced by the antigen-antibody bond—attempting to digest it through hydrolytic enzymes or to neutralize it through constituents brought in during the second phase of the diphasic

phenomenon. If it fails, the abnormal prolonged form of this response follows with characteristic lipidic liberation.

In terms of biological meaning, the formation of coagulant antibodies represents a new chance for the organism to resume the fight against antigens by using the same fundamental means, the antiheterogeneous reaction. However, since the new agent, the antigen-antibody complex, is much more noxious than the antigen alone, the intensity of the response will be much stronger and the chances of disposing of the antigen will be greater.

Allergic Reaction

The generic term "allergic" is reserved for the entire group of processes in which a coagulant antibody takes part. The reaction now against the antigen-antibody complex is a typical antiheterogeneous response. A fundamental difference exists, however, between the antiheterogeneous reaction in the primary toxic stage and the reaction which occurs when the heterogeneous factor is a complex antigen-coagulant antibody usually with more noxious character than the antigen alone. It is the nature of the antigen-antibody, the result of the bond of the coagulant and the antigen, which gives it the allergic character. This fact explains why, although we can possess specific immune serum able to neutralize an antigen alone, this will not influence the allergic response. Already bound to a coagulant antibody, the antigen cannot be bound again and consequently neutralized by another antibody. The specific neutralizing serum will have no effect upon the antigen-coagulant-antibody complex already formed and consequently will have no effect upon the processes induced by this complex. The immune serum does not influence the allergic manifestations which represent the response to antigen-coagulant-antibody complexes. This would theoretically explain the favorable effects of a specific serum upon a condition which is in the toxic primary stage, where the antigen intervenes as such, and the lack of such favorable effects in the allergic stage, where the antigen is representing only a part of a complex new noxious formation.

This mechanism would also explain why the same immune serum, although without curative effect upon the allergic stage of a condition, will have preventive activity. Before the onset of the allergic stage—that is, before the coagulant antibodies have appeared—the active immune serum will bind and neutralize the antigens still free in the organism. Under these conditions, when coagulant antibodies appear, the antigens are no longer available to be bound by them to form the noxious antigen-coagulant-antibody complexes. Without curative action, immune serum is effective as a

preventive only when administered prior to appearance of coagulant antibodies.

An important factor for the allergic response is the time of liberation of coagulant antibodies. Generally, a period of 6 to 8 days is required. Under special circumstances, as in cases in which the organism has manufactured the same antibodies in the past, the time necessary for their appearance is reduced even to 4 days. In other cases, for certain antigens or for older subjects, the time may be as long as 14 days or even longer. For certain antigens, or under special circumstances, the body appears unable to make coagulant antibodies at all. In that case, no allergic manifestations appear.

It must be emphasized that antibodies will be liberated even if the antigen is no longer present. The presence of antibodies alone does not give rise to any reaction and their appearance will pass without any manifestations. However, they can persist under certain circumstances for months or years and become a potential source of abnormality. At any time if the same antigen becomes present in the body, the coagulant antibody will form the allergic bond with it. The body will then react against the newly formed complex with an antiheterogeneous response. If this occurs in the blood or central nervous system, it can appear as an immediate violent reaction which corresponds to anaphylactic shock. It is the intensive first phase of the diphasic phenomenon which kills in anaphylactic shock. Such shock can be easily produced in animals as passive anaphylaxy by making coagulant antibodies and antigens available concomitantly in the blood.

Lipido-proteic Antibodies

Analysis of allergic antibodies indicates that they have two constituents, lipids and proteins. Electrophoretic analyses reveal that they are displaced mostly as beta globulins. Experiments show that such lipido-proteic antibodies lose their activity if broken into their constituents, neither the lipid fraction nor the protein alone being able to bind the antigen.

The study of the lipido-proteic antibodies, brought us back to consider the role of the lipids in the immediate or prolonged first phase. Most, if not all the natural antigens have lipids and lipoproteins in their structure. As we have seen above, some of the fatty acids induce defense responses. The administration of fatty acids is followed by a leucopenia—especially a lymphopenia; administration of sterols, by a hyperleucocytosis. Some fatty acids, such as those obtained from the tubercle bacilli, induce characteristic lesions such as giant cells. There are both naturally present lipoproteins and those resulting from the bond of the body's freed fatty acids to the

antigen, which seem to act as specific antigens, inducing the appearance of coagulant, allergic antibodies. Experiments, which we will discuss below, have shown that, while the specificity of the antibodies is highly related to the protein fraction, the allergic or immune character of the resulting response is due to the fact that lipido-proteins are involved in these processes. The injection of the product resulting from the action *in vitro* of the acid lipidic constituents of an organism upon proteins of another species acts as an antigen inducing the early appearance of coagulant antibodies. The repeated injections of the product obtained through the action *in vitro* of foreign lipoacid fractions of various origins upon different body proteins also induces allergic response. Just as we have connected the appearance of the first diphasic phenomenon to hydrolytic enzymes and the prolonged form of the antiheterogeneous defense mechanism to the intervention of lipids, we relate the allergic body defense to the intervention of lipido-proteic formations. The allergic stage of defense thus could be considered to be a lipido-proteic defense response against lipido-proteic antigens.

Neutralizing Antibodies

The unsuccessful fight of the organism against an antigen through the diphasic, lipidic or allergic responses often can evolve further, making use of a more effective measure which corresponds to another kind of antibody, different from the coagulant type. The characteristic of this second type is that it forms, specifically with the antigen against which it is manufactured, a new kind of bond, an antigen-antibody-complex, this time entirely nonnoxious to the organism. This complex is energetically so balanced as to correspond to the constants of respective levels of the body where it occurs. Through this new bond, the antigen is biologically neutralized in the sense that the resulting antigen-antibody-complex is entirely harmless.

This type of neutralizing or immune antibody usually appears on or after the 15th day following the moment when the organism has started to organize its defense against the antigen. It can occur whether the antigen is still present or not and whether it is free or bound to coagulant antibodies. It represents the best means through which the organism opposes the influence exercised by an antigen. The appearance of the neutralizing antibodies corresponds to the last stage, the immune one, in the defense mechanism. If the antigen has produced a clinical condition, the neutralization of the antigen by the new antibody results in the slow disappearance of morbid manifestations and a progressive return to normal. With or without prior clinical manifestations, the presence of the neutralizing antibody

in the organism provides a potential weapon to prevent the same antigen from again causing trouble. This has led us to identify this part of the defense reaction as the "immune stage" of the defense mechanism.

The action of these neutralizing antibodies has been demonstrated beyond doubt through passive immunity. Their administration confers protection against the antigen. This action is limited to the antigen so long as it is not bound by another antibody. Clinically, neutralizing antibodies have a curative value if the antigen is present, inducing the primary-toxic response. They have, also a preventive effect upon the allergic form of the disease if they are administered before the appearance of the coagulant antibodies.

Neutralizing antibodies are globulinic in nature. They are displaced in electrophoretic analyses as gamma globulins. Isolated as pure globulins, they do not lose their activity. This would differentiate them from the coagulant antibodies which, as previously noted, are lipido-proteinic in nature.

The defense resources of organisms against antigens thus can be di-dactically separated into four fundamentally distinct groups: enzymatic hydrolytic, lipidic, lipoproteinic and proteinic. They correspond to distinct stages from the point of view of reactions induced and biological meaning. The first represents a primary, direct, immediate response characterized by a rapid nonspecific digestive process and followed by the exaggerated mobilization of repair processes. If the immediate response is inadequate, a second stage as a prolonged lipidic defense follows. Although it has a certain degree of specificity, this last response is still directed against a heterogeneous constitution of the agent as such. If unsuccessful in inactivating the agent, all these responses are followed by another defense stage in which action is taken against the antigen through more specific coagulant lipido-proteinic antibodies and through the antiheterogeneous reaction to the resulting complex. With the last stage, which is characterized by the intervention of proteinic neutralizing protective immune antibodies, the fight against the antigen is usually concluded successfully. Table XI, below, summarizes this systematization of the defense response.

Defense and Hierarchic Levels

The above changes represent, in a schematic manner, what happens when a noxious agent acts directly or indirectly upon blood. The same basic patterns of defense, the substances used and the processes involved can be found at various hierarchic levels. It is easy to see that, with such a

TABLE XI
THE IMMUNOLOGICAL DEFENSE MECHANISM

<i>Defense Stages</i>	<i>Character</i>	<i>Intervening Factor Present</i>	<i>Nature Of the Processes</i>	<i>Means Used</i>	<i>Moment Of Appearance</i>	<i>Resulting Condition</i>	<i>Effect Of Immune Serum</i>
Primary Immediate	Non Specific	Antigen	Hydrolytic	Hydrolytic Enzymes	Immediate	Shock	Curative
Primary Prolonged	Lipidic Defense	Antigen (probably in lipidic frac.)	Lipidic liberation	Lipids	From minutes to days	Toxic	Curative
Allergic Immediate	Allergic Specific	Allergic complex antigen antibody	Allergic bond followed by diphasic phenomena	Lipoproteinic antibodies followed by hydrolytic enzymes	Around 6th day	Allergic Shock	Not curative but preventive
Allergic Prolonged	Allergic lipidic defense	Allergic complex	Allergic bond followed by lipidic liberation	Lipoproteinic antibodies followed by new lipidic bonds	After the 6th day	Allergic Toxic	Not curative but preventive
Immune	Healing & Protective Specific	Neutral complex antigen-antibody	Neutralizing bond	Proteinic antibodies	Around 15th day and later	Healing	

complex mechanism occurring in different individuals and against a great variety of antigens, great variations in manifestations will be evidenced.

When an antigen enters the organism and is not fully neutralized by the intervention of immune antibodies, the mechanism of defense is set in motion. This can be limited to a group of entities, to one level, or can affect more levels and entities. According to the nature of the antigen and the capacity of the different entities to respond, the different process will proceed all the way to the stage of protective immunity or it can stop at any stage. These factors, nature of the antigen, levels and entities involved and degree of response for each of them, determine the pathogenic characteristics of the resulting condition. The manifestations for a stage and a group of entities can be so exclusive as to produce a characteristic clinical disease. The ability to respond through only a part of the defense mechanism depends on the nature of the antigen and on the conditions existing in the different entities affected. The clinical manifestations furnish important information concerning the defense processes which are occurring.

Clinical Manifestations

The first stage of the defense reaction, if highly intensive, can be manifested clinically as superacute shock. This occurs minutes after the intervention of the noxious factor. If the second phase of the diphasic phenomenon is intensive, a chill with high temperature will appear. A state of shock occurs if fatty acids remain predominant, as in the prolonged first phase. A feverish condition corresponds to a predominant anti-fatty acid intervention in the prolonged second phase. While the first phase of the condition appears immediately after the intervention of the antigen, the appearance and persistence of the second phase, which corresponds to the prolonged lipidic, depends upon the nature and especially the amount of antigen present in the organism. Incubation time may vary from minutes to several days. This relatively short and variable incubation time represents an important characteristic which enables us to recognize this stage. Another characteristic of this stage is the disappearance of symptoms after administration of neutralizing immune antibodies specific for the antigen.

If the antigen by itself has no toxic effects upon the organism, its presence will not induce important manifestations. The direct response, enzymatic or prolonged lipidic, will be so limited as to have either minimal clinical manifestations or none at all.

We have seen that allergic coagulant antibodies usually appear after the 6th day following penetration of the antigen into the organism. If the antigen is still present, the appearance of the antibodies will induce an

allergic condition. In cases where the antigen already has produced toxic manifestations, the appearance of the allergic stage will be marked either by new symptoms or increased intensity of existing ones. With a nontoxic antigen, the presence of which was not revealed previously by any clinical manifestations, the appearance of the allergic complex will coincide with the appearance of symptoms and, thus, with the appearance of the clinical condition. The period before the appearance of symptoms corresponds to the time before the appearance of these allergic antibodies. This incubation time, as for all allergic manifestations, will be 6 or more days, which corresponds to the time necessary for the appearance of the coagulant antibodies. An obligatory incubation time of 6 days or greater thus is an indication that the process has an allergic pathogenesis.

If the organism has previously manufactured similar coagulant antibodies against the same antigen, this incubation period could be shortened to 5 or even 4 days. Against certain antigens, some organisms need a longer time to produce coagulant antibodies and the incubation time may run as long as several weeks.

Organization and Defense

Going beyond the defense reaction in its general aspect, that is, independent of the place in the organization where the characteristic processes occur, we considered it in relation to hierarchic organization of entities. Conceptually, it can be accepted that each hierarchic entity, having a certain degree of biological independence, will have its own problems to resolve when it faces an antigen. Each will react for itself and, for this reason alone, there will be differences in the defense mechanism at different levels. It can also be accepted that because of differences in the means at their disposal, the various entities will show individual peculiarities in their responses.

Although some information is missing, our systematization of the defense reaction according to the manifestations at various organizational levels, stands. For each stage, manifestations having common basic characteristics can be identified at different levels such as cellular, tissular, organic, and systemic. At the cellular level, the first phase of the diphasic phenomenon corresponds to a manifest increase in membrane permeability and intracellular hydrolytic processes. The changes seen in the first phase of shock can be interpreted as resulting in part from such processes. We will note here only that, for cells, the first phase is characterized by vacuolization of the cytoplasm and even of the nuclei similar to the vacuolization seen in the leucocytes in the presence of a colloidal metal. In superacute

shock, which corresponds to the first part of the diphasic phenomenon, we observed such vacuoles in central nervous system, liver and pulmonary alveolar cells. (See Shock, Chapter 9) The same process at the tissular level causes lytic changes and, if this lysis acts upon vessels, produces petechiae. For the systemic level, the first phase of the diphasic phenomenon is marked by the changes occurring in blood. The leucocytes, rich in hydrolytic enzymes, have a pronounced lytic tendency. The liberated enzymes act upon the blood constituents and can impart to this stage the acute dramatic aspect often seen in clinical hemo-shock.

The prolonged lipidic phase of the antiheterogeneous reaction will have different manifestations according to the level affected. These differences will correspond closely to the antagonistic influence exercised by the two groups of lipids, sterols and fatty acids. We have discussed previously the intervention of these lipids at different levels. We will mention briefly here their role in the different phases of the defense mechanism.

We have seen that, in general, the changes at the nuclear level correspond to prolonged youth if they are produced by the predominant intervention of sterols and to a rapid aging with karyorrhexis and pyknosis when produced by fatty acids. Similar manifestations can be recognized in this phase of the defense mechanism at the cellular level for the cytoplasm and protoplasm formations, with aging signs and necrosis induced by fatty acids, and predominance of youthful characteristics induced by sterols. For the tissular level, intervention of fatty acids induces local alkalosis and edema, while the sterols induce a local acidosis and fibroblastic reaction. Lysis of vessels with hemorrhages occurs in processes in which fatty acids predominate. The predominance of sterols leads to a marked tendency of the vascular endothelium to proliferate and this, in turn, can lead to vascular obliteration and ischemic infarcts if the vessels are terminal. At the organic level, the prolonged lipidic response is more manifest than at lower levels as the result of impaired specific function of the organ. Dualism in clinical manifestations is evident; oliguria or polyuria, diarrhea or constipation, insomnia or somnolence are examples of organic impairments seen as clinical manifestations of this stage of the defense mechanism. At the systemic level, dual manifestations are even more pronounced. Hypothermia, hypotension, cold perspiration, enophthalmia and dark-colored blood are related to predominance of fatty acids, opposite manifestations to predominance of sterols. Although these prolonged manifestations, part of the nonspecific antiheterogeneous lipidic response, can occur concomitantly at the various levels of the organization, usually they affect one or

several levels. The manifestations corresponding to the one level or several levels will predominate.

The allergic stage shows the same clinical manifestations common to the antiheterogeneous response with its enzymatic or prolonged lipidic processes. The fundamental difference in the allergic stage is the obligatory incubation period of 6 or more days. Once the allergic complex is realized, the manifestations are the same as those produced by highly active antigens inducing a direct antiheterogeneous reaction.

The qualitative differences in the capacity of the hierarchic entities to combat various noxious agents can explain the differences in the manifestations of allergic processes taking place at the cellular or tissular levels as compared to those in the blood, which is at the systemic level. At any level, the mobilization of lytic enzymes able to break down the allergic antigen-antibody complex can be so intense as to bring rapid death of the entity or can be slow and prolonged. However, at the systemic level, as in the circulating blood, the products resulting from exaggerated lysis are more rapidly and completely disposed of than in cytoplasm or interstitial fluids. In the latter, they will be present for a long time and their noxious influence will persist. If the lytic products appear in moderate amounts in blood, the organism may be able to dispose of them without any clinical manifestations.

For these reasons, the presence of antigens in the blood when coagulant antibodies start to appear will not induce serious manifestations and will even prevent them. As coagulant antibodies appear gradually in the blood, only small amounts of antigen-antibody complex will be produced at any one time. Although highly noxious in large amounts, the complexes can be resolved through lytic processes if formed gradually, and consequently will not provoke clinical manifestations.

At the tissular and cellular levels, a similar progressive appearance of antigen-coagulant-antibody complex cannot be resolved in the same way. The lytic reactions which break down this complex cannot occur with the efficiency noted in the blood. The complexes and the products of lysis will progressively accumulate and the consequent manifestations will become more and more intensive. It is for this reason that long-lasting allergic manifestations correspond to serious local conditions. Even if the antigen-antibody complexes are produced at a moderate rate, when antibodies appear and the antigen is present, they will induce little or no systemic manifestations. On the contrary, serious allergic manifestations will arise if the complexes are formed at the cellular, tissular or even organic levels. Because the defense processes at these levels cannot resolve them at the

same rate as they appear, as defense processes in the blood can do, severe local manifestations result. This may lead to necrosis and even rejection of altered cells or tissues. These represent the very important differences which exist between allergic processes which occur in the blood and those which occur at the different levels following the appearance of allergic antibodies while the antigen is still present.

The fact that there will be no reaction when small amounts of the allergic complex are progressively formed, as in cases in which antigens are present in the blood at the moment of appearance of the allergic antibodies, is confirmed indirectly by the possibility of preventing severe systemic manifestations through skeptophylactic or desensitization procedures. The introduction of very small amounts of antigen thus produces only small amounts of complex at any one time, avoiding clinical manifestations. With progressive doses however, the antigens will fix circulating antibodies in sufficient proportion to prevent the formation of important amounts of the same complexes after further administration of the antigen. The presence of the second phase of the diphasic phenomenon, with the exaggeration of constituents antagonistic to those present in the first phase, will also act to prevent the occurrence of an intensive first phase when the antigen appears anew.

The situation changes entirely when antibodies appear and the antigen no longer is present. They can then accumulate in the blood in large amounts. Thereafter, sudden appearance of the antigen in sufficient quantity will form a large amount of the allergic complex and the subsequent reaction can be so violent as to kill the subject. This occurs in anaphylactic shock. When the antigen is limited to other levels, important local changes can be induced.

The neutralizing immune antibodies, if manifestations already exist, will prevent new ones from appearing and this will permit healing processes to take place without further interference. The antibodies will prevent manifestations at the respective level if the antigen appears again.

Affinity of Antigens

In the defense processes, another factor intervenes to produce differences between responses at different levels—the special affinity of antigens for various cells, tissues or organs. This affinity will determine not only the level but also the individual entities where manifestations will occur. It has to be emphasized that the independence of the levels or of groups of entities in an organism goes so far as to allow the defense processes to progress to different stages. While defense processes at the tissular level, for instance,

cannot go beyond the stage of prolonged lipidic response, those at the organic or systemic level can arrive at the allergic stage. We will see below the importance of this unequal response of the different levels.

The unequal capacity of different tissues to manufacture allergic antibodies could be postulated to explain the propensity for local allergic conditions. The ectodermic system appears especially inclined to allergic responses, as seen for the skin. We tried to relate this to the natural richness of these organs in sterols. This would explain the fact that the brain, which is richest in sterols, seems to show the earliest allergic manifestations, which could be interpreted as resulting from early or more constant appearance of coagulant antibodies.

Besides these differences in the responses of various entities, an important factor intervenes in the induction of localized allergic manifestations. It corresponds to unequal affinity of the antigen itself for various entities. This would localize the antigen in cells, tissues or organs so that when coagulated antibodies do appear, the noxious allergic complex will be formed locally in the same entities. It seems that this localization of the antigen, such as upon nerves, kidney, lung, etc., is more important than the capacity to produce antibodies in determining predilection of pathological processes for specific cells, tissues or organs.

One of the most interesting aspects of the defense mechanism is the relationship between successive steps. We could show, generally, that an intensive response in one step represents a favorable condition for appearance of an intensive response in the next step. It is a known fact that manufacture of immune antibodies is influenced by an inflammatory process. This is the reason for the customary injection of tapioca, for instance, in horses during their immunization for the production of therapeutic sera. We could show that injections of lipids, lipid acids or insaponifiable fraction of placenta, or of organs of animals of the same species for instance, manifestly hasten the appearance of the next step in the defense against the microbe.

It seems clear that under the influence of the lipids used, the agglutinins appear in blood earlier and their amount increases more rapidly than in the control animals.

Antigenic Factors

The intervention of different mechanisms in the defense has led to the supposition that each one would be induced by relatively specific factors present either in the antigen itself or appearing during the defense processes.

An analysis of this aspect of the problem of the defense has brought further interesting information.

The intervention of the first mechanism, that of hydrolytic enzymes acting through a process similar to digestion, would have as aim to break down the antigen itself as well as the groups resulting from the bond between antigen and body constituents, especially proteins. By analogy with the process of digestion, the factor present in the body which would induce this response would correspond to abnormally low number of micelles. The low number of micelles present is revealed by a cryoscopic index near zero. The digestive defense mechanism would thus intend to lower this cryoscopic index back to its normal values or even below them.

The second mechanism, that of the lipidic intervention, would have two aims. One, to act against free lipids either present in the antigen or resulting from the hydrolytic action upon fats, and second, to bind hydrosoluble constituents into complexes with a lower hydrosolubility, and consequently with lower diffusion capacity through the aqueous media of the organism. This concerns the antigen as well as the products resulting from the lytic intervention. The bond would take place through the active polar part of the lipid molecules.

The third mechanism is characterized by the intervention of the allergic antibodies with the aim of binding the antigen in higher complexes. The lipido-proteic antibodies will oppose a lipido-proteic fraction present in the antigen itself or resulting from the bond between lipids liberated in the second mechanism and proteins of the antigen or of the body. The coagulant effect would result from the bond through the polar and nonpolar groups of the lipido-proteic antibodies and those of the lipido-proteic antigenic factors.

For the fourth mechanism, characterized by the protective antibodies, the antigenic factor would be represented by the proteic constituents of the antigen, which leads to an antireplication in the specific antibodies.

It should be noted that in the complex defense mechanism the results of the intervention of a defense process represent antigenic factors for the next step. The presence of products of the enzymatic digestion leads to the intervention of the lipidic phase, largely aimed to immobilize and inactivate them; the bond between lipids and antigen leads to the appearance of the allergic lipido-proteic antibodies. Possibly, the occurring lipido-proteic complexes would intervene, facilitating the appearance of the protective antibodies. The idea that successive antigenic factors would induce the appearance of different steps in the defense mechanism, has led to a series of studies with the aim to obtain desired reactions through the use of such

antigenic factors. We will describe here very briefly several such applications which were interesting also because of the practical results obtained.

Hydrolysis Products

We tried thus to utilize the products resulting from the breaking down of body constituents or of other materials in order to induce through their administration, the appearance of the second defense mechanism. Applying the dualistic concept, we separated thus in the products of hydrolysis of different materials, those with an acid character from the group with basic and alcoholic characters. Various materials were thus hydrolyzed using KOH, NaOH or ammonia. The soluble part, separated, was treated with an acid and a precipitate obtained. After washing it, this precipitate was redissolved by alkalinizing to a pH still below neutrality. This has represented the "acid fraction." Besides acid lipids, this fraction contains also acid protein groups and even humic acids.

The part which remained insoluble after treatment with KOH (separated from the soluble part) was treated with an acid. The part which became soluble was then separated, reprecipitated by alkali, and partially redissolved by bringing the pH, through acidification, near 7. This represents the "alkaline fraction." With different degrees of chemical hydrolysis, various fractions—more or less broken down—are obtained for both the acid and alkaline fractions. The degree of this "digestion" has appeared highly important. The amount of the products obtained decreases for an insufficient hydrolysis as well as for a too highly pushed hydrolysis.

According to the mechanism mentioned above it was expected that these fractions, resulting from the breaking down of body constituents or of the antigen and corresponding to the effect of the first enzymatic defense mechanism, would induce the second step of the defense mechanism. This would correspond in part to the intervention of the properdin system and of the lipidic defense. It has as characteristic the fact that it would appear only within a certain time. The following experiment illustrates this clearly. The "acid fractions" of human blood, hydrolyzed by KOH was obtained and then injected intraperitoneally to mice. At different intervals following this injection the mice were inoculated with 3,000,000 microbes of a fresh culture of *Bac. proteus*. In controls this inoculation would result in a 100% lethal infection. No protection was seen to appear in the 16 hours following the injection of the "acid fraction." At the 16th hour, $\frac{1}{2}$ were protected. This protection increased with time to be complete after 22 hours, when all the animals survived. This protection was still present after a few days.

The inoculation of the nontreated blood in the same proportion was

seen incapable of conferring the same degree of defense, a fact which indicates the importance of the breaking down process in this "24 hours" defense. These results are similar to those obtained by I. A. Parfentjev with malucidin, a product of hydrolysis of yeast.

Another application of the same concept was in the use of the lipido-proteic complexes.

In a group of research studies, we utilized the products resulting from the bond between an antigen and a lipid, with the intent to obtain a lipido-proteic antigen and through it, a lipido-proteic defense response. Often the mixture of the antigen with the lipidic preparations appeared sufficient.

Fatty acids, such as oleic, linoleic, arachidonic or eleostearic, acting directly upon the killed typhoid microbes were usually seen to enhance the production of agglutinins and of specific immune antibodies. The same effect was produced by lipoacids of the same species as the test animal. Lipoacids of guinea pigs were especially active in promoting the appearance of agglutinins but less potent in inducing the appearance of immune antibodies. The lipoacid fraction of bacteria such as *B. subtilis*, *coli*, diphtheria, acting in vitro upon typhoid killed microbes, led to the appearance of antibodies against typhoid microbes but produced almost no antibodies against those microbes from which the fatty acids were obtained. The lipo-acid fraction of tubercle bacilli bound to killed typhoid microbes was seen to induce agglutinins but seemed to reduce and even prevent the appearance of immune antibodies. The same influence was seen with the lipids obtained from the seeds of *Bixa orellana* but was less accentuated for the lipids from fish and squid. Butanol and especially heptanol were seen to retard the appearance of all antibodies, allergic and immune.

Allergic Precipitates

The injection of killed typhoid microbes agglutinated by a specific serum was followed by rapid production of immune antisera. The serum of rabbits injected with these mixtures prevents a lethal condition induced in mice by intraperitoneal injection of living microbes in much smaller doses than serum obtained with untreated microbes.

On the other hand, the injection of the same killed typhoid microbes, mixed together with a flocculate obtained, for instance, from egg protein, and an antiegg precipitant—guinea pig serum—produces a much less rapid appearance of antityphoid immune antibodies than injection of microbes alone.

Another form of lipido-proteic complex, utilized as agent with the aim to induce not a lipido-proteic response but a higher one in the defense

process, was that of allergic precipitates. Through a blender, we obtained from rat and mouse tumors homogenates in which it was no longer possible to see cells. After centrifugation the supernatant fluid was separated, and used as antigen. Part of it was inoculated to guinea pigs, twice at 3-day intervals. The amount of appearing precipitines was determined periodically and the animal bled when the serum had a sufficiently high titre. Using the same antigen and the obtained sera properly diluted, flocculates were obtained. The precipitate separated was injected to animals having the tumor grafted. In a high proportion of cases—in more than 70% in some experiments—the tumors started to show changes 24 hours following the injection of the precipitate, to ulcerate or disappear in the subsequent days. Similar research, using pooled human tumors, is in progress.

Intermediary Lysates and Antigens

Of interest was a special use of the intermediary lysates in order to obtain changes in the antigens, which would facilitate the defense mechanism. Microbes, tissues or other products, serving as antigens were injected, mixed with intermediary lysates from blood or other sources. This was seen to result in a more specific second day defense response. Mice injected with such a mixture of blood intermediary acid fraction plus killed microbes showed resistance to the inoculation, 24 hours later, of the same living microbes in doses otherwise lethal. The protection obtained has a marked degree of specificity.

In experiments now in course, we utilize blended tumors mixed with the intermediary acid lysate fraction, to induce a defense in animals having the same tumor grafted.

The discussion above concerns what could be called the immunological part of the defense reaction. It has to be coupled with many other processes or phenomena which can be systematized as endocrine, vegetative, central nervous or even psychological responses. Some of them could be indirectly related to the intervention of lipids, and possibly involved through them in the immunological responses.

This concept of immunological defense, even under its incomplete aspect has helped us to understand a number of important pathogenic problems, including two which have been of particular interest to us: infectious disease and cancer. Our study of the infectious diseases under this aspect was reported in a preliminary communication in 1919. (37) In 1942, this part of the research was presented at the Congress of Medicine in Mexico and published in the journal "Pasteur." (38)

INFECTIOUS DISEASES

Toxic and Allergic Conditions

In infectious disease the antigen is a micro-organism which may be a virus, microbe, protozoa, mycet, etc., or even a product elaborated by a micro-organism. The response of an organism to the presence of an infectious antigen tends to follow the same successive stages previously outlined. If the means at the immediate disposal of the organism are qualitatively and quantitatively sufficient to neutralize the antigen, the entire process will be resolved asymptotically. Otherwise, the first stage of the defense reaction, the primary toxic diphasic phenomenon, will be set into motion. According to the qualitative effectiveness of this response, manifestations will vary from simple subclinical changes to clinical reactions. If the second phase of the diphasic response cannot take place, a prolonged form of the first phase will result. It corresponds to shock, which is encountered only in very severe infections. The rapidly lethal condition resulting from transfusion of massively infected blood is an example.

The second phase brings chill and fever. If the second phase response is qualitatively insufficient, the prolonged form ensues, bringing fever, the usual manifestation of many infectious diseases. The fever persists as long as the nonneutralized antigen is present. In this stage of the defense reaction against a micro-organism or its toxins, the symptoms, although resulting from the response of the organism, are still directly related to the presence of the antigen in sufficient quantity. The quantity necessary to induce the clinical manifestations can be reached within a short time after the penetration of the antigen into the organism. The toxic reaction thus can appear in a few hours. Consequently, there is no specific obligatory incubation time. The manifestations will disappear when the amount of antigen is decreased sufficiently. For some microbes, antibiotics have such action, resulting in a decrease in the amount of the antigen present, and consequently in the disappearance of the clinical manifestations. A similar decrease in the amount of the free antigen present can be obtained by its neutralization through specific immune sera, if available. Consequently, such sera have curative effects in infectious diseases characterized by a primary toxic pathogenesis.

Allergic antibodies will appear after an obligatory incubation period of 6 or more days. If the antigen is still present, it may be destroyed by the new defensive antiheterogeneous responses mobilized against the resulting allergic complex. In this case, the appearance of the allergic antibodies re-

sults in a kind of clinical crisis which can lead to the cessation of the disease. However, if this effect does not occur, the appearance of allergic antibodies will cause an increase in symptoms or in their gravity.

In cases asymptomatic prior to the appearance of the allergic antibodies because of low direct toxicity or insufficient quantity of the antigen, the disease will become clinically apparent only with the appearance of the allergic manifestations. The clinical condition thus will have an obligatory incubation of 6 or more days, since this represents the time necessary for the coagulant antibodies to be produced. Since the manifestations in such cases are due to the allergic complex and not to the direct action of antigen, they will be nonexistent or minimal during the incubation time. Due to the allergic complex, the condition will not respond to specific immune sera able to neutralize the antigen but ineffective against the allergic complex. Specific immune sera are not curative for these infectious conditions of allergic pathogenesis. As already noted, only when administered before allergic antibodies have appeared, during their incubation period, do these sera have a marked preventive effect.

Thus the pathogenesis of an infectious disease can be toxic or allergic in nature. The two pathogenic mechanisms can be identified easily through incubation time of major clinical manifestations. *An infectious disease which appears shortly after the entrance of the antigen without an obligatory incubation has to be considered, according to our concept, to be of toxic pathogenesis while one which appears after an incubation time obligatory greater than 5 or 6 days has to be considered allergic.*

Applying this concept, we have separated the clinical infectious diseases into two groups, toxic and allergic, using incubation time as the criterion. We wish to note here the great similarity in the incubation time for the diseases in each group. Most of the allergic group have an obligatory incubation time ranging from 6 to 14 days, which coincides with the usual time needed for the appearance of the allergic antibodies. The incubation time is independent of the fundamental nature of the etiological agent—virus, microbe, protozoa, etc.—or of the nature of their products—exotoxins, endotoxins, etc. This indicates that the principal factor in the incubation time is the allergic pathogenic mechanism itself.

Based upon the criterion of obligatory incubation time, the following diseases with brief incubation time have been considered as having a toxic pathogenic mechanism: diphtheria, botulism, anthrax (*Bac. anthracis*), meningococcal infections, cholera, some streptococcal infections, dysentery (especially Shiga Kruse bac.), plague, scarlet fever, pneumonia, etc. In the allergic group, with an obligatory incubation time above 6 days, we find:

typhoid, typhus, tetanus, pertussis, rabies, measles, poliomyelitis, glanders, etc. (TABLE XII) In both groups, there are varied etiological agents. Thus, in the allergic group, for example, the antigens include a microbe with an exotoxin (tetanus) with an endotoxin (typhoid), a rickettsia (typhus), and a virus (rabies).

TABLE XII
INFECTIOUS DISEASES

Incubation

<i>Low</i>	<i>Obligatory above 6 days</i>
Diphtheria	Typhoid
Anthrax	Tetanus
Botulism	Pertussis
Gaseous Gangrene	Glander
Plague	Tularemia
Erysipelas	Leprosis
Dysentery	Typhus
Meningococcic Inf.	Rabies
Cholera	Measles
Pneumococcus Inf.	Mumps
	Poliomyelitis
	Smallpox
	Chickenpox
	Recurrent fever

The concept of toxic and allergic pathogenesis for these diseases is impressively confirmed when we consider the effects of specific immune sera upon their evolution. The specific sera demonstrate curative properties for all diseases in the first group with brief incubation time, considered in our concept because of this incubating time as toxic. Not one of the conditions of the second group, considered as allergic on the basis of their incubation time alone, can be cured by immune sera. Still more impressive is the fact that, in spite of the lack of curative effect, the same sera have a marked preventive effect upon the same allergic conditions if administered before the onset of the symptoms, that is, during the incubation period. This confirms our explanation that the therapeutic inefficiency of the sera in the second group is due to the allergic pathogenesis of the disease and not to a lack of active antibodies. Moreover, the same sera have a curative action upon infections with brief incubation periods induced experimentally in animals with the same agent. The concept has been confirmed in most of the infectious diseases and we will discuss some of these diseases briefly.

Before discussing this aspect of infectious diseases in more detail, we want to mention another occurrence which can be interpreted also through

the concept of allergic conditions. It concerns a kind of recurrence of symptoms seen often around the 7th day after the beginning of the clinical condition in infectious diseases which, by themselves, have allergic pathogenesis such as typhoid, mumps, measles, pertussis, etc.

While in these cases the condition itself can be considered an allergic manifestation against the infectious agent as antigen, a 7th day exacerbation in the course of the clinical condition can be interpreted as a second allergic reaction. This time a new antigen has to be considered. This appears to occur with the first allergic manifestation, the new reaction appearing 7 days later. The complex antigen-coagulant-antibodies responsible for the clinical manifestations of the allergic condition could represent this secondary antigen. Besides the antiheterogeneous reaction—enzymatic and lipidic—which determine the symptoms of the condition, this complex induces the appearance of a new group of coagulant antibodies against it. Around the 7th day after the beginning of the clinical condition when these new coagulant antibodies against this secondary antigen appear, they induce the exacerbation seen.

The existence of these secondary allergic reactions toward secondary antigens, often themselves of allergic nature, explains many of the tardive manifestations seen in the course of infectious conditions.

Pneumococcic Pneumonia

This disease, which appears after a very short incubation period, has the characteristics of a primary or toxic condition, with chill marking the beginning of the clinical manifestations. Antipneumococcic immune sera, corresponding to the type or even to the subgroup of the etiologic microbe are curative when administered in time and in adequate doses. In the natural evolution of the disease, a crisis usually appears on the 8th day. This corresponds to a marked aggravation of symptoms which can be so intense as to lead to death. The term "crisis" indicates this characteristic exaggeration of the manifestations. The coincidence between appearance of the crisis around the 8th day and the moment when allergic manifestations generally occur has suggested an allergic nature for this crisis.

Usually, the course of the disease changes suddenly after the crisis. Most of the marked manifestations disappear in a short time. This allergic crisis, with an initial increase in the severity of the condition, is not seen in patients who have received specific serum which has acted to prevent the crisis as allergic reaction. In pneumonia the crisis has its beneficial effect, a fact which accords with the concept that allergic intervention provides a new opportunity to resolve the intervention of the noxious agent. As we

have seen, the allergic stage represents a second, more complex method of combatting antigens. In the case of pneumococcic pneumonia, this allergic defense effort is often successful. The evolution of the disease is stopped.

Diphtheria

We considered diphtheria, because of its characteristic short incubation time, to be a typical primary-toxic disease. This is also confirmed by the curative effect of its immune serum. However, the disease has a manifestation in which we recognize an allergic nature: diphtheria paralysis. With a usual obligatory incubation period of about 8 days, and always of more than 6 days, diphtheria paralysis is a typical allergic condition. Once present, it resists diphtheria immune serum, yet it can be efficiently prevented by the same serum. In animals such as guinea pigs and hamsters, such paralysis can be induced and its allergic character clearly recognized. The heating of toxin at 56°C reduces its direct toxicity without impairing its antigenic properties. Administration of even huge amounts of heated toxin does not produce any immediate toxic effect. Yet the heated toxin, even though it has lost its toxic effect, induces paralysis.

Classically, this paralysis has been related to a hypothetical thermostabile fraction of the toxin, with a high incubation time. This view does not agree with the results of our experiments. When different amounts of the same heated toxin are injected in guinea pigs of the same sex, age and weight, the incubation time for paralysis, although always above 6 days, changes, becoming paradoxically longer when higher doses are used. With great amounts of the heated toxin, corresponding to 20,000 lethal doses of the nonheated toxin, incubation time becomes as long as 14-17 days in contrast to 8-9 days for relatively small amounts. TABLE XIII shows this relationship.

TABLE XIII
Changes in the incubation time of paralysis induced
by different amounts of heated diphtheria toxin

<i>Amount used</i>	<i>Incubation time— average of 4 animals</i>
.5 LD	8.33 days *
1 LD	8.25
5 LD	8.00
20 LD	8.25
100 LD	9.75
1000 LD	11.00
5000 LD	13.75
20000 LD	15.75

* One in four animals did not show paralysis.

If the paralysis were induced by direct action of a thermostable fraction of the toxin, then higher doses of the fraction should reduce, or at least, not increase the incubation time. This paradoxical fact can be explained simply through the mechanism of allergic pathogenesis. It is a fact common to immunological reactions that an organism has greater difficulty in manufacturing any antibody when very large amounts of antigen are present than when smaller amounts are involved. This difficulty is translated into a longer time necessary for the appearance of the antibodies. As seen in our experiments, in the case of an allergic reaction, this difficulty in the manufacture of coagulant antibodies would result in a longer incubation time.

The localization of the allergic manifestation as paralysis can be explained in part through the affinity of toxin as antigen for nerves and in part through the participation of the nerves in the allergic reaction. The levels at which the diphtheria toxin acts seem to be tissular, organic and systemic, with preference for the adrenals, inducing characteristic suprarenalitis. When coagulant antibodies appear, no manifest systemic allergic reaction will occur with the antigen still present in the blood. The allergy will be manifest, however, at the lower tissue level and especially in the nearby nerves. Antigen must be present in the nerve at the moment of appearance of the coagulant antibodies if paralysis is to occur. This can be demonstrated by using sensitizing and triggering injections of toxin in animals.

We sensitized guinea pigs to heated and unheated toxin by injecting relatively small amounts intravenously. On the sixth or seventh day, another small quantity of the same toxin was injected, this time near the sciatic nerve. The total amount of toxin was far below the lethal dose. Two or three days later, paralysis developed in the injected limb in a high proportion of animals while no such paralysis could be observed in animals injected only intravenously or with the same total amount of toxin at once in the limb. In other experiments, the daily injection of small amounts of toxin, whether heated or nonheated, near the sciatic nerve, induced paralysis although the total quantity of toxin was much lower than that which ordinarily would induce paralysis in any similar animal. Paralysis appeared in these cases after an incubation period of about 12 days. The animals sensitized by one or more injections of heated toxin responded to the non-heated as triggering injections and vice versa, indicating that antigenic properties were responsible for the paralytic allergic manifestation.

In humans, anti-diphtheria serum, effective against toxic manifestations, had no effect upon paralysis once it had appeared but is very effective in

preventing it. The same was true in animal experiments. Injected 24 hours before the appearance of paralysis, the serum had a consistent preventive action. This fact confirms again the allergic pathogenesis of the paralysis.

In another experiment, we showed that administration of cortisone, with its anti-allergic action, also reduces the incidence of paralysis without having the same effect upon the direct toxic action. It can be noted, too, that among small laboratory animals, diphtheria paralysis can be induced readily in guinea pigs, less readily in hamsters, and not at all in adult rats and mice. In addition to the sensitivity of guinea pigs to diphtheria toxin, this can be related to the great capacity of these animals in general to produce allergic antibodies and thus to be subject to anaphylactic reaction. Based on these considerations, we can classify diphtheria paralysis as a typical localized allergic reaction.

Typhoid

Typhoid, as seen in humans, is an allergic condition with an incubation period obligatory longer than 6 days. However, in experimental animals the same microbe induces a condition with a short incubation period, a fact which indicates a primary toxic pathogenesis. This difference can explain the striking difference in results with immune sera. The literature emphasizes great efficacy in experimental animals for various immune sera prepared against this microbe and its endotoxin (Chantemesse, Besredka, Kitasato, Wassermann, etc.), but no efficacy in the human disease.

In our experiments, when a sufficient amount of microbes, alive or dead, was injected at once, a primary-toxic condition was induced in guinea pigs. The incubation period was brief. With the same microbe, alive or dead, we were able to obtain the allergic form in guinea pigs with repeated, daily injections of small amounts. The allergic form similar to that seen in humans was induced. After about 12 days, temperature started to rise and usually remained high for more than two weeks, even with the dead microbe if the injections were continued. If living microbes were used, the condition continued even without new injections. We even obtained positive hemoculture at this time. Under similar conditions, the same allergic form of typhoid also was induced in rabbits although much less consistently than in guinea pigs. An antityphoid serum obtained from rabbits showed activity against the toxic form of infection induced in guinea pigs. It was entirely ineffective against the allergic form when that was already present, although the total amount of microbes injected over a period of many days was smaller than was used to induce the toxic form. Injected before the

8th day, the same serum prevented the appearance of the allergic form of the experimental disease.

Tetanus

In the light of our concept, we studied tetanus pathogenesis in an effort to explain the classically emphasized separation between the so-called small and big animal disease. (39) In mice, tetanus has a short incubation period and is manifested by localized contractions, while the disease of so-called "big animals" starts with trismus after an incubation period of more than 6 days. Based upon incubation times, we considered tetanus to be the toxic form in small animals, the allergic form in large animals.

We could, in fact, induce a condition in mice manifested by trismus and epistotonus, and having an incubation period longer than 8 days, by daily repeated intravenous injections of small amounts of toxin. The special affinity of this toxin for the nervous system, and the strong bond between nervous tissue and toxin, limited the response to the antitetanic serum even of the primary form. If injected in time, however, the antitetanic serum controlled this primary form. The same serum appears to be highly effective in the prevention of the condition in animals which have been prepared for the allergic form, provided it is administered before the allergic manifestations have appeared. The same serum is totally inactive once the allergic condition is present.

Rabies

Under all circumstances, rabies needs an incubation time of more than 5 days. From our point of view, therefore, it has to be considered an allergic condition. When the rabies virus was passed repeatedly through the brains of rabbits, incubation time became continuously shorter and ultimately was fixed at 6 or even 5 days. Classically, this progressively shortened incubation time is interpreted as being due to progressively increased virulence of the virus after these passages. In the light of our concept of the pathogenesis of infectious disease, a reduction of incubation time is the result of increased virulence only in cases of primary direct toxic pathogenesis. In the allergic condition, which has an entirely different pathogenic mechanism with the incubation period related to the time necessary for the body to produce coagulant antibodies, a shorter incubation would correspond to a different change. It results from a greater facility of the organism for manufacturing antibodies against the infectious agent. In the case of rabies, a short incubation period of 5 days for a "fixed virus" would mean that the organism is able to manufacture allergic antibodies more easily,

and consequently earlier. Apparently, antibody production is more difficult for the "street virus" which has a longer incubation time. The fixed virus consequently appears to be not only a brain-adapted virus, but also a weaker antigen, against which the body and especially the nervous system is more easily able to manufacture allergic antibodies.

The possibility of using the fixed virus as a vaccine in an individual already infected with street virus can be explained by differences in the relationship between the organism and the two viruses. Usually the street virus has a much longer incubation time, indicating that the body needs much more time to manufacture the allergic antibodies. The same as the animal is able to make allergic antibodies in a short time against the fixed virus, the vaccinated individual will be able to manufacture more rapidly also the protective neutralizing antibodies against the same changed fixed virus. The neutralizing antibodies will thus appear earlier and act against the "street virus" before the organism in general and the nervous system in particular has made allergic antibodies against it.

It is possible, however, that an additional factor may intervene in this case. In vaccine, we use an allergic complex as it is present in the nervous system. This corresponds to a further step in the general process of immunity and its presence could shorten still more the time necessary for the appearance of protective neutralizing antibodies.

The concept of rabies, clinical and experimental, as an allergic condition has recently received confirmation through the results obtained with a specific antirabies serum (Koprowski). With no curative capacity, this serum is able to prevent the disease if it is injected before the appearance of the clinical condition, even if only shortly before. It helps in cases where no more time is left for active immunity to be established by the body itself as a response to the vaccine. This passive immunity is consequently indicated for the case in which vaccination starts late. The serum, with no curative effect once the clinical condition has started, has a preventive effect, a fact which accords with the concept of rabies as a condition with allergic pathogenesis.

Syphilis and Tuberculosis

Syphilitic chancre has the characteristics of an allergic condition. The first lesion, often a small blister on a nonindurated base, shows a minimal reaction in spite of its richness in treponemas. It is only after an incubation of about 9 days that the intensive reaction appears, with the characteristic induration. Because of this, the chancre can be considered to be a specific allergic manifestation. The positive lutein reaction also corresponds

to an allergic response. The appearance of secondary manifestations also can be indicative of allergic pathogenesis, but with another antigen than the treponema involved.

Several possible antigens have to be considered. One would consist of constituents of the microbe itself against which the body needs almost one month to manufacture specific coagulant antibodies. Another antigen would correspond to constituents of the body itself becoming heterogeneous under the influence of the treponema. A lipido-proteinic antigen seems plausible. Complement fixation, flocculation, and other diagnostic tests for syphilis use antigens which are not directly obtained from the microbes but usually correspond to lipido-proteic fractions of organs, such as heart. In the original reaction of Wassermann, the antigen was an extract from organs rich in treponema, such as the liver of stillborn infants with heredosyphilis. This would favor the hypothesis that a secondary antigen is involved and that it has its origin in the body constituents heterogenized through the influence of the spirochetae. A similar antigen would account for the pathogenesis of the secondary manifestations. By extending this concept, the tertiary lesions and parasyphilitic manifestations can also be seen to be of similar allergic pathogenesis, with other newly formed antigens involved. Secondary antigens would conceivably develop in tuberculosis as well. While the primary tuberculous chancre can be seen as an allergic manifestation having the lipido-protein of the microbe as antigen, the cavern formation can be attributed to a secondary antigen.

It appears highly probable that it is these "secondary" antigens, together with the inability of the organism to manufacture efficient immune antibodies, that keep the defense in the allergic stage and impart to both tuberculosis and syphilis not only their chronic character but also their clinical gravity. The allergic pathogenesis explains also the inefficiency of all the tentatives to obtain sera against these conditions.

Streptococcal Infections

Erysipelas and many other streptococcal infections appear as primary toxic diseases with a short incubation. Active immune sera in sufficient amounts injected in time give good results. Often a marked change in the symptoms is seen toward the 8th day, a fact which could be considered to indicate passage into the allergic phase. Many other manifestations of streptococcal infections, such as those seen in rheumatic fever, can be considered allergic.

The glomerulonephritis which appears as a complication of scarlet fever or of pharyngeal streptococcal infection is especially interesting. While a

change in general symptoms in these infections is seen toward the 8th day, this complication usually appears toward the 24th day. The intervention of a secondary antigen resulting from the bond between lipids and the renal tissues can be hypothesized in the light of studies concerning immunological defense processes against tissues, which we present in the following pages.

We do not want to leave the problem of infectious diseases without a few more words about the use of lipids in the defense mechanism against microbes. The fact that lipids liberated in the first defense responses are bound to microbes and intervene in this complex form to promote the appearance of higher defense processes has led us to use similar bonds in order to stimulate this defense. We have seen above how lipids other than those offered by the infected organism can be used. The injection of killed microbes treated with lipoacid from heterogeneous sources, such as from species naturally refractory to the microbe, has enhanced the defense mechanism. Microbes treated with lipoacids of the tubercle bacilli or of *Bixa orellana* were seen to induce a strong specific allergic response.

Interesting results were obtained through the use of insaponifiable fractions bound to the microbes. The fractions obtained from refractory species appeared to be most effective in enhancing the defense mechanism in general. The insaponifiable fractions obtained from the entire body of rats, animals refractory to most infections, gave the best results for most of the infections studied.

In these investigations, in addition to using killed microbes treated with lipids *in vitro*, we employed another method to treat the microbes. Lipoids were added to the media in which the organisms were grown. Some of the lipoids were seen to increase, and others to decrease, microbial virulence. Killed and used as vaccines in cases of resistant infections, these lipoid-treated microbes were seen to induce more effective immunization.

Experiments in progress indicate the possibility of using such microbes—and even viruses so treated—to obtain long-lasting immunity. Microbes with very reduced virulence are used as live vaccines. Their capacity to induce effective defense responses in a short time also has led to their use as "late" vaccines, *i.e.*, vaccines which can be administered during the incubation time of an infection. As these studies are still in progress an evaluation of the results is not yet possible.

An interesting aspect of the influence exerted by lipids upon microorganisms is their use in producing qualitative changes in antibiotics. Preliminary research shows that the addition of lipids of the microbes against

which more active antibiotics are sought seems to alter the antibiotics so that they have a higher degree of specificity against these microbes.

IMMUNOLOGICAL DEFENSE AGAINST CELLS AND TISSUES

Heterogenization of the Transplants

It is known that the introduction in a normal subject of cells or tissues from an animal of another species or even a transplant from the same species will induce the appearance of defense processes. These differ with the degree of heterogeneity of the transplant. Experimentally, we can vary this degree of heterogeneity of transplanted cells and study the different responses in the frame of the normal and abnormal defense mechanism.

For the highly heterogeneous transplant, such as cells or tissue of a strange species, a primary response occurs, with liberation of hydrolytic enzymes and lipids. If sufficiently strong, this response will destroy and eliminate the transplant. If the transplant is moderately heterogeneous, such as one from an individual of the same species, the primary reaction is milder so that the transplanted tissue survives this attack. It will, however, be killed and rejected with the appearance of the second defense stage, *i.e.*, that of the allergic reaction. The damage to the transplant can be attributed to the antiheterogeneous reaction, which this time appears to be directed toward the product resulting from the bond between the transplant and tissue allergic antibodies.

For a still less heterogeneous transplant, such as one from young animals of the same species, the two defense responses are mild. However, the transplant is often destroyed through a later intervention of immune antibodies. This is seen to occur after some months for organs or for cell transplants such as bone marrow cells for the treatment of severe radiation damage. In these cases, the defense mechanism which intervenes months after grafting can be correlated to the immune stage. An autograft, which is a perfectly homologous transplant, usually will survive. The fate of a transplant thus appears to be determined by its heterogeneity. This heterogeneity, however, does not result only from the differences which exist between donor and receiver. Even an autotransplant can be heterogenized by surgical manipulation, heat or other treatment, or by changing its organizational relationship to other entities, to such a degree as to be destroyed by an immune, allergic or even a primary defense response.

The heterogeneity of the transplant—*intrinsic* or induced by the appli-

cation of external agents—represents only one factor which determines the nature of the defense processes. Another factor corresponds to the changes in the antigen or constituents induced by the intervention of primary, allergic or even protective immunological reactions. The study of the defense reaction against the organism's own tissues or cells heterogenized by previous immunological responses is of special interest, in view of the role of such heterogenized entities in a more complex defense mechanism. The organism often heterogenizes its own entities through the agents used in the defense against foreign entities. Primary, allergic and even immune reactions induce various degrees of heterogenization of the organism's own constituents at various levels of the organization.

Through the intervention of hydrolytic enzymes, lipids, allergic antibodies or even neutralizing antibodies, different changes in an organism's own entities can be induced. From these, the heterogenization of body entities by lipids was studied in particular. The heterogeneous effect of lipoacids could be shown in many experiments, as in the following: Suspensions of cells of different organs of guinea pigs, in a concentration of 1 gram of cells to 10 cc. of saline, were prepared. At the same time lipoacid suspensions in saline were obtained starting from 2% solutions of different lipoacids in alcohol. Four weekly administrations to guinea pigs of the separate cell suspensions or of the lipoacid suspensions were not followed in most of the animals by any serious manifestations. A heterogenization of the cells was obtained through the action of the lipoacid suspension upon the cells. While one single injection of the so-treated cells showed no noxious manifestations, consecutive injections at weekly intervals were seen to induce, in less than a month, important changes generally concerning the respective organs from which the cells derived.

The lipoacid-cell complex acts as an antigen, with the type of cell determining the organ where the abnormal changes will occur, and the lipid determining the character of the occurring reaction. Depending upon the lipoacid, the effect will vary from minimal tissular lesions all the way to massive degenerative changes leading to death.

The degree of heterogeneity of the lipoacid appears to be one factor which determines the stage of defense induced. Oleic and linoleic acids, and the lipoacids from human placenta, cow liver or total body of guinea pigs had a slighter effect in inducing organ lesions than the lipoacids obtained from Bixa orellana and especially from the tubercle bacilli which led to serious damage in the respective organs. Tuberculin acting upon the cells had the same effect as lipids obtained from tubercle bacilli.

Through variations in the nature of the autogenous factors—hydrolytic

enzymes, lipids, allergic antibodies or even immune antibodies—a graduated series of changes in an organism's own entities can be induced. Of the factors which intervene in the heterogenization of such entities, we have studied the lipids in particular. An antigenic role for lipoacids could be shown in many experiments. For example, suspensions of red cells or cells of different tissues of guinea pigs in a concentration of 1 gram of cells to 10 cc. of saline were prepared. At the same time lipoacid preparations were obtained in the following manner. 5 cc. of a 2% solution in alcohol of different lipoacids or mixtures of lipoacids were added to 110 cc. of water and the preparation boiled under low pressure until reduced to 100 cc.

The cell suspensions were administered to guinea pigs in four injections at weekly intervals with no serious manifestations. The same was done for the lipoacids above. A preparation was obtained through the action of the lipoacids upon the cell suspension in the following manner. 5 cc. of the colloidal lipoacid aqueous suspension were added to 5 cc. of the suspension of cells of different tissues. To 5 cc. of red cells, only 1 cc. of the lipidic suspension was added. The mixture in each case was incubated for two hours at 37°C and centrifuged. The cellular residues, separated from the supernatant fluid, were resuspended in saline and kept frozen. While one injection of the so-treated cells showed no noxious manifestations, consecutive injections at weekly intervals were seen to induce, in less than a month, manifest changes in the respective organs. With the red cells, a marked anemia was induced. Oleic and linoleic acids, and the lipoacids from human placenta, cow liver or total body of guinea pigs had only a slight effect in inducing organ lesions. The lipoacids obtained from Bixa orellana and especially from the tubercle bacilli led to serious damage in the respective organs and resulted in death usually in less than 3 weeks. Tuberculin in these cases had the same effect as lipids obtained from tubercle bacilli.

The degree of heterogeneity of the lipoacid appears to be the factor which determines the stage of defense induced. The lipoacid-cell complex acts as an antigen, with the type of cell determining the organ where the abnormal changes occurs, and the lipid determining the character of the occurring reaction. Depending upon the lipoacid, the effect will vary from minimal tissular lesions all the way to massive degenerative changes leading to death.

The intervention of a bond between cells and lipids appears evident when acid lipid preparations are injected repeatedly at weekly intervals in the same organ. Lesions are obtained which are similar to but less intensive than those produced by the cell-lipoacid complexes.

It was highly interesting to note the differences in lesions depending on the origin of the cells injected. Zones of necrosis, often with subacute cellular degeneration and even with inflammatory processes, were induced by not too heterogeneous fatty acids. Acute glomerulonephritis, liver degeneration, pneumonia, enteritis or encephalitis resulted from repeated injections of cells from kidney, liver, lungs, intestines and brain treated in vitro with bixin, lipoacids of tubercle bacilli, lipoacids of fish or even fatty acid mixtures of cod liver oil.

Against tissue transplants, injections of the host with lipoids with negative character—such as fatty acids, mixture of lipoacids of different origins, and lipids with SH or SeH as polar groups—have exaggerated all phases of defense processes. Skin transplants between siblings, which usually give a high percentage of accepted grafts, were rejected completely after treatment with some of these agents. The degree of heterogeneity of the agents appeared particularly interesting. With lipoacids of the same species, very high doses were required to induce only minimal changes. On the other hand, preparations of lipoacids of fish, mollusk, molds and microbes produced marked effects. Transplants treated with these preparations were rejected or absorbed after eight or more days. Seldom was an immediate rejection seen.

Even more interesting were the results obtained by direct action of the lipids upon transplants, achieving a bond between them. For these experiments, the agents were used in oil solutions as well as in saline suspensions. Transplants were dipped into different preparations. Even autografts if treated with lipoacids of the same species often were rejected. This took place even after more than three weeks. When more heterogeneous lipoacids were used, such as those obtained from other species, autografts were rejected as completely as transplants of the same species, *i.e.*, around the eighth day. This also occurred with relatively heterogeneous agents, such as the lipoids of microbes, especially those of the tubercle bacilli. With still more heterogeneous agents, such as lipoids with SH or SeH polar groups, the treated transplants were rejected through an immediate direct inflammatory reaction.

The influence exerted by injections of the opposite group of lipoids with positive character was in the opposite direction. The percentage of accepted transplants was increased.

By dipping skin transplants of animals of the same species in preparations of the insaponifiable fractions of the species, the percentage of persistent grafts was highly increased. In some experiments all the transplants between siblings were positive. Even between different strains of mice,

such positive results were obtained. The treatment of transplants with butanol alone was not effective. Adding butanol to the preparation of insaponifiable fractions, however, enhanced the effect of the latter.

The most interesting results were obtained by cross-treatment—in which the transplant was treated with the insaponifiable fraction of the strain of the host and the host with the insaponifiable fraction of the donor. An unusual number of positive grafts were obtained between strains of mice and, in exceptional cases, even between species when a mixture of the two preparations of insaponifiable fractions of the donor and host was used for the treatment of both transplant and host.

Even more interesting results were obtained when, in addition to these treatments of transplant and host, another treatment—that of the "bed" of the transplant—was added. The wound receiving the transplant was soaked with the mixture of insaponifiable fractions. Often after the graft, treatment with the fractions was continued through small injections into the bed of the transplant. Injections into the transplant itself, if possible, increased the number of positive results.

Before pursuing further the study of these interesting problems, an analysis of another aspect of the response to heterogenized material has appeared necessary. It concerns the intervention of different levels of the organization in the defense mechanism. This was seen to vary according to the degree of heterogeneity of the transplant. The defense processes thus can be limited to the heterogenized entity or to entities of the same level, or they can extend far into the hierarchic organization. With a highly heterogenized material, a broad hierarchic reaction occurs with several superior levels intervening. In these cases primary enzymatic or prolonged processes involve the tissular, organic and systemic levels. With less heterogenization, the ensuing primary reaction is not strong enough to destroy and eliminate the heterogenized entities and an allergic reaction takes place. This involves other levels such as tissular and even organic. With still less intensive heterogenization, the defense remains localized at the affected level itself and is weaker. With the defense inefficient in its primary or allergic stages, a protective stage becomes necessary in order to take care of the heterogenized entities.

The analysis of many conditions indicates the importance of the different factors for the development of the clinical manifestations.

Seventh Day Manifestations in Trauma

We have studied these defense reactions for trauma, the degree of intensity of the trauma indicating the extent of the exogenous heterogeniza-

tion. Very intensive trauma can produce a lethal superacute shock which corresponds, as we shall see below, to a generalized primary response. A less intensive trauma may induce a tissular necrosis with consecutive sloughing as a localized primary defense. A still less intensive trauma may induce an allergic tissular response. The importance of these changes for clinical manifestations is such that it appears necessary to emphasize them. After surgery, for instance, a slight temperature elevation is often observed between the 7th and 9th day. This has to be interpreted as an allergic reaction. When intensive enough, this allergic response with the ensuing lytic action passes from the tissular level to the higher level of the blood vessels. Along with inflammation and pain, local hemorrhages often appear. Severe hemorrhages occur at this time after various traumatic incidents. The most disturbing complications for plastic surgery of the nose, for instance, are the severe "7th day hemorrhages." The fact that they start at this critical moment indicates their allergic pathogenesis. The study of these allergic changes has shown that they occur in the evolution of all traumatic lesions. They can occur and remain clinically inapparent and uneventful, as seen in the following experiment.

In groups of rats of the same sex and age kept under similar conditions, parallel skin incisions 3 cm. long at $\frac{1}{2}$ cm. intervals were made. The lesions were excised at different times and chloride content determined. Fig. 78 shows the curve of average values of the total chloride content of these skin wounds in groups of six animals for each day. It can be seen that intensive local chloride retention occurs with the first defense reaction, with values as much as four times greater than those of normal tissues. On the third day, chloride content falls. It goes below normal tissue values after the fifth day during the healing process. However, in an otherwise regular curve, there is a distinct temporary increase in chloride content on the 8th day. Its occurrence at this time, when coagulant antibodies appear, indicates its allergic nature.

The same allergic pathogenesis explains the exacerbation of symptoms seen about the 7th day in many conditions. In patients who have suffered a myocardial infarct, for example, recurrence of pain is often seen the 7th-8th day after the infarct.

Part of the effects of chemical, physical and hormonal agents could be interpreted in terms of influence exerted upon the different factors which intervene in the defense mechanism. Some agents such as opium derivatives were seen to affect the liberation of hydrolytic enzymes while others interfere with the manufacture of allergic or immune antibodies. The influence exerted by radiation upon the defense mechanism can be related to its

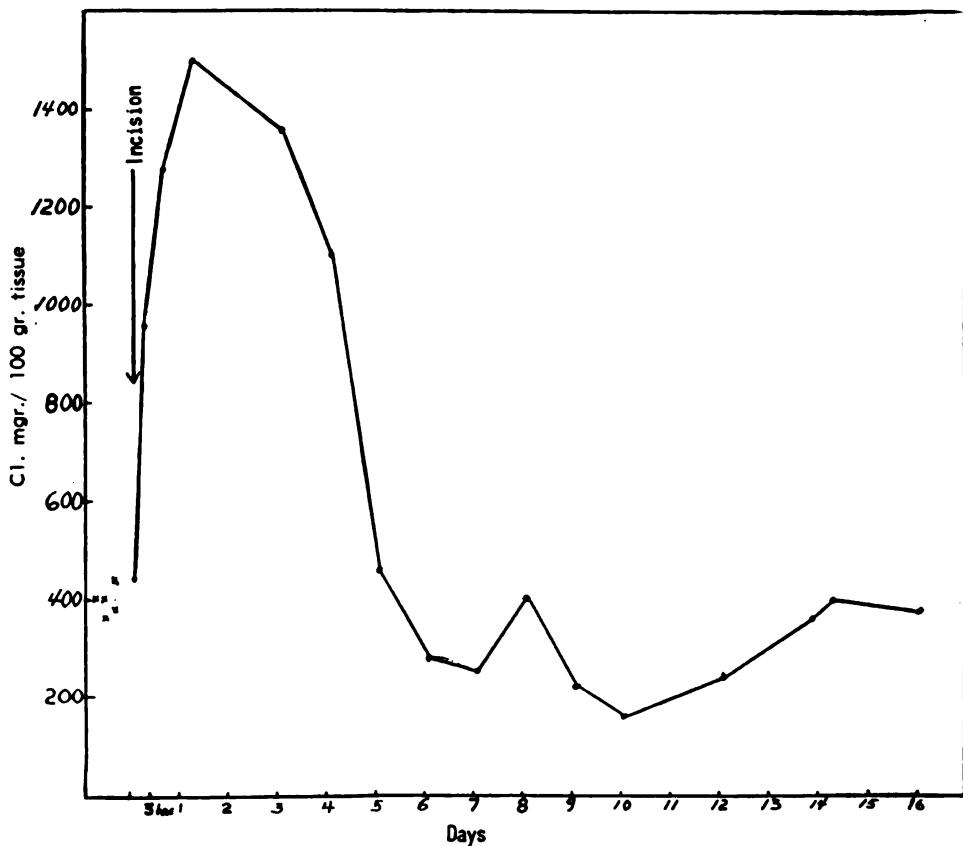


FIG. 78. *Wound chloride curve.* The curve of the amount of the chlorides present in skin wounds in rats corresponds to the average value obtained in 6 rats for each figure. A first phase, with high values corresponds to the offbalance D. This is followed by a second phase characterized by an offbalance A. A variation in the curve corresponding to the 8th day is constantly seen as corresponding to an allergic reaction. The values represent mgs. of chlorine per 100 gr. of weight of the wet material.

effect upon granulocytes and lymphocytes while neoglucogenic corticoids affect the connective tissue and lymphocytes.

Research in all these directions is still in progress and the results will be communicated in further publications.

For the time being, they have brought more information and suggestions of research in the special case of the immunological problem of cancer.

IMMUNOLOGICAL PROBLEMS IN CANCER

We used the data obtained from the analysis of the defense process against cells and tissues in the study of the immunological problem of cancer. The different cancerous hierarchic entities, as defined previously may be con-

sidered to correspond, up to a certain point, to heterogeneous entities—the grafted tumors to transplants and the spontaneous tumors to heterogenized entities of the individual.

We tried, in a first series of experiments, to follow the intervention of the different mechanisms of defense on grafted tumors in animals, employing transplants of various degrees of heterogeneity. Different types of tumors were used. Highly heterogeneous tumors obtained from species other than the host would not grow when transplanted. The death of the transplant, even its rejection if mechanically possible, occurs in a short time. The necrosis of the transplant and the relatively wide inflammatory process that develops around it immediately after the graft indicate the intervention of the first stage of the defense reaction from the cellular to the organic level.

With a second group of tumors, usually from the same species or even moderately heterogeneous, the grafts take and the tumors grow for a time. Often, around the 8th to the 15th day, the transplant starts to show profound changes. The changes affect the entire tumor which involutes rapidly and is often expelled. That this is due to allergic reaction could be shown by the following experiment. Fragments of the same kind of tumor which had been obtained from different animals were transplanted at 2 to 3 day intervals in the same host. In spite of the different ages of the transplants, the death and rejection of all occurred at short intervals and in the same manner, indicating the intervention of a mechanism taking place in the host and relatively independent of the evolution of the transplant itself. Such a mechanism would be the intervention of allergic antibodies.

In a third group of grafts of low heterogeneity, the tumors continue to evolve for an even longer period of time, and it is only in a few animals that these tumors are entirely rejected. This change, which consists of cytolysis of the tumor, takes a certain time to be completed, which indicates that it probably results from the intervention of protective antibodies. The first and the second defense mechanisms appeared to be inadequate to conquer the tumor and it was the third stage, with formation of protective antibodies, which apparently was able to accomplish it. This mechanism is confirmed by the fact that later grafts are negative from the time they are transplanted, through cytolytic changes in the tumor and not through an intensive inflammatory process as seen in the primary reaction. The immunological nature of the defense in these cases could be seen also through the passive immunity which could be induced in other animals with the serum of the host.

Grafts in Humans

The grafting of cancerous cells in normal humans usually leads to the appearance of a growing tumor which (315) after a period of days, almost always suffers the same fate as a moderately heterogeneous transplant: death followed by resorption or expulsion. The time when this process occurs indicates the allergic nature of the defense mechanism. It is highly probable that, without the intervention of this efficient allergic defense, the cancerous process would have continued to evolve.

Such continued growth occurs if grafts are made in subjects already having their own cancerous process. This would indicate that the allergic defense mechanism against the graft is no longer operating in these cases. The fact that a cancerous subject accepts a new tumor graft while the normal one rejects it indicates that defense processes are different for the normal and this cancerous subject. The inability of these subjects to reject a grafted tumor through an allergic response appears to be the major immunological difference between the normal and the cancerous subject. Still more important is the fact that in cancer patients, the anomaly would correspond to *a loss* of the capacity to reject the grafted tumor which the normal subject seems to have.

In trying to determine the nature of the immunological anomaly in the evolution of a spontaneous cancer in patients, we have to relate it to this loss of the defense processes as seen above.

In studying this occurrence in general, a loss of defense against an antigen can be conceived to occur for any of the three different mechanisms involved in defense: primary, allergic or protective. In cases when this takes place, the loss of the protective stage will take place first. The allergic defense will be affected next and finally, the primary response. This explains why the inability of an individual to achieve one stage of the defense leaves the defense resting in the immediately previous stage. The inability to manufacture protective globulinic antibodies, for instance, will leave an individual in the allergic stage which, in the development of defense, precedes manufacture of the immune antibodies. This results in a potential allergic condition if the antigen is absent or an actual allergic condition if the antigen is present. We have seen that this occurs in most of the chronic infectious conditions. Similarly, with the inability of an organism to manufacture coagulant antibodies, the defense remains in the previous defense stage, the primary lipidic one.

Before going further, we have to discuss a factor believed by many authors to be involved also in the defense mechanism against cancer. A few

years ago, the defense mechanism in general had been related to the properdin system. However, when considered in terms of the systematization of the defense processes, properdin has to be regarded as a nonspecific direct antinoxious reaction. It appears to be involved in the antiheterogeneous defense response, appearing after the enzymatic hydrolytic attack and at the beginning of the prolonged lipidic intervention.

The decrease in the properdin content of the blood of subjects with cancer has caused various authors to try to explain through it the differences in the reaction of cancerous and normal subjects toward a new transplant. The analysis of the conditions under which this occurs, however, has shown us that the anomaly does not reside in the antiheterogeneous processes of defense, which are the same against antigen and allergic complex, but in the allergic reaction itself. From the immunological point of view, the difference between a normal subject and one with invasive cancer resides in the loss of capacity to induce the second type of defense, the allergic, toward the cancerous tissue. Corresponding to an inability to manufacture coagulant antibodies, this deficiency would explain the lack of respective antiheterogeneous reaction toward the antigen-coagulant-antibodies complex and consequently the low blood content of properdin seen in these cases.

Failure of the allergic defense mechanism specifically against cancer entities need not mean general failure of allergic defense. The failure may be limited to inability to manufacture allergic antibodies against a specific antigen. We have seen, especially for the infectious diseases, that primary and allergic processes can occur with great intensity and still not be qualitatively efficient. The agent, the microbe, for instance, can still remain present despite even violent allergic reactions. The mere presence of defense processes does not implicitly mean successful defense; they may be qualitatively insufficient.

In cancer, if the allergic defense is insufficient, two eventualities have to be considered: either the organism in general cannot pass into the allergic stage of defense and therefore is unable to manufacture allergic antibodies, or this response is only qualitatively insufficient. In the latter case, the general and even local reactions could be quite intensive but still be ineffective. This seems to occur only in certain forms of cancer such as those with a high inflammatory process; for instance, in the inflammatory form of breast carcinoma. As this cancer starts and evolves as an acute mastitis, very intensive defensive processes, apparently only of the primary stage, occur. But they are unable to check the disease which usually evolves even more rapidly in these cases. This is also true for other cancers where fever is

present, indicating a prolonged primary, toxic stage. The lack of local reaction seen at the site of the growing transplant in the cancerous subject at the time when the normal individual kills or rejects the transplant points to the fact that the anomaly resides in qualitative inability to manufacture allergic antibodies.

The next problem was to investigate the reason for the failure of allergic defense against the tumors. We could show that the cancerous subject has not lost the capacity in general to manufacture coagulant antibodies. Even subjects with very widely spread cancer were able to respond with a local skin allergic reaction to a second injection of an antigen (proteins from mollusks) made more than ten days after a first preparatory one. (*Note 7*) Their inability to fight transplanted cancer cells through a similar allergic reaction indicates that the loss of this capacity is not general but relatively specific toward the cancerous cells. The lack of an intensive inflammatory process, as well as the existence of high amounts of lipids in the cancerous tissue, also would indicate indirectly an inability of the cancerous subject to resolve the existing immunological problem of fighting cancer through an allergic reaction. The presence of large amounts of lipids indicates that the defense mechanism has been arrested in the stage of pronounced lipidic predominance. Abnormal amounts of lipids thus could represent an indirect means of recognizing the failure of an allergic response to cancerous entities.

The next problem was to try to determine where in the organization the failure occurs. The different levels of the organization are independent to a certain degree and passage of an abnormality from one level to another induces hierarchic progression of the condition. This has posed the problem of the progressive loss at the different levels of the natural capacity to defend against cancer. Recently many investigators have shown that cancer cells pass into the lymphatic system and into the general circulation in a much higher proportion than had been suspected before. Malignant cells in the circulation are destroyed, however, by the defense means which are not lost at this level. The same patient thus may still have an actively growing cancer at the tissular level, indicating that this defense process, although successful for the higher levels of the organization, does not intervene at this lower level.

The hierarchic progression of cancer can be seen as a progressive loss of the immunological defense capacity. While the organism conserves the capacity to fight at a higher level, a lower hierarchic entity no longer opposes the cancerous condition. It is not the absence of cancerous cells in blood or organs which explains the lack of an explosive spread of the dis-

ease, but the presence of efficient defense means at these levels which keeps a cancer still localized.

Metastases

The relative independence in the loss of the defense capacity of different entities would explain one of the most baffling problems of cancer—why certain cancers tend to metastasize to certain organs or tissues. Some cancers show bone metastases, others spread to many organs, while still others spread only to certain specific organs. This can be explained by a loss of the defense capacity at the organ level. While some organs lose, others still maintain their allergic defense capacity. The circulating cancer cells will induce multiple metastases in the first but will not be able to take hold in the latter.

A similar mechanism can also explain the persistence of inactive cancer cells for years after an operation. The defense mechanism, while it is not able to affect the cells and destroy them, is still sufficiently active at the tissular level to prevent the condition from progressing at this level. The cancer cells will start to invade this level only when the tissular level is unable to defend itself further through an allergic response against the invading cells. By losing its allergic response capacity, the tissular level will even exaggerate the corresponding lower primary stage of defense, that is, the prolonged lipidic phase with the consequent changes which this brings on. Among them would be the appearance of pain.

Under these circumstances, the general immunological condition favorable to the hierarchically progressive development of cancer has to be regarded as the loss of the capacity of the different levels for an effective allergic response toward cancerous entities. The immunological problem of cancer consequently appears in a special light, different from all the other known conditions where an unsuccessful immunological response is present. In the other conditions, the problem of the inability to conquer an antigen is one involving the incapacity to mobilize or develop an effective immunological response. In cancer, the body appears to have lost a previously existing capacity that was present before the disease appeared. In other diseases, the immunological problem is to create a new and favorable condition in the fight against an antigen, by developing means which do not exist in the normal individual. In cancer, the immunological problem would be to prevent the loss of a property possessed by normal subjects or, if already lost, to find some means to regain it.

These considerations and the study of the different factors involved in the development of the progressive defense stage has led us again to the

role of the lipids in these processes. The appearance of a stage in the defense mechanism seems to be strongly related to the fulfillment of the qualitative requirement for the previous stage. Deficiency of essential factors in one stage represents an impediment for the next stage. In the case of cancer, failure of the specific allergic phase thus could be traced to a qualitatively inadequate preceding lipidic phase. A level is unable to surmount an allergic defense because the lipids which can mobilize are qualitatively inadequate. Even abnormal richness in lipids thus could be interpreted as resulting from their qualitative inadequacy. This very excess indicates their importance.

Immunological Therapeutic Approach

The fact that effective defense resources are present at a higher level does not mean that they inevitably will act at a lower level.

This view of the abnormal immunological processes in cancer has pointed the way for some new therapeutic approaches. From the therapeutic point of view, the problem becomes one of how to induce the body to regain, at the necessary level, the lost immunological capacity, and through a specific allergic defense, to combat the cancerous entities. Furthermore, since this specific allergic defense capacity is lost independently by the various levels of organization, the immediate problem would be how it could be recovered for the particular level where the loss occurs. The existence of adequate defense capacity at a higher level, such as the systemic, does not provide a solution for cancer present at lower levels such as the tissular, because of the independence which exists between levels. Only the manufacture of coagulant or immune antibodies against cancer at the proper level would put the individual in a sufficiently active defense phase to enable him to resolve the condition at that level.

The problem of immunity at the proper level thus appears to be critical for any immunological attack against cancer. It is evident in other conditions as well and has inspired the use of local vaccination in localized infectious conditions. (307, 308)

The study of immunity against viruses has permitted us to recognize the importance of immunity at different levels. Virus infection is a typical cellular condition, the virus multiplying only within a cell. Theoretically, an immunity at all levels can be induced for viruses. According to the view discussed above, however, a systemic immunity with circulating antibodies will not insure a cellular defense. It would intervene only when the virus is passing through the systemic level and its activity would last only during the time when circulating antibodies are present. During this time, the

virus will be prevented from reaching the cells. Once the circulating antibodies are no longer present, the cells cease to be protected. For an efficient defense against viruses an immunity within the cell appears thus to be indispensable. The use of dead virus vaccine will induce only systemic immunity, which can be recognized through the circulating antibodies. It is unlikely that the killed virus enters the cells. It does not affect them, and consequently does not induce cellular immunity. Even a mild cellular infection with living virus will give the necessary long-lasting cellular immunity. This would explain the need for living and not killed vaccines for viral infections, as first postulated by Pasteur.

A similar level immunity can explain the differences seen between the immunity resulting from the use of microbial vaccines and that produced by natural disease. Typhoid infection gives lifelong immunity; the vaccination, only relative and temporary immunity. An explanation can be found in the fact that, in the disease, along with the septicemia, manifest changes occur in organs and tissues. Spleen and lymphatic tissues are highly affected in typhoid and it is possible that the development of the defense at their level would explain the lifelong immunity that follows the natural infection.

In cancer, the problem would be to induce not a systemic defense, which is still present for invasive cancer, but an effective tissue or even cellular defense. Immunological treatment of cancer would have to make tissular and possibly cellular levels regain their capacity to defend themselves through efficient allergic responses. The immunological prevention of cancer would lie not in the creation of this defense or in increasing it quantitatively but enhancing it qualitatively. A successful allergic defense at this level apparently would have a preventive and even curative effect. The use of lipids in the induction of the defense mechanism against tissues has an interesting application in cancer. A systemic treatment with lipids or lipoids can change the defense response so that it can be effective at a specific level where it is otherwise inadequate. For invasive cancer, the lipid activity must be induced at the cell level. The active lipoids for this purpose are those with a high affinity for the cancerous cell.

As abnormal cells in general show similar capacity to bind the lipoids administered, this general affinity becomes a handicap if abnormal entities other than cancerous cells are present. These considerations have led us to attempt to use methods which will insure the activity of lipids at the cell level.

In one of these methods, the chosen lipoids are brought directly into contact with cancerous cells through local injections into the tumors. Single injections produce only limited changes in tumors. Local injections re-

peated so as to insure the presence of the lipoids once, and then again 15 days later, are required to induce an effective response. The lipoids or lipids are so chosen that, when bound to body constituents they will induce allergic or immune defense responses. The acid lipids of tubercle bacilli, bixine or guinea pigs are especially prone to induce allergic reactions, while the lipids of microbes—such as coli, typhoid or diphtheria—produce immune responses.

In another method, lipids chosen were bound to cancerous entities in vitro. Cancerous cells were obtained and treated in vitro with lipids under whose influence the body is able to manufacture allergic or immune antibodies. Colloidal suspensions of the lipids or lipoids were prepared as mentioned above, mixed with suspensions of cancerous cells, kept at 37°C for a few hours, then separated from the non-fixed lipids and injected into patients. In order to obtain good results it was necessary to inject this material at least twice, at an interval longer than two weeks, in order to insure an allergic reaction against the cell-lipid preparation. While a single injection produced good results only in a very small number of cases, repeated injections were manifestly more effective. When cancerous tumor cells could be obtained through biopsy from the patient, we used them for the in vitro treatment with lipoacids. When biopsy material was not available, we used cancerous cells of similar origin as the tumor of the subject, preferably pooled.

The condition for success of these methods has appeared to be the presence of the cell-lipid complex at the moment of appearance of antibodies. This is assured only by the repetition of the injection. Another interesting aspect of the immunological problem in cancer, related to loss of the natural defense mechanism, is the loss by cancer entities of their capacity to utilize certain elements known to intervene in the defense mechanism. The role of magnesium in the properdin system, copper in cytochrome oxidase, of calcium in general defense, suggests a correlation between their deficient utilization in cancer and the loss of the defense. We will discuss this problem below, after reviewing the pharmacological aspect of these elements.

CHAPTER 8

THE CORRELATION BETWEEN THE BASIC CONCEPTS

HERE IS A CLEAR interrelationship between the four basic concepts previously discussed which permits us to consider them together and to establish a unified viewpoint. For all four can be seen to represent different parts of the same fundamental problem in biology: the manner in which an entity resolves energetic differences between itself and the environment.

We have seen that, in the framework of fundamental laws governing nature, matter can be considered to correspond to islets of heterotropy opposing the homotropic trend of evolution. Conservation of an existing entity appears to be the principal means by which heterotropy can be achieved. And heterotropy is fulfilled, specifically, through maintenance of the constants of entities as values different from those of the environment.

Hierarchic Organization

The continuous tendency of nature to progress toward maximum homotropy has made the conservation of existing entities a persistently acute problem. The problem posed by the progressively changing environment cannot be solved through changes within entities themselves. Any "adaptation" of the entity itself would affect its constants and, consequently, would be contrary to the fundamental purpose of heterotropy. Nature has resolved the problem in an entirely different way. Since the entity itself must remain unchanged, and yet the influence of the environment must be counteracted, nature has made use of hierarchic organization. Secondary parts, reproducing the immediate environment, are joined to existing entities, often surrounding them and acting as buffers against environmental influences. Through these added secondary parts, hierarchic entities are organized so

that they reproduce the characteristics of the environment present at the time of their formation. Through this means successively repeated many times, an entity can be kept unchanged, in a medium similar to the original one, despite continuing changes in the environment.

Hierarchic organization thus represents the main mechanism through which the heterotropic achievements, represented by entities, counteract the influence exerted by the homotropic force. Conceptually, hierarchic organization can be seen to represent a form of defense developed in time by entities against a specific factor, progressive homotropic changes in the environment. The successive steps of the hierarchic organization respectively the hierarchic entities, reproduce in short, the evolution of the relationship which has been developed between the entity and the changing world. Hierarchic organization condenses the phylogenetic evolution of this specific part of the defense between the entity and the changing environment. Through this view, we can integrate organization in the general defense, the hierarchic organization being part of the mechanism used against progressing homotropy.

Constituents

In the same manner, we can further integrate into the same defense mechanism the various constituents which form the secondary parts of the hierarchic entities. We have thus tried to correlate these constituents more directly to the successive environments in which entities evolved. We have seen above, how this applies to elements which are common to, and predominant in, both the entities and the environments which correspond to the media in which these entities evolved. Through the correlation between elements which enter into hierarchic organization at various levels, and their positions in the periodic chart, the successive phylogenetic passage from one environment to another has a specific meaning. In media formed by elements with lower atomic weight, the influence exerted by progressive homotropy is less manifest. The changes in the elements as body constituents can be thus also integrated in the same defense mechanism.

Besides the elements, other constituents can be similarly integrated into the defense against the changes of the environment. In the immediate defense process against noxious agents, we have seen the successive intervention of different constituents—enzymes, lipids, lipido-proteins and proteins, in that order. The high degree of individuality and independence of the entities in the hierarchic organization has permitted us to conceive of these constituents as participating with a certain independence for each entity. The presence of all these constituents in each higher biological

entity, which is part of the complex organism, suggests that these constituents entered into the formation of these entities as the result of their intervention in the defense mechanism. Thus, it can be conceived that, in its phylogenetic development, each entity has passed through a succession of defense phases in which specific groups of constituents—enzymatic, lipidic, lipido-proteic and proteic—have been predominant. In actual organization, while all higher entities contain fundamentally the same constituents, different substances are predominant at different levels. This can be explained by the predominance of a particular defense mechanism at a particular level. According to this view, this defense is principally in the first stage, that is, of enzymatic nature, for most endodermic formations. It is in the prolonged lipidic stage for ectodermic formations, lipido-proteic for the reticuloendothelial system, and proteinic for cells. Through these correlations, constituents can be more completely integrated in defense.

The kind of special defense developed for the different levels of organization, through predominant specific constituents, has not been followed by a total discard of the other constituents, which do not have such roles. Instead, the latter have been retained in the entities in smaller amounts and in inactive forms. This confers upon the entity the capacity to mobilize these constituents and use them when the need to respond to an acute emergency arises. Pre-ferments and even ferments in mitochondria; fatty acids and anti-fatty acids bound as esters; lipido-proteins and proteins in various combinations—all these are inactive constituents which can be changed easily into active agents. When fighting a new noxious intervention, an entity will resort to liberating or activating these constituents kept in reserve. Each entity and level of organization does this independently of other entities and levels, yet constituents activated at one level can act at other levels, too. The success or failure of defense especially in its first stages, depends not only on the intrinsic value of the constituents available, but also on the capacity of the afflicted entity to utilize these means by activating them. Although activation processes become strikingly evident in abnormal conditions similar processes seem to be important even in the maintenance of existing entities.

Dualism, as we have seen, characterizes both normal and abnormal physiology. That which is considered "normal" is the result of an alternating intervention of two groups of opposite constituents, producing an oscillatory movement and a dynamic balance. The dualism seen in abnormalities, when one or the other opposed factor is persistently predominant, is related to hierarchic organization and the defense mechanism.

Dualism results from the intervention of two fundamental forces in

nature—homotropy and heterotropy. Even the simplest analyses make evident either the homo- or the heterotropic character for many manifestations and processes. For instance, an ulceration or an enzymatic hydrolysis of a protein has to be interpreted as an homotropic effect while a growing tumoral mass or the synthesis of a protein can be seen as an heterotropic one. For other manifestations, this character appears less immediately evident and it is through further analysis that it can be recognized. Dualism, like the other concepts, thus can be integrated in the defense of entities against an environment progressively changing toward maximum homotropy.

We have used this conceptual fundamental view in studying many problems in biology. It has aided us to formulate helpful working hypotheses. Despite its shortcomings, when applied to particular situations, this basic concept has served as a guide in correlating specific problems with the fundamental laws governing nature. It has also engendered helpful new interpretations of available data. Through the relationship of the four concepts discussed above and the fundamental defense mechanism, we have been able to analyze many problems without reverting to empiricism. Certain of these problems, to whose better understanding this approach appears to have contributed, are discussed in the pages that follow.

CHAPTER 9

SHOCK

IN SPITE OF THE PROGRESS realized in the last decade, shock remains one of the most challenging problems in medicine. That lipids have a critical role in shock pathogenesis seems clear from a long-term study which began with an investigation of the activity of fatty acids in the induction of the abnormally dark color of blood seen in shock. The results of this study will be discussed here not only because of the intrinsic interest of the problem of shock itself but also because shock often represents the terminal phase of cancer as it does of many other diseases. In this presentation, we will try to remain as much as possible within the framework of our direct contribution to an understanding of shock. A portion of these researches was published in 1943. (40)

In studying the very complex phenomenon of shock, one has to consider a series of well-defined problems. Shock has been related not only to a large number of causes but also to a series of very varied clinical manifestations. An initial problem was to determine whether there is any common relationship between the different types—between the shock, for instance, which kills a subject within a few minutes after a severe sudden trauma, and the shock that kills in days through profound systemic metabolic impairments. What is common to, and what is different between them, from the point of view of pathogenesis? What constituents intervene and how, in shock? These and many other problems have been approached systematically.

Types of Shock—As a starting point, we attempted to classify the types of shock and found an interesting relationship according to the time of their appearance, that is, the interval between application of the noxious stimulus and onset of manifestations. Three types could be identified with this criterion.

There is an immediate type of shock which appears within a few minutes after the application of the noxious agent. It is induced experimentally in animals by intravenous injection of a noxious substance, by scalding the animal in hot water, or by strong mechanical trauma. It has predominant central nervous system manifestations, including exophthalmia and paralysis of the posterior limbs, followed by clonic convulsive movements, and usually is terminated by death. A similar *superacute* type of shock is occasionally seen in humans following transfusions of blood with an incompatible group. It also may be seen following very severe trauma. In the case of bullet wounds, for example, large calibre bullets may bring rapid death. Neither immediate hemorrhage nor any organ impairment is sufficient, in itself, to account for the speed of death in many of these cases. However, it can be explained by the rapid and intensive participation of the central nervous system in this superacute type of shock. Sometimes such shock is not lethal in animals or humans and is followed by a period of prostration and ultimate but slow recovery. We called this type of shock the "superacute."

In a second type of shock, more frequently encountered in humans, the manifestations appear after a certain period of time. Such shock often is seen after direct transfusions, when the rate of injection has been too rapid or when the syringe and tubes have not been well coated with oil or paraffin, or when there has been a subgroup incompatibility between donor and receiver. The patient usually experiences a severe chill within 30 minutes. The chill is succeeded by a rise in temperature which usually lasts 15 to 60 minutes or more. The patient next experiences diaphoresis, after which the episode usually is concluded. In some cases the symptomatology is different. At about the same time—30 minutes—after transfusion, for instance, hypotension with hypothermia, cold and clammy perspiration, and intensive dyspnea are noted. In these cases death can follow in a short time. The same reaction is sometimes seen to occur, usually also in about 30 minutes, after the release of a tourniquet. We have employed the term "*acute shock*" to describe this second type characterized by its appearance at approximately 30 minutes after the noxious intervention.

A third form, the "*state of shock*," is considerably slower in onset and persists much longer. Characterized by hypotension, impairment of circulation, cold and clammy perspiration and marked exophthalmia, it may lead to death after several days during which the condition progressively increases in severity. It can, however, also end in recovery. This is the form

most often encountered in clinical medicine, in cancer and many terminal conditions.

The next problem was: could a common pathogenic mechanism be recognized despite the greatly varied manifestations of these three forms of shock?

Shock Mechanism

We saw one primary correlation between the three clinical types of shock in the fact that sometimes one type is followed by another. Superacute shock, if not lethal, may be followed by acute shock which, in turn, can change into a state of shock.

But it was the chemical analysis of blood, organs and entire bodies of animals killed by any of the three types of shock which indicated the possibility of a mechanism common to all three. A low antitryptic power of the blood, and the presence of substances resulting from protein hydrolysis were found to characterize all 3 types of shock. Additionally, an increase in the amount of free fatty acids, and the presence of abnormal members, occurred in all three types.

Fatty acids were studied from the point of view of the reciprocal position of their double bonds, through the oxidative fission method mentioned previously. The appearance of oxalic acid following oxidative fission indicates the presence of conjugated double bonds. The oxalic acid index obtained indicates the proportion of these conjugated double bonds. In normal rats, this oxalic acid index usually is zero in the total amount of fatty acids; in normal mice, values below 1 are seen. In all animals in shock, even in cases of superacute shock followed by sudden death, the oxalic acid index is invariably much higher. Furthermore, the death of an animal in acute shock or state of shock appears to be related to the presence of a critical oxalic acid index, indicating a concentration of abnormal fatty acids incompatible with life. Whether it appears in a relatively short time as in acute shock, or many days after the noxious intervention as in the state of shock, the oxalic acid index found in dying animals is between 14 and 17. Such high values are not found in superacute shock but the oxalic acid still is markedly increased. Thus, the presence of hydrolytic processes together with abnormal fatty acids appears to be a common pathogenic factor for the different forms of shock.

Pathological Changes

The three types of shock—because of the presence in all of hydrolytic processes and abnormal fatty acids—could be related to the first phase of

the immediate diphasic defense phenomenon or its prolonged form. The next problem was to determine what other factors might influence the development of differing manifestations so as to make shock appear in three forms.

The study of pathological changes characterizing each of these forms was undertaken. We found cellular vacuolation a characteristic lesion in animals in superacute shock. Vacuoles are present in the parenchymal cells of the liver, to a lesser extent in the alveolar cells of the lung, and to a still lesser extent in kidney cells. Of special interest was the fact that these vacuoles are often seen in the cytoplasm and even in the nuclei of brain cells. These findings explain the predominance of the neurological symptoms in this form. In a publication in 1943, we described this vacuolation as a characteristic of the superacute shock. The fact that the characteristic pathological change encountered in superacute shock is the presence of vacuoles in different cells suggests that this form of shock occurs principally at the cellular level.

In the acute type of shock, which usually appears half an hour after noxious intervention, there may be some evidence of cellular vacuolization, but the principal changes are at the tissular level. The changes are largely localized in the immediate areas damaged by the noxious agent and are manifested by vascular and interstitial pathology such as marked edema or capillary hemorrhage. Splanchnic vasodilatation and petechiae at the surface of pleura or peritoneum appear when the noxious agent acts indirectly in the blood or is applied directly to it through intravenous injection. The degree of generalized vascular damage corresponds to the degree of direct participation of the blood. We have discussed previously, in the chapter on defense, the changes occurring in the blood which characterize hemoshock. The characteristic leucolysis, which is followed by hydrolytic digestion, explains the high degree of breakdown of blood constituents and vessels observed in this kind of shock. While the participation of the cellular level—and especially of the central nervous system—characterizes superacute shock, participation of the tissular level leads to the acute form.

We consider pathologically characteristic of the state of shock—in addition to the changes seen in blood, such as hemoconcentration, dark color, tendency to form sludges, etc.—two other specific manifestations; milliar lesions in the gastric mucosa leading to hemorrhage and ulceration, and a manifest fluid accumulation in the first portion of the small intestine. Since the various changes in the state of shock affect the blood and two organs, the stomach and duodenum, they can be considered to involve the organic and systemic levels.

This analysis has permitted us to continue to develop the hypothesis that all three forms of shock stem from the same fundamental mechanism—the appearance of abnormal fatty acids as part of the first phase of the diphasic defense reaction. The differences in manifestations between the forms of shock are due to the level at which the mechanism operates, cellular for superacute shock, tissular for acute, and organic and systemic for the state of shock.

The study of a special condition, hemoglobinuria "a frigore," or paroxysmic hemoglobinuria, has helped us to understand the time factor in shock. In this condition, immersion of the hand in ice water, for instance, induces hemoglobinuria and violent chill about half an hour later. We have been able to demonstrate that in the development of such a manifestation, two or often even three hemoshocks occur, each one characterized by a diphasic phenomenon. The first shock appears within ten minutes after immersion of the hand in icy water. Usually the first sensation and chill are very slight and while a reduced hemoglobinemia is present, hemoglobinuria is almost nil. It is the second hemoshock, appearing approximately 30 minutes later, which is usually very intensive with manifest hemoglobinuria. The third shock, which appears about two hours after immersion in ice water, is usually clinically inapparent and is revealed only by blood analysis.

The study of this condition has indicated that in the appearance of the three episodes of hemoglobinuria, besides the changes in the red cells under the influence of cold, which are characteristic for the condition as seen in the Donath-Landsteiner test, the important factor is the leucolysis occurring as part of the hemoshock. The subsequent hemolysis leads to free hemoglobin in the blood which, if in sufficient amount, passes into the urine. The changes induced in leucolysis will determine the degree of consequent hemolysis. The suppression of leucolysis by administration of morphine or other opium derivatives prevents any manifestation, while physical exercise undertaken following the immersion of the hand in icy water induces, in addition to a very intensive leucolysis, exceptionally intensive clinical manifestations. The time when the three hemoshocks appear also marks the time when the three forms of pathogenic shock—superacute, acute and state of shock—are seen. The intervention of three different noxious heterogenized constituents appears plausible. (*Note 1*)

Fatty Acids and Sodium Chloride in Shock

We noted that in all three types of shock, abnormal fatty acids can be found. A study of the role of these fatty acids permitted us to further un-

derstand the mechanism involved in these three types of shock. Since these same fatty acids have been seen to figure in abnormal metabolism of sodium chloride, the next logical step was to investigate the correlation between the latter and shock. Following this line, efforts were made to see if the differences between NaCl metabolism at different levels of organization would help explain the peculiarities of the different types of shock.

As we have noted, when abnormal fatty acids impair sodium chloride metabolism, two processes occur. First, there is abnormal fixation of chloride ions by abnormal fatty acids; then, sodium ions, freed following this chloride fixation, become bound to carbonate ions, resulting in alkaline substances. The pathological nature of chloride fixation results principally from the fact that the binding taking place at the conjugated double bonds is abnormally strong. Occurring in two steps, with a displacement of the double bond in the first, the bond between the conjugated fatty acids and chloride ions appear nonreversible. (*Note 8, Chapter 6*)

The great inequality in the ability of chlorides and sodium ions to pass through membranes can serve to separate, anatomically, the fixed chlorides from the free remaining cations. When this occurs, two distinct processes can be recognized, one involving the binding of chloride ions by abnormal fatty acids, the other involving the binding of carbonate ions by sodium ions and the resulting appearance of alkaline compounds. In the cells, the two processes take place separately, the sodium alkaline compound inducing the appearance of vacuoles. In tissues, the chloride fixation takes place predominantly in the cell, while the binding of carbonate occurs in the interstitial spaces. This leads to a localized intercellular alkalosis with consequent edema.

The same mechanism is involved in the changes associated with the state of shock, except that these processes occur at the systemic level. It is the part played by the sodium chloride of the blood in normal physiology, especially in the process of digestion, which explains the abnormal changes seen as characteristic of the pathological manifestations in the state of shock.

Normally, chloride ions are excreted into the stomach, where they are bound to hydrogen to form hydrochloric acid. An almost equal amount of sodium ions, bound to carbonate ions, is eliminated in a second step into the intestines via the pancreatic and intestinal secretions. The chloride and sodium ions are later liberated to form sodium chloride which is entirely reabsorbed in the distal portion of the intestinal tract, the colon. The sodium and chloride ions are not simultaneously secreted in the digestive tract. The interval between the excretion of chloride ions into the stomach

and of sodium ions into the intestines accounts for the physiological "alkaline tide" associated with digestion.

When chloride ions are pathologically fixed to abnormal fatty acids in the blood, they can no longer be dissociated and secreted by the stomach in the form of hydrochloric acid. Instead, they remain bound to the fatty acids and accumulate in this form within the gastric mucosa. The multiple milliar gastric mucosal ulcerations in the state of shock results from the intervention of these abnormal fatty acids brought into the mucous membrane by the chloride ions to which they are bound. The ulcerations are caused by the catabolic action of fatty acids. Thus, the first phase of abnormal sodium chloride metabolism leads to the characteristic multiple gastric ulcerations.

The second phase is related to the metabolism of sodium. The sodium ions are secreted as carbonates by the pancreas and intestinal mucosa in the first part of the small intestine. In the state of shock, because they do not encounter the chlorides normally coming from the stomach, they remain as carbonates. As sodium carbonate is accumulated in the first portion of the small intestine, a local alkalosis occurs, leading in turn to an important local retention of water. It should be noted that this is a very different situation from achlorhydria or hypochlorhydria in which, while the chloride ions are not secreted into the stomach, no excesses of sodium ions appear in the blood or in the intestines, and consequently no local alkalosis or fluid accumulation occurs.

The difference between the systemic and tissue processes in shock lies in the localization of the abnormal sodium chloride metabolism. In tissue anomaly, the separation of sodium chloride takes place between the cells and the pericellular structures. At the systemic level, it occurs between the stomach and intestines, with the blood serving as intermediary. This mechanism explains the larger amounts of water which distend the upper parts of the intestine, as observed in autopsies of animals which have died in this form of shock.

The close similarity between the abnormal processes that take place in sodium chloride metabolism at the tissue and systemic levels provides the basis for another working hypothesis concerning the mechanism in superacute shock. We have seen that the production of vacuoles in cells characterizes this latter form of shock. The unequal cellular permeability for chlorides and sodium in their dissociated form is known. Chloride ions can circulate much more easily between cells and the pericellular spaces than can sodium ions. An initial effect of the intervention of abnormal fatty acids in cellular pathology is the fixation of chlorides. At the same time, an

increased permeability in membranes occurs. This would permit more sodium ions to pass through cell membranes and to accumulate intracellularly, inducing a liberation of potassium, the cellular cation. As the chloride ions are bound to fatty acids in the cells, the sodium ions in the cells liberate potassium and join it to form alkaline compounds. Isolated in vacuoles, these compounds also accumulate water.

Thus, we have a concept of single pathogenesis for all three forms of shock based upon abnormal sodium chloride and water metabolism, with the abnormality taking place at different levels of the organization, cellular for superacute shock, tissular for the acute form, and systemic for the state of shock. The displacement of potassium by sodium in cellular physiology contributes to the increase in serum potassium found in all forms of shock.

Water Metabolism

The localized retention of water, prompted by the alkaline sodium compounds which result from abnormal sodium chloride metabolism, occurs in the cells, tissues or intestines in the different types of shock. Many of the differences in manifestations between the three forms of shock can be explained in terms of localization of this abnormal water metabolism. The sensitivity of the cells of the nervous system to intracellular changes explains the predominance and severity of the nervous system manifestations in superacute shock. Abnormal tissue water metabolism explains not only the predominantly local character of the manifestations seen in acute shock, but also the hemoconcentration values in these cases. As often seen in burns, important amounts of water are driven out of the blood into the damaged tissues.

The abnormal water metabolism however, appears to be the principal manifestation in the state of shock. Upper intestinal water accumulation, rather than a general unlocalized fluid loss, can be demonstrated in the pathogenesis of this form of shock. In opposition to the local lesion with a high retention of water, the general subcutaneous tissues sustain a loss of water rather than an accumulation during shock. This would not occur if there were a general increased permeability of all capillaries, allowing water to pass freely. The role of water accumulation in the first portions of the intestine due to the abnormal loss of systemic water was demonstrated in animal experiments. When the small intestinal tract had previously been removed, and a state of shock was later induced by trauma, no hemoconcentration occurred.

It is the participation of one or another of the three principal levels of the organization—cellular, tissular or systemic—which explains why the

same pathogenic process, abnormal sodium chloride and consequent abnormal water metabolism, produces such different manifestations in the various types of shock. It must not be forgotten however, that in the last analysis, the abnormalities in sodium chloride and water metabolism result from the intervention of abnormal fatty acids. Fatty acid intervention, together with the abnormal sodium chloride and water metabolism confirm the unitary pathogenesis of the three forms of shock.

Other Changes

Other changes associated with shock also can be related to the influence exercised by abnormal fatty acids. The appearance of rouleaux of red cells may be easily explained by fatty acid intervention. It is the replacement of the nonpolarity normally present at the surface of the red cells by a dipolarity which results in the formation of the rouleaux. This can be induced by fatty acids *in vitro*. Sludge formation would represent a still more advanced step in this same process and would appear to result from a poly-polarity at the surface of the red cells. Sludge formations have been induced *in vitro* by fatty acids added in larger amounts to plasma. (*Note 2*) They contribute to the circulatory impairment considered to be an important factor in the tissular respiratory troubles seen in shock.

We have noted that the richness in free fatty acids interferes with the ability of the red cells to keep oxygen fixed, a fact which would impair its transport. This, together with hemoconcentration and circulatory impairment, has been found to account for the black color of the blood in shock. (*Note 3*) The clinical manifestations are characteristic of offbalance D.

Experimentally Induced Shock

The hypothesis that the three types of shock are caused by the intervention of the same factor—abnormal fatty acids—has been further confirmed experimentally. The cellular changes that characterize superacute shock can be induced by the rapid introduction into the blood stream of even minimal amounts of fatty acids in preparations in which they are bound to plasma constituents.

Pooled heparinized plasma of mice was treated by stirring it in a nitrogen atmosphere for one hour with a preparation of conjugated trienic fatty acids. The nonbound fatty acids were separated through short centrifugation. The plasma was injected intravenously in mice. For control, plasma treated under the same conditions with stearic acid was used. While control animals did not show any apparent discomfort, the mice injected with the plasma treated with conjugated fatty acids died immediately, in most

cases even during the injection itself. With such preparations, superacute shock was induced in what we consider a direct way, the sudden death contrasting with the cases of hemoshock where death occurs usually after an interval of a few minutes. This characteristic of direct immediate death is consistent with the pathogenic role of fatty acids in superacute shock.

The tissue changes that characterize the acute type of shock also may be induced by local administration of abnormal fatty acids with the condition that sufficient amounts are used. (*Note 4*) The systemic changes that typify the state of shock can also be produced by prolonged absorption of fatty acids, as when they are repeatedly introduced intraperitoneally. (*Note 5*)

The relationship between shock and lipids can be further seen in the antagonistic effect exercised upon shock induced with standardized trauma by two groups of lipids with positive and negative characters. We have utilized the Noble-Collipp drum on a large scale to induce shock in rats. In some groups of animals shock induction was constant; in other groups under the same conditions, shock could be induced only in some animals. Nevertheless, it was still possible to recognize opposite effects induced by the administration of the two groups of lipids. In some animals even apparently little influenced by the trauma, the injection of a mixture of conjugated fatty acids immediately after trauma brought death within a short time. In no other animals, traumatized under the same conditions, have we seen death occurring within the same short interval of time. This also applies to animals injected before trauma. In these cases, the animals died even during the trauma, that is, in the drum. (*Note 6*)

Conversely, the administration of sterols, especially preparations of the insaponifiable fraction of human placenta, before induction of trauma prevented lethal shock almost without exception, whereas under the same conditions the same trauma produced death in a high proportion of the controls. Even when injected immediately after trauma, this sterol preparation prevented the development of lethal shock in a high proportion of cases. (*Note 7*)

The different forms of shock, although resulting from the same fundamental abnormal process, appear to respond differently to therapeutic agents—again because of the localization of the abnormal processes at different levels. Adrenalin and related compounds, when administered in time, are able to control superacute shock, but they are almost entirely without influence upon the other forms. While acute shock can be influenced by the administration of a large amount of sterols and butanol, superacute shock is unaffected, possibly also because of the slow absorption of

the sterols. None of these agents is of significance in the treatment of the state of shock which is only mildly influenced by butanol and certain cortical hormones such as hydrocortisone, especially when introduced directly in the circulation.

In order to act upon the fatty acids and sodium which produce the abnormal water metabolism, we have utilized glycerophosphoric acid administered in large amounts parenterally. Diluted with saline, it was usually injected intravenously. The good results obtained are discussed later.

The use of heptanol and of polyunsaturated alcohols has also led to interesting results. It was however with preparations having several of these agents, working at different levels of the organization, that the best results were obtained.

The measurement of the chloride index and of the surface tension of the urine have represented valuable means to judge the changes occurring in shock, in their clinical evolution and especially in the action of the agents in relationship to the occurring recovery.

The study of shock has contributed to the knowledge of the therapeutic problems of cancer and other conditions. The cause of death, when a predominance of fatty acids occurs as a systemic manifestation, corresponds to the state of shock. The possibility of successfully influencing this form of shock would furnish a valuable tool for the treatment of all severe manifestations related to predominance of fatty acids.

CHAPTER 10

RADIATION

THE STUDY OF THE effects produced by irradiation upon all biological entities has resulted in the accumulation of a large amount of data. Being unsystematized, this information has helped but little to resolve the many physiological and therapeutic problems connected with radiation. New light on these problems can be provided by relating them to the basic concepts we have been discussing.

Other factors also have led us to study the problem of radiation. Its widespread use for the treatment of cancerous lesions with indisputable success in many cases, and the fact that some of radiation's effects appear to be quite similar to those induced by the administration of different lipids, led us to investigate the mechanism through which radiation works and, especially, the possible relationship between radiation and lipids. We will discuss here briefly, some of the results of this investigation.

Irradiation of Lipids

We began by trying to determine the effects of radiation upon normal lipids *in vitro*. As always, we tried to guide the research by theoretical considerations. Investigation of *in vivo* and *in vitro* effects of radiation upon proteins in general showed that histones, protamines and alkaline amino acids are most sensitive. These constituents of complex protein molecules have positive electrical character. This relationship between sensitivity to radiation and positive electrical character provided a clue as to where to look in fatty acid molecules for changes induced by radiation. Several positive centers are present in the energetic structure of fatty acid molecules. One is represented by the carbon of the carboxyl. Its positive character is due to its bond to two oxygen atoms. This would explain the exaggerated ionization which takes place at the level of this carboxyl group.

Other positive centers also can be recognized. We have mentioned previously that the positive character of carbon propagates through the chain in an induction effect that causes alternate odd carbons to be positively charged, although the strength of the positive character decreases rapidly with distance from the carboxyl. Since a double bond greatly enhances the energetic character of the carbons linked by it, induction will result in a center in which a more intensive positive carbon is present. Study of the reactions that take place at the double bond in a fatty acid molecule confirms this view, since an electrophilic character predominates

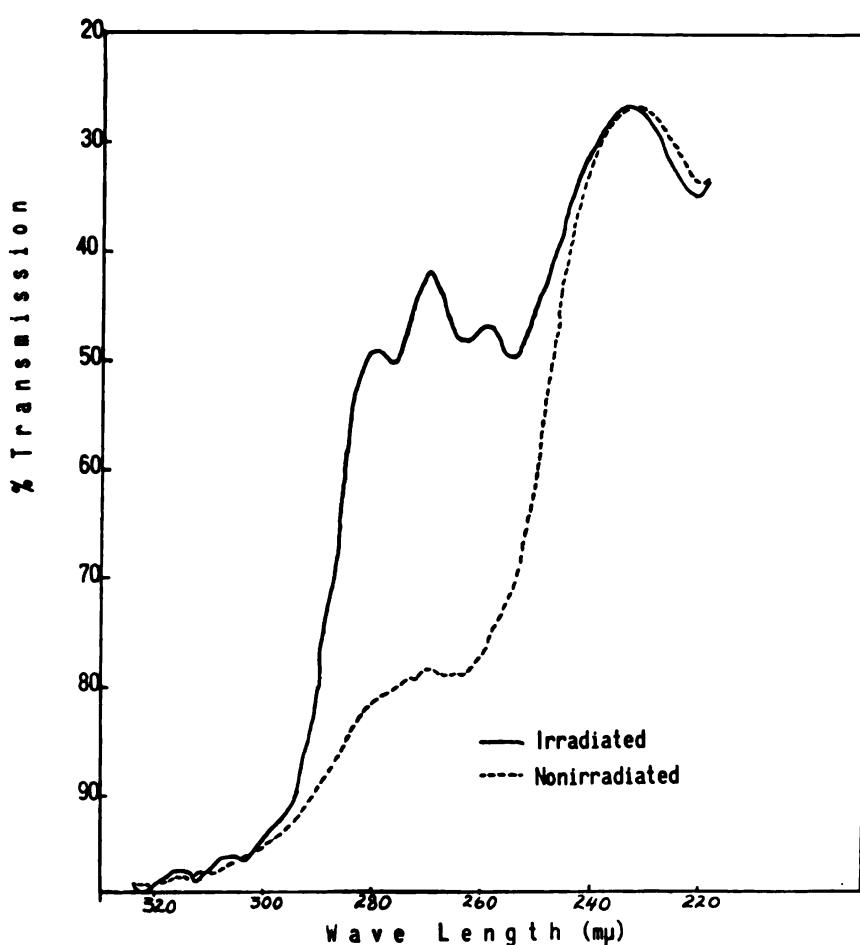


FIG. 79. *Irradiation and conjugation in vitro.* Spectral analyses in ultraviolet of samples of commercially available linoleic acid (with small amounts of linolenic acid present) irradiated with gamma rays from 80 mgr of platinum filtered radium/10 cc, for 6 days at room temperature. For the analyses, dilutions of 0.002% in alcohol, with alcohol as reference, were used. The absorption spectra of the irradiated (—) linoleic acid compared with the nonirradiated (....) shows the appearance of conjugated trienes recognized through the characteristic peaks.

at this point. When treated with sodamine, carbons forming double bonds combine selectively with it, indicating the positive electrostatic character of this formation. Consequently, we thought that the effect of irradiation would be most likely to occur here. This has been confirmed experimentally. We could demonstrate that radiations cause changes especially in the reciprocal position of the double bonds in the molecule.

The results of this research were originally presented before the Fifth International Congress of Radiology in London in July 1951. We will limit ourselves here to a short resume of the procedures and findings:

Irradiation in Vitro

a) Radiation of polyunsaturated fatty acids in vitro induces a conjugation of their double bonds, which increases quantitatively with the intensity of the radiation. This has been shown by spectral analysis and by the oxidative fission method. (*Note 1*) Samples of commercially available linoleic acid which, through analysis, have been found to contain variable amounts of linolenic acid, or cottonseed oil were treated with radiations of different sources, such as radium in platinum needles for gamma radiation, in monel metal for beta radiation, thorium X for alpha radiation and X rays. Table XIV shows the results of analysis of the oxalic index. Figs. 79 and 80 show the direct spectral analysis of the samples before and after irradiation as well as the result of their chemical conjugation.

Comparison of direct spectral analyses shows the appearance of an important amount of conjugated trienes in the irradiated samples. Analysis after chemical conjugation of irradiated and control samples shows a greater amount of trienes in the irradiated sample indicating that a process of desaturation also has taken place through irradiation.

A direct relationship was observed between conjugation and amount of radiation. (TABLE XV) The quantity of conjugated isomers, determined by spectral analyses and measured by the oxalic index was seen to increase as radiation was increased either by prolonging exposure time or increasing the amount of radium used.

b) Irradiation of fatty acids appears to induce the appearance of conjugated trienes. When a mixture of polyunsaturated fatty acids, such as those found in cod liver oil, was exposed to a radiation source consisting of platinum filtered radium, the changes were limited to the appearance of conjugated trienes. Conjugated dienes were seen in only some experiments and then only in small amounts. The presence of conjugated members was recognized through their characteristic absorption peaks in ultra-violet anal-

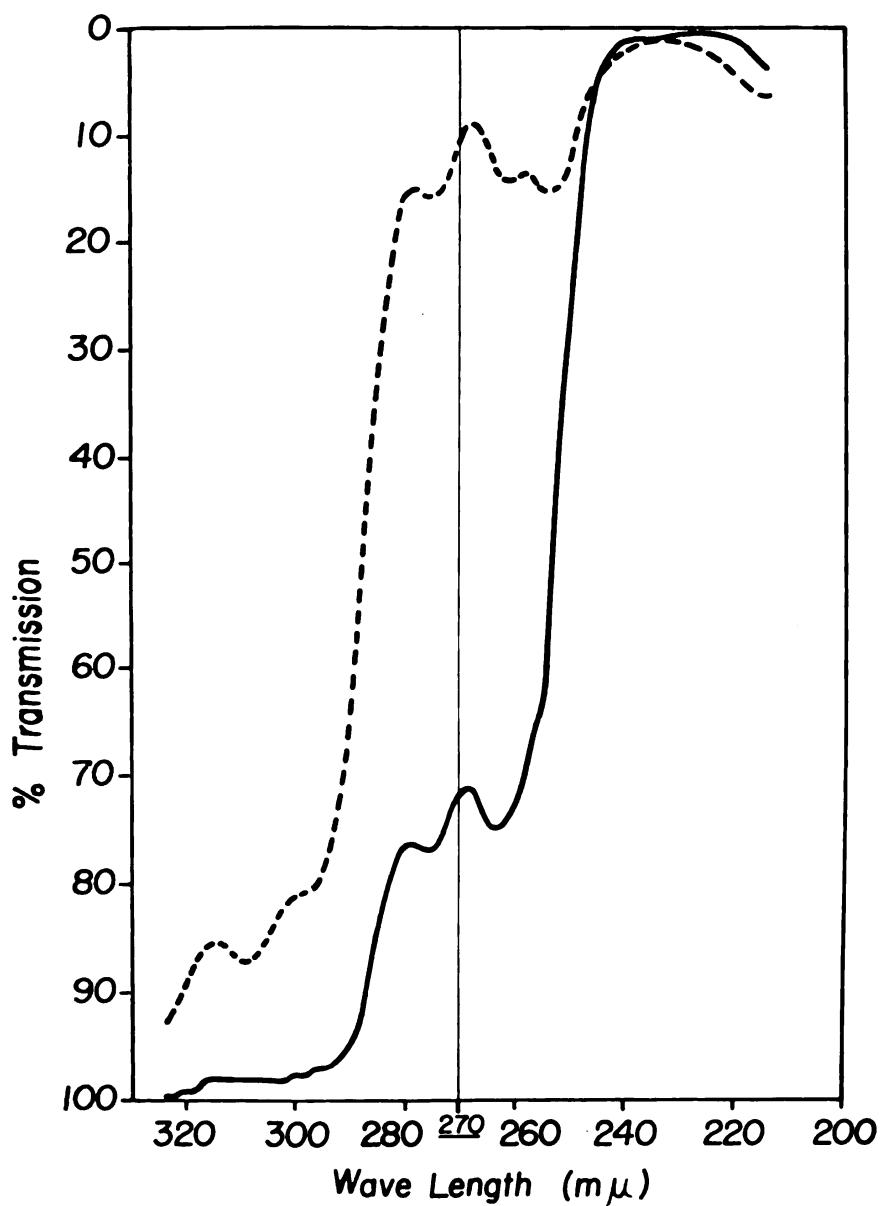


FIG. 80. *Irradiation and desaturation.* Spectral analyses in ultraviolet (0.002% in ethyl alcohol with alcohol as reference) of untreated sample (—) and of the radiated sample (....) both after alkaline isomerization. They show that the irradiation has induced also an increase in the amount of trienes present, which indicates that a desaturation also occurred.

ysis. When the same mixture of fatty acids was treated by the usual chemical methods employed to produce conjugation, *i.e.*, with potassium hydroxide in ethylene glycol or glycerol (41), the spectral analysis showed that the preparation contained fatty acids having between 2 and 6

TABLE XIV

Fatty Acid	Type of Radiation	Source of Radiation	Exposure Time (Days)	Oxalic Acid mg/g Fatty Acid
Linoleic acid	—	—	—	0
" "	gamma	50 mg Radium	6	8.3
" "	gamma	120 mg Radium	8	9.9
" "	gamma	120 mg Radium	15	13.3
" "	beta	25 mg Radium in Monel metal	4	9.85
" "	alpha	150 uc Thorium X	7	4.3
" "	x-ray	5000r daily—deep therapy machine	27	6.8
" "	x-ray	5000r daily—deep therapy machine	53	12.4
Cottonseed oil	—	—	—	0
" "	gamma	80 mg Radium	5	5.2
" "	gamma	80 mg Radium	8	8.0

double bonds. Figure 81 shows the curves of spectral analysis for such an experiment in which 3 cc. of a cod liver oil fatty acid preparation were treated for six days with 100 milligrams of radium filtered through platinum. In curve "a," of the untreated sample, it can be seen that there is no absorption due to the presence of conjugated members. Curve "b," for the irradiated fraction, shows the typical conjugated trienes, while Fig. 82 shows the result of the chemical conjugation of the nonirradiated preparation with members having from 2 to 6 double bonds.

TABLE XV

EFFECTS OF IRRADIATION UPON THE QUANTITY OF OXALIC ACID PRESENT AFTER OXIDATIVE FISSION

Fatty Acid	Source of Radiation	Expo- sure time: Days	Oxalic Acid mg/g Fatty Acids
Linoleic Acid	0 120 mg Ra	0 4	0 4.5
10 cc.	"	6	6.2
	"	8	9.9
	"	15	13.3
	"	20	14.4
10 cc.	150 mg Ra	6	8.2
	"	15	16.3

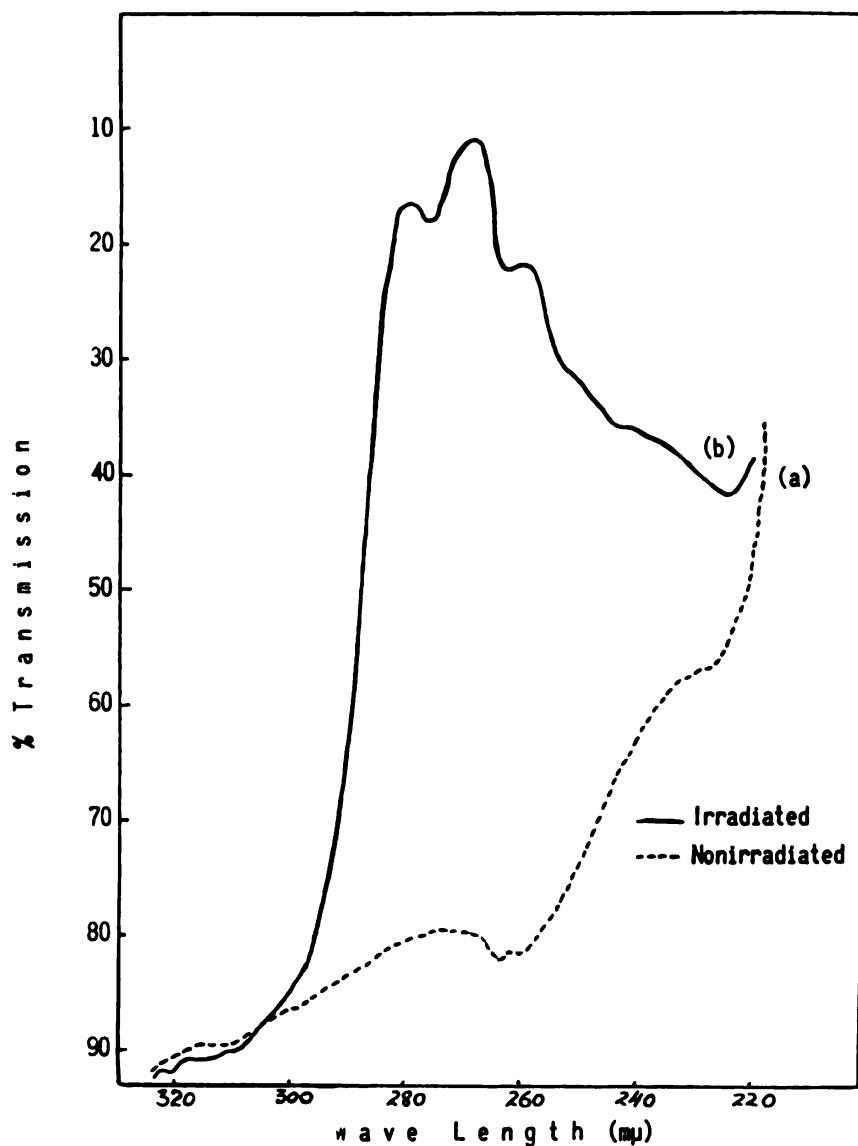


FIG. 81. Spectral analysis (0.002% in alcohol) of a mixture of *fatty acids from cod liver oil*, untreated (....) (a) and irradiated (b) (—) with 100 mg platinum filtered radium/3 cc, at room temperature for 6 days. The analysis shows that the conjugation which takes place leads to the appearance only of conjugated trienes, in spite of the presence of di-, tri-, tetra-, penta- and hexaenic unsaturated fatty acids as shown by the absorption spectrum of the same mixture after chemical conjugation with potassium hydroxide in ethylene glycol. (c) as seen in Fig. 82.

c) The changes induced in fatty acids are essentially the same regardless of radioactive source. Thus the effect upon a linoleic acid preparation containing some linolenic acid was the same with alpha particles of Thorium X, beta rays from radium in monel metal, gamma radiation from platinum

filtered radium, and X-rays generated by a 400 kw. therapy unit. Figures 83 and 84 and Table XIV show these results.

Irradiation in vivo

d) Irradiation of the body of normal animals killed by decapitation, under ether anaesthesia, produces small amounts of conjugated fatty acids. In early experiments, different organs obtained from slaughterhouses were irradiated and fatty acids examined. In general, even after intensive radia-

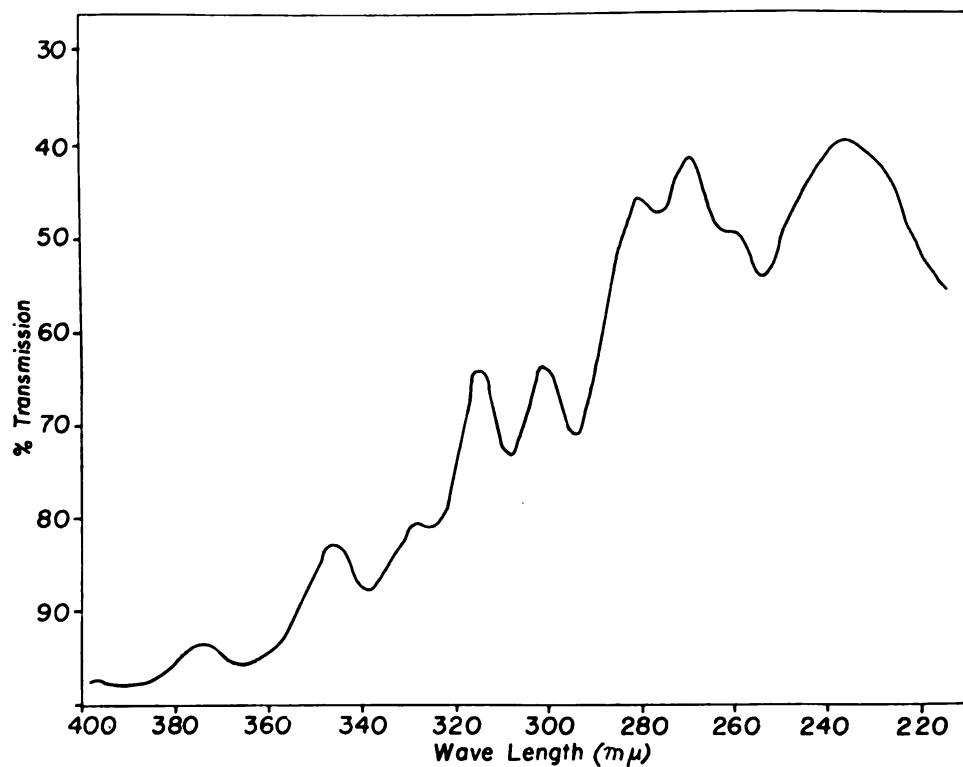


FIG. 82. Curve of spectral analysis of the cod liver oil fatty acids after chemical conjugation, shows the presence of di-, tri-, tetra-, penta-, and hexaenic members.

tion, corresponding to 4,000 r. in one treatment, the oxalic index of fatty acids was never found to be above 1, corresponding to 1 mg. of oxalic acid obtained from one gram of fatty acids.

e) On the other hand, the amount of conjugated fatty acids in the bodies of living animals receiving radiation increases significantly. The following experiment is illustrative. Eighty rats of the same sex, age and weight (about 180 gms.), separated into several groups, were given 1500 r., delivered by a therapy unit with no filter. Four control animals were killed

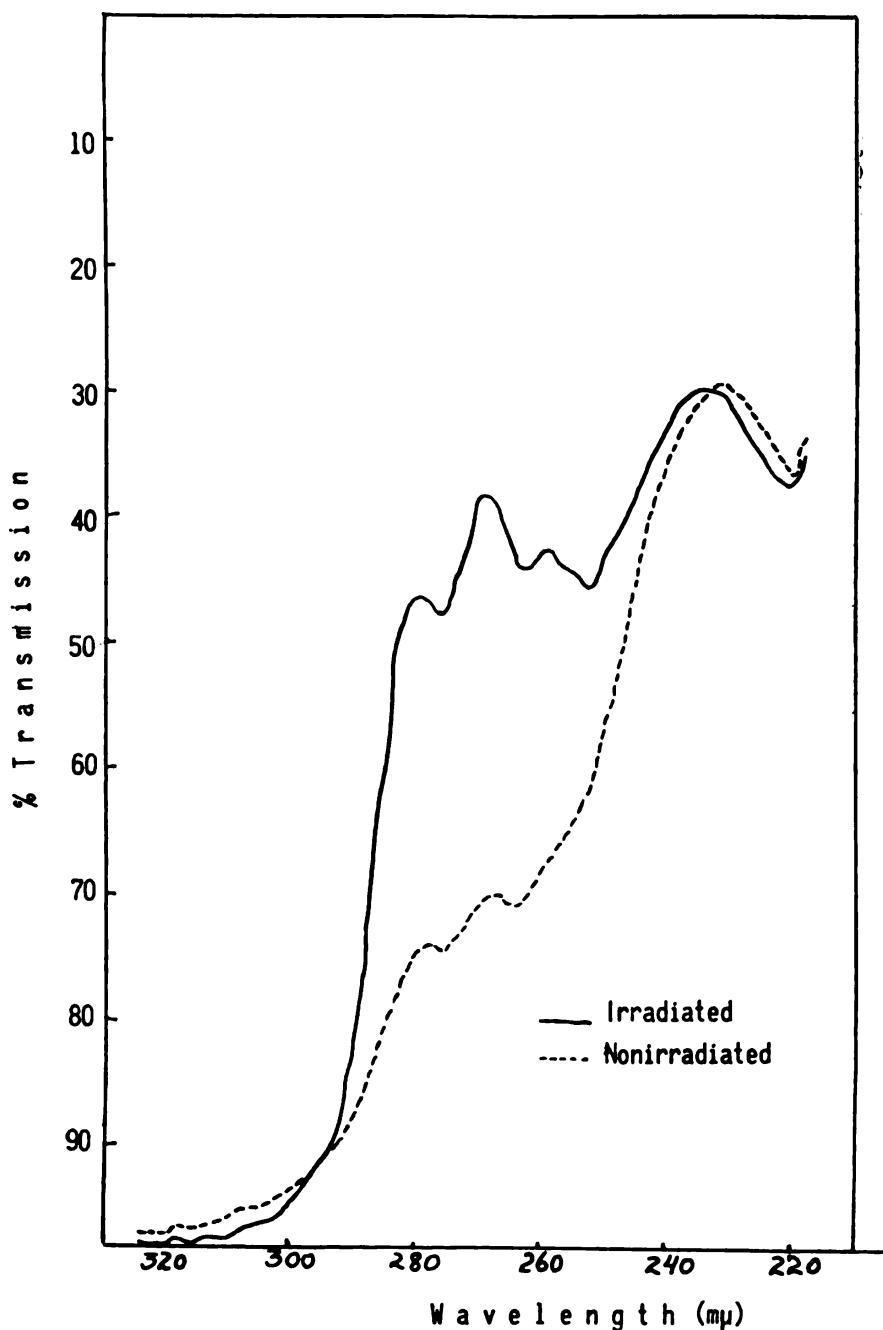


FIG. 83. Absorption spectra (0.002% in ethyl alcohol/ethyl alcohol) in ultraviolet of commercially available linoleic acid (with small amount of linolenic acid) non-irradiated (....) and irradiated (—) with beta particles from 25 mg monel metal filtered radium/10 cc at 37°C for four hours. Some conjugation occurs in the control when kept in the incubator.

before exposure. Groups of four treated animals were sacrificed periodically, starting immediately after irradiation, at 2, 6 and 24 hours after irradiation, and each day thereafter until all animals died or had been killed.

During this time, nontreated control animals, kept under the same conditions, were also sacrificed. The quantity of conjugated fatty acids

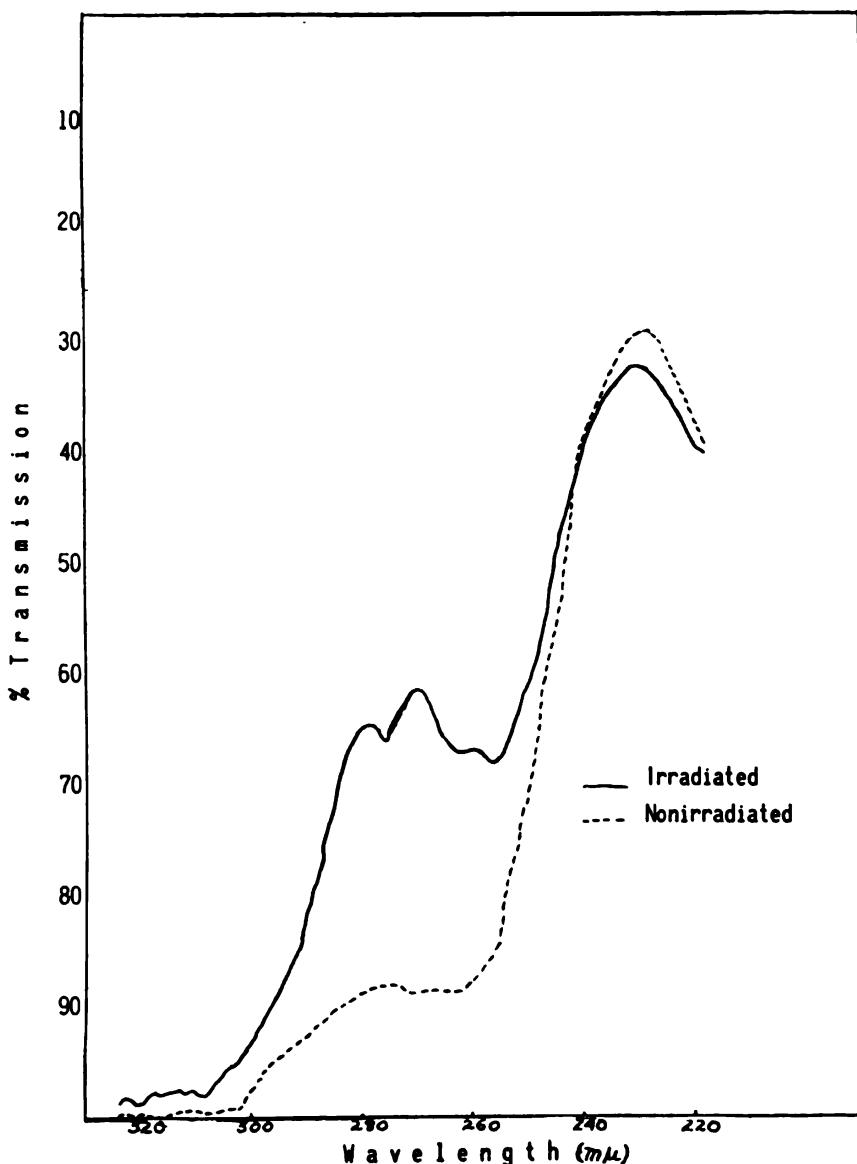


FIG. 84. Absorption spectrum of the same material as in Fig. 83, non-irradiated (....) and irradiated with *alpha* particles from 150 mc Thorium X/10 cc at room temperature for 7 days. (—)

found in the entire body of each individual animal at the time of death was determined by means of the oxalic acid index method.

The oxalic acid index for fatty acids in the bodies of untreated control animals was usually zero. Occasionally there was a variation from 0 but it was always below 0.6. Irradiated animals killed within the first two days showed an irregular increase in conjugated fatty acids, with oxalic acid index values of between 0.6 and 5.1. Three days after irradiation, the oxalic acid index was above 3 in all the dead or sacrificed animals. The index rose

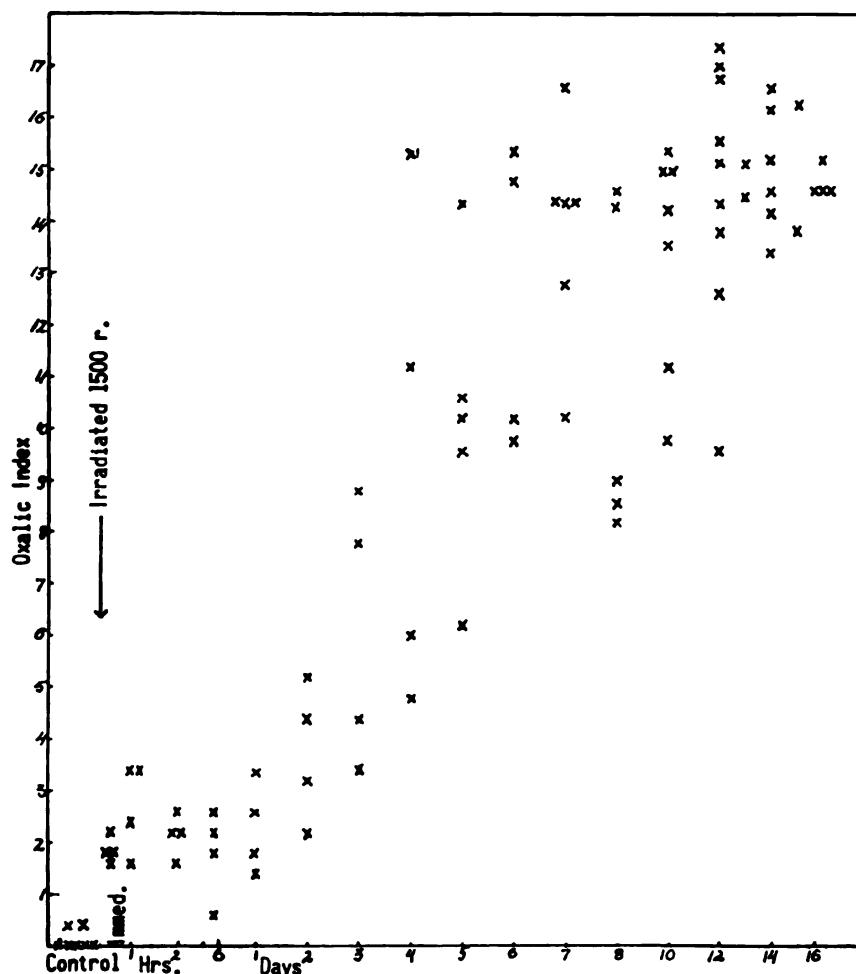


FIG. 85. Fatty acid conjugation induced by irradiation *in vivo*. Changes in the oxalic index of total fatty acids of rats irradiated with a lethal dose (1500r). Sacrificed at different intervals, the oxalic index of their fatty acid shows progressively increasing values. The animals die when the index has arrived at a critical value between 14 and 17.

even in animals which showed no visible ill effects at the time they were killed.

The index increased continuously with the passage of time until the animals died. By the fifth day, it was above 6 for all animals and, by the seventh day, with few exceptions, it was around 10. After the twelfth day, it had risen above 12 in most of the animals. In all animals which were

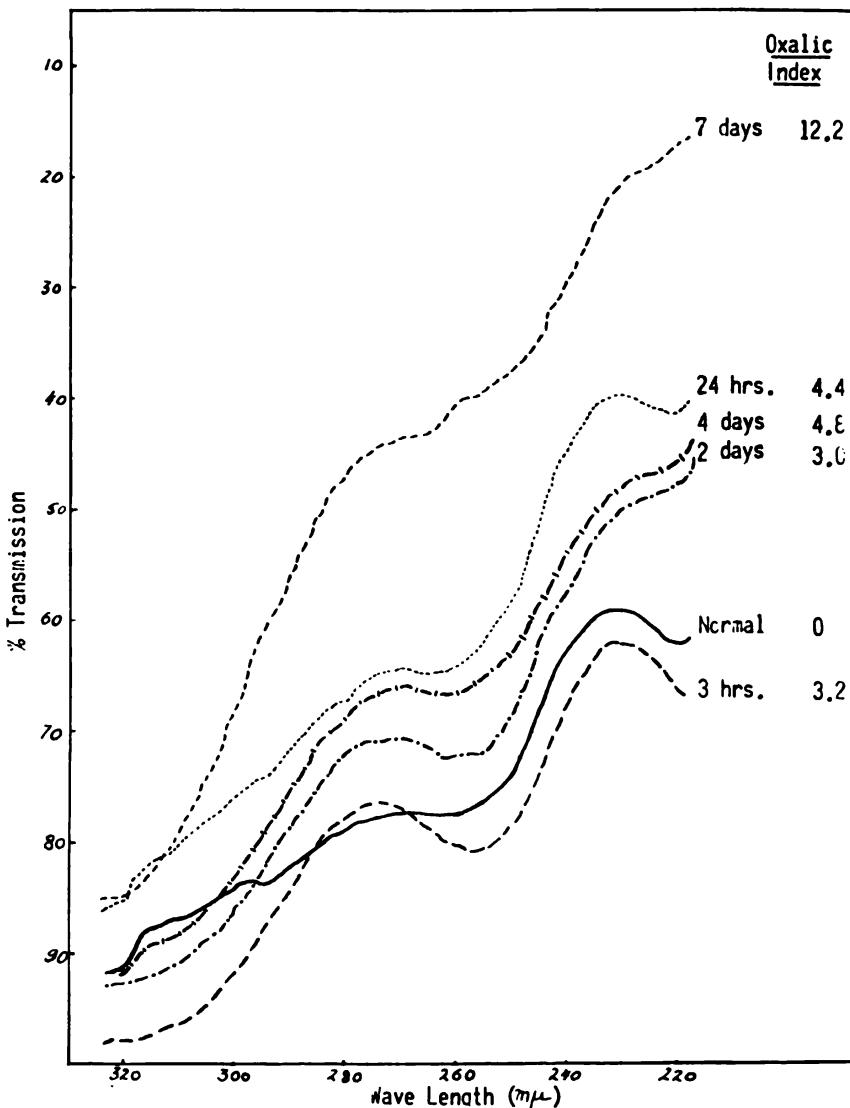


FIG. 86. Spectral analysis (.01 in ethyl alcohol) of the total fatty acids obtained from the body of rats irradiated with 1500r shows changes more manifest around 270 m μ . The oxalic index of the preparation is indicated and shows a parallel increase with the changes in the curve.

sacrificed after the 13th day or which had died at any time, the index showed values between 14 and 17. (Note 2) Figure 85 shows these results in the group of rats described in this experiment. These changes were observed when the same procedure was repeated in other groups of animals. These experiments clearly indicated that the quantity of conjugated fatty acids progressively increases in the days following the exposure in animals

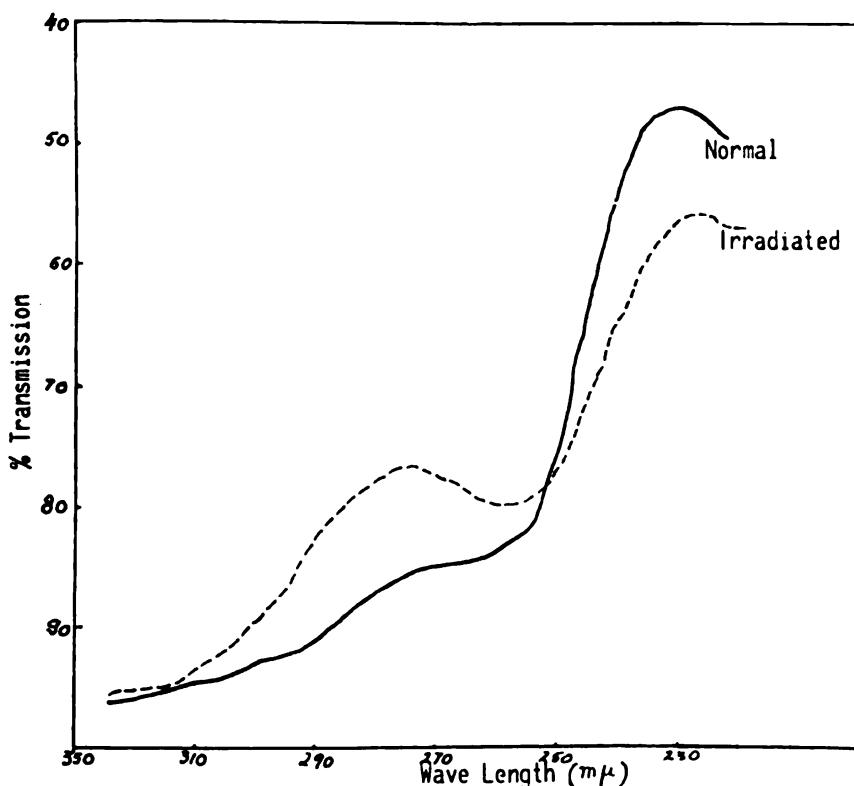


FIG. 87. Spectral analysis (.01 in ethyl alcohol) of the fatty acids of the total body of a mouse irradiated with 1500r, shows an increase in fatty acids with the absorption corresponding to 270 mμ., as compared with the control.

treated with one lethal dose of X-ray. Death occurred when the amount of conjugated fatty acid reached a critical level equivalent to an oxalic acid index value between 14 and 17. The spectral analysis of fatty acids of animals treated with radiation showed changes corresponding to the presence of conjugated isomers. These appear in the samples of fatty acids obtained from the entire body of these animals. (Figs. 86 and 87) Still more evident were the conjugated trienes in the fatty acids of organs. Figures 88, 89 and 90 show the difference in such analyses as compared to corresponding un-

treated controls. The presence of conjugated trienes appears clearly in the characteristic peaks.

The concept of a critical value for the oxalic index is supported by other studies in which the same value is found in animals dying after adrenalectomy or after thermal, chemical or traumatic states of shock. Even in animals dying in superacute shock, within 3 to 5 minutes after being severely scalded in hot water, the level of conjugated fatty acids is higher than in controls.

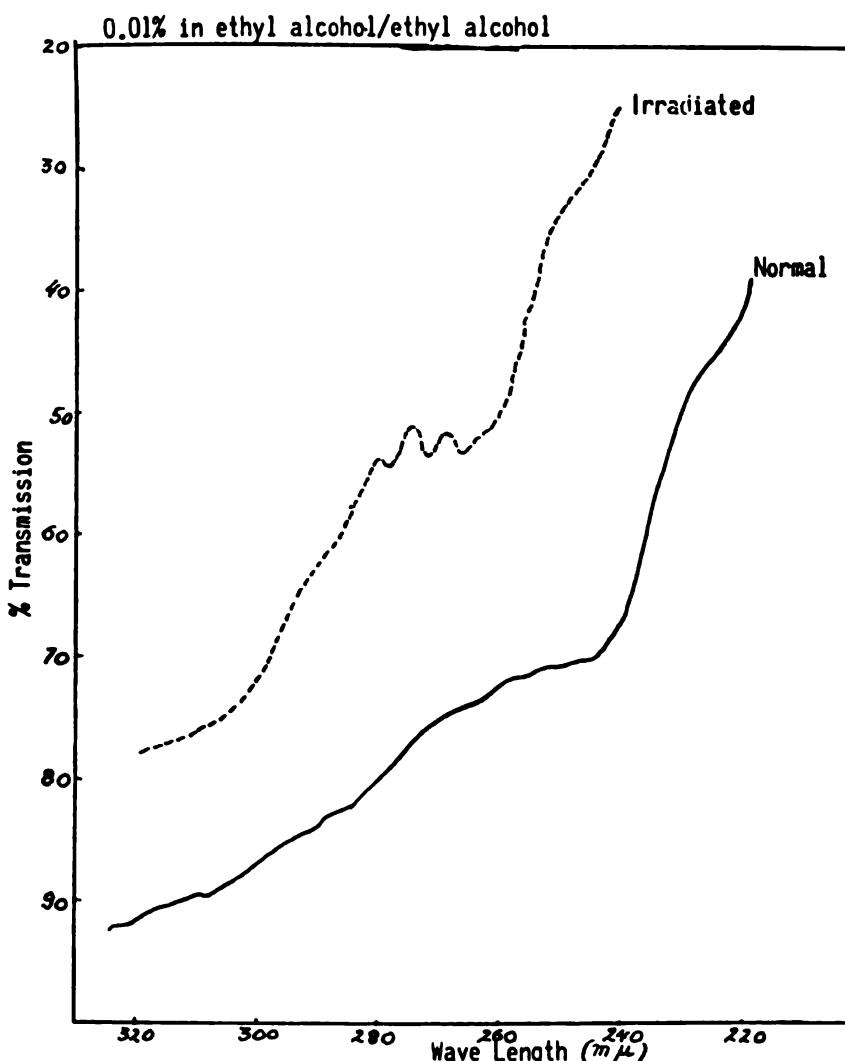


FIG. 88. Spectral analyses (.01 in ethyl alcohol) of the fatty acids of the kidney of a normal rat and of a rat irradiated 6 days previously with 1500r. The peaks characteristic for conjugated trienes are seen.

When the irradiated dose was not a lethal one, that is, below 600 r. in our experiments, the oxalic index increased at first but decreased after about two to three weeks. It never reached the critical value of 14-17. (Fig. 91, Note 3)

Local Effects

f) We completed the studies of the effects of radiation upon fatty acids *in vivo* by considering them at the local tissue level. The first requirement

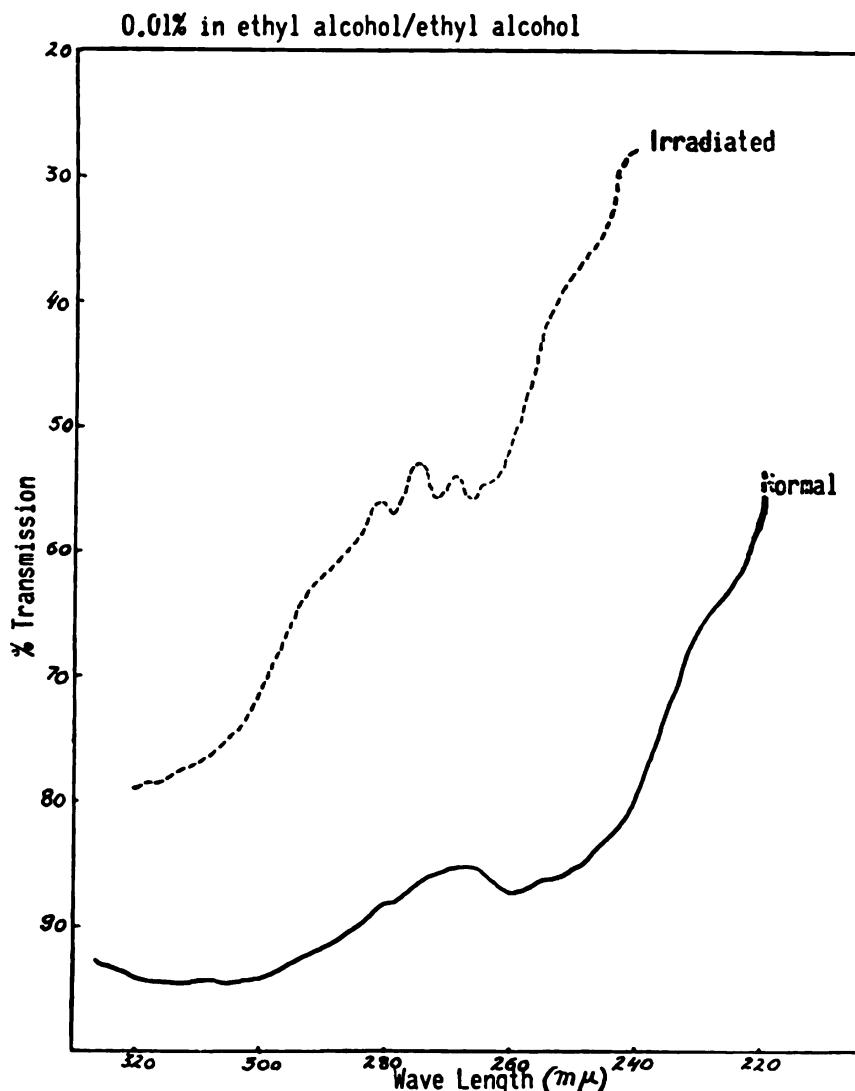


FIG. 89. The spectral analyses (.01 in ethyl alcohol) of the fatty acids of the liver of a normal rat and of a rat irradiated 6 days previously with 1500r shows the appearance of the characteristic peaks of conjugated trienes.

was to establish a radiation procedure which would produce a standardized lesion. When radiations were applied directly to the skin of animals, the individual differences in response were quite marked. These could be explained in part on the basis of age and particularly of sex, the difference

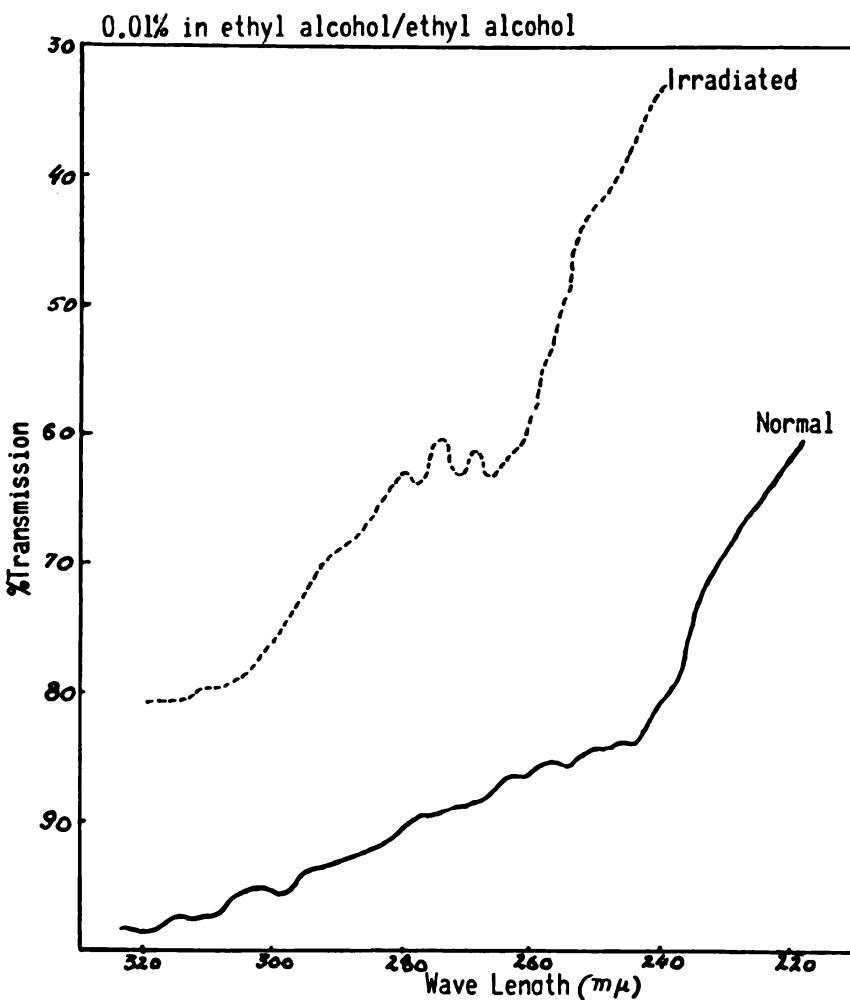


FIG. 90. Shows the spectral analyses (0.1 in ethyl alcohol) of the fatty acids of the lung of a normal rat and of a rat irradiated with 1500r 6 days previously. Peaks corresponding to conjugated trienes are present.

between the skin of male and female rats being manifest. However, there were also pronounced individual differences in animals of the same sex, age and weight living under the same conditions, so that even when the experimental procedure was carefully controlled, the same amount of radiation caused reactions that varied widely from slight erythema to ulceration.

The problem of variability was satisfactorily resolved by radiating abnormal tissues, such as those of a wound, instead of normal tissues. Standardized lesions were first produced and then irradiated. We used the following technique: an area of the skin on the back of male rats weighing around 200 grams was epilated and, under ether anaesthesia, a 2 cm. long incision

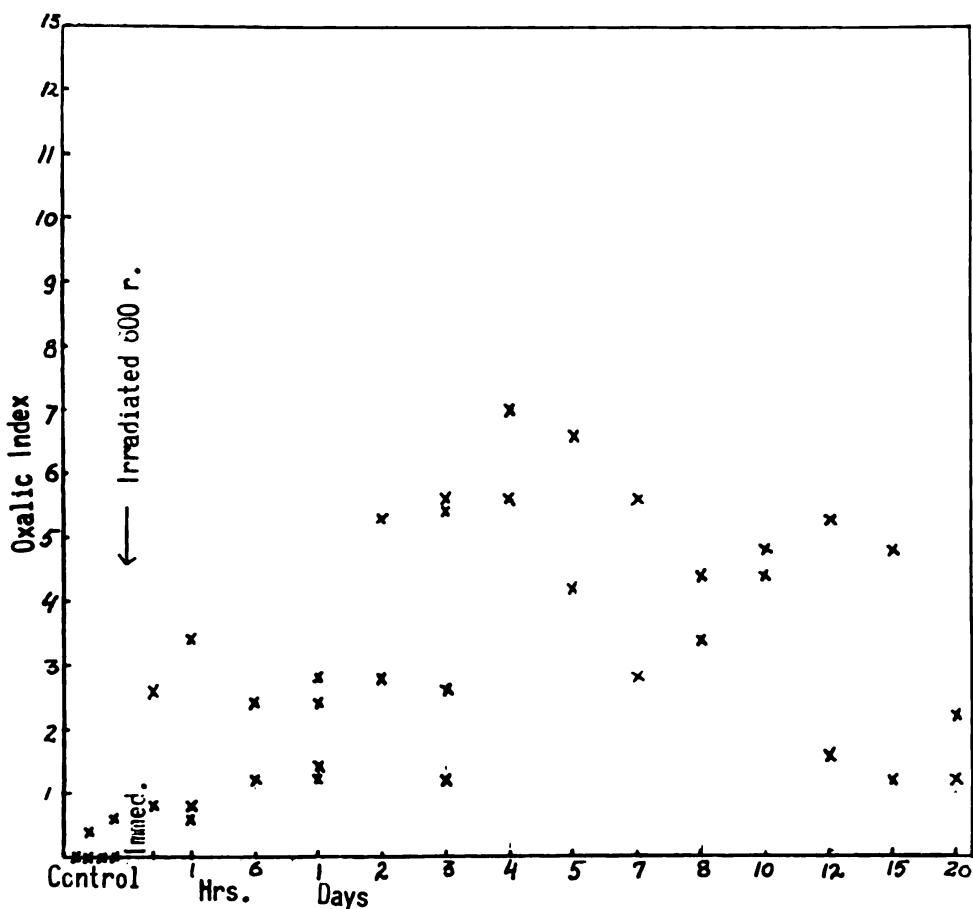


FIG. 91. Fatty acid conjugation and irradiation *in vivo*. The changes in the oxalic index of total fatty acids of rats submitted to sublethal irradiation (600r.). Only a temporary increase in the oxalic index of the fatty acids of the animals is seen, the amounts not reaching the critical values.

was made, penetrating the skin and subcutaneous tissues down to the dorsal aponeurosis. A needle containing radium was then placed between the lips of the cutaneous wound. A thread passed through the hole at one end of the needle was used to fix it to the skin. Two retaining sutures were also used to maintain the radium needle between the lips of the wound. The needle was left in place for the desired length of time and then easily

removed with the help of the thread passed through the hole of the needle. The retaining sutures were also removed and the wound left open and undressed.

The length of time that the needle was left in place varied with the amount of radium, the nature of the filtering metal, and the radiation burn desired. We found that 10 mg. of platinum filtered radium had to be left in place for about 90 hours in order to produce a standardized ulceration that would last about four weeks before healing. The same effect was obtained when 25 mg. of monel metal filtered radium was kept between the lips of the wound for only two hours. When monel metal needles were used for only one hour, too great differences appeared between the ulcerations obtained and the time necessary for their healing. A two-hour exposure caused an ulceration which usually required 4 to 5 weeks to heal in control animals. If the needle was left in place for 3 hours, the ulcerations were quite uniform but they required over two months to heal and more than half of the wounds never healed. Failure to heal and more extensive necrosis resulted for periods of exposure beyond 3 hours.

Therefore, we utilized 10 mg. of radium in platinum for 90 hours in one group of experiments, and 25 mg. of radium in monel metal for 2 hours in another group, in order to produce standardized ulcerations that would generally heal spontaneously after 4 to 5 weeks. This technique has been used in several hundred animals for various experiments. The fatty acids of these standardized radiation lesions were studied.

Days after irradiation, the ulcerated lesions were removed along with about one cm. of surrounding tissue and submitted to analyses. It was always necessary to use as many as 5 or 6 lesions to obtain the quantity of fatty acids needed for an oxalic index determination. The lesions were found abnormally rich in conjugated fatty acids. Commonly, indices as high as 40—and in exceptional cases as high as 65—were found (TABLE XVI) in comparison with 0 or 0.3 for normal skin with its subcutaneous tissues.

Lipids and Radiation Burns

g) The appearance of conjugated fatty acids as an effect of radiation has posed the problem of the role of these abnormal fatty acids as intermediary agents in the biological changes induced by radiation. In trying to solve it, we compared the effects obtained by administration of conjugated fatty acids with those of radiation at different levels of organization. This study was facilitated by considering the changes which take place in

TABLE XVI
OXALIC INDEX OF FATTY ACIDS OF RADIATION BURNS

	Elapsed Time	Average
Normal Skin		0.1
Non-treated wound	24 hours	2.2
	48 hours	3.9
	72 hours	2.3
	6 days	1.8
Wound with 25 mg. radium in	2 hours	1.2
monel metal for 2 hours	24 hours	6.1
	48 hours	13.9
	4 days	19.1
	1 week	31.0
	2 weeks	46.0
	3 weeks	49.4

the cellular cytoplasm and nuclei as induced by various substances designated as radiomimetic agents.

It could be seen that apparently all agents which induce radiomimetic effects are lipoids with negative polar groups. The effects of higher polyunsaturated fatty acids, and especially the conjugated isomers, appear to be the same as those of known radiomimetic agents. The similarity between the effects of these fatty acids and those of radiation makes it logical to consider that at least some of the radiation-induced changes result from the intervention of these abnormal fatty acids.

We have seen that the changes induced by fatty acids upon cell metabolism are in large part due to an increase in cell membrane permeability. A similar change of cell membrane permeability could be recognized among the effects of radiation. Following radiation, it could be seen that sodium of the interstitial fluids penetrates into the cells more readily. This was observed when radioactive sodium was used. (42) The cellular vacuolization seen to follow radiation, especially higher doses, represents a corollary of the abnormal penetration of sodium into the cells which partly results from the increase in membrane permeability.

h) At the tissular level, the influence exercised by radiation upon pain was seen to greatly resemble that induced by administration of fatty acids. Radiation efficiently relieves pain that has an acid pattern but it may increase pain of an alkaline pattern. Furthermore, pain which appears following radiation has an alkaline pattern and consequently is increased by further radiation, or administration of unsaturated fatty acids. (*Note 4*)

i) At the tissular level, it could be seen that the area of ulceration of the standard lesions obtained through irradiation of skin wounds was

increased by the administration of polyunsaturated fatty acids in general. In some cases the ulceration doubled in size as compared to controls. The administration of fatty acids also markedly delayed wound healing. When the quantity of fatty acids administered was great enough, the wounds did not heal at all. Six daily subcutaneous injections, each of 1 cc. of a 10% oily solution of cod liver oil fatty acids, prevented healing. (*Fig. 92*) The area of ulceration was even greater when only $\frac{1}{4}$ cc. of a 10% solution of the conjugated fatty acid isomers, obtained through an in-vitro conjugation of the preparation of cod liver oil fatty acids, was administered under the same conditions. This showed that conjugated fatty acid isomers had a more manifest effect upon radiation wounds than the unconjugated acids obtained from the same source.

j) We followed effects of intensive radio and radium therapy in humans at organic levels. In cases with radiation-induced proctitis, mucositis, or epidermitis, the changes observed were seen to correspond to the pattern encountered with fatty acid predominance, and especially to the pattern induced by abnormal fatty acids. The appearance of oxidizing substances in the urine is frequently observed in patients with radiation burns after extensive X-ray therapy. They were almost consistently seen in those cases in which radiation lesions were produced. The administration of fatty acids, and particularly of conjugated fatty acids, to these patients increased the intensity of the lesions.

k) Systemic changes induced by intensive radiotherapy were also analyzed. Here again, the changes followed the pattern observed when there is a predominance of fatty acids, particularly of abnormal fatty acids. The appearance of oxidizing substances in the urine was frequently noted after intensive X-ray therapy and, as mentioned previously, was consistently observed in those cases in which a radiation lesion was produced. Other systemic effects of intensive radiotherapy were seen to include an increase in urinary excretion of surface active substances, an increase of potassium in serum, a retention of chlorides and water, and particularly, an increase in the sulphydryl index indicating an exaggerated excretion of this group. These changes following intensive radiation are, as previously noted, similar to those seen when a predominant intervention of fatty acids occurs.

Certain of these changes appear to have prognostic importance for radiation therapy. For example, in several cases with very low urinary surface tension, high retention of chlorides and absence of urinary peroxides. The continuation of irradiation led to death. (*Note 4*) This is consistent with the findings in animals that lethal effects of irradiation are directly re-

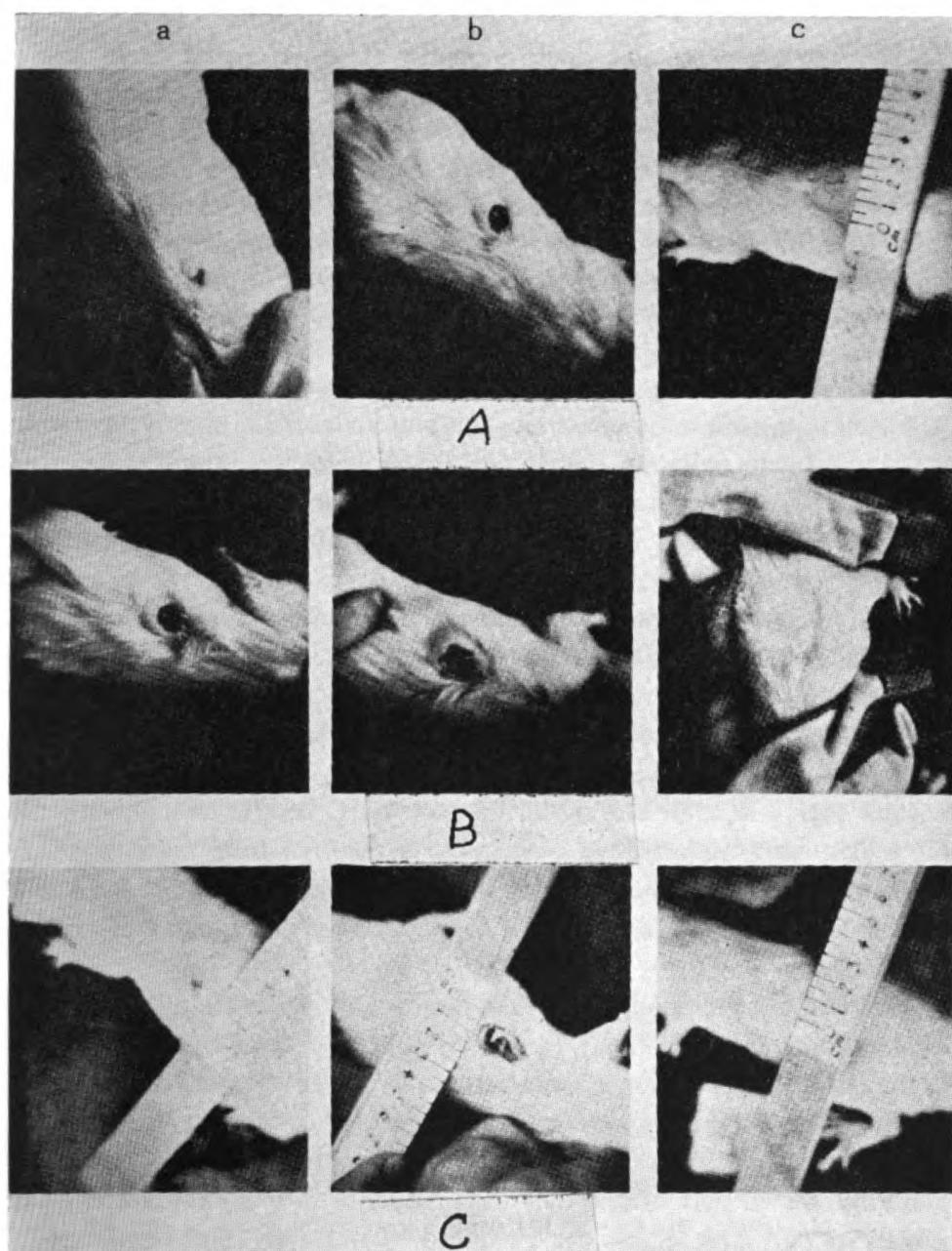


FIG. 92. Lipids and radiation wounds. Radiation wounds 5 weeks after exposure to 10 mgr. radium in platinum—for 96 hours. (a) Untreated controls; (b) treated daily with 1 cc of a cod liver oil fatty acids 10% solution; (c) treated daily with 0.5 cc of a 10% solution of unsaponifiable lipid fraction extracted from human placenta. The treatment with fatty acids results in larger lesions than in controls, with no tendency to heal. The treatment with the unsaponifiable fraction leads to a healing of the lesion in around three weeks.

lated to the appearance of large amounts of surface active substances in the urine.

ROLE OF ANTI-FATTY-ACIDS

The study of the biological effects of radiation also has revealed an important role for anti-fatty-acid agents. The intervention of these substances in the physiopathological processes that occur in the organism under the influence of radiation can be considered to be reactional. They correspond to a response of the organism to changes induced by radiation upon the constituents such as fatty acids.

This antagonism is clearly shown in experiments with animals. The administration of sterol preparations not only reduces the size of ulceration in standard skin radiation lesions but also significantly improves the rate of healing. Insaponifiable fraction preparations from human placenta, beef liver, spleen or blood, as well as from butter, produce such favorable effects. (*Fig. 92*)

If sterols are administered 24 hours after the radium is inserted (or later), the influence upon the dimensions of the ulcer that develops is reduced and is further reduced with increased delay. In some of the animals treated with 1 cc. of a 5% oily solution of the insaponifiable fraction of human placenta in sesame oil for seven days a week, healing with a normal scar was complete within two weeks. Controls, treated with the oil vehicle only, required an average of more than four weeks to heal. Similar effects were obtained with the administration of 1 cc. of a 7% solution of butanol in saline twice a day, beginning with the day of radium application.

The use of small amounts of radiation has, in general, a different effect to that of intensive radiation from this point of view. This can be attributed to the reactive intervention of anti-fatty acids. An exaggerated scar-forming effect, prolonged fibroblastic reaction, exaggerated connective tissue formation, and vascular sclerosis and thrombosis resulting from endothelial proliferation are all part of this long-term response to moderate amounts of radiation. The same effects are produced by anti-fatty acid preparations. All the manifestations are opposite to those obtained with high doses of radiation or fatty acids.

From a clinical point of view, the administration of the insaponifiable fraction preparation had a beneficial radiation effect. Even in lesions that had persisted for years, the pain was observed to disappear after a few days with t.i.d. doses as low as 1 cc. of a 5% solution of the insaponifiable

fraction of placenta in oil. In several cases, chronic lesions three to five years old healed in only a few months of treatment.

1) The opposite clinical response to high or low doses was frequently observed in the systemic changes in patients receiving X-ray therapy. While high doses led to a manifest lowering of the surface tension of urine and an increase of the sulphydryl index, together with the other changes corresponding to offbalance D for small doses of radiation, certain opposite effects related to a predominance of sterols were noted. Of particular interest was the absence of oxidizing substances in the urine, and the changes in urinary surface tension. In all cases treated, a first reaction to radiation was a higher sulphydryl index and low surface tension corresponding to a fatty acid predominance. When small or moderate amounts of radiation were used, this reaction was very slight and rapidly disappeared in favor of a second change corresponding to a predominance of sterols with high urinary surface tension, for instance. It is interesting to note at this point that this secondary response has been observed especially in those patients for whom radiation also has had a limited therapeutic effect. As we will see, the dualistic interpretation of data furnished by urinary analysis in patients undergoing radiation therapy can be used to guide this therapy.

Role of Adrenals

m) The study of the systemic secondary anti-fatty acid response to radiation has led to an evaluation of the intervention of the different anti-fatty acid agents and to the role of the adrenals. It is known that adrenal hormones have a peculiar effect upon the lymphatic system. They induce a shrinkage of the thymus, spleen, and lymph nodes, along with blood lymphopenia. Since similar effects are produced by irradiation, the problem of the part played by an intervention of these adrenal hormones in the radiation response is of interest.

When the adrenals were removed, shrinkage of the thymus and spleen and lymphopenia still occurred after radiation, but was markedly reduced as compared to nonadrenalectomized irradiated controls. Since shielding of the adrenals during irradiation does not alter the effect upon the lymphocytes and lymphatic organs, the role of the adrenals appears to be an indirect one. Adrenal hormonal secretion appears thus to be a response to the systemic changes induced by irradiation. Adrenalectomy would eliminate this secretion and thereby diminish the degree of lymphopenia and the involution of lymphatic organs. However, the secretion does not result directly as an effect of irradiation of the adrenals since shielding does not influence it. Another factor seems to intervene to stimulate adrenal hor-

monal secretion. The differences between the effects seen in adrenalectomized and nonadrenalectomized irradiated animals corresponds thus to the adrenal response to the systemic changes.

In experiments on rats, we have shown that polyunsaturated, and especially conjugated, fatty acids induce changes in the number of lymphocytes a short time after their administration, and that this is followed by involution of the thymus, spleen and lymph nodes. This seems to occur through the intervention of the adrenals since it takes place to a greatly reduced degree when the same amounts of fatty acids are administered to adrenalectomized animals. The abnormal fatty acids seem to influence the adrenals and their response elicits lymphopenia and involution of lymphatic organs. However, this indirect action through the adrenal glands is only part of the story. Large doses of the same fatty acids will directly induce a certain amount of lymphopenia and involution of the lymphopoietic organs since these changes also occur when these fatty acids are administered in large doses to adrenalectomized animals.

n) All of this research indicates that two of the mechanisms through which radiation acts upon the organism involve changes in lipids. In one, the action is directly through fatty acids; in the other, as a response to these fatty acids, anti-fatty-acid agents intervene. The role of the adrenals appears to be still more interesting considering the nature of the fatty acids produced by radiation. As seen above, the conjugated trienes appear almost specifically as a result of irradiation of mixtures of fatty acids. It was also seen that the corticoids intervene specifically against these conjugated fatty acids. This correlation seems to represent the link between radiation, conjugated fatty acids and the adrenal response. (Ch. 6, Note 17)

Direct Action of Radiation

o) In spite of the importance of fatty acids and anti-fatty acids, they represent only one part of the mechanism through which radiation acts. The direct and indirect action of radiation on other constituents also must be considered. The influence exercised upon these constituents can be largely related to various changes. There is a quantitative relationship, for instance, between induction of mutations and the direct impact of radiation on proteins. Changes in fatty acids also are the result of such a direct impact. It appeared interesting to ascertain how much and which of the pathological changes that follow irradiation are due to the direct impact upon lipids and how much to the impact on the other constituents.

The three kinds of biological activity of radiation—through other constituents, through changed fatty acids, and reactional through anti-fatty

acids—could be studied at different levels of the organization. We note here a few of the results of these studies.

Below the cellular level, the influence of lipids seems to decrease, causing the direct effect of the radiation on other constituents to appear predominant. For nucleo-proteins and below them, only this last effect seems to occur, the changes induced apparently affecting histones and alkaline amino acids. The close mathematical relationship between the amount of radiation and mutation would seem to indicate that, even at the gene level, only the effect upon constituents has to be considered.

The introduction of anti-fatty acids into the medium in which tetrahymena or suspended cells (as from ascites tumors) were irradiated, served to distinguish the direct effects from those induced through fatty acids. In the presence of anti-fatty acids, vacuolization and even changes in the nuclei seen in the irradiated controls are prevented. The fact that these changes, which characterize the radiomimetic effects, were reduced by anti-fatty acids, indicates the role of fatty acid changes in the pathogenesis of these effects. Among other agents tested, the insaponifiable fraction of organs, and especially of placenta, appeared to be most effective in preventing radiomimetic effects. The high alcohols or glycerol also showed such influence, but to a lesser extent. In complex organisms, the difference of the effect of radiation on fatty acids and on other constituents is increased at the higher levels. At the systemic level, this effect is almost limited to the fatty acids.

The introduction of polyunsaturated fatty acids to the medium greatly increase the toxic effects of radiation, as compared to controls exposed to radiation or fatty acids alone. The proportion of mutations was not changed, however.

The ultimate effect of radiation at different levels depends upon the relationship between three factors: changed fatty acids, other changed constituents, and the intervention of adrenals. The effect of the adrenals is progressively more manifest at the higher levels of the organization. At lower levels, the direct intervention of the abnormal lipids becomes more important than the adrenal response, the latter being less able to act at these levels. At the cellular level, the influence of lipids is still predominant. At the tissular level, the direct lipid effect is still striking, while the influence of the adrenal response is limited to the connective tissue. Although the effect upon the lymphatic constituents (as part of the adrenal response) is important at the organic level, the steroid response becomes more important at the systemic level.

p) On the other hand, it appears possible to vary the amount of the

lipidic effect by changing the nature of the radiation. The use of more penetrating rays or of different corpuscles has to be investigated in terms of the relationship between influence upon fatty acids and the effect on other constituents. It could be seen that, in corresponding dosages, the less penetrating radiations had a greater influence upon fatty acids than the more penetrating. The fact explains the reduction of radiation burns directly related to the intervention of fatty acids. Similarly, in a systemic procedure, such as teleradiotherapy, the effect on other constituents is reduced as compared with the direct influence exerted upon the fatty acids.

It is possible that radiations using neutrons would induce an increased direct impact on other constituents without a correspondingly increased effect upon the systemic fatty acids. The skin effect, which is minimal with these radiations, would indicate little intervention of fatty acids.

The unequal part played by lipids at the different levels can be utilized to obtain variations in radiation effects. If effects upon the lowest levels of the hierarchic organization, such as upon histones and basic amino acids, are desired, radiation could very well be the tool to be chosen, because of the small amounts of lipids present at these levels. If the influence could be limited to such action, radiation could be considered ideal for such therapeutic effects. Unfortunately, this is not possible even when very penetrating radiation is used, and the effect of radiation upon lipids still constitutes one of the principal factors which must be considered when radiation is used as a therapeutic weapon.

Thus radiation is not the ideal means for affecting the subchromosomal level, in spite of the fact that it may, through its effect upon proteins, have a profound influence below this level. Its ability to cause a conjugation of fatty acids represents the serious obstacle to its use. In view of this, the effect of radiation upon lipids actually can be considered as an undesirable epiphenomenon whenever the purpose of the therapy is to achieve a local effect at the lower levels. Frequently, the changes which require discontinuation of radiation therapy can be recognized to correspond to abnormal local or systemic metabolism produced by the abnormal fatty acids.

It must, however, be recognized that the appearance of abnormal fatty acids has some advantages even upon protein effects, since indirectly they can make local tissues more sensitive to radiation. We have previously noted that abnormal fatty acids cause changes in the tissue and cellular metabolism which lead to local alkalosis. This local pH change may have favorable results by acting upon the amphoteric proteins and by increasing the positively charged members which apparently are the only ones sensi-

tive to radiation. Indirectly, the intervention of the abnormal fatty acids will thus increase the sensitivity of tissues to radiation.

Before going further, we wanted to emphasize an aspect of the offbalance D for which the study of shock and radiation brought an important contribution. A separation can be made between two phases of offbalance D, one in which oxygen is principally fixed and another in which chlorine is fixed. The first phase, "oxygen," has as characteristic the appearance of peroxides in the urine, and clinically has little noxious manifestation. The other phase, "chlorides," with fixation of this ion leads the serious manifestations as seen, for instance, in shock. For this reason, in radiation the disappearance of urinary peroxides with persisting offbalance D, as seen in the other patterns, will indicate a passage from phase "oxygen" into phase "chlorides," which corresponds to the appearance of a serious condition. (See also Note 4, Chapter 10)

Radio-Therapy

The above considerations appear important in the radiotherapy of tumors. The tissular and systemic changes related to the intervention of fatty acids, especially when these changes are sufficiently intense, in themselves can act upon tumors. However, when abnormally intense, they can constitute a serious limitation for continuation of radiation. The manifestations that result from the pathogenic effect of abnormal fatty acids, if intense, can prevent the use of large doses of radiation which would otherwise be necessary to influence a tumor through a direct effect upon the lower levels of the biological organization, histones, nucleo-proteins and even genes. Consequently, the appearance of abnormal fatty acids, which represent an important factor in the biological effect of radiation, can be considered as a favorable effect when we seek to bring about systemic changes and influence pain and metabolism, particularly at higher levels. At the same time, they can also represent a principal obstacle to the more effective use of this same therapeutic agent when one wants to obtain an effect at lower levels.

As for the effects obtained through the influence exerted by fatty acids, they can be decreased by changing the antagonistic relationship between the abnormal lipids and the defense mechanism of the adrenals. With small amounts of radiation separated by long intervals, the intervention of the adrenals, as long as they function normally, can overcome the effect of the fatty acids. With higher doses applied more often, the fate of the irradiated individual depends upon whatever antagonistic factor predominates. With high doses or with a relative adrenal insufficiency, the direct effect of

the abnormal fatty acids can become predominant. In that case, the type D offbalance will be more pronounced. It is in such offbalance that subjects die from too intensive radiation. These factors can be of major significance in the intervention against accidental radiation as well as in guiding the therapeutic use of radiation.

Because of the intervention of abnormal fatty acids, systemic radiation does not seem to be the best procedure unless a very intense systemic effect is sought. If this effect is desired, it can be obtained through a method other than radiation. Furthermore, as we have noted above, the conjugating effect of radiation upon fatty acids is almost entirely limited to the production of trienes and dienes. The biological effects of such conjugated fatty acids are more apparent at the tissue level and above it. The energetic value of conjugated trienes and dienes seems to be too meager to permit them to act intensively at levels lower than the cells or nuclei.

In order to have a manifest fatty acid effect, it appears necessary to have an adequate application of radiation. Since an exaggerated systemic action of the abnormal fatty acids may even induce lethal effects, radiation does not appear to be the therapeutic method of choice for an influence exercised through fatty acids. Radiation, however, is more compatible even with a desired localized effect through the limitation of the field in which the changes in fatty acids are induced. In this case, fatty acids may intervene with a lower systemic influence. This accounts for the analgesic action of radiation which probably is related to an effect exercised by local fatty acids. Even here, however, the appearance of an alkaline pattern of pain can lead to undesired changes. In this case, radiation will increase the intensity of pain. This fact reduces the indications for use of radiation even at the tissue level.

Biologically-Guided Radio-Therapy

The knowledge of the important roles played by abnormal fatty acids and anti-fatty acids in the biological effects of radiation has suggested a biological guide for radiotherapy. Urinalysis, by reflecting various systemic changes, can serve as a valuable indication of manifestations and processes present in subjects undergoing radiation. The persistence of a pattern related to predominance of fatty acids indicates that the patient has passed into an imbalance that can only be increased by further irradiation and, if it becomes sufficiently intense, may even prove to be lethal, causing the patient to die with symptoms of severe shock. In contrast, a pattern corresponding to the predominance of sterols could be considered as being consistent with a preponderant reactive response, which would indicate that

higher amounts of radiation could be used without danger. From a practical point of view, the information given by the urinary surface tension has appeared very valuable. The moment when, and the amount of, irradiation to be given can be determined by these analyses. A low surface tension would contraindicate administration of radiation while high values would indicate that radiation should be increased.

The administration of lipids or lipid-like substances would represent a method of controlling undesirable processes and allowing more effective use of radiation. If the reactive intervention of adrenals appears too strong, a lipoid with negative character could be added to counteract this and, consequently, could increase the desirable effect of the radiation. Subjects receiving fatty acids or sulfur preparations along with radiation have shown intensive local effects with very small doses of radiation. Epidermitis and mucositis were seen in such patients even with doses as low as 600 r. The same intensive effect could be seen in the tumors. The use of lipoids appears indicated when an intensive effect through fatty acids is sought, as in lymphatic tumors. On the other hand, if the effect of fatty acids is higher than can be accepted, and represents a handicap for the desired effect on proteins, then adrenal hormones or other anti-fatty acids must be added. By reducing the effect of abnormal fatty acids, it becomes possible to obtain a more intense impact on proteins and, at the same time, to avoid the otherwise inherent undesired side effects. The choice of the anti-fatty-acid agent must be guided by the level at which the effects of the abnormal fatty acids would make themselves felt. While corticoids act especially upon systemic and organic levels, sterols and other positive lipoids act upon the lower levels. Butanol and similar agents are effective upon local changes, such as pain.

The guidance of radiation therapy, as an example of how this new view may be used to improve therapeutic approaches, will be discussed later.

CHAPTER 11

PROBLEMS IN CANCER

NEW INSIGHTS INTO many pathological problems—and those of cancer in particular—are offered by the concepts we have been discussing. Let us take, for instance, the problem of just what cancer is. Classically, one is entitled to speak of a condition as cancerous when cells with cancerous character are present in the body. Whether, on the one hand, only cancer-in-situ cells are identified or, on the other hand, the patient is dying and has almost no organ or function left untouched—the condition is considered cancerous. Yet, so long as the concept of cancer is associated implicitly with the concept of malignancy, to consider clinically healthy individuals to be cancerous only because of the presence of cells with cancerous nuclear characters, when most of them will never show further development of the disease, is entirely confusing. It is essential to separate the two concepts, the presence of cancerous entities and actual malignancy.

The fact that the hierarchic levels of the organism participate in the various manifestations of cancer puts the problem in its true light. A cancerous condition does not implicitly mean malignancy when it involves only the presence of an entity with cancerous character. Other attributes must be considered. In the hierarchic progression of cancer, malignancy begins to be manifested when the cellular level participates and induces invasive cancer. With malignancy an attribute of only some of its phases, cancer can be seen to embrace many changes, beginning with those of the lowest hierarchic entities and terminating with the systemic lethal condition.

The plurality of phases of cancer, with the broad variations in time and other factors which determine the passage from one phase to the other, logically raises several immediate questions.

In view of the multiple phases, one cannot speak of pathogenesis of cancer in general, but rather of pathogenesis of the different phases. Con-

sequently, even postulating the existence of some specific original cancerous change, such a change would not, alone, induce the entire disease and determine the passage through successive phases. Different pathogenic factors must be considered to intervene in order to have cancer pass from one phase into another. The evolution of the cancerous condition has to be related to these different factors, some of them possibly more important than the original specific change. The passage of a cancer from the non-invasive to invasive phase, or from tissue to systemic, is surely more important than the appearance of a low level cancerous entity. An original change at a lower level appears, in fact, to be of very little importance, not only because of its ubiquity but also because it is not implicitly related to malignancy. From this point of view, then, cancer no longer can be defined as some single specific change in a cell, nucleus, chromosome, gene or other biological entity.

Carcinogenesis has to be conceived of in an entirely new way, in terms of plural factors and their relative values. Accepting the phases above invasive cancer as the only ones which correspond to clinical malignancy, they have to be regarded as the end result of a series of cancerous changes developed at progressive levels, with the intervention of many factors, not just one.

Diagnostic Tests

This view puts the problem of diagnosis of cancer in a new light. The recognition of a cancerous condition by itself, although important, has little clinical meaning. The presence of "cells with cancerous characteristics" in the prostate of almost all men 40 years of age and older, and in the thyroid, lung and stomach in a high proportion of the population, has failed to produce a general feeling of despair only because such findings are commonplace. While they still mean cancer, they do not implicitly indicate malignant disease. It appears very clear that a diagnosis of cancer is incomplete without immediate qualification as to its phase. We can no longer speak of cancer with any degree of practical meaning unless we add a descriptive adjective—noninvasive, invasive, tissular, organic or systemic.

And the search for a test to detect cancer will have no meaning as long as we have not defined in advance the information we want. A test, biochemical or immunological, which indicates the existence even of a specific anomaly in the noninvasive phase or before, while interesting, will have little significance since this anomaly exists in so many subjects and for most does not go beyond the noninvasive phase. The test will not indicate malig-

nant cancer in the clinically frightening sense. On the other hand, the processes which are added to a noninvasive phase form of cancer and turn it into the invasive, tissue, organic or systemic phase, have no character of specificity. Similar growth changes, or the appearance of lipidic predominance, which represent added factors are seen in many other conditions. By using them for diagnostic purposes we will not recognize the cancerous condition but only nonspecific intervening factors. While these factors are responsible for the changes, malignancy develops only when, and because, these factors operate on already abnormal entities, *i.e.*, cancerous entities. This explains the nonspecificity of many proposed tests and the misleading positive results obtained in conditions such as pregnancy where one of these added factors, (active growth processes) is always present.

A test for cancer, to have clinical value, would have to indicate two things: one, the specific early change which is widely distributed but represents the essential condition for the potential development of malignancy; and, two, the concomitant presence and concomitant operation of the non-specific factors which can cause the actual development of malignancy. This kind of diagnostic test undoubtedly will come from further systematized study of biochemical changes induced by the simultaneous action of the two groups of factors.

Immunological studies represent an approach of value for diagnosis. The different phases of cancer can be interpreted, in the final analysis, to correspond largely to the intervention of the defense mechanism at different stages at the different levels. As mentioned above, a change in a phase results also from a change in the defense stage at the respective level. We have seen that the immunological aspect of cancer cannot be understood without accepting a relative independence of the levels in their different stages of defense. This view explains some seemingly paradoxical occurrences.

Cancerous cells are frequently found circulating in the blood yet this does not indicate generalized cancer. While the organism defends itself successfully at the systemic level against cancerous cells, the cancer can still progress at the lower level of the tissues. The loss at this low level of an effective defense, principally primary or allergic, which is still persistent at the systemic level, explains why the cancerous cells invade the tissues. A test indicating the presence or absence of any immunological reaction would consequently have value only when related to hierarchic levels. It must furnish indications only of what is happening at a specific level. The nature of the immunological reaction in cancer is also different from the reaction in other conditions. Defense capacity—natural defense capacity—

at different levels is lost as the respective level participates in the disease. This is in distinction to the immunological processes in other conditions in which the normal individual lacks specific immune bodies. An immunological test for cancer would have to reveal the loss of a previously existing defense mechanism rather than the appearance of an immunological response. This loss can be revealed in different ways. In one, the response to a cancerous antigen is investigated, and its lack would indicate the existence of a cancerous condition at this level.

In a study now in progress, we utilize pooled human tumoral tissues as antigen, and try to see if an allergic reaction can be induced with it, in two administrations, sensitizing and trigger. Two intradermic injections at the same site are made 12 days apart. They induce an allergic reaction in normal individuals, but are without effect in patients with active malignancy. If the effect is negative, a third injection is given 15 days later. A similar test is made for the conjunctiva, with sensitizing and trigger instillations of a similar antigen. No allergic reaction indicates a positive result, while a reaction is considered to be normal.

Another test which we are studying is based on the same lack of efficient defense mechanism at the tissue level. Such a lack of defense would permit a cancerous antigen to be present without the body offering a sufficiently effective defense against it. The presence of such an antigen in the tissues is revealed by inducing an allergic reaction, through the administration of specific coagulant antibodies. Sera of guinea pigs injected with pooled human tumors and having a high precipitin content are used in intradermic injections or in eye instillation. An immediate reaction indicates a positive result. As control, we use normal guinea pig sera. The studies are now in progress and the diagnostic value of these tests will be reported in a later publication.

Circulating Cancer Cells and Surgery

These immunological considerations have appeared important in considering a problem related to the use of surgery in cancer. The existence of a veritable flow of cancerous cells in the general circulation, largely induced by the manipulations inherent in operative procedures, has produced grave doubts as to the value of the measures taken by surgeons to prevent local spread of cancerous cells through the surgical act itself. Paradoxically, however, these precautions have been followed by good clinical results. Analysis from the point of view of the defense mechanism involved can clearly explain this situation. In the invasive phase, the systemic defense mechanism is still adequate to insure destruction of cancer-

ous cells which get into the blood. This is not true at the level of the interstitial formations, that is, at the tissular level, where such defense means are failing. The real danger during surgery consequently is not so much the presence of cancer cells in the blood, since the blood can still take care of them, but the spread of these cells at the tissular level where the defense capacity has been lost, and where a cancerous cell consequently has every chance not only to remain alive but also to grow.

The independence of the defense mechanism at different levels also must be taken into account in explaining the differences in the events which follow the appearance of a spontaneous tumor and those which occur after experimental tumor transplantation.

EXPERIMENTAL CARCINOGENESIS

The problem of carcinogenesis appears in a new light when cancer is considered under the concepts presented above. Classically, the experimental induction of cancer is judged successful only if the result is a tumor in the invasive phase, that is, with abnormal cells invading normal surrounding tissues. This is considered to correspond to a fundamental specific change which transforms the normal cells into cancerous ones. The entire disease is held to stem from the relationship between these abnormal cells and the organism. (290, 291, 303) To these simple views of the abnormality, we have proposed another one.

In our view cancer represents a hierarchically organized condition. Its invasive form is only one phase in a long series of changes which transforms successive hierarchic entities into cancerous entities. Carcinogenesis, thus, is not simply a change of a normal cell into a cancerous one but a step by step progressive hierarchic development. A cell is cancerous only if it has a cancerous nucleus just as a nucleus is cancerous only if it is formed by cancerous chromosomes which, in turn, are cancerous if they have cancerous genes. With the same reasoning, it is possible to go far down in the organization, below genes even to nucleo-proteins or still lower to histones or even alkaline amino acids, to find that the first changes, which can be considered to be specific for cancer, take place at the bottom of the organization of the biological realm. In other words, a cell becomes cancerous after specific cancerous changes have occurred in all the hierarchically inferior entities that compose it. Thus, a successful experimentally induced cancer, *i.e.*, one that is already in the invasive phase, means that changes would have affected the entire series of hierarchic entities, including the cells, whose participation results in the invasive character. Seen under this

aspect, carcinogenesis no longer can be accepted as a simple process occurring in the cells, but must be regarded as a succession of organized processes.

This becomes still more interesting when it is realized that changes in the constituents at the lowest levels of the organization can occur on a statistical basis, that is, independently of the direct intervention of external agents. As these changes have to be developed for many successive hierarchic entities, it takes a certain time for them to be realized. This would explain why most cancers appear after a certain age. Cells with cancerous nuclei, *i.e.*, in the noninvasive phase, frequently are present, in older people, in many organs without producing clinical manifestations. Conceptually, in order for an agent to be considered a successful carcinogen, it must act upon these noninvasive entities to such an extent as to change them into invasive ones. It can thus act upon entities which have already progressed, by themselves, far enough in the hierarchic development of a cancerous process and have arrived at the noninvasive phase without any manifestation. The excessive length of time necessary, even for the most active agents, to induce invasive cancer would suggest, however, that more than a simple passage from an already existing noninvasive cancer into an invasive one is involved. A plurality of changes must be induced, some or all at levels below the cell.

We are inclined to favor this last hypothesis which obliges us to consider that a carcinogen induces changes at different levels of the organization. It is supported by a series of facts. In addition to having the capacity to induce invasive tumors, carcinogenic agents also induce precancerous lesions which correspond to cancerous entities below the invasive phase. Cells with abnormal nuclei or with only abnormal chromosomes are almost constantly seen in induced carcinogenesis. Even agents which produce a high proportion of invasive cancer consistently induce such changes at lower levels as well. For carcinogens which induce a low proportion of invasive cancers, the effects often appear to stop at lower levels. Such activity at subnuclear levels of the organization is seen in the capacity of most of the carcinogens to induce mutations and monstrosities.

In the concept of hierarchic organization, mutations are considered to result from changes taking place at the gene level, with lower levels left unaffected. Monstrosities result from changes at the chromosome level. Comparison of carcinogenesis with mutations and monstrosities has led us to consider that cancerous changes begin at levels much below those involved in mutations and monstrosities, possibly at the nucleo-protein level or, even below. The complex cancerous condition to which the invasive

form corresponds can thus be seen to be the result of a series of anomalies which have taken place at different levels below the tissular. Carcinogenesis at the invasive phase is conditioned by the existence of changes at all the lower hierarchic levels. While they can appear as the result of the development of the organization, conceivably these changes can be hastened or even induced by the carcinogens.

The concept of multiple changes in carcinogenesis has caused us to search for multiple factors in carcinogens themselves. The possibility that such factors might be found was suggested by the existence of so-called co-carcinogenic agents. These are substances without carcinogenic activity of their own but capable of inducing such activity in cases in which some carcinogens are administered in doses too small to induce invasive cancers by themselves. This peculiar intervention of co-carcinogens can be explained through the multiple factors in carcinogenesis.

It can be conceived that the factors present in a carcinogen do not have equal activity. The differences appear evident when the carcinogen is administered in very small amounts. While some factors still have sufficient potency in these small amounts to accomplish their part in the complex process of carcinogenesis, others are quantitatively insufficient and do not induce changes. The total effect is an incomplete set of changes. Under these circumstances the addition of a co-carcinogen can replace the action of the quantitatively inadequate factors, and consequently complete the plural action necessary to produce an invasive cancer. Because any one co-carcinogen can replace only certain factors, co-carcinogen activity has a certain specificity.

With the hypothesis of multiple actions in the same carcinogen, the next step was to try to recognize them. A study, identifying different active energetic centers in the structures of carcinogens, has substantiated the hypothesis.

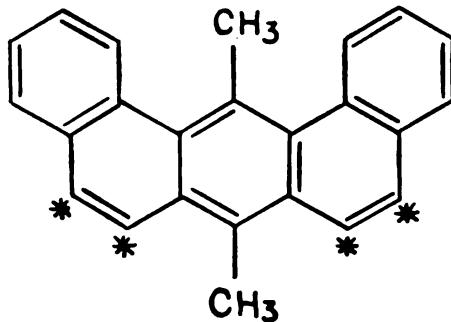
We attempted, as a first step to systematize the analysis of such energetic centers in carcinogens. A short resume of this study is presented here.

Energetic Factors

A well known and generally accepted concept tries to correlate carcinogenic activity with the presence of one energetic factor, identified as a "condensation of electrons," at certain regions of a molecule and revealed by the physicomathematical approach offered by Pulman and Dawdel.

Studies of the role of electron distribution in carcinogenesis were started by Otto Schmidt (43), which showed that an electron density exceeding $0.44e/a^2$ in the meso region of the molecule appears necessary to confer

carcinogenic properties. This concept was partially modified and amplified by Pulman, Dawdel and their co-workers (44) who have shown, by quantum analysis of various carcinogens, that the density of the π electrons is increased in certain preferred regions of the molecules, the *K* regions. They showed that, when electron densities exceed 1.292e at these regions, the substances have carcinogenic properties. Figure 95 shows such a *K* region.



9:10 Dimethyl 1:2:7:8 Dibenzanthracene

FIG. 95. The regions *K* in carcinogenic molecules.

From our point of view, a tentatively interesting aspect of this condensation of π electrons lies in two facts: the presence in some carcinogen molecules of more than one such *K* region, and the presence of different values for these *K* regions in different molecules. It would be the presence of more than one *K* region in the same molecule which would result in intervention in more than one process and thus contribute to plural activity.

Further analyses, however, suggested that the condensation of π electrons in *K* regions would represent only one of the factors that would induce activity in these agents. We have identified another energetic factor in the presence of two atoms having the same electrical sign and being bound together within the molecule.

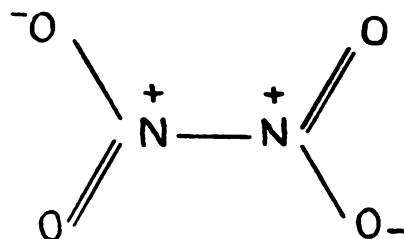
Twin Formation

We have considered the existence and importance of these "twin formations" as indications of energetic activity in the course of studies on electronic molecular arrangements. In a molecule, an alternation of successive atoms results in part from the alternating polarity of these atoms within a molecule and in part from the opposite characters conferred upon the

two carbon atoms when they form acetic acid, an important precursor in biological syntheses. It is through alternate polarity that an induction effect of an energetic center in the molecule propagates itself along the chain. The presence of any energetic center in the molecule represented by polar groups or a lateral chain, for instance, will enhance this alternate polarity. When one or more such inductive effects are propagated through the chain, two adjacent atoms may be found to possess the same electrical sign for their charge or ionoid character. The twin formation which results represents a center of increased molecular reactivity. This reactivity can be so intense as to lead to breaking down of the molecule, something which occurs often in inorganic substances. This has led Pauling to believe that this condition, called "adjacent charge rule," cannot exist.

"Pauling has pointed out that the mutual potential energy of two electrical charges of the same sign is so high that a canonical structure having net residual charges of the same sign on any adjacent atoms would have too high an energy level to contribute appreciably to the real molecular structure." So notes William A. Waters in "Physical Aspects of Organic Chemistry." (45)

The form suggested for nitrogen peroxide (N_2O_4), (Fig. 96) would appear to be impossible because of the high energy developed at the two positive nitrogens.

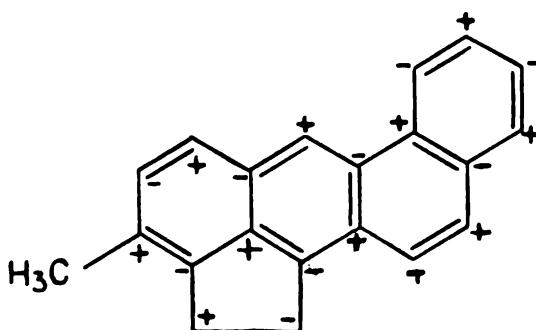


Nitrogen Peroxide

FIG. 96. The existence of nitrogen peroxide molecule is prevented by the high energy developed at the two adjacent positive nitrogens.

However, the forces that exist in most of the organic molecules are much weaker, so that the resulting "twin formations," although energetically potent, are not strong enough to induce the breaking down of the molecule. Consequently, they would exist and represent important energetic centers.

We have studied a number of carcinogenic agents, seeking twin formations. Analysis of the ionoid character of the carbons of the methylcholanthrene molecule reveals the presence of twin formations which could be localized at various points of the molecule. Figure 97 shows the energetic aspect of methylcholanthrene and the ionoid character of its carbons. It is the presence of the cyclopentane group in the molecule that induces the same sign in two adjacent carbons. The presence of the methyl group would determine the electrical character of C₂₀ and consequently the succession of alternate signs. On the other hand, the double bonds will determine the probable localization of these twin formations in the molecule at the K formation itself, that is, at C₅ and C₆.



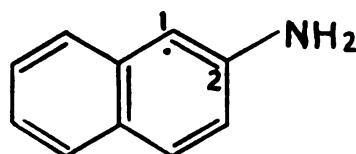
20-Methylcholanthrene

FIG. 97. The energetic aspect of methylcholanthrene, with twin formations.

Twin formations can be found in many carcinogens. It must be emphasized, however, that unequal energetic values can be recognized easily for different twin formations and would explain differences in their activity, a fact which would confer possible plural properties upon this group of qualitatively similar energetic formations.

Another aspect of the relationship between these formations and carcinogenesis appears to be even more interesting. While no twin formations can be found in several agents, the formations are present in the substances resulting from metabolism of these agents in the body. The relationship of twin formation to carcinogenic activity can be suspected when such changes appear simultaneously with carcinogenicity.

For example, no twin formation occurs in 2-naphthylamine, (Fig. 98) whose direct carcinogenicity is questioned, but such a formation appears in heterocyclic 3:4:5:6 dibenzcarbazole, one of its intermediates (46), which is known for its carcinogenic properties. (Fig. 98bis) This is also true for

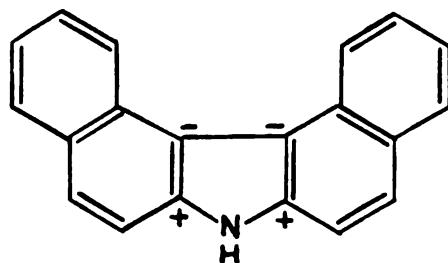


(a)

2-Naphthylamine

FIG. 98. No twin formations exist in 2-naphthylamine.

aminofluorene, which is also related to 2-naphthylamine. (Fig. 99) The existence of a twin positive carbon group or a twin negative in the same molecule can further explain the diversity of the tumors produced by this carcinogen and its acetyl derivative, which has the same energetic picture. (47, 48, 49, 50, 51, 52)

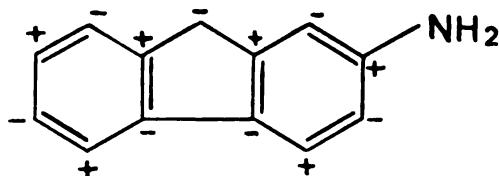


(b)

3:4:5:6 Dibenzcarbazole

FIG. 98bis. A twin formation appears in the intermediate 3:4:5:6 dibenzcarbazole.

Twin carbons can be correlated with the degree of carcinogenicity of the sulfur isosters (53) in each of which a thiophene nucleus replaces the benzene ring of 9:10 dimethyl 1:2 benzanthracene. This also applies to the azo compounds with twin formation at the level of the azo bond. Figure 100 shows the presence of a twin nitrogen at the level of the azo bond, due to the influence exerted by the symmetric rings.

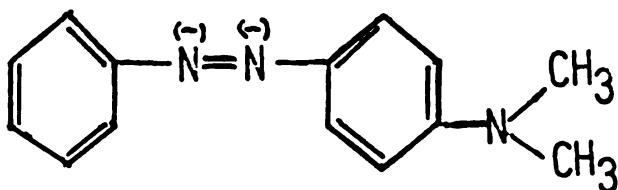


2-Aminofluorene

FIG. 99. A twin carbon group is present in aminofluorene.

Furthermore, it is the relationship of twin formation to carcinogenicity which indicates the need for considering the metabolism of various carcinogens in the organism.

Dimethylamino-azobenzene, butter yellow, which has a twin formation and is an active carcinogen, can become still more active through the metabolic changes occurring in the body which lead to products with twin carbons. The same 2:2'-azonaphthalene, with a twin formation, becomes more active because of its transformation into amines passing through hydrase compounds. 2:2'-diamino-1:1'-dinaphthyl, with twin car-



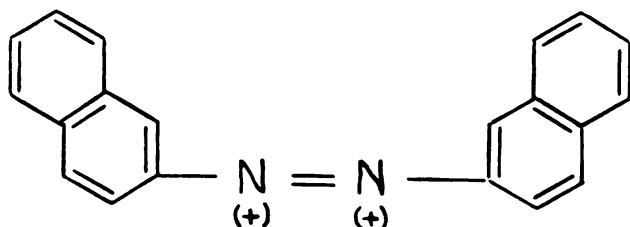
4 - Dimethylamino azobenzene

FIG. 100. A twin formation is present in 4-dimethylamino-azobenzene at the level of the azo bond.

bon formation, is more active than the precursor, 2:2' Azonaphthalene. (54), (Figs. 101 and 102)

It is possible that benzidine rearrangements of the hydrazo derivative determine twin formation and thus explain its carcinogeneity.

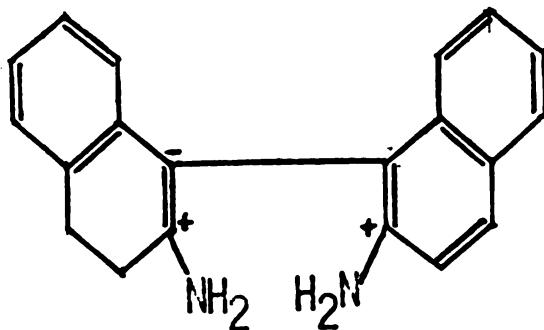
The similarity in kinds of tumors produced by the derivatives of 4-aminostilbene (Fig. 103), and the aminofluorene derivatives (55), makes us think that twin formations can appear in this case through changes occurring in the organism.



2:2' Azonaphthalene

FIG. 101. 2:2' Azonaphthalene has only a slight activity.

Some artificial estrogens of high potency (56) diethylstilbestrol and triphenylethylenic acid, (57), (*Figs. 104 and 105*) are known to have carcinogenic activity. While a twin carbon is present in both, such a formation is assumed to appear more active in the latter, as the result of metabolic changes in the body.



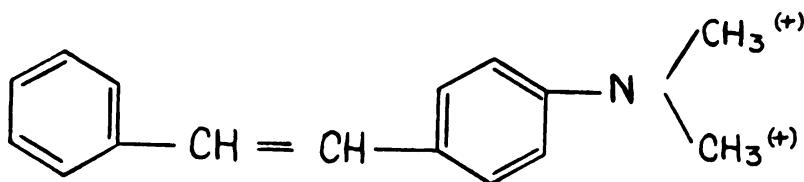
2:2' - Diamino 1:1' - dinaphthyl

FIG. 102. The passage of 2:2' azonaphthalene into the active 2:2'-diamino 1:1'-dinaphthyl results in the appearance of an active twin carbon formation due to the influence exerted by the amino-group.

An interesting aspect is furnished by urethane and other esters of carbamic acid. Figure 106 shows that no twin formations can be seen directly or through a change in the molecule. This accords with these substances' lack of capacity, noted by many authors, to induce cancerous lesions or even tumors. (58, 59, 60) Orr (61) relates lesions produced by carbamic

acid esters to chronic inflammations, noting their regression when treatment is discontinued. (Note 1)

From analyses of the substances able to induce invasive cancers, it can be observed that many present a twin carbon or nitrogen formation, usually activated by the induction exerted by a polar group or by double bonds. Some of these substances originally without twin formation become carcinogens only when changes occur in the body leading to the appearance of a twin formation.

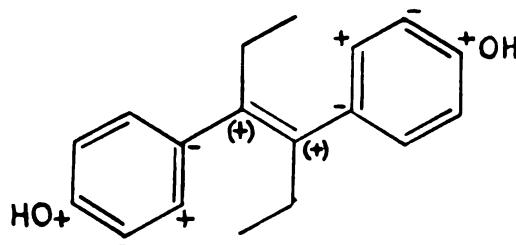


4-Dimethylaminostilbene

FIG. 103. 4-Aminostilbene derivative.

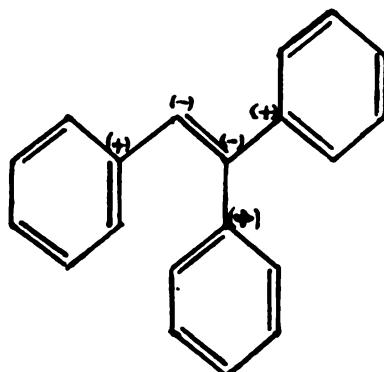
It must be emphasized, however, that according to the concept of plural factors in carcinogenesis, twin formation does not appear to be an obligatory condition for carcinogenic activity; other factors can produce such activity.

It is interesting to note that in most carcinogens, especially in the hydrocarbons, the twin formation is electrophobic due to its richness in electrons. For the present, we wish to stress only that in substances considered to be actively carcinogenic, *i.e.*, capable of inducing invasive cancer, twin



Diethylstilbestrol

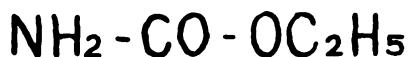
FIG. 104. A twin formation exists in diethylstilbestrol.



Triphenyl-ethylene

FIG. 105. The position of the twin formation in triphenyl-ethylene.

formation appears to be an added factor which insures complex activity. Intervention of groups of two energetic centers with the same character, in carcinogens, places in a special light a group of agents which, under particular circumstances, induce tumors. One group with alkylating activity, is formed by the nitrogen mustards, diepoxides, polyethylene amines and dimethanesulfonyloxyalkanes. One of the physicochemical characteristics of this group is the presence of two electrophilic centers near enough to each other to permit joint action. Still more important seems to be the fact that, through changes in all these substances, new formations may appear which energetically could be ultimately considered similar to twin formations. Through this character, their activity could also be parallel to that encountered in the carcinogens mentioned above.



Urethan

FIG. 106. Urethan has no twin formation and apparently—according to many authors—no *direct* carcinogenic activity.

Nitrogen Mustard Derivatives

The most representative and better studied substances of this group are the nitrogen mustard derivatives, characterized by the 2-haloethyl amine group (*Fig. 107*) attached to a radical which can be aliphatic or aromatic. It seems that it is through hydrolysis that the compound becomes biolog-

ically active, and Haddow has shown that activity is present only if hydrolysis is sufficiently high. (62) The inequality of hydrolysis in different members can be related to the influence exerted by the radical bound to the nitrogen. It seems that the presence of a stronger energetic center, as it appears in positively or negatively charged atoms bound to the cyclic radical, reduces the dissociation of the chloroethyl group. Generally, nucleophilic groups would retard the dissociation. Sufficient evidence exists to show that biological activity follows the elimination of the chloride ion and the appearance of a carbonium ion as a reactive intermediate. A further passage into the ethyleneimmonium ion, considered more stable and

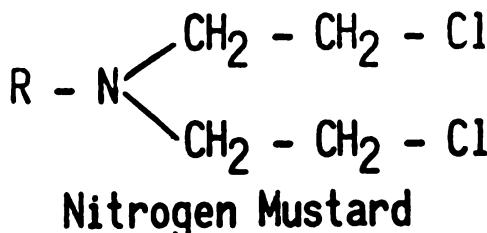


FIG. 107. The nitrogen mustard derivatives.

therefore less reactive, seems to complete the transformation. Figure 108 shows these changes.

The haloalkyl side chains in the molecule appear indispensable for biological activity. (63, 64, 65, 66) They lead to the immediate appearance of two positive electrostatic energetic centers. This does not represent a minimal condition, according to Landing and co-workers. (67, 68) These investigators have shown that in nitrogen mustards, cytotoxicity increases with the number of haloalkyl side chains. It is to be noted that a double electrophilic center is found not only in the two original haloalkyl side chains, but also in the later product, the ethyleneimmonium ion. In view of the more frequent appearance of this ion also for other agents, the analysis of the relationship of this group to twin formation appears interesting.

In the ethyleneimmonium group, while a negative charge can be seen at the nitrogen, a positive charge appears to be present between the two CH_3 , providing a certain polarity. With two carbons positively charged and in a relatively fixed position, this group is similar energetically to a positive twin carbon group. A two-step change, with the imonium group in the first, and a carbonium in the second, can explain, as we shall see below, the strange biological activity of the nitrogen mustards which have a carcinogenic activity only through changes which take place in the organism.

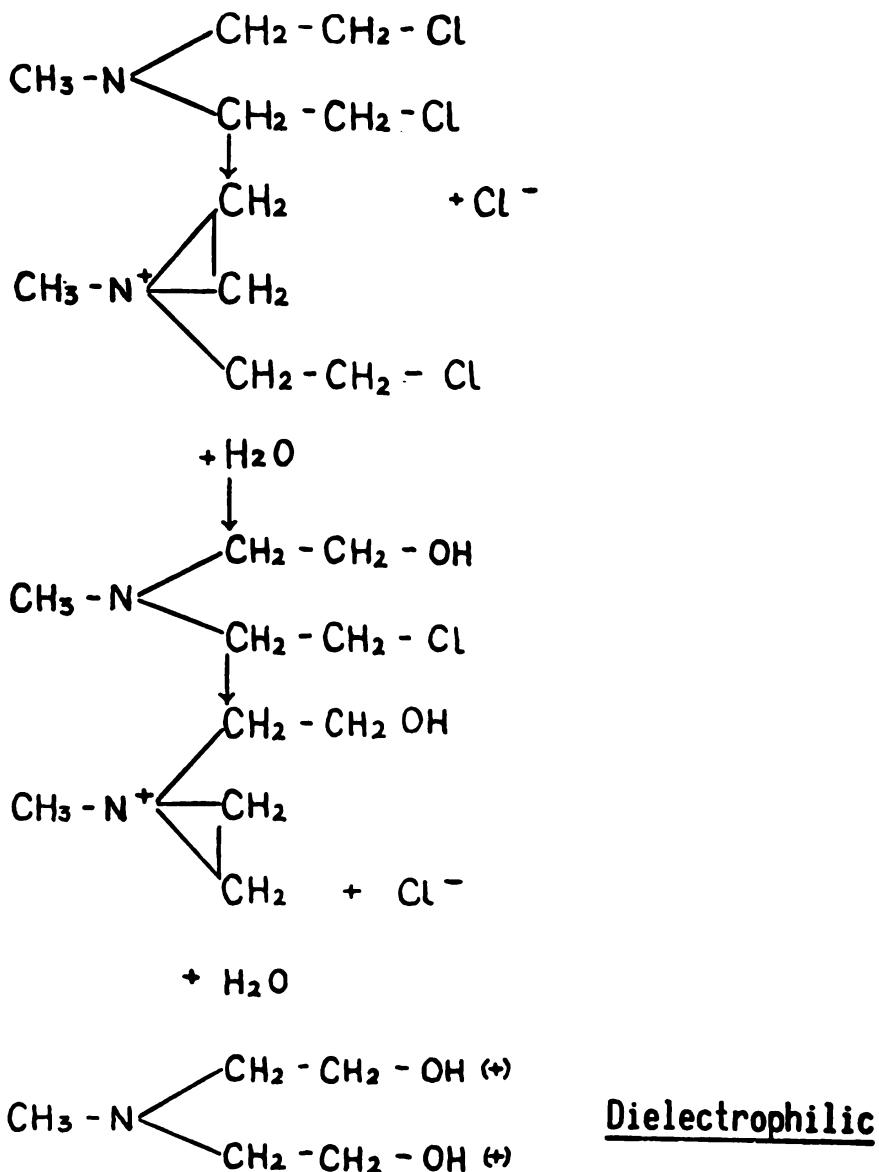
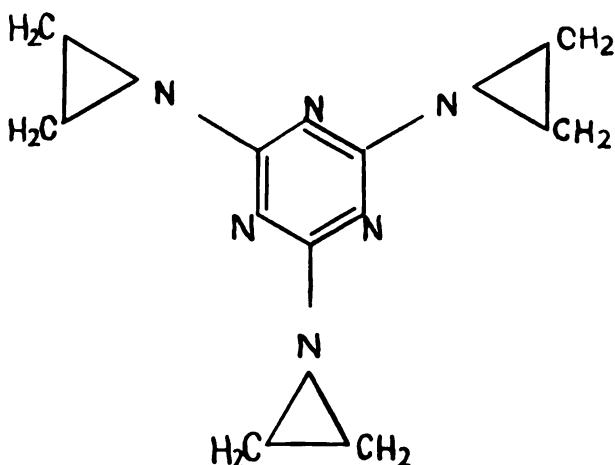


FIG. 108. The changes occurring in the nitrogen mustard leads to ethyleneimmonium in which an energetic aspect similar to that of a twin formation is present.

This agrees strongly with the nature of the more recently studied relatively active carcinogens, the ethyleneimines, where similar centers are seen. (Fig. 109) The biological effect of the ethyleneimine group has been considered to be related to a reactive intermediate.

Generally, if sufficient influence is exerted by another center in the molecule, the imine group becomes active. This center can be a nitro group as in 2:4 dinitrophenyl-ethyleneimine, or other ethyleneimine groups as

in methyleneimine 1:3:5 triazine. (Fig. 110) Through the influence exerted by these centers, the ethyleneimine group can have its carbons charged sufficiently to become a dielectrophilic formation. The possibility of a reactive intermediate and a more stable electrophilic form thus appears common to the two groups, mustards and ethyleneimines.



Triethylenimine 2-4-6-Triazine

FIG. 109. Ethyleneimines are active carcinogens, probably related to their energetic aspect with a formation energetically similar to the twin formation.

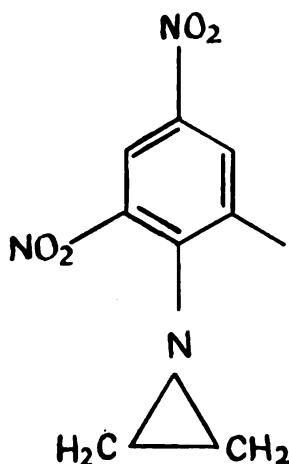
Epoxide Carcinogens

A similar condition is also found in the epoxide carcinogens. Carcinogenic activity has been recognized for substances having two epoxide centers in close proximity in the molecule. The epoxide center by itself can lead to a formation similar to that of carbonium ion, as seen in Figure 108 and thus to the same formation found in mustards and ethyleneimines. The analogy goes still further. The energetic center appears insufficient to accomplish biological changes without an inductive activation. In the case of epoxides, this is usually brought about by another similar epoxide group in the same molecule.

As no carcinogenic activity has been found in substances with only one epoxide center or with two epoxide centers far apart, the inductive centers seem to be of primary importance. The two energetic centers forming the epoxide group, similar to those of the ethyleneimines, do not alone appear

sufficiently reactive to induce important changes. Only when enhanced by reciprocal induction is their reactivity adequate to induce either the appearance of a reactive intermediate or a sufficient charge in the ethylene carbons to produce biological activity. These changes can be measured by the reaction with thiosulfate ion and consequently can be related to the reciprocal positions of the two epoxide centers.

The biological activity of dimethanesulfonoxyalkanes can also be related to a similar energetic formation. Such a formation appears when the



2:4 Dinitrophenyl Ethylenimine

FIG. 110. Through the influence exerted by the nitro group upon the ethylenimine, the imine group of 2:4 dinitro-phenyl-ethyleneimine, becomes dialectrophilic.

molecule is metabolized, with the difference that the two CH_2 in this instance seem to come originally from other chains. (Fig. 111) For the methylolamides, it is possible that a similar process occurs during the changes that take place in the organism.

Some corroboration can be found in the fact that two forms can be observed in these last groups of carcinogens. One is electrostatically active; that is, it has a certain ionic character. The second has a dual electrophilic activity which can be related to a twin formation with molecular reactivity.

Thus, twin formation, with its special reactivity, appears common in many carcinogenic agents. To be biologically active, the twin formation has to be sufficiently strong and this is insured by an induction effect exerted

by other formations in the molecule, such as double bonds in parallel position or polar groups. A twin formation as energetic center in the molecule would exert a molecular field effect. It would thus represent a center of molecular reactivity which has to be considered as such in the analysis of plural activity.

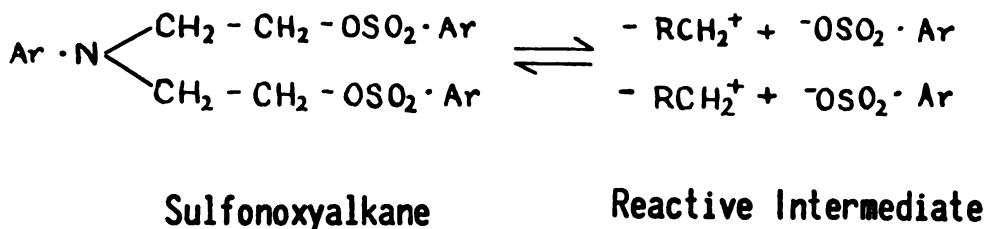


FIG. 111. Changes occurring in sulfonyloxyalkanes leading to two active CH₂ centers.

Synjugated Formations

The study of various carcinogens has permitted us to recognize and relate to complex carcinogenic activity another energetic influence exerted by two or more double bonds when present in a parallel reciprocal position in cyclic molecules. This led us to the concept of "synjugated formations" with 2, 3, 4 or more such parallel double bonds.

In studying methylcholanthrene, one of the most potent of the known carcinogenic agents, the curve of its absorption in ultraviolet light was considered. This curve is shown in Figure 112. The place and form of the peaks could be interpreted in a peculiar way when conjugated double bond formations were considered. In the curve of methylcholanthrene, we could recognize portions that correspond to an inverse of the curves obtained from various conjugated polyenes. Furthermore, the curve obtained through the spectral analysis of methylcholanthrene can be considered to have high similarities to the inverse of the curve of a mixture of conjugated polyenes. Figure 113 shows the spectral analysis of conjugated cod liver oil fatty acids, while Fig. 114 shows the inverse curve of mixture of conjugated fatty acids of cod liver oil in which conjugated di-, tri-, tetra-, penta- and hexaenes are identified. Figure 115 shows the comparison between the curve of methylcholanthrene and the inverse of the peaks of the mixture.

We were thus led to consider the conceptual interpretation of these curves in terms of the special relationship that exists between double bonds in the same molecule. In the classical concept, two double bonds are considered conjugated if two of their carbons are joined by a single bond. In the zig-zag representation of aliphatic molecules, the conjugated double

bonds fulfill this condition. (*Fig. 116a*) Applying this relationship to cyclic molecules, what was considered to correspond to conjugation, according to this criterion, did not show properties similar to conjugated aliphatic members. (*Fig. 116b*) This made us consider, as the condition for the properties present in conjugated formations, another character: the reciprocal parallelism between double bonds present as they appear in the aliphatic mole-

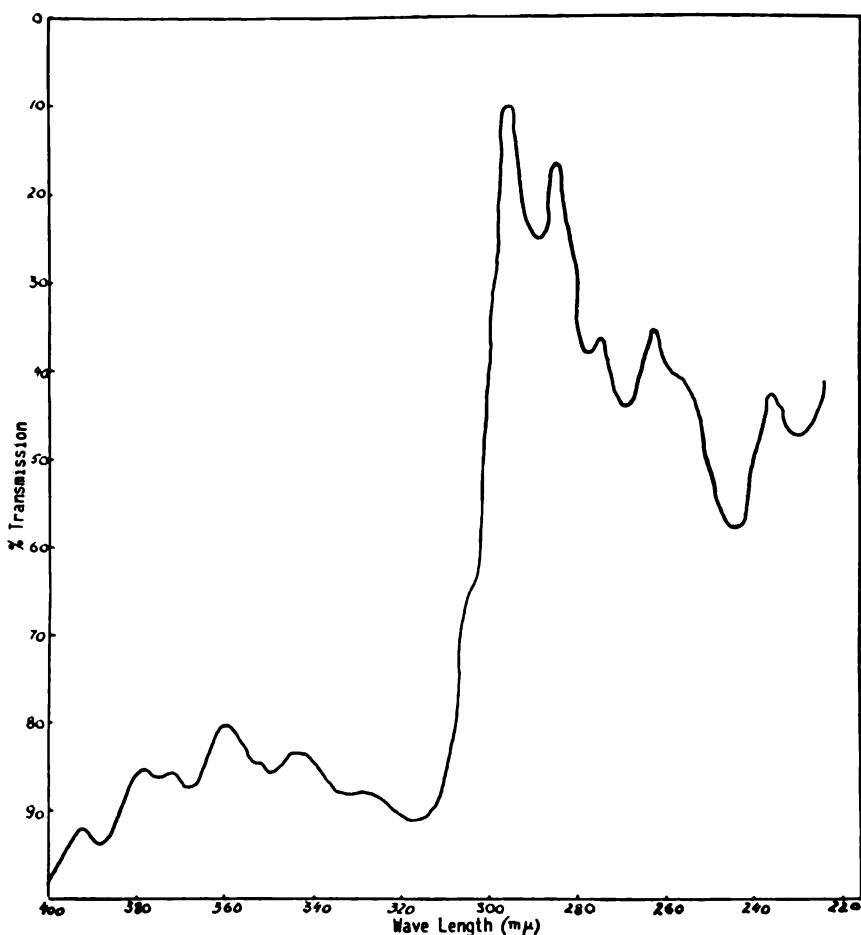


FIG. 112. *An interpretation of the spectral analyses of methylcholanthrene. Curve (a) shows the spectral analysis in ultra-violet of methylcholanthrene.*

cule. Two or more double bonds in a cyclic molecule would thus realize a similar kind of energetic formation when parallel, and would do so independently of the number of the single bonds present in-between. (*Fig. 116c*) For didactic purposes, we have applied the term "synjugated" to energetic formations resulting from parallel double bonds separated by more than one single bond.

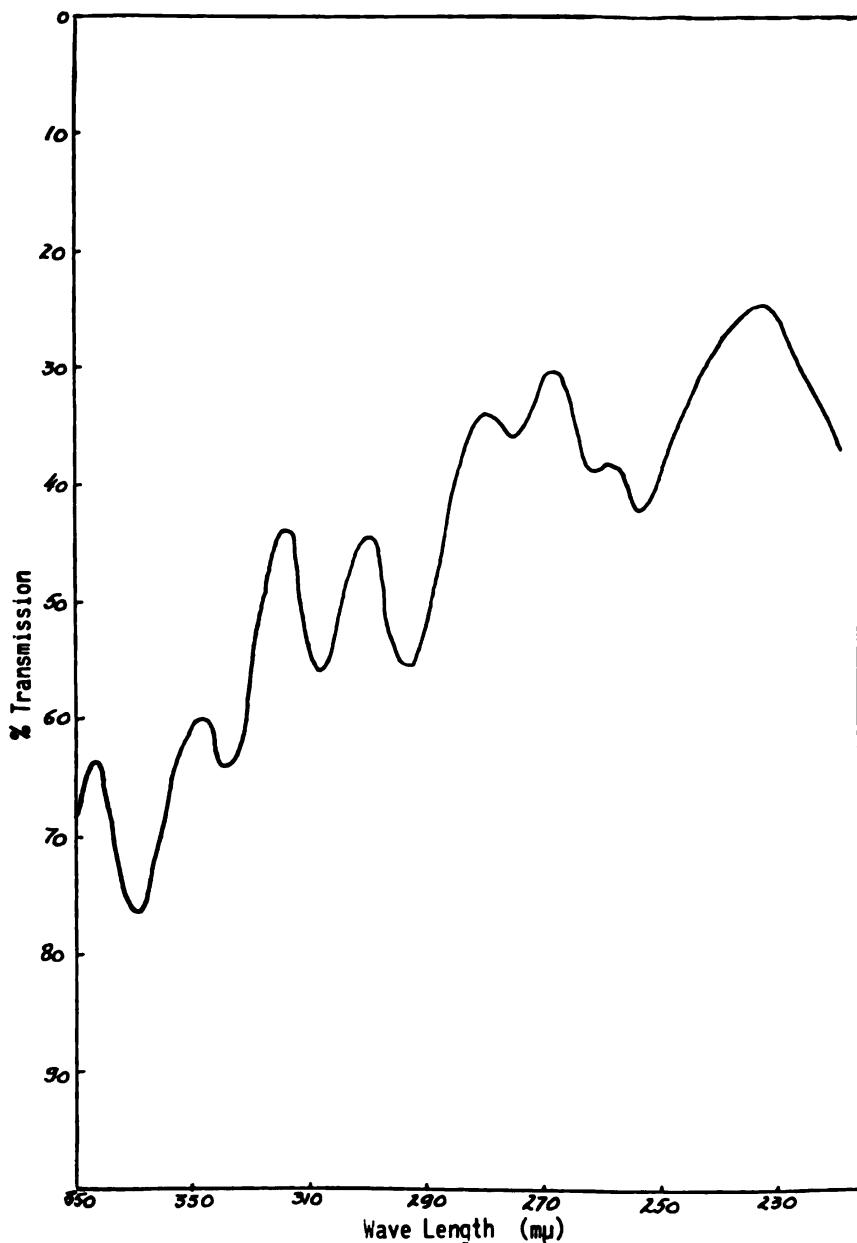


FIG. 113. The curve shows the spectral analysis of the mixture of conjugated fatty acids with members having from 2 to 6 double bonds, as obtained by treating cod liver oil fatty acids with KOH.

Thus, in the methylcholanthrene molecule, there exist formations composed of two, three and four parallel double bonds (Fig. 117), which we call di-, tri-, and tetraenic synjugated formations. It is logical to assume that they are important in determining the energetic aspect of this molecule

when the relationship of its spectral analysis to the curve corresponding to the inverse of conjugated di-, tri- and tetraenes can be recognized. From the point of view of its relationship to the plurality of factors determining the carcinogenicity of a substance, the presence of parallel double bonds,

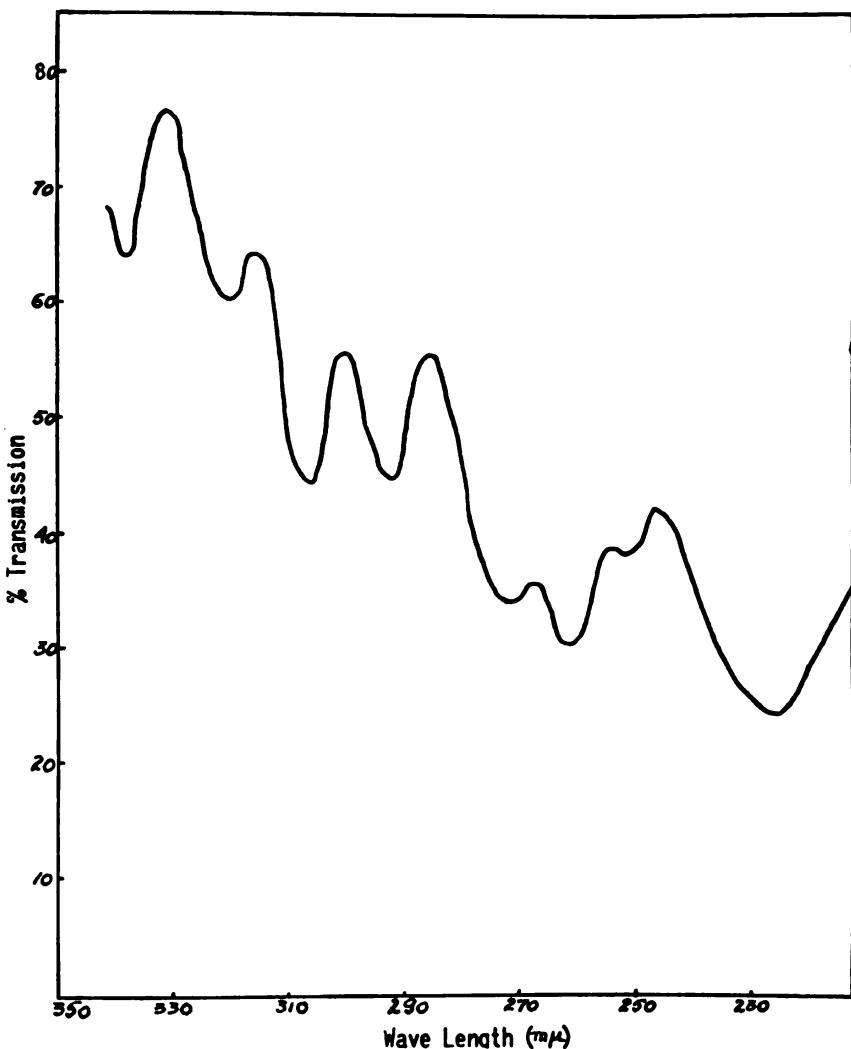


FIG. 114. This curve is the inverse of the curve of Fig. 113.

and the synjugated formations which they constitute, is interesting. Theoretically, each one of these synjugated formations would by itself represent a reactive possibility. Although qualitatively similar, they would show manifest quantitative differences. It must be noted that, while they are not present in all carcinogens, they are in most active, realizing di-, tri-, tetra- and

even penta-synjugated formations. According to the concept of plural activity in carcinogenesis, synjugation, while not indispensable for carcinogenetic activity, would represent one of the factors that can make it possible.

Together with the condensation of the π electrons in the K regions and the presence of polar groups, the twin and synjugated formations would

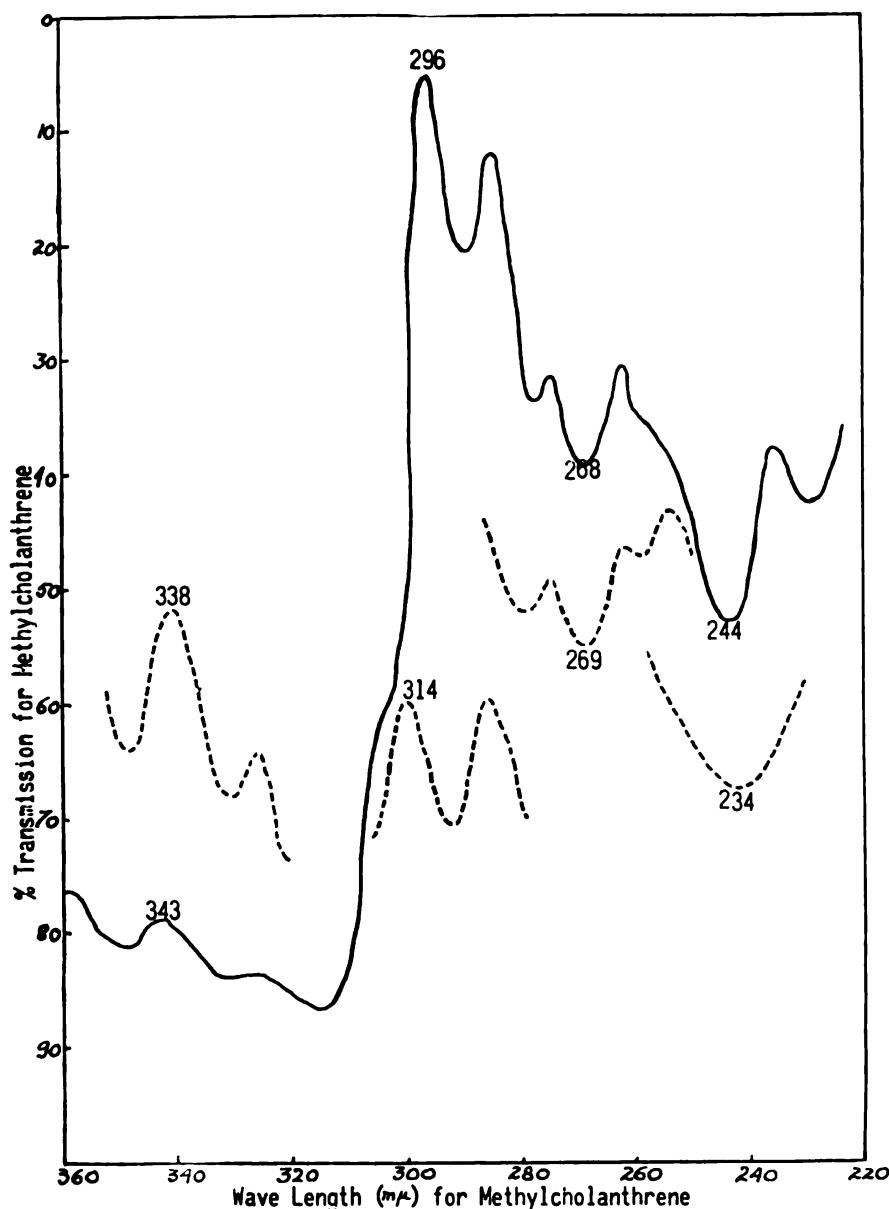


FIG. 115. Direct comparison between the curve of the spectral analysis of methylcholanthrene and the inverse of the peaks characteristic for the different conjugated fatty acids as seen in Figs. 113 and 114.

confer high plural activity upon the molecules of active carcinogens. An energetic spectrum of a carcinogen can be established in which these factors can be presented systematically.

Figure 118 shows a spectrum for 9:10 Dimethyl 1:2:7:8 Dibenz-anthracene.

In the light of this analysis, it appears logical to conceive that the carcinogenicity of a chemical compound is a result of many factors, and that the great differences in carcinogenic properties of various agents is the re-

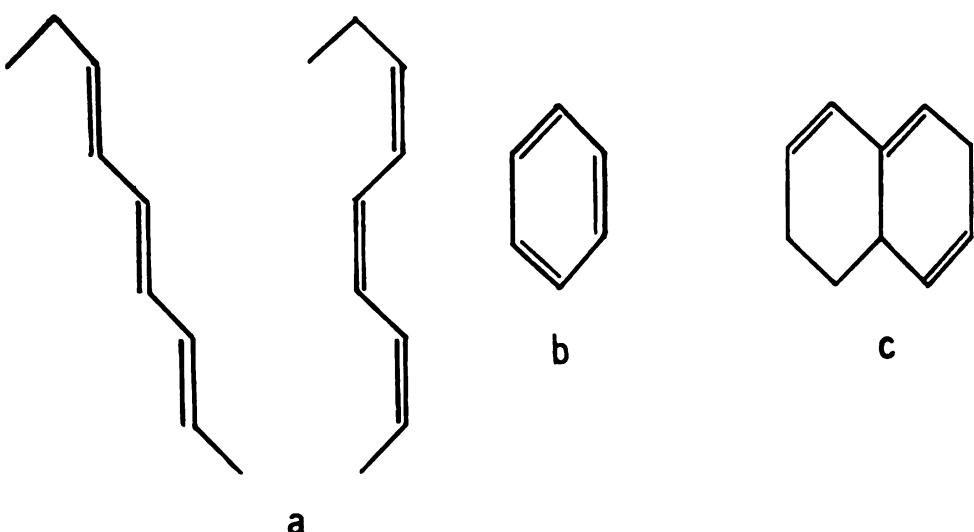


FIG. 116. *Conjugation and synjugation.* In the aliphatic chain (a) the presence of single bonds between the double bonds induce the parallel position of double bonds. It is this parallelism, which through the reciprocal influence exerted, induces the energetic characteristics of the conjugated formations. In the benzene molecule (b) where the double bonds, although separated by single bonds, are not parallel, the lack of this parallelism explains the lack of the properties characteristic to the conjugated formation. The parallelism when present in cyclic molecules (c) realizes the "synjugated" formations.

sult of differences in their energetic spectra. The differences are consequently qualitative as well as quantitative. From this viewpoint, it is possible that the great carcinogenic activity recognized for some substances would correspond to the presence in them at once of a great number of energetic factors.

The study of the correlation between the presence of various energetic centers and carcinogenesis has been facilitated by relating carcinogenic changes to levels of organization. Taking place at different levels, the induced processes can be seen to correspond to an entire series of manifestations which, while present also in invasive cancer, often can be recognized

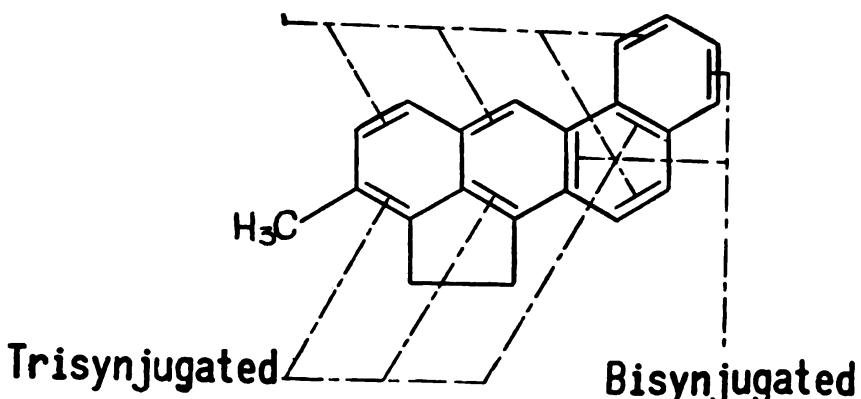
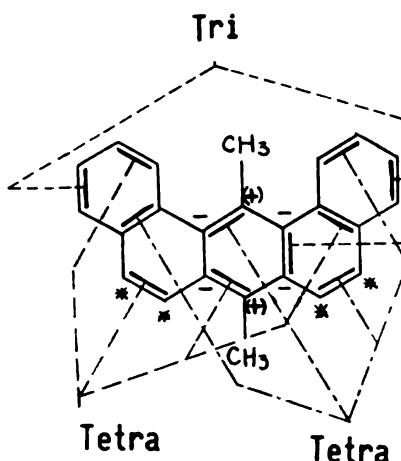
Tetrasynjugated**Synjugation in 20-Methylcholanthrene**

FIG. 117. The parallel position of the existing double bonds in methylcholanthrene corresponds to a bi-, tri-, and tetrasynjugation.



- Two K Regions **
- Two Twin Formations
- One Trisynjugated Bond
- Two Tetrasynjugated Bonds

9:10 Dimethyl 1:2:7:8 - Dibenzanthracene

FIG. 118. The energetic picture of 9:10 dimethyl, 1:2:7:8 dibenzanthracene, shows the presence of two K regions, two twin negative formations, one trisynjugated bond and two tetrasynjugated bonds.

in cases in which an invasive cancer is not induced. Following this view, it can be expected that carcinogenesis is the summation of a whole series of actions induced in the organism, some exogenous and others endogenous.

Consideration of the plurality of the factors which intervene in chemical carcinogenesis and make it a complex process leads us to consider viruses in the etiology and pathogenesis of cancer in a similar light.

VIRUSES AND CANCER

More than fifty years ago, Borrel presented his hypothesis of viral origin of cancer based primarily on analogies. Since then, although an enormous amount of material on this subject has accumulated, much of it has been contradictory and it has appeared to be impossible to arrive at any clear-cut concept of the role of viruses in the pathogenesis of tumors. However, it seemed that an attempt to correlate most of the data with information furnished by the study of chemical carcinogenesis in the light of the concept of cancer as a complex hierarchic disease might be of some value for an initial simplification of the problem. (293, 312) (*Note 2*)

Some theoretical considerations have helped in systematizing the data and in indicating the probable limits of viral intervention in carcinogenesis. Just as with chemical carcinogens, it could be assumed that virus intervention may bring to bear multiple factors. An analysis of the processes which occur under viral influence indicates that this hypothesis is plausible.

Even more than chemical carcinogens, viruses are able to act only at certain levels of organization. Their intracytoplasmatic and often intranuclear development conditions the intervention of these agents at these levels. Fundamental differences in carcinogenic effect could be expected if viruses are able to influence the subnuclear levels, or the nucleus, or, on the other hand if their activity is limited to the cytoplasm. This view has permitted us to understand the striking difference in influence exerted by various viruses which, although recognized by any worker in the field, has not been the subject of any special consideration.

Two Types of Carcinogenic Effects

The difference lies in the time needed for a virus to produce carcinogenic effects. Inoculation of fowls with purified Rous sarcoma virus, for instance, has been seen to produce a clear-cut, immediate effect. Changes have been recognized within 48 hours at the site of inoculation. They take place in the nuclei of fibrocytes and consist of swelling, appearance of a more distinct nuclear membrane, cleared nucleoplasm, margination of chro-

matin and one or more enlarged nucleoli. In as few as one or two days, cytoplasmic changes are also evident. There is manifest basophilia with swelling of the cell which becomes greatly enlarged. Concomitant with these changes, the abnormal cells invade the fibrillar tissue. The tumor which develops has the character of the classical spindle cell Rous sarcoma.

Thus a cancerous tumor in the invasive stage, with typical nuclear and cytoplasmic cell characters, is induced in only a few days at the site of inoculation. This is characteristic of one type of viral carcinogenesis, the extremely active one. Integrated in the concept of complex carcinogenesis presented above, it would mean that the entire series of changes—from those at the lowest level which determine the cancerous character to the cytoplasmic changes which produce the proliferative, invasive cancer—has been achieved by the virus in this short time. In fact, this tumor grows rapidly, is palpable even at the fifth day and fatal in two to three weeks.

Almost diametrically opposed to this type of carcinogenesis are tumors which represent another type of virus intervention, such as certain mammary cancers in mice. Viruses that produce such tumors can be obtained from various organs, even from those of animals without apparent tumors. They induce the appearance of tumors but only under very characteristic circumstances. Preferably introduced in the first days of life—subcutaneously, intraperitoneally or even orally—they will produce their effect only after many months or even after one or two years, as tumors of a specific organ, such as the mammary gland, for example. However, such tumors appear almost only in females who have had one or more pregnancies. In this case, the virus acts only upon highly differentiated cells and acts independently of the site of inoculation. The extremely long period without manifestation, the fact that the virus can be found to some extent in various organs which show no change, and the specific localization in a highly differentiated organ such as the mammary gland, would indicate that the carcinogenic intervention of the virus is highly related to a specific character of these cells, their particular differentiation. This would place virus intervention at the cytoplasmic level where differentiation occurs.

Under this interpretation, the length of time necessary for tumor appearance would be related to the time needed for a natural evolution of the mammary cells to the point where they are sufficiently differentiated. It appears probable that this length of time corresponds to that needed by abnormal hierarchic entities of the mammary cells to have arrived, independently of the virus, at a state corresponding to that of precancer or noninvasive cancer. Intervention of the virus at the cytoplasmic level would then transform the relatively advanced but still noninvasive cancer cells

into invasive cancer cells. Viruses would act, in this case, as cytoplasmic carcinogens.

These two types represent the extremes of carcinogenesis in which viruses play a role. They help to interpret many of the other data furnished by experiments. For didactic purposes, we shall regard as "broad-scale" viruses those which act from very low to high levels of the organism, and as "cytoplasmatic" those which act at the higher cellular level only.

Some of the rapidly acting broad-scale viruses will induce evolving tumors in a much shorter time than any known chemical or physical carcinogen, a fact that can be interpreted as meaning that these viruses are more capable of inducing not only the cytoplasmatic carcinogenic changes but also the entire scale of preparatory changes leading to invasive cancer. Viruses differ from the usual active chemical carcinogens in their special capacity to induce changes easily at the cytoplasmic level where they are particularly capable of multiplying and acting. This would contrast with most chemical agents which generally have low carcinogenic activity at the cellular level. Chemical and virus carcinogenic activity would complement each other. This is in accord with experiments of Russian scientists, which have shown that cultures of cells treated with methylcholanthrene *in vitro* become highly carcinogenic when inoculated in animals if a cancerous virus is also added.

This view of the activity of cytoplasmatic viruses at the higher cellular level, as contrasted with many chemical carcinogens usually more active at lower levels, appears also to be in agreement with the experiments of Rous and Kidd (69, 70) which demonstrated the capacity of coal tar extracts to localize the Shope papilloma virus. The high cytotropic character of this virus is well known. It would easily act upon cells already transformed from normal into noninvasive form by chemical agents which are active at the lower levels. These chemical agents thus "localize" the viral activity. This is in accord with the ability of chemical agents in the Rous and Kidd experiments to increase the percentage of invading carcinomas as compared to the papillomas present. According to the view presented above, the papilloma as a benign tumor would represent changes similar to those seen in cancer but limited exclusively to the higher levels, without cancerous entities at the lower levels. The addition of an agent with a broad-scale of carcinogenic activity, that is, acting also at the lower levels, such as the chemical agent, would give the resulting lesion the entire cancerous scale, that is, the character of malignancy.

The integration of viral carcinogenesis in the concept of cancer as a complex condition and recognition of the two extreme types of viral car-

cinogenic activity permits us to understand the reserve of most authors over the viral etiology of cancer. For instance, many have refused to accept as a carcinogenic factor the virus shown by Bittner (71) to be present in maternal milk and to influence the appearance of mammary carcinoma in mice. The refusal is based upon comparison of this virus with that of the first type seen in fowl tumors. With the systematization presented above and the concept of broad-scale and cytoplasmatic carcinogenic viruses, this reason is not valid. Furthermore, a carcinogenic virus should not be considered to be the indispensable factor able to induce proliferative cancer in animals which usually have a virus cancer. This would explain why, in certain breast carcinomas in mice, a viral agent could never be found. (72)

The specific capacity of a virus to act upon a differentiated cytoplasm explains the fact that a virus may be widely distributed among organs but does not induce tumors except in special cells. Previous preparatory changes seem necessary for the cytoplasmatic virus to intervene. This is in accord with a low incidence of tumors in certain strains of mice despite an abundant presence of the "milk factor" virus. (73)

These facts shed a new light on the entire problem of the relationship between viruses and tumors. Viruses can multiply in organisms without inducing cancer. The virus of mammary carcinoma in mice can be transmitted to females through spermatozoa and can be found in large amounts throughout the organism. The virus is present a long time before any cancerous lesions are seen and is present in organs that will never have tumors and even in animals that never develop cancer. The development of this virus, like all viruses, takes place in the cell cytoplasm which does not necessarily mean the induction of carcinogenesis as long as other factors are not present. No tumors appear as long as the cell has not undergone the prior changes required if the cytoplasmatic carcinogenic effect is to take place. Without the previous changes, the virus will not influence the cell any more than many other noncarcinogenic viruses. It is only in the presence of an advanced cellular change that the virus will produce an invasive cancer.

Plural Activity

The capacity of a broad-scale virus to induce an invasive tumor in a short time through plural activity at different levels has been related to its richness in lipids. Its analysis makes us suspect the presence of several parts in the virus, each one able to act at a different level, as in the case of active chemical carcinogens. A similar plural influence can be seen

exerted by viruses other than those with carcinogenic activity. The study of bacterial viruses has shown the existence of such plural parts. (*Note 1*)

Luria (74) has shown, after irradiating a bacteriophage with ultra-violet light, that if lytic activity can no longer be obtained by the intervention of a single one of these particles, it can be induced with two or more of them. They act as though several parts, which usually are present in the virus but which were unequally inactivated by the irradiation, would be necessary in order to induce the process of lysis. This agrees with the experiments of Debruck and Hershey (75), which have shown that new types of viruses with new properties can be obtained when units of the same phage strain or related strains are mixed together. The new properties are combinations of those of the mixed units. (298)

Similar changes in the plural constitution of the viruses would explain other peculiarities observed in bacterial phages. Bacteria can carry phages for generations before any lytic activity occurs. The lysogenic strains (76) of bacteria are examples. It is possible that a virus may undergo temporary changes under certain circumstances; this would explain the frequent impossibility of finding a virus immediately after it infects a bacterium, and the "disappearance" of some viruses in animals immediately after infection. Since, in both cases, the virus is found later, a change which makes it unable to act and thereby be detected is plausible. The "masked" virus would be one with only some of its plural properties present. The possibility of recovering a lost property was demonstrated in the Berry-Dietrich phenomenon, when a heat-inactivated myxoma virus recovered its lethal capacity if inoculated along with a fibroma virus.

This concept of plural activity finds further application in the explanation of many phenomena observed in viruses in general and in variations in carcinogenesis. The "self-sterilization of the neuro-infections" described by Levaditi has to be regarded rather as partial inactivation of the viruses especially if the viruses can be reactivated. This occurrence must be separated from cases where a total destruction of the virus can be supposed to have taken place.

The lethal infection induced in mice injected intraperitoneally with salivary gland viruses of certain strains is an example of the latter. The presence of the inclusion bodies in liver and other organs, and the total inability to produce the disease in other mice (77) can be interpreted as a sign of a destruction of the viruses in the organism. The inclusion bodies can be interpreted as resulting from an agglutination of the viruses themselves as shown by Nicolau in herpes. (78) In other cases, such as protracted herpes infection in rabbits (79), or vaccinal infection in rabbits

(80), only partial inactivation can be considered to occur since electrophoresis, repeated passage, or even dilution restores pathogenicity. The restorative factor can be of varied nature. Cases in which pathogenicity is restored by a nonviral agent—activation of the virus of swine influenza in the presence of *Hemophilus influenza* (81), for example—are most revealing.

Virus and the Host

This concept of plural activity explains the relation between tumorigenesis and destruction induced by viruses. Often "neoplastic" infection and "destructive" infection are induced by the same virus. (82)

The herpes virus thus induces necrotic lesions in the chick embryo when introduced in early stages, but if the embryo is more developed, the same virus produces proliferative changes. (83) The myxoma virus induces more proliferative lesions if attenuated than does the unchanged virus. (84) Under special circumstances, such as in older animals, sheep pox virus induces papilloma instead of pustular infection (85). It must be remarked that these different results are not limited to viruses; they occur with radiation or even with other infectious agents. (86) *Bartonella bacilliformis*, which induces often-lethal Oroya fever, seems to be the cause of "verruga peruviana," a fibroangiomatous tumor often seen in subjects recovering from the acute disease.

The differences in activity of the same virus appear to be related to the age of the host. Generally, youth of the host increases the virus' capacity for acting at more levels. The virus can produce lethal destructive disease in young animals but only a neoplastic response in adults, as seen for the fibroma virus in rabbits. Furthermore, the neoplastic response also occurs in young animals but only if a small amount of virus is inoculated, or if an attenuated virus, such as a long-stored one, is used. (87) This is clear in the case of the Rous sarcoma and other chicken tumors.

When injected into very young animals, Rous sarcoma and other chicken tumor viruses produce a hemorrhagic lesion (88) but they will induce tumors in adult animals. The destructive effect can be repeated with repeated passages of the virus in very young animals but in adults each passage produces the neoplastic response. This is also true for some strains of lymphomatosis virus (89) which induce tumor formation in adults and necrotizing processes in young animals or embryos. It is also true for the virus of neurolymphomatosis (90), and of gliomas. (91) These viruses, although selective for the nervous system, induce inflammatory or neoplastic lesions according to the age of the infected animal.

In a general way, it has been postulated that for viruses, as for bacteria, the young animal represents a favorable terrain, while a certain resistance is encountered in the adult. Waters and Bywaters (92) have shown that the filtrable agent isolated by Prickett and Belding (93) is not transmitted spontaneously if the animal is older than 40 days. It is transmitted through the eggs, although months elapse before there are manifestations. (94)

The problem cannot be limited to the host, since, by passing the virus through young or old animals some of its properties can be changed. Gross (95) has shown that a cell-free extract obtained from leukemic mice of the AK strain, would induce the condition in C₃H mice, provided the inoculation is given within a few hours after birth. The results published by Gross and the inability of other authors to reproduce them (96) could be explained by differences in the virus strains used. (97)

This was shown in the experiment of F. Duran-Reynals (98), in which the Rous sarcoma virus undergoes changes during passage in the adult chicken, which make it adaptable to another species, namely ducks. The virus growing in young chicks seemed unable to induce the disease when inoculated in ducklings or in older animals. Tumors obtained even through cell suspensions, some of them very large tumors, could not be transmitted for more than one or two generations. However, filtrates of tumors from older chickens, when injected into ducklings no more than a few days old, induced tumors which easily could be passed to young as well as adult ducks. This change in the virus was strictly conditioned by the age of the chicken; it occurred only if the animal was between three and ten months old. If the animal was more than 19-20 months old, injection of the filtrates was always unsuccessful, and injection of the cells only rarely induced tumors.

These changes in the virus are explained by mutation. Among various resonance forms which appear on a purely statistical basis, one different from those previously predominant finds favorable conditions for its development—conditions that are not favorable for the predominant forms. These experiments have permitted us to further correlate the intervention of viruses with the influence exerted by several chemical factors upon the complex tumor pathogenesis.

Virus and Lipoids

In studying the influence exerted by lipoids upon viral activity, we could show that the presence of free fatty acids, especially polyunsaturated, induced changes opposite to those induced by the anti-fatty acids.

In rabbits, administration of various preparations of fatty acids, espe-

cially polyunsaturated, induced an unusual degree of resistance. Animals previously given subcutaneous injections of fatty acid preparations showed a reduced general response to chicken pox inoculation as compared with controls, and practically no response in the skin at the site of the fatty acids injection. On the other hand, administration of insaponifiable lipid fractions obtained from tissues of receptive species was followed by manifest responses localized in the zone of injection, even in species otherwise refractive to viral infection. It is under this special influence of lipoids that we have further investigated the intervention of the viruses in carcinogenesis.

Cells vary in their content of lipids. We could see that richness in sterols of a group of cells increases their receptivity to, and favors the development of, viruses in general, while richness in fatty acids, especially polyunsaturated, has an opposite effect. The local increase in a tissue's richness in sterols makes it more susceptible to the localization and development of a virus, as is shown in the following experiment.

In rabbits, intracutaneous or subcutaneous injections of a colloidal suspension of cholesterol were made on epilated skin at several sites. Twenty-four hours later, the animal was injected intravenously with suspension of smallpox vaccine. Characteristic lesions were observed to develop at the sites of the cholesterol injections.

The general effect of sterols upon receptivity to viruses, noted in many experiments in animals, was also recognized in humans. The following observation appears interesting. Mrs. D. R. had always appeared refractory to smallpox vaccines. Until the age of 40, repeated inoculations produced constantly negative responses. She was treated at that time with a cholesterol preparation for precordial pain, receiving daily 2-3 cc. of a 2.5% solution of cholesterol intramuscularly. After three weeks of this treatment, she was obliged to go abroad and it was necessary for her to have the routine smallpox vaccination. For the first time in her life, a characteristic positive result was obtained.

The relationship between sterols and viruses, which would explain the affinity of most viruses for the nervous system and skin, since both are of exodermic origin and particularly rich in free sterols, would also explain why young cells similarly richer in sterols are more susceptible to viruses, and the facility with which almost all viruses develop in embryos, such as in chicken embryos.

Changes in richness in lipids were observed under natural circumstances other than those related to age. Thus seasonal changes could be noted, the cold season leading to an increase of fatty acids while the summer season

brought an increase of sterols. This would help to explain the seasonal changes usually observed in naturally occurring viral infections. (99)

The epidemiology of poliomyelitis may be related to the organism's richness in sterols in the summer, particularly on hotter days. Seasonal changes were noted in naturally occurring tumors in which a viral etiology is seen. A certain resistance appears in the fall and increases in the winter in the case of leukoses and possibly in other natural viral tumors. (100) This would explain the manifest seasonal changes observed by us in the transplanted Walker tumor in rats or in grafted tumors in mice in general, and in the induction of tumors through carcinogens. Similarly, the induction of teratomas in testes through local administration of zinc chloride was noted to be influenced by the seasons. (101)

The influence exerted by sterols would explain the fact that viruses able to act only at a higher level, as in the cytoplasm, tend to develop in animal cells abnormally rich in sterols. It is highly probable that once it has penetrated, a virus will develop within a cell only under favorable conditions, and these are insured by the presence of sterols. The virus will persist, interfering little with the fate of the cell until other changes occur at lower levels. These other changes take many months or even years to be completed, and only then would the influence of the virus be apparent through its activation of the noninvasive abnormal cell. Activation can occur regardless of seasonal changes in sterol richness. It seems superfluous to note that this relationship holds more for cytoplasmatic viruses than for those with broad-scale activity. The latter are also more active in young animals.

Changes in age of the host and other circumstances can modify the character of viral carcinogenesis, leading to rapid or very slow development, or even to complete lack of response. This was often noted for Rous sarcoma. In young animals, small amounts induced rapidly growing tumors with multiple hemorrhagic metastases rich in filtrable virus. In adult animals, despite the large amount of virus necessary, tumors took months to appear, seldom metastasized, and could be transmitted with difficulty, or not at all, by filtrates or even by cells. (102)

The relationship between viral carcinogenesis and lipids has been the basis for a group of experiments in which we tried to influence the carcinogenic activity of a virus by administration of sterols. Experiments still in progress, using sterols obtained from chicken embryos, seem to indicate that lipids can strongly change viral carcinogenic activity. In general, they induce an increased response to viruses.

Many other peculiarities of the relationship between viruses and carcinogenesis have been analyzed in terms of intervention of lipids as an

intermediary factor. The capacity of a virus to induce tumors in different organs—as seen for leukemic tissue cell-free extracts in mice, which induce peculiar salivary gland tumors (103), or tumors in the adrenal gland or in the subcutaneous tissues (104)—can be explained by certain peculiar affinities of the viruses for differentiated tissues, possibly related to certain specific lipids found in these tissues. A similar affinity for the salivary gland is seen for rabies virus. There are also the affinities shown by the neuro- and dermatotropic viruses, and by the neoplastic viruses for mammary gland, lymphatic tissue, neuroglia, etc. While affinity for the adrenal gland could be related to its richness in sterols, affinity to other sites could be related to other lipids.

The plurality of localizations of viruses also can be related to affinities of mutated agents for different cells. The experiments of Gross (105), which show the possibility of separating out of the same filtrate the agents responsible for salivary gland tumors and for leukemic changes, would indicate that a change in the virus must be also considered. However, the change can be interpreted as a mutation and can be related to the influence exerted by lipids upon the virus. Treatment of virus with lipids has shown the possibility of inducing changes in its behavior. Data showing the influence upon tissular receptivity of such changes will appear in future publications.

With this concept of the role of viruses in the pathogenesis of cancer, it seems possible to explain other peculiarities that have led to confusion in this field.

It has been noted that viruses act as factors determining the change to a cancerous entity which, once induced, can continue to develop without need of further intervention of the virus. This poses the problem of the relationship between viral carcinogenesis and development of the virus in the tumor itself. Even in a tumor, the multiplication of the virus has to be separated from that of the growth of the tumor. Although often interrelated, they must be considered as two different processes. The growth and even direct transmissibility of the tumor can continue, independent of the presence of the agent that originally induced it. When tumors have been induced by a chemical carcinogen, they can be transmitted in continuous generations over many years, producing large tumors each time, a fact which would preclude any possible direct intervention of the agent in these later tumors. Similarly, tumors once induced by virus can be further transmitted by cells, the virus no longer being apparent in the tumors. A tumor induced by a virus often serves as a medium for the multiplication of the virus. However, even while the tumor can continue to grow, it can become an adverse medium for the further multiplication of the virus.

This explains the peculiar fact that tumors induced by a virus can be rich in or can lack an appreciable amount of virus, as often seen in a Shope papilloma (106), or even in tumors in fowl (107), which pass through periods when transmission through cell-free filtrates becomes impossible, while transmission through the transplant of tumor cells still continues. The virus multiplication capacity can vary not only with the host but with the virus itself, thus explaining the changes noted above.

The results of the interesting studies of Bryan, Galman and Maloney (108) who have investigated the relationship between richness in virus of an induced Rous sarcoma and the percentage of positive results in induction easily can be interpreted under this view. The chances of inducing tumors increase in cases in which the host is also a favorable medium for the multiplication of the virus, and vice versa. This would explain why these authors found little or no active virus in cases in which the injected material produced less than 50% positive results, but cases originated by a material that induced a large proportion of positive results were rich in active virus. The capacity to multiply after inoculation in the host itself thus increases the ability of the virus to act as a carcinogenic agent, which seems logical. The relative independence of the two processes—the multiplication of the virus and the induction of tumors—appeared clearer in the cases mentioned above, where the virus develops in the entire body of mice, even in successive generations, without inducing tumors.

The presence of viruses in the organism, even without inducing tumors, helps to explain the rather puzzling experiment in which a transmission through filtrates, considered characteristic for viruses, was seen to occur for tumors induced by chemical carcinogens. Carrel has claimed to have transmitted through filtrate passages tumors induced by arsenic, tar preparations and even indoles. These tumors were of the Rous type obtained in fowl. More recently, similar tumors transmitted through filtrates were observed by McIntosh and Selbie (109), Maisin, Haddon and Haagen (110), and Oberling and Guerin (111) after injection of methylcholanthrene, especially in fowl. The considerations presented above furnish a logical explanation for these observations.

A first factor to consider is the presence, in animals regarded as normal, of a virus able to intervene to produce a neoplastic effect under special circumstances. We have noted that such a virus can be present without inducing tumors. Fowl appear to be especially susceptible to viruses (112), statistics showing that viral lymphomatoses are responsible for 50% of the malignancies in chickens. Even while some species display an inborn resistance to viral infection, others are highly receptive, as seen for the viruses

of sarcomas (113) and lymphomatosis. (114) Viruses have been found in as many as 10% and even 20% of chickens, according to some reports. (294)

The number of animals with viruses and no tumors must be considered still higher when presence of viruses is revealed by antibodies. Duran-Reynals, in collaboration with the East Lansing Agricultural Experiment Station (115), has shown that, while not one of 23 chickens kept isolated and free of lymphomatosis showed antibodies in the blood, hundreds of chickens taken at random did have the antibodies. This fact makes it highly probable that the presence of the viruses in the chickens used for carcinogenic studies was independent of the administration of the chemical carcinogen. Furthermore, the role of chemical agents acting alone as carcinogens can be discounted because their effects differ widely. In the series of McIntosh, the tumors appeared far from the site of injection which is very unusual for methylcholanthrene. Furthermore, the agents used by Carrel, except tar extracts, generally have little or no carcinogenic activity.

The two hypotheses—one, that chemical carcinogens alone can induce filtrable tumors; the second, that this is only coincidence and the tumor is entirely of viral origin—can be reconciled under the concept of plural intervention. Thus the chemical carcinogen would induce only part of the process; the remainder, at higher levels, would result from viral intervention. The change that occurs in the cells through the influence of the chemical carcinogen could also favor the change in the virus, making it not only more active but also of neoplastic character. The concept of plural changes needed to induce active carcinogenesis permits us not only to integrate the intervention of viruses in the concept of carcinogenesis in general, as presented above, but also to consider this intervention in relation to other factors.

Hormones can play a part; they are needed to induce the degree of differentiation that is a condition for viral co-carcinogenic intervention. Inoculated intraperitoneally, the Bittner milk factor, although active, will rarely induce mammary tumors in virgin females although the virus can be proved to be in the body. The hormonal changes related to pregnancy and lactation influence the mammary gland and cause a differentiation. Since this differentiation represents a condition for viral neoplastic activity in these cases, hormonal intervention can be integrated as an added factor important for the viral carcinogenic activity. The hormonal factor would appear to be an indirect co-carcinogen, and it is under this aspect that its role in carcinogenesis has to be studied.

The concept of plural co-carcinogenic intervention permits us not only

to relate the different pathogenic factors involved in carcinogenesis; in addition, by relating these factors to various levels of organization, it allows us to obtain new insight into the genetic factor.

GENETICS AND CARCINOGENESIS

The genetic factor in carcinogenesis can be understood in terms of phylogenetic hierarchic development. Such development results from a series of progressive changes which lead to successive hierarchic levels of organization. As we have seen, for each level of the organization a series of different solutions are available when a new hierarchic entity is to be realized. This results first from the fact that various numbers of entities take part in the constitution of the principal parts. Since different constituents can form the secondary parts of these new hierarchic entities, the number of solutions is increased. The resulting solutions can be considered on a statistical basis. Many of these new entities will die immediately; others will subsist as such; still others will progress. Their fate results largely from their interrelationship and the conditions present in the environment in which they find themselves.

The striking similarity to the resonance process studied in the lowest levels of organization, such as atoms or molecules, has led us to consider that changes at higher levels are of the same fundamental nature. Of all the resonance forms that occur at each of the levels, there are some that, on a statistical basis, persist and develop. These persisting resonance forms make up the normal organism. The favored resonance forms are determined by heredity and also by environmental conditions. While the resonance forms appear on a statistical basis, the environmental condition can vary and new resonance forms will mark the intervention of external factors. As a normal entity is composed of the persistent resonant forms, abnormality occurs when such an entity persists. The characteristics of any individual are provided by the resonance forms which have developed phylogenetically and also ontogenetically. These predominant forms are "isotropic." For didactic purposes, we called the others "allotropic."

Allotropic Resonance Forms

It must be accepted that, originally, it was the intervention of allotropic resonance forms which permitted the appearance of new forms able to respond well to the environmental changes. The phylogenetic development of different phyla, species, strains and even individuals, can be seen as resulting from such different solutions for the same problems. When, how-

ever, an allotropic resonance form appears, during ontogenetic development, it results in an anomaly. At the level of genes or chromosomes, it produces a mutation or monstrosity. At a still lower level, such allotropic resonance forms may result, not in monstrosities or mutations, but in cancerous entities. The concept of cancer as a hierarchically organized disease accords with this view of allotropic resonance forms. A first cancerous entity would, therefore, develop when an allotropic resonance form occurs at a low level of the organization. Under favorable conditions, the allotropic entity would develop hierarchically, passing on through the different levels of the organization, and realizing allotropic chromosomes, nuclei, cells and tissues.

In order to have an invasive cancer, it would thus be necessary that an entire succession of favorable conditions be present insuring the development of a continuous line of hierarchic cancerous entities. These favorable conditions can occur spontaneously at each level, and both cancerous and normal entities may have many allotropic forms. Carcinogenesis would correspond to the creation of these favorable conditions, and, as seen above, it can take place at the different levels of the organization. The result is a hierarchic succession of the persisting forms of the allotropic series of respective cancerous entities.

The long time usually necessary for a cancerous condition to appear accords with this mechanism. The intervention of any external factor considered capable of inducing, by itself, the development of cancerous allotropic forms, has to be regarded as favoring the conditions necessary for the persistence and development of the succession of allotropic cancerous entities. (304)

This would also explain the relationship between hereditary factors and carcinogenesis. Just as the individual has the capacity to realize the successive allotropic forms, so a strain or even a species can inherit the tendency to develop such allotropic forms. This explains the persistence of mutation forms. It applies to the development of strains with high or low incidence of spontaneous tumors. It would also explain the vast differences among different species and strains in their response to carcinogens.

The concept of cancer as corresponding to a series of allotropic resonance forms at the successive hierarchic levels is of importance in terms of the intervention of external factors. Such environmental influences can establish conditions favorable for the development of allotropic forms at successive levels, permitting the progression of the allotropic line. The inequality of their action at different levels accounts for the big differences

seen between the various carcinogens. It is under this special aspect that we saw above the important problem of induced carcinogenesis.

The relationship between carcinogenesis and the defense mechanism can be understood in terms of the differences in defense capacities of allo-tropic and normal entities. From the study of cancerous and normal subjects, it appears that the latter have the capacity for defense responses. It appears highly possible that the characteristic of the "normal" resonance forms resides in their ability to resolve noxious interventions. The allo-tropic forms lack this ability. The loss of the defense mechanism at various levels, which is characteristic of cancer, can be regarded, up to a certain point, as being the result of the intervention of allotrophic resonance forms, which would appear to be fundamentally inadequate to oppose the hierarchic progression of cancer. Incapable of responding with the full defense mechanism when confronted by continuous noxious interventions, cancerous entities use the simpler, primitive defense forms, and especially the lipidic prolonged form. This lipidic predominance represents the principal factor in producing the actual manifestations of cancer, with their dualistic nature.

The intervention of noxious factors in carcinogenesis is well known. Trauma and microbial viral infections in particular are such factors. Co-carcinogens such as croton oil, and some solvents such as benzene and toluene, can be considered noxious factors. (295)

This view of a plurality of factors intervening together to realize the hierarchically complex condition of cancer has another value. It helps to reconcile various explanations of the pathogenesis of cancer, each attributed to a different etiological factor, and each based on uncontested evidence. According to our view, with the possible exception of the broad-scale virus, which leads to the appearance of cancer cells in a couple of days, in all other cases a number of factors of different nature intervene. To resonance changes, would be added chemical, viral, metabolic, hormonal or defense influences, at the same or at different levels, in order to provide the necessary circumstances for cancer development. The fact that, regardless of the nature of agents used to induce them, cancers, once induced, differ very little or not at all makes plausible the hypothesis of plural exogenous factors acting to bring about the necessary favorable conditions.

The above presentation—a resume of our research—must still be considered to be a working hypothesis.

Other aspects of the cancer problem have been analyzed anew in the light of the concept of pathogenesis discussed above.

Lipids and Carcinogenesis

As we have seen above, the recognition of the fact that a series of successive changes take place in carcinogenesis has invalidated the concept that in order to consider a substance active in this field, it has to induce the entire series of changes by itself, including the passage into the phase of invasive cancer. An agent can be considered active if it induces only a part of the successive series of changes. Didactically, we can thus consider changes concerning the subnuclear, nuclear, cellular and metazoic levels. We have investigated the intervention of lipids in carcinogenesis from this specific point of view.

The coordination of data from various observations and experiments has indicated that some of the lipids would act especially at the cellular and tissue levels.

Statistical data have indicated a greater proportion of cancer of the cervix in non-Jewish women as compared to Jewish women. This was tentatively related to the circumcision of the respective males and this correlation was confirmed by statistical data concerning other groups of population practicing circumcision, such as the Moslems. The probable role of smegma was seen in experiments in animals made by different workers. It was reported that in mice, smegma, sterilized or non-sterilized, introduced in the vagina of mice followed by the suture of the vagina, would induce papillomatous and cancerous lesions of the cervix.

Statistical studies (324) showed a similar correlation between cancer of the prostate and circumcision, with a lower proportion of cancer in circumcised individuals than in those not circumcised. Entirely different results were obtained by other workers. Studies made by the group of Memorial Hospital in Cleveland, concerning cancer-in-situ, showed no differences between Jewish and non-Jewish women. Similarly, several workers have reported that the prostates in individuals over 40 years of age, who died of conditions other than cancer, have present in high proportion cancer-in-situ cells. No correlation with circumcision could be found in these cases, the same proportion being found in all the ethnic groups examined. (325)

We tried to interpret these totally discordant conclusions of the two groups of statistics and found the explanation in the concept of plural intervention of carcinogens. In the first group of statistics in the cases of both females and males, the cancerous processes considered were those in the invasive cellular stage, while in the second group of statistics, the non-invasive cancer phase was considered. It appears thus quite clear that the intervention of the circumcision and respectively of the smegma exists but

has to be placed at a precise point in the progressive changes in the evolution of the cancerous condition, at the passage from noninvasive into invasive cancer. Without any influence upon the appearance of the cancer-in-situ, the smegma would act manifestly by changing the proportion of active cancer present. Its influence appears thus to be exerted at the cellular level, where the occurring changes result in the passage of the noninvasive into the invasive cancer, and at the tissue level where the loss of the capacity to defend itself against the cancer cells permits their invasion. This led us to the hypothesis that the cancerous process in the cervix and prostate, evolves independently of circumcision until the cancer-in-situ step, but will make the next step toward invasive cancer predominantly under the influence of the smegma of noncircumcised males. Without such intervention at this stage, such a change may occur but in an impressively lower proportion. The invasive cancer of the cervix is thus almost entirely non-existent in virgin nuns. This consideration and the richness of smegma in positive lipids has permitted us to go farther and consider the problem of the role played by these lipids in carcinogenesis.

We have tried for a long time, but with little success, to induce cancer in animals through the administration of unsaponifiable fractions alone. The several positive cases obtained in mice with repeated injections of the unsaponifiable fractions of placenta, chicken embryos, eggs or butter, have not appeared sufficiently consistent to warrant any conclusions.

In our attempt to investigate an intervention of the positive lipids for the specific change, corresponding to the passage of the noninvasive to the invasive cancer, we carried out the following experiment. We selected female mice with at least two previous pregnancies. After a third pregnancy, when in lactation, the mammary gland was injected with small amounts of the above mentioned unsaponifiable fractions. The results seem to indicate a higher proportion of mammary carcinoma in these mice than in controls.

Similarly, we have tried to influence cells of the cervix in mice, through the introduction of the same different preparations of unsaponifiable fractions in the vagina of ex-breeder mice, followed by suture of the vagina. The first results have shown a high proportion of malignant tumors. The concept of the intervention of abnormal lipids in carcinogenesis has led us to utilize in similar experiments, instead of the above preparations, unsaponifiable fractions considered to be heterogenized by being heated above 320°C. At the moment, these experiments which are in progress seem to indicate the existence of such an influence.

It is of even greater interest to note the influence exerted by the unsaponifiable fractions upon animals that had received urethane, according

to the experiments of Berenblum. By combining three factors, urethane for the first changes in the amino-acids, several pregnancies for the changes until the noninvasive phase and unsaponifiable fractions especially heterogenized for the passage into the invasive phase, a high proportion of invasive mammary and cervical cancers seems to be induced in the first experiments. Of importance appears the factor that a certain lapse of time is necessary between each two factors which are applied in the above mentioned succession. This concords with the concept of plural successive changes in carcinogenesis discussed above.

Carcinogenic Activity of Urethane

The interesting research of Berenblum has brought an important contribution not only for the largely debated role of urethane as carcinogen, but also for the problem of carcinogenesis in general. The fact that croton oil, applied to the skin, induces the appearance of malignant tumors in animals previously fed with urethane, concords largely with the concept of plural changes taking place in carcinogenesis. The analysis of the influence exerted by carbamic acid upon amino-acids would place the intervention of this agent at the first members of the biological realm. It can thus be seen that the bond between the amino-acid group and the carboxyl and amine groups of carbamic acid occur in a way similar to that which occurs between two amino-acids with the big difference that in the first case it would result in the appearance of the CNCN formation. (*Fig. 201*) As mentioned above, this CNCN formation represents the group which characterizes the first biological entity. The place of this CNCN group, not at the end of the molecule opposed to the carboxyl as in the alkaline amino-acids, but as corresponding to the bond which results in polymers, represents the anomaly, which according to the work hypothesis we advance, would correspond to the first cancerous entity. The fact that the specific activity of urethane takes place at the lowest levels of organization, explains the necessity that a certain time separates its intervention from that of croton oil, which would act only at the higher levels, probably inducing the passage from noninvasive to invasive phase. This time is necessary for the first cancerous changes to build up the series of cancerous hierarchic entities since the cocarcinogen, croton oil, would act only in those more evolved cancerous entities. In experiments in progress, the passage of the urethane-induced noninvasive cancerous entities into invasive cancer, is successfully obtained by treatment with preparations of unsaponifiable fractions of placenta or eggs.

INTERVENTION OF PSYCHOLOGICAL FACTORS IN CANCER PATHOGENESIS

The role of psychological factors in the pathogenesis of cancer, although still obscure, has been of increasing interest in recent years. Various theoretical considerations (*i.e.* the relationship between the known effect of emotions upon hormonal and biochemical balances and the possible effect, in turn, of the latter upon neoplasms), as well as a number of clinical reports and experimental and statistical studies, point toward psychological influence in cancer pathogenesis, but little is actually known in this area.

In order to explore this matter further, a research program has been carried on in our Institute since 1952 by Dr. L. LeShan. This study has included the evaluation of projective personality tests given to over 300 cancer patients; interviews of 2 to 8 hours each with over 150 patients; and extensive exploratory psychotherapy (of from 60 to 400 hours) for 25 patients. Control groups were also included in each category. For patients undergoing psychotherapy, regular comparisons were made between the personality picture and various biochemical activities reflected in blood and urine analyses.

A "back-and-forth" method between the three techniques of personality evaluation has been employed. Hypotheses formulated from data obtained with one technique have been evaluated, refined and clarified by data from the others. When a hypothesis was consistently supported by all three approaches, an attempt was made to formulate it in terms permitting it to be subjected to critical test by experimental or statistical technique.

As an example, an hypothesis was developed that the cancer patient, more often than chance would allow, had lost a major emotional relationship, and had been unable to find a satisfactory substitute, some time before the first apparent symptom of cancer. This hypothesis appeared to be validated by data from all three techniques. It was then formulated in terms by which it could be tested. If the hypothesis were true, then certain social groups which, *a priori*, had known higher rates of such losses should also have a higher cancer mortality rate. Thus, for example, if marital status were taken as the only variable, then, after age was cancelled out, we should expect the highest cancer mortality rate in the "widowed," the next in the "divorced," the next in the "married" and the next in the "single." Published data, such as census material, could be used to explore the accuracy of this prediction. Various predictions of this type—all based on the hypothesis—were made. When tested against published statistical data, all were demonstrated to be valid. (116, 117)

At this point of the research, one general, emotional pattern has been found in over 50% of the 300 studied cancer patients and in approximately 10% of the equated controls: An early life history with much self-doubting and some anxiety over relating to others; the establishment of one personal relationship that afforded a high degree of satisfaction, meaning and validity to the individual and provided him with a "raison d'être"; and the loss of this relationship, followed by inability to find a substitute, and a period of intense (if often concealed) depression. This has been elaborated upon, and case histories presented in various publications. (118, 119, 120, 121, 122)

In summing up this research in a recent paper (123), the following conclusions could be reached by LeShan:

1. There seems to be a correlation between the existence of neoplastic disease and the persistence of certain types of psychological situations.
2. The most consistently reported, relevant psychological state has been the loss of a major emotional relationship. Often the psychic state resulting from this loss could be traced to a period shortly before the first noted symptoms of cancer.
3. There appears to be some relationship between personality organization and the evolution of the cancerous condition.
4. There may be some relationship between personality organization and the type or location of a cancer.

It would seem as if future research in this area, to be as useful as possible, must focus upon the chemicophysiological changes which result from variations in psychic states in general. It is highly probable that these changes are mediated through the endocrine system. Through the linking of psychic states with hormonal changes, we may be able not only to integrate psychological factors with the many other factors influencing development of cancer, but also to relate them to certain levels of organization. It may be possible to establish the relationship of psychological factors to other influences which favor or even induce passage of cancer from one phase to another. This may permit a more complete understanding of cancer and help in finding new points at which some therapeutic value might be expected from psychological intervention.

The relationship between the adrenals and psychic states on the one hand, and between the adrenals and the lymphatic system on the other hand, could explain why the influence of a psychologically unresolved problem is most evident, among all cancerous conditions, in lymphomas. LeShan has been able, by analyzing a sizable number of these lymphoma cases, to

recognize more clearly and more consistently than in other conditions the existence of a pattern of psychological changes occurring prior to clinical illness. Recurrences of symptoms, or periods of exacerbation, also could be connected to events which had deep repercussions upon the psychological state.

Research along these lines, seeking information on psychic factors in the pathogenic mechanism of cancer, is being pursued actively by LeShan and his co-workers at our Institute.

CHAPTER 12

PHARMACODYNAMIC ACTIVITY

IN OUR RESEARCH, we have investigated the pharmacodynamic activity of a series of substances in terms of the physiopathological concepts discussed above: the level of organization at which they act, the dualistic nature of their activity, their relationship to body constituents, especially to lipids, and the changes they induce in the defense mechanism. We will limit ourselves in this presentation only to those of the substances investigated which are of therapeutic interest.

The dualistic concept has permitted didactic separation of agents into two groups with antagonistic properties. In the last analysis, this separation could be related to the two fundamental tendencies in nature, homotropy or heterotropy. For inorganic agents, this criterion appears to be directly related to the elements present. However, for organic agents, especially for lipids and lipid-like substances, this simple criterion is less valid. Another, the positive and negative electrical character of their active polar groups could be used. It must be emphasized, however, that some of these polar groups show either positive or negative properties depending upon the medium in which they work. An alcohol, for example, can act as an acid under special circumstances, in which case it forms metal alcoholates; or it can act as an alcohol forming esters with acids. For this reason, in considering these substances, we have limited the sphere of changes studied to those corresponding to the conditions present in biological entities.

Since, as seen above, substances with negative polar groups actively intervene in various processes, while those with positive polar groups control the activity of the first, we will start this presentation with the former.

AGENTS WITH ACTIVE NEGATIVE POLAR GROUPS

Among agents which have negative polar groups, a further division can be made according to the nature of these groups. Under biological conditions, the most important of negative polar groups is the carboxyl. Losing a proton when dissociated, the carboxyl confers acidic property to the molecule into whose structure it enters. We will consider, first, such carboxylic acids. Among such agents, the individual differences seen in biological activities have to be related to their nonpolar parts.

In the frame of our research, with organic acids, we were especially interested in those with a predominance of the nonpolar group; that is, those having lipoidic properties. They are, principally, fatty acids, which, according to the presence of one or more double or triple bonds in the nonpolar part, can be separated into saturated and unsaturated.

Fatty Acids

Saturated Fatty Acids

The lipoidic character in this homologous series starts with the five carbon valeric acid, although caproic acid is the first lipoidic member found in animals. The role of the saturated fatty acids in organization is largely related to the activity, as caloric metabolites, of the members with even carbon numbers. These saturated fatty acids are absorbed, circulated and stored as triglycerides, and it is also this bond to glycerol which apparently favors their biological role as caloric metabolites.

As previously noted, only the members with a relatively short chain, less than 12 carbons, can be directly metabolized through Knoop beta oxidation. Longer chain saturated fatty acids undergo a breakdown before further metabolic degradation. Members with 16 and more carbons also play a limited constitutional role, usually together with an unsaturated member, when bound to glycerophosphoric acid to form phospholipids. Even when free—that is, when their polar group is not neutralized—they do not have a manifest functional role and, consequently, show no important pharmacodynamic activity.

The higher fatty acids exert no demonstrable influence upon microbes nor upon viruses such as bacteriophages. No influence has been observed upon monocellular organisms, cells or tissues *in vitro*. No changes were seen in the respiration of liver slices or of Sa 180 ascites cancerous cells in the Warburg microrespirometer. Similarly, no influence can be seen on red blood cells nor on leucocytes treated *in vitro*. No changes in the chloride

content of tumors or wounds are induced by administration of these acids. The fact that no change could be induced in the second day wound crust pH explains the lack of any effect upon pain.

Even large doses, such as 20 cc. of a 10% solution of these acids in sesame oil, do not change the intensity of pain of alkaline or acid pattern in humans. No influence has been noted upon tumor evolution, even with the direct technique of dipping tumor transplants in the agent, repeated for successive transplants. Existing edema or ulceration is not influenced. At the organic level, no changes can be seen in the function of various organs or upon liver regeneration rate. These fatty acids do not influence convulsions induced by thiamine in rats even when administered in very large doses, such as 5 cc. or more of 10% solutions for 100 gr. of body weight. The same lack of influence is seen on systemic metabolic manifestations, as recognized through various analyses. Except for palmitic acid, which shows an adrenal defense index of 12, the index for the other members of this series is below 6, indicating only a certain participation of the adrenals in the defense mechanism against these acids.

No changes can be found in the number or character of leucocytes in animals treated for a few days with these acids. No change in the sodium or potassium content of the blood or in urinary analyses is induced by these substances even in subjects whose analyses reveal abnormal patterns. No action upon body temperature and no influence upon the evolution of the defense mechanism can be observed.

It is interesting however, to note the effect of the sodium salts of these acids, especially on *in vitro* lysis of different cells. (*Note 1*)

Unsaturated Fatty Acids

Of the unsaturated fatty acids, oleic acid is the most widely encountered in nature. As previously noted, *monoethenoids* have a group of 9 carbons either toward the carboxyl or methyl end of the molecule. With the double bond between C₉ and C₁₀ in a molecule of 18 carbons, oleic acid appears to satisfy both tendencies, which could account for the ubiquity of this acid.

Oleic acid circulates, is deposited as reserve, and is used as caloric metabolite especially when bound to glycerol. In considerably lesser amounts, when bound to glycerophosphoric acid, it takes part as a phospholipid in the formation of the lipidic system of the organism. It is only slightly active in oxidation processes. For this reason, large doses are necessary to influence abnormal processes at different levels. Even then, only limited changes are induced. In *in vitro* studies, oleic acid produces no changes in bacteriophages. A certain influence is noted for receptivity of

dermotrope viruses. Injected subcutaneously in rabbits, oleic acid induces in the skin, at the site of injection a zone of low receptivity for smallpox virus. Oleic acid has a reduced effect upon microbes, causing the appearance of some gram negative individual forms of *Bac. subtilis*, for instance. Mixed directly with blood, oleic acid induces hemolysis. When plasma is treated with this acid, and then mixed with red cells, the influence exerted upon red cells because of the small amount of acid fixed upon plasma, is reduced. Although oleic acid may influence the pH of the second day wound crust, causing an increased local alkalosis (*Fig. 119*), its influence

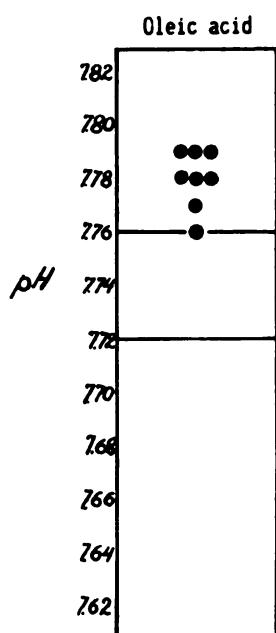


FIG. 119. The second day wound crust pH for oleic acid shows the constant presence of a change toward more alkaline values.

upon pain is almost nil. Little or no change is seen in standardized radiation wounds in animals treated with the acid. A limited influence can be observed on tumor growth by using the technique of dipping transplants for successive generations. Use of a 10% solution of oleic acid in tricaprylin before grafting, repeated for successive generations, impairs growth of Ehrlich mouse adenocarcinoma after the sixth or seventh generation in some experiments, even later in others. Negative passage takes place between the eighth and tenth graft. Under the same condition, very little or no changes are noted in other tumors, and no changes are seen in tumors in animals treated with this acid even though changes have been reported by some authors. (124) Suspensions of cells of different organs, treated *in vitro* with colloidal suspension of oleic acid and injected in animals of

the same species, induced no changes with a single injection. Repeated injections after 3 weeks induced lesions in the respective organs.

At the organic level, some effects can be obtained by using high doses of oleic acid. For example, to prevent convulsions induced by thiamine chloride, doses as high as several hundred milligrams per 100 gr. of body weight are required and even then this effect is not constantly seen. Systemic changes, recognized through blood and urine analyses, are almost nil, even with large doses of oleic acid. The compound however, does prolong liver regeneration time.

Among the *diethenoic*s, we studied linoleic acid. The caloric activity of the uncombined fatty acid is reduced while constitutional and functional activities are increased. Linoleic and linolenic acid appear to be organizational rather than functional fatty acids because of their preferred bond to glycerophosphoric acid. They are absorbed from the intestines, mainly as phospholipids. No effects upon phage can be seen. The refractivity to smallpox virus induced on rabbit skin is more manifest than for oleic acid. However, the effect upon microbes, such as *subtilis*, is less evident than for oleic acid. Crenelated red cells, with increased tendency to conglutinate and increased sedimentation rate, are found after linoleic treatment of the blood in vitro. To avoid hemolysis, the acid is not added directly to the blood but to the plasma which is then reunited with the cells. Crenelated cells also appear *in vivo* in rats injected intraperitoneally with large amounts of this acid, such as 10 cc. of 10% in oil. A definite shift toward alkalosis is found in the second day wound crust pH, which explains the influence seen upon pain. Linoleic acid increases pain of an alkaline pattern and decreases pain of an acid pattern, though only slightly. Only in relatively large doses (2-400 mg./100 gr. animal), does it prevent the convulsions induced by thiamine.

In lesions induced by radiation, the administration of small doses of this fatty acid often produces a favorable healing effect. This effect can be related to the growth-stimulating action of essential fatty acids in small quantities. The effect is just the reverse of the unfavorable influence on healing observed when larger doses are administered. An effect upon tumors in animals can be achieved only through repeated treatment of successive transplants or through the treatment of successive generations of the host. The effect of repeated injections of tissue cells treated in vitro with a suspension of linoleic acid was more manifest than that obtained with oleic acid. The effect upon the systemic level, although more manifest than for oleic acid, still is limited, even under abnormal conditions. Blood and urinary analyses are only slightly and briefly changed toward the D type of

offbalance even when large amounts are administered. Blood eosinophiles are decreased and potassium content increased, both only slightly. Body temperature is slightly depressed.

Preparations especially rich in triethenoid *linolenic acid* were used and no differences between their biological activity and that of linoleic acid could be noted.

We obtained preparations especially rich in *arachidonic acid* from salmon oil. The caloric contribution of this acid can be considered almost nil compared to its functional role. It is absorbed, circulated and stored, principally as esterifying sterols. Although this acid is present in the body in relatively small amounts, it represents more than 25% of the fatty acids of the adrenals. In view of the highly functional role of the adrenals, it is logical to suppose that arachidonic acid's abundance in the glands is not merely coincidental. A liberation of this acid, together with other higher polyunsaturated fatty acids from the adrenals appears to take place during the first phase of the diphasic defense phenomenon. In the first minutes following a noxious intervention inducing shock, a depletion of fatty acids in the adrenals occurs, coinciding with increased amounts in blood.

Besides their role in the defense mechanism, the adrenal fatty acids seem to intervene in normal physiology. Successive liberations seem to occur, alternating with liberations of sterols, as well as corticosterones. Together, these produce the diphasic oscillations which characterize the physiologic dynamic balance.

Preparations rich in arachidonic acid seem to act at different levels. No manifest influence upon phages is observed. The influence upon smallpox viruses and upon microbes is similar to that of the linoleic preparations. The *in vitro* and *in vivo* effects upon red blood cells, such as crenelation, conglutination and increased sedimentation rate, are more manifest than for linoleic acid. Leucopenia also is seen. The effects upon the pH of the second day wound crust and upon pain are, however, the same as for linoleic acid. The intensity of acid pain is reduced while that of alkaline pain is increased. In doses much smaller than for linoleic acid, arachidonic acid preparations are able to prevent convulsions induced by thiamine. But they do not seem to change the evolution of radiation lesions. The influence upon tumors is very similar to that of linoleic acid, and only limited changes are observed after treatment of successive generations, either through the dipping of transplants or through treatment of successive hosts. Organ cells treated *in vitro* with suspension of this fatty acid were seen to induce changes in the respective organs, if injected twice at 3 weeks interval. Systemic influence is not manifest even under abnormal conditions.

Blood and urine analyses are slightly and temporarily changed toward the pattern of fatty acid predominance.

Continuing these studies, we have investigated *polyunsaturated fatty acids* with more than four double bonds, particularly clupanodonic acids from cod liver and sardine oils. Most of the studies were made by using the fractions which when brominated are soluble in acetone at a low temperature, *i.e.*, around -63°C . The different fractions obtained were identified through iodine number, neutralization value and spectral analysis after conjugation through treatment with KOH.

All the biological effects upon viruses, microbes, cells, etc., are more accentuated than for linoleic or even arachidonic acid. Changes in red cells and leucocytes are much more apparent. At this point we must emphasize the preference of these fatty acids for red blood cells over plasma both in vitro and in vivo treatments. (*Note 2*) They have greater effects upon pain than do linoleic and arachidonic acids, reducing pain of an acid pattern and exacerbating pain of an alkaline pattern. The local pH, as determined by second day wound crust measurements, also is shifted toward increased alkalinity. Convulsions induced by thiamine are influenced by much smaller doses than those required with other unsaturated fatty acids. For some preparations with iodine indices around 350, doses as low as 35 mg./100 gr. of body weight are sufficient to prevent convulsions. Changes in the evolution of tumors are more striking with these preparations, especially when the transplants of successive generations are dipped in the preparation. With this technique, negative results are obtained even at the third transplant for Ehrlich mammary adenocarcinoma. An obvious effect is noted on radiation-induced lesions, with ulceration increased and healing slowed. The influence upon cholesterol levels in the blood and upon hypertension is greater than for arachidonic acid. The effects upon systemic changes, observed through analyses, are temporary and no greater than for linoleic and arachidonic acids.

Acid Lipidic Fractions

Bearing in mind the role of acid lipidic constituents in the physiology of various biological entities, preparations containing these fractions were obtained. Tissues, organs, organisms, and organic products were saponified and acid fractions soluble in ether were isolated. We called them "acid lipidic fractions," or "lipoacidic fractions." Their analyses revealed, in addition to various fatty acids, other substances with lipidic and acid character. Some were identified as porphyrinic acids.

Significant differences related to the sources of these preparations could

be recognized in biological effects at different levels. Preparations obtained from intestine, for instance, had no obvious effects on any of the manifestations; there were no systemic or organic changes, no effects upon pain, red cells or leucocytes *in vitro*, and no influence on tissue respiration. Treatment of successive transplants showed no apparent effects, even after ten passage generations. No effects upon organs through repeated injections of cells treated *in vivo* by these preparations were seen.

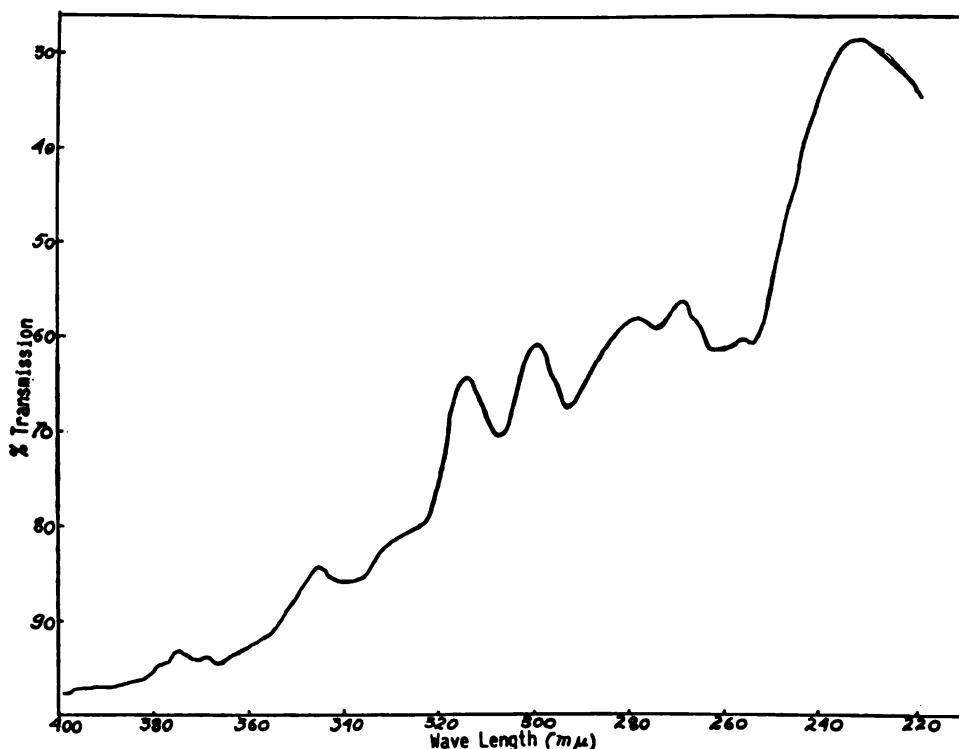


FIG. 120. Spectral analysis of rat liver fatty acids after chemical isomerization shows the presence of fatty acids with 3 and 4 double bonds. (0.002% in ethyl alcohol)

On the other hand, other preparations from placenta, liver, blood, etc. did show activity upon all manifestations, including pain. They induced negative results on tumor growth after transplant dipping for just two or three generations.

The differences in activity could be related to the richness of these preparations in polyunsaturated fatty acids. It could be shown that it was not the total number of double bonds present, as determined by the iodine number, that was significant but rather the relative abundance of higher

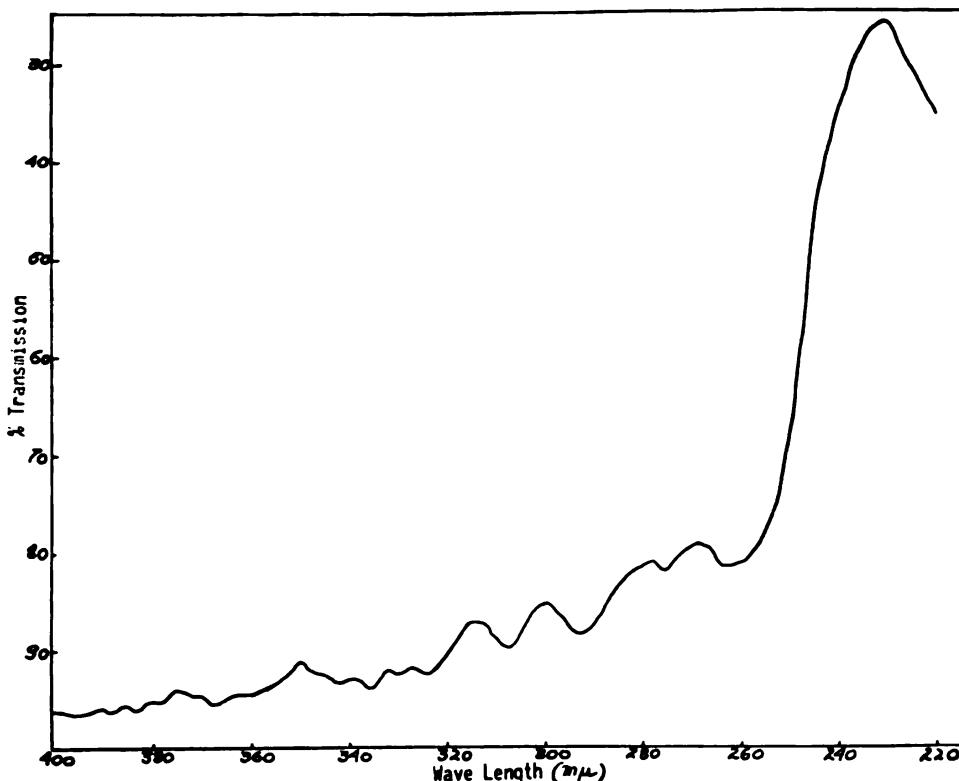


FIG. 121. Spectral analysis of the intestinal fatty acids of rats after chemical isomerization shows minimal amounts of members with 3, 4 or more double bonds. (0.002% in ethyl alcohol)

unsaturated members, as recognized by special analysis after adequate chemical conjugation. Figures 120, 121 and 122 show such analyses.

Abnormal Fatty Acids

Because of the role of abnormal fatty acids in the pathogenesis of the offbalance type D, particularly related to radiation, a study was made of preparations of acid lipids obtained from abnormal tissues, organs and organisms. In an initial group of researches, animals that had died of radiation sickness, shock, acute infections or after adrenalectomy were used. Guinea pigs infected with *B. anthracis* and mice infected with strep hemolyticus were used as sources of abnormal lipids in a large number of experiments. Acid lipids obtained from autolysates of tissues were employed. We also used the fatty acids of cod and sardine oil, which may be considered to correspond not to natural but to altered fatty acids since they were obtained after autolysis of cod liver and whole sardine bodies.

After having determined that conjugation of double bonds is the basis

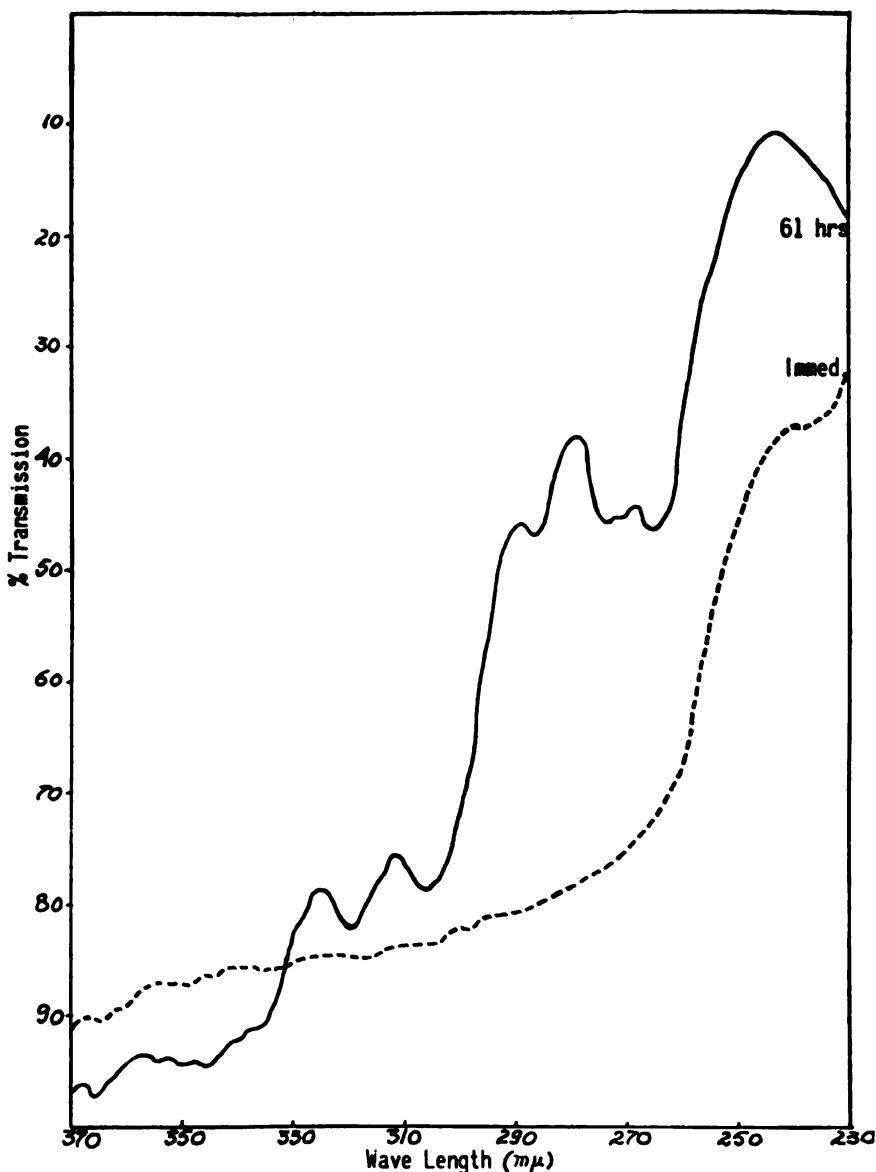


FIG. 122. Spectral analysis of the fatty acids of the entire body of rats, after chemical isomerization shows the presence of di- and triethenic members, and little of members with more double bonds. (0.002% in ethyl alcohol)

of abnormality in pathogenic fatty acids, conjugated isomers of different fatty acids were prepared and studied. Eleostearic acid obtained from tung oil was used extensively in animal and clinical research. Parinaric acid was obtained from nuts of parinarium laurinum and was used to a lesser degree. Various conjugated fatty acid isomers, recognized through spectral analysis and oxalic indices, were obtained in a higher proportion from

different lipoacidic preparations by using a modification of classical methods of conjugation. (Note 3) Many mixtures of naturally occurring fatty acids found in saponifiable fractions were conjugated by the same method. Conjugated di, tri, tetra, penta—and hexaenic fatty acids were further separated from these mixtures and studied. The unexpected relationship of these conjugated fatty acids to oxygen was of interest. While the treatment of linoleic acid at 37°C with oxygen leads to progressive increase in the amount of peroxides present, this does not occur for the conjugated isomer (Fig. 123) apparently because of repeated destructions of the peroxides formed.

In general, the effects produced by conjugated fatty acids at different levels of the organization are more intensive and persist longer than those

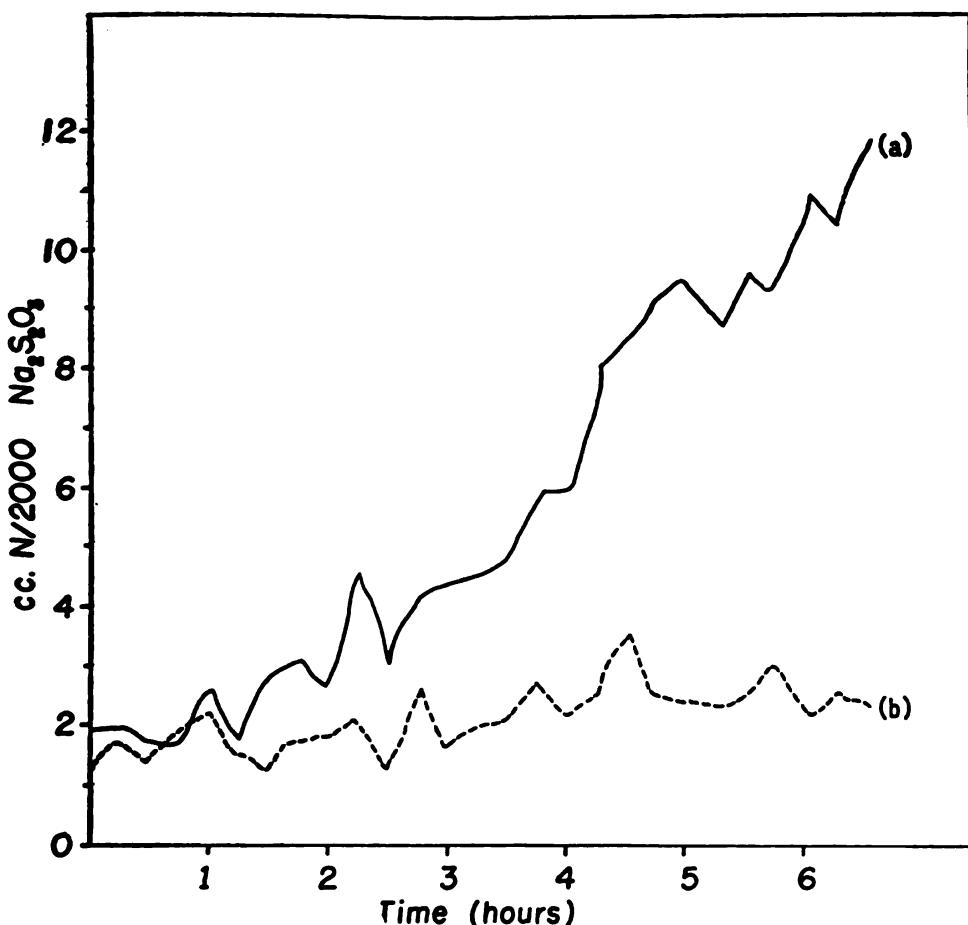


FIG. 123. Curves of peroxides of samples of linoleic acid (a) and its conjugated isomer (b) induced through the passage of oxygen (100 ml per minute for 30 cc of sample) at 37°C. While peroxides are progressively increasing in the linoleic acid preparation, they do not change in the conjugated isomer.

of the nonconjugated isomers. It is interesting to mention here the influence exerted by these preparations upon viruses. Although there are no changes for bacteriophages, a marked influence is seen *in vivo* upon receptivity of the organism to viruses. Subcutaneous injection of conjugated fatty acids in rabbits establishes a zone of refractivity toward inoculation of smallpox vaccine in the skin, which is greater and more persistent than that observed for nonconjugated isomers. The effect upon microbes also is clear. Gram negative strains with manifest morphological changes were obtained from *B. anthracis*, and persisted as such for 6-15 passages before the old morphological and tinctorial characters returned. The effect upon red cells and leucocytes *in vitro* is more apparent than for corresponding nonconjugated isomers. This is also true for the influence upon the respiration *in vitro* of tissues or ascites cells.

The difference in the effects of conjugated and nonconjugated fatty acids is particularly striking in certain manifestations. For example, pain with an acid pattern is more easily changed to alkaline by treatment with conjugated fatty acids than with nonconjugated isomers. Once the alkaline pattern appears, it persists for a long time. A manifest effect is seen upon lesions induced by radiation. Standardized radiated wounds in rats, which heal in about four weeks in control animals and require six to eight weeks to heal when treated with nonconjugated fatty acid preparations, fail to heal at all when treated with corresponding doses of conjugated isomers of the same fatty acids.

The isomers of fatty acids also differ in their effect upon animals in shock. When anaesthetized animals are scalded by immersion up to the level of the xiphoid in water at 90°C, immediate fatal shock occurs if the immersion lasts for more than four seconds. With a three second scalding the animals die after several hours. The administration of fatty acid preparations markedly reduces the survival time, and this effect is more manifest for conjugated members, especially for eleostearic acid. In general, animals in shock induced by any means, such as by the Noble-Collip drum, show a special sensitivity toward conjugated fatty acid preparations.

The effect of conjugated fatty acids at the systemic level, as recognized by blood and urine analyses, is in the same direction as that for the nonconjugated but is more manifest.

There are significant differences between the effects of conjugated and nonconjugated fatty acids upon the evolution of transplanted and spontaneous tumors. In a small proportion of mice, a mixture of nonconjugated fatty acids prepared from cod liver oil or sardine oil prevents the appearance of methylcholanthrene-induced tumors. The conjugated fatty acids

obtained through the treatment of these preparations show this preventive effect in a large proportion of mice. These experiments are interesting from several standpoints and are presented in some detail in Note 4.

Conjugated fatty acids produce an increase in the chloride content of wounds in animals. Values are 40% higher than in untreated lesions. The same increase of chlorides occurs in tumors. When Dba mice with adenocarcinoma were treated for ten days with conjugated fatty acid preparations, and tumors were removed and chloride content determined, values were up to 180% higher than for controls with untreated tumors. (*Note 5*) An interesting effect was noted in two rat tumors. For years, passages of Guerin's rat tumor and of sarcoma induced in our laboratory by the injection of benzpyrene have shown a peculiar character. Although they grow to large size, the tumors have no necrotic zones. After treatment with conjugated fatty acids, large necrotic zones appeared, leading to ulceration and death. The appearance of these zones of necrosis corresponds to changes in the fundamental character of the tumor. Transplants of fragments of these treated tumors, although taken from nonulcerated regions, or young tumors, resulted in tumors showing early central ulceration. This character persisted without further treatment in succeeding generations. The ulcerating tumors appear to be a mutant of the original and the mutative change can be related to intervention of conjugated fatty acids. This was confirmed by the fact that similar changes were constantly obtained in the same tumors with the same agents. A similar but less manifest and less constant effect is obtained with preparations of cod liver oil fatty acids administered repeatedly in large amounts. The same effect was obtained with the injection of the fatty acids directly in the tumor itself.

The second day wound crust pH shows marked changes toward alkalinity under the influence of conjugated fatty acids. The effect upon regeneration time of liver is also manifest; cells full of fatty droplets do not appear at all or appear much later than in untreated controls. In rats weighing 200 grams, with a large enough dose, such as 2 cc. of 10% solution of polyconjugated fatty acids obtained from cod liver oil and repeated for two days, the adrenals show complete depletion of fats. They become small and red in color and contain no sudanophil material. The liver regenerates with small cells with compact cytoplasm and almost entirely bare of fatty droplets.

The effect of conjugated fatty acids upon the lymphatic system is manifest. A marked involution of thymus, spleen and lymphatic gland follows the injection of conjugated fatty acid preparations, particularly of a mixture of conjugated cod liver oil fatty acids. The effect upon tumors is irregu-

lar. In some animals a marked retardation occurs, in others no effect can be observed. In convulsions, no greater effect is seen than that produced by nonconjugated isomers. Blood *in vitro* assumes a color darker than when other fatty acids are used. Eosinophiles are markedly reduced after administration of conjugated fatty acid preparations. Important changes in organs were obtained with repeated injections of the respective cells treated *in vitro* with suspensions of these acids. Changes in the analytical values of urine, however, are not greatly different from those obtained with nonconjugated isomers. With sufficient amounts of conjugated fatty acids, a frank hypothermia is obtained.

Bixine

In the same group of fatty acids we can place bixine, a member of the polyterpene family with 9 conjugated double bonds which we obtain through saponification of the seeds of *Bixa orellana* and have studied widely. The changes produced in microbes are similar to those with other polyconjugated fatty acids. Changes in connective tissues in animals appear to be particularly interesting. The first reaction to subcutaneous injection of an oily solution of 1% bixine in rats or mice is an inflammatory process, with the injected material dividing into hundreds of tiny droplets. Some, however, melt away and the unabsorbed injected material again forms one or two big drops. The wall containing the drops is very thin and transparent and appears to be made up of very few connective cells which have extremely long fibrils, representing the highest degree of differentiation of these cells.

The effect upon pain is similar to that of conjugated fatty acids. The second day wound crust pH shows a manifest change toward alkalosis. The effect upon radiation wounds is the same as for conjugated trienes. In animals injected with convulsant doses of thiamine, only a few milligrams of the bixine preparation are required to prevent convulsions. Of all the fatty acid preparations used, bixine appears to be the most efficient in its anticonvulsant action. The iodine number of 430, found in our preparations, confirms, thus, in this case too, the correlation seen between anticonvulsant effect and richness in double bonds of the fatty acids.

The distribution of bixine following administration is interesting. Chromatographic study of blood constituents after hydrolysis shows that almost all the bixine is in the red cells, with minimal quantities in plasma. Lesions such as wounds or tumors, after the administration of bixine, become particularly rich in this substance in comparison to normal tissues. Changes in evolution of tumors also are manifest. The administration of this agent

often leads to rapid necrosis and edema. In animals and humans, we saw massive tumors become ulcerated in a few days after use of only a few milligrams of the substance. The ulcerating effect of conjugated fatty acids upon tumors reaches its maximum with bixine. In animal tumors which, in successive transplants, had never shown spontaneous ulcerations, the injection into the host of only a few milligrams of bixine in oily solution produced, in addition to ulceration, a change in the tumor which can be considered to correspond to a mutation. Further transplants consistently developed ulcerating tumors and the ulcerative character persisted in succeeding generations. Massive degenerating changes were obtained in organs after repeated subcutaneous injections of suspensions of cells obtained from these organs and treated *in vitro* with bixine.

The changes toward offbalance D induced in various systemic patterns in humans by bixine are similar to those produced by conjugated fatty acids. However, once induced, these changes are very persistent, and often remain uninfluenced by anti-fatty acids. It is this characteristic of resistance to further changes which represents a certain handicap for therapeutic use of this agent.

Fading Response

In contrast to the relatively persistent changes induced by bixine, the striking character of the effects upon pain or systemic manifestations obtained with fatty acid preparations, and especially with the conjugated isomers, is their short duration. Furthermore, at the beginning of their administration, these agents, even in relatively small doses, exert intense effects, but such effects cannot be obtained later without continuously increasing the amount used. After a certain time, even large doses have very little effect.

An explanation of the fading character of the results obtained with these and many other agents can be found in the fact that the organism defends itself against any factor able to influence its balance. In the case of conjugated fatty acids, this defense seems to be provided mainly by the adrenal glands. E. F. Taskier has studied this aspect of adrenal defense in our laboratory and this research is presented in *Note 17, Chapter 6*. An adrenalectomized animal is usually less resistant toward the administration of many agents than a normal or sham-operated animal. This drop in resistance is expressed as an adrenal defense index, as the ratio between the minimal lethal dose for the normal and for the adrenalectomized animal. For most fatty acid preparations, this index is between two and three, and becomes greater for the conjugated fatty acids. Fatty acids with three con-

jugated double bonds, however, have an adrenal defense index of 120. Eleostearic acid is 120 times less toxic for sham-operated controls than for adrenalectomized animals, indicating a specific adrenal defense against these acids. Progressively increased intervention of the adrenals would explain the fading effect mentioned above.

Alpha Hydroxy Fatty Acids

Alpha hydroxy fatty acids were obtained by fixing an OH at the carbon adjacent to the carboxyl. Some of these acids exist in nature—in significant amounts in the brain and skin, and in very small amounts in the kidneys. In our research, they were originally prepared from natural sources, such as animal brain and skin. Most of the studies however, were made with synthetic alpha-hydroxy-fatty acids. For experimental purposes in animals and humans, we principally used pure synthetic alpha hydroxy fatty acids. Mixtures of these members obtained through the treatment of acid lipidic preparations also were employed.

In animals and humans, alpha hydroxy fatty acids induce less systemic, organic, tissue and cellular changes than do the corresponding untreated acids. However, we would like to mention a striking exception: the response of lymphosarcoma 6C3HED in mice to the administration of alpha hydroxy-caprylic acid. Although this tumor uniformly kills control animals within 10-12 days, it disappears in over 80% of animals treated with alpha hydroxy caprylic acid, even if treatment is instituted late, that is, when the tumor has already grown to 1 cm. in diameter, a size usually reached 2 or 3 days before death. In the few animals in which the tumor does not disappear, its growth is so slowed down that survival time is extended to three or more weeks. (*Note 6*)

Other alpha hydroxy fatty acids close to caprylic acid, such as alpha-hydroxy-caproic and capric acids, show no influence upon evolution of this tumor. We could not obtain similar effects with any of the other saturated alpha hydroxy fatty acids that have chains with 4-20 carbons, nor with alpha hydroxy-oleic or linoleic acids. Nor did alpha hydroxy-caprylic acid or any of its homologues appear to have any influence upon other transplanted tumors in mice, the Walker tumor in rats or, spontaneous mammary tumors in mice.

Other Fatty Acids

The fading effect seen with naturally occurring fatty acids is so great a handicap for therapeutic use of these substances that we searched for heterogeneous fatty acids which the organism does not normally encounter

and against which it would not be prepared to defend itself. This brought us to the study of fatty acids having different nonpolar groups than those of the normal and abnormal constituents. While most of these were prepared synthetically in the laboratory, we utilized on a substantially large scale two natural fatty acids which exist in plants and are sufficiently heterogeneous, ricinoleic and crotonic acids.

Ricinoleic acid has a double bond between 9 and 10 and a hydroxyl at carbon 12 instead of the second double bond found in linoleic and linolenic acids. As a result of the induction effect propagated from the carboxyl through the chain, the C₁₁ is a positive carbon. The positive character is enhanced by the adjacent double bond between C₉ and C₁₀, and by the hydroxyl bound to C₁₂. C₁₁ thus is very strongly positive. We related the intense local alkalosis with consequent water excretion corresponding to the alkaline watery diarrhea to the effect of ricinoleic acid liberated in the intestine, and have considered it to correspond to a local organic offbalance similar to that induced in tissues by unsaturated fatty acids. This would explain the intensive laxative effect of castor oil.

We utilized ricinoleic acid parenterally with the aim of obtaining a similar effect in abnormal cellular and tissue lesions. The oily solution of ricinoleic acid has low toxicity when administered parenterally. However, no manifest effect upon tumors or at different levels of organization was obtained. Crotonic acid did not show the expected influence at these levels.

Other heterogeneous agents, polyhydroxy fatty acids, were studied. They were prepared by adding one or more OH groups to the nonpolar groups at the double bonds of unsaturated fatty acids. 9, 10 dihydroxy and 9, 10, 12, 13 tetrahydroxy fatty acids were no different than the corresponding unsaturated fatty acids in their effects upon pain or systemic analyses in humans, or upon tumor growth in animals and humans.

Peroxide Fatty Acids

Peroxides and epoxides of fatty acids were prepared and studied. They showed more manifest effects upon viruses and bacteria in vitro and in vivo than the other fatty acids. Investigations of the effects of these fatty acids upon higher levels have been limited until now. It seems that the effects upon systemic and organic manifestations are somewhat different than those obtained with use of the fatty acids from which the peroxides and epoxides were derived. Influence upon pain and upon tumors was greater for the corresponding unsaturated fatty acids. This research—especially with polyepoxide fatty acids—is still in progress.

Halogenic Compounds of Fatty Acids

The study of changes in lesions where abnormal fatty acids are present has shown the importance of fixation of chloride ions in these substances. Considering its place in the periodic chart, chlorine is an element with heterotropic character. Consequently, it could be conceived of as being antagonistic to fatty acids, counterbalancing their homotropic character. This was confirmed by pharmacological study of fatty acids to which chloride ions were added at the double bonds. We were particularly interested in conjugated fatty acids in which effects of treatment with chlorine could be followed through spectral analysis.

When a mixture of conjugated fatty acids from cod liver oil was treated with chlorine, the peaks in spectral analysis progressively disappeared. (Fig. 124) This did not lead, as expected, to increased toxicity. Thus far, in early trials, the different preparations, from 9-10 dichloro-stearic acid to the polychlorinated mixture of fatty acids from cod liver oil, have not shown effects greatly different from those of conjugated fatty acids upon tumor evolution, systemic analyses, and pain. No difference has been noted between the derivatives and their corresponding fatty acids in animal

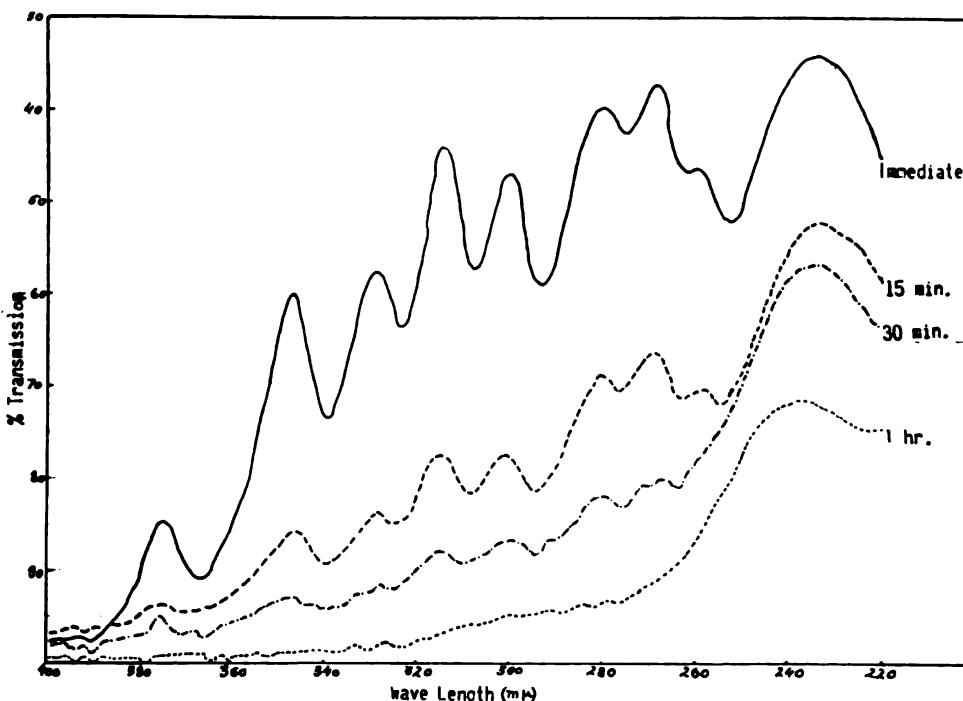


FIG. 124. Influence exerted by chlorine gas upon the conjugated fatty acids. A parallel decrease in the amount of all the members is seen. (0.002% in ethyl alcohol)

experiments or in research in humans. Experience with these products, however, indicates that they may induce milliar gastric ulcerations which we consider to result from the influence exerted by the fatty acids upon the gastric mucous membrane where they are brought by the chloride ions to which they are strongly bound. Research in this direction confirms the part which these fatty acids, solidly bound to chloride ions, take in the pathogenesis of the state of shock.

An over-all analysis of the pharmacological activity of the fatty acids mentioned above shows a similarity in fundamental effects obtained with most of these preparations. This can be interpreted as resulting principally from the fact that all have in common, their lipoidic character and the same polar group with acid properties—the carboxyl. As a result, these substances are fixed in the same position in abnormal entities, a fact which seems to represent the most important factor in their pharmacodynamic activity. The further biological differences seen between the influence exerted by the various members studied would be related to a secondary effect of these substances resulting from the intervention of the nonpolar groups. This finding—that the fundamental pharmacological activity of fatty acids is connected with the site of activity which is determined by the lipoidic character and the polar group present while the proper pharmacological activity is due to the intervention of the nonpolar group—has been of capital importance not only for understanding the activity of these substances but also for determining the path of our further research. Because of this, we investigated, in a second step, lipoids with other negative polar groups.

LIPOIDS WITH OTHER NEGATIVE POLAR GROUPS

Lipoaldehydes: With a carbonyl as polar group, the lipoidic properties appear with propanal in the homologous series of the aliphatic aldehydes. With the carbonyl less dissociated than the carboxyl, the lipoaldehydes represent *negative* lipoids able to act for a longer time than the respective acids. We were especially interested in three groups of aldehydes. In one, with a nonsaturated short nonpolar chain, we searched a conjugated formation between the double bond of the oxygen of the carbonyl and the double bond of the nonpolar chain. Another group of the lipoaldehydes corresponded to long chain fatty acids, while the third was formed by saturated short chain aldehydes with an odd number of carbons. From the energetic point of view, there were the first and especially the third groups which appeared as the most interesting. In the last group the opposite influ-

ence exerted by the carbonyl and methyl groups upon the intermediary carbons of the chain gives the entire molecule an especially high reactivity. This opposite influence is seen at its maximum in propanal, where C₂ suffers the influence energetically opposite of the carbonyl and methyl group. The fact that, due to its relatively high solubility in water, propanal—which is a lipoid—can be administered in aqueous solutions and still act upon the lipidic system—makes of it an especially interesting agent.

We have investigated these groups of lipoaldehydes from the point of view of their influence exerted upon the two offbalances. In the group with unsaturated short chains, we studied acrolein and crotonic and maleic aldehyde without seeing any special effect upon the other levels than the cellular one, where a vacuolation was obtained. Furthermore their toxicity has represented an handicap. More interesting has appeared the group of the saturated short molecules with odd number of carbons. While with heptanal we have obtained besides an influence upon pain, also a manifest inhibitory effect upon the growth of experimental tumors, it was propion aldehyde which has shown the most interesting effects upon pain.

This was seen for the group of aldehydes with aliphatic saturated chain such as *propionic* and *heptylic aldehydes* or with *cyclic*, as *salicylic aldehydes*. In adequate doses—from $\frac{1}{20}$ cc. to 2 cc. of the 10% solution of propionic aldehyde, or of the 1% solution of the heptylic or salicylic aldehydes—a manifest influence was obtained upon the systemic condition as well as upon pain. Patients in offbalance A with pain and general discomfort, were seen to have a decrease of the symptoms after the administration of propionic aldehyde. The effect upon tumors was reduced and propionic aldehyde did not change the evolution of the tumors in spite of the marked improvement of the general condition and even of the cessation of pain.

Lipoids with Thiol Groups

Mercaptans: According to the systematization of lipoids presented above, a thiol group, acting as a polar group, will form a lipoid when bound to an energetically preponderant aliphatic or cyclic nonpolar group. In the homologous mercaptan series, even the lowest members are lipoids because of the weak electrostatic forces of the thiol group.

Although methyl mercaptan is a lipoid according to our classification, this substance is too volatile to be used. Therefore, the first low member of this homologous series to be investigated was ethyl mercaptan.

The effects of ethyl mercaptan upon microbes were more limited than those seen for fatty acids. To determine the effects at the different levels

of the organization, ethyl mercaptan had to be administered parenterally. As for all other members of this series, we utilized ethyl mercaptan in 5 or 10% oily solution in cottonseed oil. In acute toxicity tests, the lethal dose was found to be 145 mgs./100 grams of body weight for mice, and 153 mgs./100 grams for rats. The immediate effects upon nuclei were similar to, but less intense than those of conjugated fatty acid preparations leading to caryorrhexis or pycnosis in abnormal cells. No abnormal mitosis was seen in organs with high mitotic activity, such as the intestinal mucosa or bone marrow, although appreciable changes were observed in mitosis in animal tumors. A secondary effect, an exaggeration of aging processes,

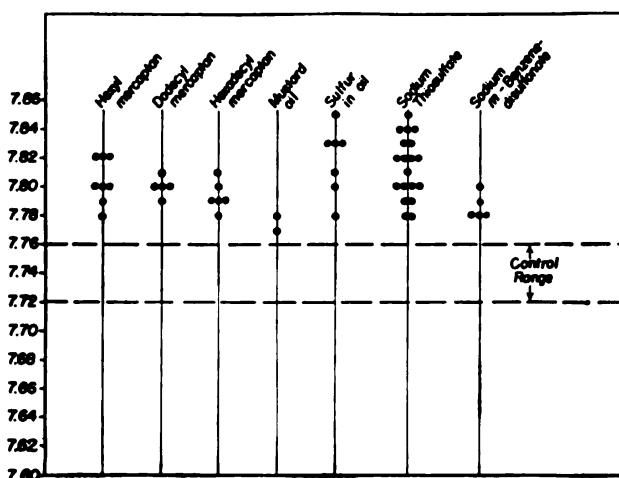


FIG. 125. Second day wound crust pH shows a constant change toward more alkaline values for all the substances having sulfur in their polar group.

was first recognized, several days after administration in the changes in granulocytes at the site of injection. The average number of nuclear lobes of the leucocytes was often very high, even above seven. This was also true for leucocytes in circulating blood when the product was injected intraperitoneally or even subcutaneously in rats. The immediate effect of such injections was a prolonged leucopenia, especially a lymphopenia.

The chloride content of tumors and wounds was especially increased through treatment with mercaptans. At the tissular level, local pH of the second day wound crust was increased, an effect characteristic for all lipoids with sulphydryl as the polar group. (Fig. 125) This explains the direct effects of ethyl mercaptan upon pain and other alkaline or acid symptoms. These effects are qualitatively similar to those seen for the polyunsaturated fatty acids but much slower to appear. While placenta acid lipid prepara-

tions, for instance, produce an effect upon pain—an increase in intensity for an alkaline pattern and a decrease for an acid pattern—even within 5 to 8 minutes after parenteral administration, the effect of mercaptans is reduced and appears after half an hour, or later.

The effect upon tumors in animals was especially manifest upon a rat sarcoma originally induced by benzpyrene in our laboratories and passed through successive transplants over a period of many years. Throughout this period, this tumor showed 100% positive takes, characteristically growing to huge size, at times as large as the rest of the animal, but without ever showing either spontaneous regression or necrotic areas. When ethyl mercaptan was injected subcutaneously in daily doses of $\frac{1}{2}$ cc. of a 5% solution in cottonseed oil into animals with these tumors, interesting changes were observed. If the tumors were already large, above 6 cm. in diameter for instance, only a few regressed (5/20 for tumors of 6 cm. in diameter). In tumors that did not regress, large necrotic areas developed and were followed by ulceration. Most became infected, leading to death. Tumors smaller than 6 cm. in diameter frequently regressed rapidly and then disappeared (between 9/20 and 20/20 in different experiments). No such striking results were observed in any of the other tumor strains in rats or mice treated with ethyl mercaptan, although in several, growth arrest occurred or necrotic zones appeared. Ethyl mercaptan injected in the tumors themselves induced the same necrotic changes in most animals. (*Fig. 126*)

The effect upon lymphatic organs was manifest. Spleen, thymus and lymph nodes were involuted in animals treated for a few days with mercaptans. The effect upon convulsions was irregular. Even with small doses, convulsions were prevented in some cases but, in general, the effect was less constant than with fatty acids. Eosinophiles decreased rapidly in animals or humans treated with this substance. All urinary analytical data were influenced by administration of mercaptan, with changes toward the patterns of type D offbalance.

Because of the very disagreeable odor of ethyl mercaptan, we were obliged to discontinue its use so that effects of this agent upon some analyses, such as surface tension, sulphydryl and calcium excretion, could not be studied in humans.

Other Mercaptans: Superior homologues of the mercaptan series were used. They were divided into three groups. The first included propyl, butyl, amyl and hexyl mercaptans; the second, heptyl and allyl mercaptans; the third, members with more than 10 carbons. The first group produced much the same effects, which tended to diminish as the number of carbons in-

creased. For example, effects were considerably reduced for hexyl mercaptan as compared with ethyl mercaptan. The second group—the heptyl and allyl mercaptans—produced more intensive effects. This was especially true for allyl mercaptan. Members of the third group—with longer carbon chains such as dodecyl and hexadecyl mercaptans—produced effects so slight that they seemed almost nonexistent, except upon pH of the second day wound crust, which showed values far above the range of the controls, just as with other members of this homologous series.

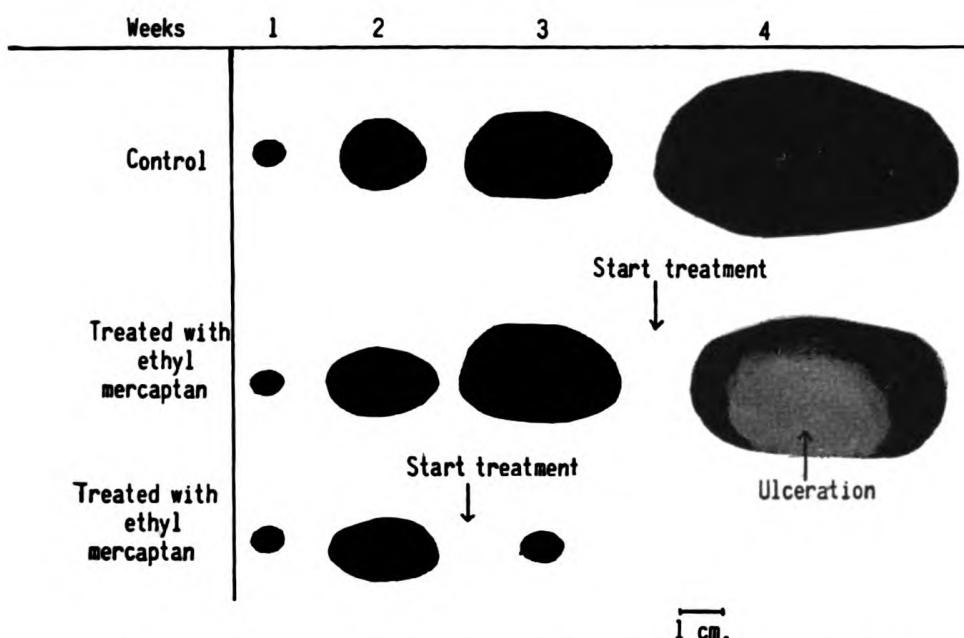


FIG. 126. Influence exerted by ethyl mercaptan upon a sarcoma induced by benzpyrene. For bigger tumors it induces constantly an ulceration, while for small tumors, their disappearance.

With the idea of using thiol as a polar group and having another center in the molecule as a secondary center, we studied a series of other substances. One was dimercapto-propanol (the B A L preparation) often used for heavy metal poisoning. It proved to be completely without effect on pain, tumor growth and systemic changes. It had less activity than the higher mercaptans, which themselves were less active than polyunsaturated and abnormal fatty acids.

The difficulties encountered in administering mercaptans, mainly related to offensive odor of lower members and inactivity of the less obnoxious members, led to a search for other chemical agents that might be active without being evil smelling. Extensive study was made of various prepara-

tions that appeared to have a bivalent sulfur bond at the nonpolar group. We investigated colloidal sulfur which, if introduced in the organism, seemed to undergo changes similar to those of bivalent sulfur. We found, thus, that the sulfur absorbed after being administered in colloidal form, was almost entirely eliminated after oxidation in the form of sulfates. In animals, no pharmacodynamic influences were observed. Only the pH of the second day wound crust was increased when these preparations were administered parenterally in suspension or were given orally mixed with food, in proportions up to 4%. There was no evident change in tumor evolution in animals or humans.

Hydro-Persulfides

Another sulfur compound, so-called "sulfurized oil," in which sulfur and fatty acids appear to form a hydopersulfide, (*Note 7*) was tested. This hydopersulfide preparation, although it exhibited no influence upon viruses *in vitro*, did induce a good degree of local resistance of the skin to smallpox infection. The effects upon microbes were reduced. There was little direct influence exerted upon cells. The preparations with 0.5 to 1% sulfur bound to cottonseed oil were well tolerated locally when administered intramuscularly or intraperitoneally.

The effect of parenteral and oral hydopersulfide upon pain was slow to appear, in contrast to the effect of fatty acids and even of mercaptans. However, it persisted for a long time. Pain of the acid pattern was eased; pain of alkaline pattern was exacerbated. The influence upon the second day wound crust pH was marked. The local pH increased to values even higher than 7.80. In radiation lesions, the dimension of ulceration increased and healing was retarded or even prevented. In some tumors in animals, the rate of growth was slowed. This latter effect was not uniform in the different types of tumors tested and even in the same type of tumor in different groups of animals. Systemic changes also varied. Doses corresponding to 5 mgr. of sulfur were not toxic for 30 gm. mice in a single injection. Nor were 15 mgr. doses in 200 gr. rats. Chronic toxicity studies showed that 0.2 mgr. daily injections in mice and 5 mgr. injections in rats for as long as three months did not induce pathological changes. High doses such as 1 to 2 cc. of a 1% preparation injected several times a day in humans was almost uniformly followed by a rise in temperature, usually after a few days.

Other Compounds with a Thiol Group

The results obtained with hydopersulfide preparations led us to seek other compounds with sulfur bound to fatty acids instead of triglycerides as in those mentioned above.

Sulfur compounds were prepared from various conjugated fatty acids such as conjugated linoleic acid and eleostearic acids and from mixtures of conjugated fatty acids obtained from cod liver oil, fish oil, human placenta, blood and various organs. While active in smaller amounts, they were not qualitatively different from hydopersulfide preparations obtained from cottonseed oil, producing the same pharmacological effects in most tests, especially upon pain, systemic manifestation and evolution of experimental tumors.

The fact that sulfur bound to the nonpolar group, as in hydopersulfides, produced less manifest results in animals and humans than mercaptans, which have a thiol group as a polar group, led to the study of other substances in which thiol radicals instead of sulfur were added in similar positions, and consequently were considered to act as secondary energetic groups. A series of preparations, in which one or more thiol groups were fixed at the double bonded carbons in various conjugated or nonconjugated polyunsaturated fatty acids, were obtained. These substances differ fundamentally from the fatty acids mentioned above in which sulfur atoms were fixed not at the carbons bound by double bonds but at the carbon adjacent to the double bond. 9-10, dithiostearic acid, 9, 10, 12, 13, tetrathiostearic acid, as well as polyunsaturated and conjugated fatty acids with thiol groups fixed at their double bonds, were obtained. In general, they showed no marked biological effects on animals, no influence upon pain or systemic patterns similar to those observed for the other lipidic products with bivalent sulfur.

The hydopersulfide group was added to soaps. Sodium and ammonium soaps were obtained through saponification of the triglycerides of fatty acids on which sulfur was already fixed. Effects at the different levels of organization were markedly reduced. There was no influence upon pain, organic or systemic manifestations. However, a striking effect was noted on many microbes. Growth of *Bac. anthracis* was prevented in some experiments, even with dilutions of 1/2,000,000. For *staphylococcus aureus* and *streptococcus hemolyticus*, a similar effect was obtained with dilutions higher than 1/200,000. In animals, even oral administration in drinking water in a dilution of 1/500 and, in some experiments even 1/1000, con-

trolled infection caused by these microbes. The antibiotic activity appeared to be similar to that of penicillin. (*Note 8*)

Tetrahydronaphthalene Persulfides (Sulfurized Tetrarine)

We utilized the known marked tendency of tetrahydronaphthalene to fix oxygen with the resulting explosive peroxides, to fix sulfur in similar combinations. Persulfides of this substance were thus obtained and their pharmacological activity studied. While only a limited effect was noted upon viruses and microbes, the influence upon *tetrahymena pyriformis* approached that seen with active polyunsaturated fatty acids. The product with 5 gm. sulfur fixed for 100 grams of tetrahydronaphthalene, showed a relatively low toxicity in normal animals, 75 mgr./100 gr. in mice and 125 mgr./100 gr. in rats being well tolerated in intraperitoneal administration. The influence upon wound healing in animals, upon pain, and upon the systemic analyses in humans, was similar to that seen for the mercaptans. Although the immediate effect on pain was limited, prolonged administration was effective. The analytical changes of the urinary surface tension and blood potassium were the most manifest. The toxicity was highly increased for animals with ascites tumors (Sa 180, Ehrlich and Krebs). However, the influence exerted upon transplanted and spontaneous tumors in animals was one of the most favorable ones, compared to the effect of other tested agents.

Thiosulfates

We investigated thiosulfates with the intention of studying agents which, in addition to a manifest reduction effect, would act through bivalent sulfur liberated in the body. The elimination of part of the sulfur of thiosulfates as mercapturic acid has led to the supposition that the bivalent sulfur ion, separated from the thiosulfate ion, would act through combinations similar to those found in the metabolism of thiolipoids. It is for this indirect action that although hydrosoluble, without any direct connection to lipoids, we investigated the biological activity of thiosulfate together with and under the same specific aspect as the lipoidic sulfur compounds considered above.

There was a very limited effect upon microbes and viruses, no effect upon phages, and no change in the receptivity of rabbits to smallpox virus. With either oral or parenteral administration, the immediate effect upon pain was manifest. Oral administration of $\frac{1}{2}$ cc. of a 10% solution of sodium thiosulfate in water, or intramuscular injection of $\frac{1}{2}$ cc. of the 4% solution, was usually followed by a definite effect upon pain in less

than 10 minutes. As this was found to be opposite for the two patterns of pain, sodium thiosulfate was used even for diagnosis of pain pattern. Pain of an alkaline pattern increased while a decrease occurred in pain of an acid pattern. The second day wound crust pH increased manifestly with the use of this substance.

Cellular and nuclear changes following administration of thiosulfate preparations were similar to those produced by mercaptans. The effects upon the lymphatic system and on liver regeneration, however, were minimal. Convulsions induced by thiamine were controlled well by thiosulfate in doses of 120 mgr. per 100 gram of body weight. Injected simultaneously with administration of thiamine, thiosulfate prevented convulsions in a high proportion of animals (17/20).

The effect upon radiation lesions was less manifest. An increase in wound size and prolonged ulceration occurred only with use of relatively large amounts of sodium thiosulfate daily. Doses above 40 mg./100 gm. of body weight were needed to obtain these effects. The healing of a simple wound was retarded only with large doses, around 50 mg./100 gm. of body weight. On the other hand, when very small doses were administered, such as 5 mg./100 gm., the healing effect was enhanced. Effects upon tumors were less manifest in animals. Slight and inconsistent changes were seen in grafted tumors. Very often in the same experimental group, tumors disappeared in some animals while in others the growth rate was only slowed or remained unchanged. The erratic results on tumors in animals produced by thiosulfates were similar to those seen for many of the sulfur preparations, and appeared as characteristic for this group. Repeated injections of thiosulfates in tumors were seen to induce the disappearance of the tumor if growth was slow enough to permit such injections for several weeks. At the systemic level, the most marked effect, other than that on sulphydryl index, was on surface tension which usually dropped with the administration of a sufficiently large amount.

Most of the research on thiosulfate was done with sodium salts. In a few cases, very high dosage, such as 6-10 grams daily, produced moon-face and slight leg edema, apparently related to sodium retention. This disappeared with cessation of the medication.

Changing the cation of the thiosulfate from sodium to magnesium appeared to increase, sometimes markedly, the results obtained in our experiments. Potassium thiosulfate seemed to be more effective, especially against pain. Its use however, has been limited by disadvantages. When administered parenterally, it causes considerable local pain at the site of injection as most potassium salts do. If administered to patients having

pain or other symptoms of an alkaline pattern or systemic manifestations corresponding to type D, a more marked increase in intensity of symptoms occurs than for the sodium or magnesium salts.

We also investigated sodium tetrathionate. Except for lower dosage requirements, no other advantages were found in its use. Its relative instability is a handicap.

Alpha-Thio-Fatty Acids

In other studies, we tried to introduce sulfur into the fatty acid molecule, this time changing the polar group itself. With the sulfhydryl replacing the hydroxyl of the carboxyl group, a bivalent negative sulfur was introduced, thus realizing a thionic group. (R-COSH)

We prepared several members of this thionic acid series corresponding to various saturated, polyunsaturated and even conjugated fatty acids. We studied in particular the effect of hexylthionic acid, corresponding to caproic acid. The results observed were essentially the same as those seen with the other bivalent sulfur containing lipoids mentioned previously. In addition to influencing pain and systemic changes, hexylthionic acid produced some interesting effects upon experimental tumors, reducing the growth of a few of them. However, there were no important differences from the effects of the other sulfolipoids.

Another entire series of products was prepared by introducing a thiol group at the C₂, or alpha position, of various fatty acids, with the intention of creating a more complex polar group similar to that present in alpha hydroxy or alpha amino compounds. Alpha-thio-fatty acids were thus obtained for the entire homologous series of saturated, and for many of the nonsaturated, fatty acids. Some members of this series of alpha-thio-fatty acids, such as caproic, caprylic and myristic, were studied extensively both in animals and humans. From the biological point of view, however, they showed no manifest differences over the thiolipoids previously discussed.

Thioglycolic Series

All these researches with limited biological results brought us to the study of lipoids in which the thiol represents a polar group but in which a secondary polar center is present in the molecule. Many such synthetic thiolipoids were prepared in our laboratory with the hope that they would prove biologically more effective and would have alkylating activity as well. Two series appeared to be interesting, since they were being active particularly at lower levels of organization. This led us to utilize them also on a larger scale in clinical work. While consistent results were obtained

on pain and systemic changes, the influence upon animal tumors was erratic and no different from that of other preparations with thiol groups or the sulfur compounds mentioned above. There were marked effects in some animals with tumors; in others with the same tumor treated identically, there were no effects at all. In humans the effects on pain, tissular, organic and systemic levels were similar to those of many other sulfolipoids.

Starting with these substances, derivatives were prepared. One group comprised derivatives with a special character. In order to have only one active polar group, one of the two polar groups had to be blocked. For thioglycolic acid which we studied, either the thiol or the carboxyl group could be blocked, leaving the uncombined radical as the active polar group. Since we were interested in substances having the thiol group as active polar radical, the carboxyl group was blocked by replacing its hydrogen with a methyl group. Methylthioglycolate has been thoroughly studied in our laboratory. Its pharmacological activity is similar to that of the other thiol preparations mentioned above.

Other thioglycolate esters with ethyl, propyl or butyl instead of methyl, were prepared and studied but showed no advantage over the methyl ester. We tried to obtain the allyl ester in order to have a more potent secondary center but we were unable to synthesize it.

In the same group of agents we studied another substance, beta mercaptopropanoic acid, having a thiol and a carboxyl as polar groups. Used uncombined, it could be seen that here again, as with thioglycolic acid, it is the carboxyl that acts as active polar group while the thiol acts as a secondary energetic center at the nonpolar group. This acid is very toxic in animals, producing as a peculiar effect, manifest muscular spasms. The compound also produced abnormal muscular rigidity, seen immediately after death. In nontoxic doses, it showed a marked influence upon tumor growth. Many tumors disappeared; in many others, a reduction in size occurred. Impressive results were obtained in spontaneous mammary carcinoma in mice where a fairly high proportion of tumors disappeared (28/40). Repeated injections into these animals gave good results if the growth was slow enough to permit treatment for a period of at least a month. The preparation, however, showed toxic effects in animals with tumors, producing weight loss similar to that produced by the thioglycolic series. To change the polar group and have the thiol act as such, we blocked the carboxyl with a methyl in some experiments and with an allyl group in others. But the influence upon tumors in animals was reduced through these changes.

Relationship to Sulfur Metabolism

The study of lipoids with sulfur posed the problem of the relationship between their structure and biological activity. We mention this here because it not only explains the influence exerted by these agents but also because it indicates the manner in which this research has had to be developed. We have seen that, according to the systematization of the elements, sulfur represents a nonmetal anti A element, active especially at the metazoic level. It is its action as an isolated element that appears interesting, in addition to the metabolic changes which it induces in the organism when present in a negative lipoid.

It seems that the organism generally metabolizes bivalent negative sulfur by changing it ultimately to the hexavalent form. Ferguson and du Vigneaud have studied the metabolism of methionine and cysteine which are the principal sources of sulfur in the organism. (125, 126) While the evidence we have on this subject is too limited to provide more than a working hypothesis, it would indicate that other compounds with lipidic character—the thiol-containing lipids—have more important biological activity.

The metabolism of these compounds varies among individuals, especially those with abnormal conditions. The study of the excretion of sulphydryls through the urine, expressed as the sulphydryl index, has served us as a guide in studying the metabolism of sulfur up to a point. The high excretion of the thiol group seems to be related not only to low oxidation but to an abnormal form, as mentioned above, since with the exaggerated excretion, the thiol level in the blood is reduced. This form is to be considered as an excremental one. This seems to be confirmed by the fact that the administration of bivalent sulfur, even in a large amount, is not followed consistently by its elimination in the form of thiols in the urine. In some subjects, an impressively high proportion of the thiols administered appears in the urine, and this is true even for relatively small doses of thiols or of bivalent sulfur as in thiosulfates. In other subjects, on the contrary, even when larger amounts are administered, the increase in elimination is minimal or does not occur at all. The abnormality in sulfur metabolism, which appears to be a limited capacity to oxidize it to the hexavalent positive form, also means an exaggerated intervention of the thiol group as such in the economy of the organism. This occurs along with symptoms and signs, previously noted, corresponding to an exaggerated oxidative intervention of fatty acids, in which processes the thiols probably take part.

We tried to study the capacity of the organism to fully oxidize thiolic

sulphur by following the response to the administration of a known amount of sulfur in bivalent negative form. The change of the sulfhydryl index of urine would serve as a tolerance test for thiol metabolism.

After injections of 80 mg. of sodium thiosulfate, the differences in the capacity of various organisms to metabolize it could be seen and related to pathological conditions. This concept of thiol metabolism can be the basis for understanding an abnormal form of thiolic sulfur which may be involved in the pathogenesis of abnormal conditions. Substances containing a thiol group, such as methionine, cysteine and particularly glutathione, are present in sizable amounts in the organism, but it is not this form of thiol that intervenes in the abnormal metabolism. A large amount of the normal form of thiol is present in the blood of subjects with a low urinary sulfhydryl excretion. When another form, the abnormal one, intervenes, it is excreted in the urine. The organism eliminates this "abnormal" compound with sulfhydryls. It seems quite probable that this abnormal thiol compound is in a lipidic form since the sulfhydryl-containing compound is readily extracted by ether from the urine. Its affinity for the lipidic system would explain the influence exerted upon fatty acids and the oxidative processes occurring in the lipidic system. The thiolipoids intervene catalytically in the oxidation of the fatty acids, as seen in experiments *in vitro*.

Thus, the thiol group in lipoids containing bivalent sulfur rather than metabolized sulfate would increase catabolic metabolism. Although the thiol in this abnormal form is largely eliminated by the urine, apparently as a defense mechanism against its pathological activity, some of it is probably retained in the cellular lipids where it continues its activity. Circulation of sulfur in thiolic lipidic form, with consequent impairment of its change from the bivalent negative sulfur into the hexavalent positive sulfur, would thus appear as the fundamental source of the participation of sulfur in the abnormal pattern. The influence exerted by administration of thiosulfates upon the sulfhydryl index can serve as an indication for these specific changes.

Sulfur is an anti-A element and it is active as such in all the forms in which it exists in the organism, although the intensity of its action varies at different hierarchic levels. The activity of thiol as an anti-A factor can be related to the influence exerted by carcinogens or other agents upon the biological activity of this radical, especially when it is taking part in the formation of enzymes. This relationship explains the results obtained by repeated injections of organ or tumor cells treated *in vitro* with agents having a thiol as polar group. The heterogenization induced leads to the appearance of severe changes resulting from the allergic reaction.

It is interesting to note that sulfur has an anti-A tendency even in the hexavalent positive form in which it appears as sulfate. The sulfate ion has a capacity in the organism to inactivate lipoids of a positive character. The sulfate becomes bound to such substances, thereby tending also to facilitate their excretion in urine. Many of these substances are eliminated in combination with the sulfuric radical in the forms called "sulfo-conjugated."

In view of this, the effect of sulfate ions appears to parallel that of the fatty acids. Both oppose substances having a positive polar character; that is, both are biologically antagonistic to anti-fatty acids. In the sense that they oppose antifatty acids, both the bivalent negative and hexavalent positive sulfur have anti-A activity, the first directly and the last indirectly.

The characteristic influence exerted by sulfur in its different forms is based on its action as an agent inducing changes toward increased homotropy, by acting at levels above the cellular. This is a typical example of the relationship between an element's activity and its place in the periodic chart. Sulfur is a member of the series with homotropic action; it belongs to the period which corresponds to the metazoic compartment and thus acts above the cellular level.

Selenium Lipoids

The influence exerted by bivalent sulfur upon oxidation processes in which fatty acids participate, served as a guide for further research. Seeking agents that would act at a still lower level of the organization, even below the cells, we considered other substances which also affect oxidation processes. Theoretically, at least, it appeared possible to induce changes at a compartment below the metazoic, at which sulphydryl-containing compounds act.

We have discussed previously the systematization of the biological activity of elements, their fundamental anti-A and anti-D influence, their distribution among the various levels of the organism, all related to their atomic structure and their place in the periodic chart.

All of this led us to investigate selenium which is the nonmetal element next to sulfur in the sixth series of Mendeleeff's periodic table—the series with an anti-A character in which oxygen is the first member. According to its period, selenium belongs to the cellular compartment.

The first problem was the nature of the compound in which it would be active. We were particularly interested in using bivalent negative selenium because of the activity of bivalent negative sulfur. However, we did investigate the selenic and selenious acids. These acids or their sodium salts

have limited effect upon viruses and microbes. An interesting effect is seen in *Tetrahymena pyriformis*, where a manifest cellular vacuolization is induced. The influence upon tumors, pain, organic and systemic levels is less manifest and toxic effects are great. Therefore, we prepared lipoid compounds with a predominant nonpolar group and with a negative bivalent selenium. We utilized, on a larger scale, hexyl and heptyl diselenides synthesized in our laboratories by M. Bier.

Hexyl and Heptyl Diselenides

Studies have shown that hexyl and heptyl diselenides are lower in acute and chronic toxicity than selenic and selenious acids and their sodium salts. In wounds and tumors, these selenium preparations induce a relatively limited fixation of chlorides, the increase above controls being only about 16%. In only a very few cases could any direct effect upon pain be observed within a few hours. However, the long range effects after several days of administration, seemed to be superior to those of various fatty acid preparations. This was true both for the decrease in intensity of pain with an acid pattern and the increase of pain with an alkaline pattern. The effects persisted for many days. In animal tumor experiments, there were relatively slight changes in growth or survival time.

No manifest influence was seen at the organic level. With relatively large amounts of these agents, an involution of the lymphatic system was obtained. Thymus, lymph glands and spleen were markedly reduced in size in animals dying after acute toxicity tests, and adrenals were small and appeared to be depleted of their sudanophilic content. In rats, a frank lymphopenia followed administration of large doses of these preparations, and eosinophilopenia also was uniformly seen. Changes in urine analyses also were obtained with high doses.

It is noteworthy that administration of diselenide to a subject with a type A pattern induces the appearance of oxidizing substances in the urine, as one of the first changes.

Effects at the cellular level are seen even with microgram dosages. Vacuolization occurs in the cellular cytoplasm. It is interesting to note that, despite the cellular vacuolization, pericellular edema occurs. The fact that these selenium compounds are active in small doses may be an indication that they act entirely at the cellular and not the metazoic level. This effect at the cellular level is confirmed by the fact that almost constantly the administration of selenium if in sufficient amount is followed by a manifest increase in serum potassium values, and a decrease in the amount in red

cells. This change in serum potassium is apparent before any other change, and is generally obtained with relatively very low doses of selenium.

The effects upon cells of another lipoidic selenium preparation, with selenium this time as the polar group, warrant mention. The preparation, synthesized in our laboratory, is hexylselenoic acid in which the hydroxyl of the carboxyl group has been replaced by a SeH radical. A manifest effect is produced by this agent in animals with ascites tumors. Intraperitoneal injection leads almost constantly to the disappearance of such tumors, even if the compound is used after ascites is already present. We used this product to bind selenium *in vitro* to cancerous cells as will be seen below.

Tetrahydronaphthalene Perselenide

The fact that in the first phases of the defense mechanism the organism uses fatty acids acting largely through their products of oxidation, has directed us—as we have seen above—to search in the therapeutic approach for agents having as pharmacodynamic activity an intervention of peroxides. In a further step, parallel to lipidic peroxides, we investigated similar products in which, instead of peroxides, persulfides were present, sulfur being the element immediately above oxygen in the VIth series of elements. We studied thus the persulfides, among which the tetrahydronaphthalene persulfide has been an interesting compound. Its activity was explained, according to the biological systematization of the elements in which oxygen corresponds to the organism level, while sulfur represents a metazoic element. Following the same line, we searched similar compounds for selenium—an element still higher in the VIth series, which corresponds to the cellular level. We thus prepared and studied perselenides by bounding selenium to tetraline in the same way as was done for oxygen and sulfur.

The effects of the perselenides on microbes or animals were similar to those of the other selenium preparation discussed above. Tetraline perselenide showed low toxicity in animals, $\frac{1}{4}$ cc. of the 10% solution of the product obtained having 25 mg. selenium %, was not toxic in intraperitoneal injections in mice. Administered orally in humans, in doses from $\frac{1}{50}$ -2 cc. of the solution containing 25 mg. of selenium per 1 cc., repeated even several times a day, did not show toxic effects. The influence exerted on pain and systemic changes took some time to appear as with the other selenium preparations. The influence exerted upon the growth of experimental tumors in animals was more manifest than for the other selenium preparation. Similar results were obtained with the perselenides of naphthalene and other aromatic hydrocarbons.

An investigation of the influence exerted by the immediately heavier member of this VIth series, tellurium, is in progress.

The foregoing data on lipoids with negative character indicate that their activity generally is related to changes in processes in which ultimately an intervention of oxygen takes place. This brings us to the first member of the sixth series, oxygen, a nonmetal with D inducing biological activity.

We studied the effects of ionic oxygen, using compounds which liberate oxygen readily. These included hydrogen peroxide as well as peracids and their salts, such as perchloric, perboric, persulfuric and periodic.

The changes induced by these substances upon microbes, viruses and cells are similar to those obtained with polyunsaturated fatty acids and all are catalogued as radiomimetic. This fact tends to confirm the importance of oxidation changes in the pharmacodynamics of fatty acids. The effects upon pain and at organic and systemic levels also were similar to those of polyunsaturated fatty acids. It is interesting to note in the same frame of activity the appearance of oxidizing substances in the urine following the oral administration of these agents in higher doses.

We investigated the effects of turpentine oil which is known to induce the appearance of peroxide in vitro. Highly oxidized through treatment with oxygen or especially bound to sulfur, turpentine oil has shown interesting pharmacological activity. An old therapeutic device was parenteral administration of turpentine oil to stimulate the defense mechanism in cases of septicemia. However, we saw no such stimulating effect in the fight against cancerous cells. The influence exerted upon the cellular level of the organization was quite reduced. The action of atomic oxygen appears to be different from that of the molecular, as we will see later.

ALKYLATING AGENTS

We investigated, as compounds with negative character, certain alkylating agents, choosing from the large number available those which also showed lipidic properties. We were especially interested in two members, sulfur mustard and epichlorohydrine. Sulfur mustard contains, along with one active polar chloroethyl group, a second represented by the bivalent sulfur polar group. It has the effect at different levels of organization of producing an offbalance with predominance of the acid lipids. We will discuss briefly here some of the experiments in which this influence upon the body lipids has been observed.

Sulfur Mustard

In studying sulfur mustard, we were first interested in its effects upon body lipids and, through them, upon the lipidic system of organisms. In inducing sulfur mustard's characteristic skin lesions, an interesting relationship was observed. Pure sulfur mustard was applied to mechanically epilated skin of rats. If a sufficient amount—2 to 3 drops—was used and spread on one square cm., the animal died. However, the time of death varied. If the lesion showed a massive necrosis, followed by deep ulceration, similar to a burn of the third degree, the animal died in about three weeks. However, if the lesion was only erythematous, similar to a burn of the first degree, the animal died in only 3 to 4 days. It seemed as if the lesion itself intervened secondarily in the pathogenesis of changes leading to death. Sick but still living cells appear to have an activity which is highly detrimental. The abnormal cells apparently produce substances which are responsible for rapid death of the animal. In widely necrotic tissues, these abnormal but still living cells are limited in number; in an erythematous lesion, they form the lesion itself. This correlation of toxicity with local lesion was confirmed by the fact that excision of the lesion itself, if performed in time, prevented death in some animals. The administration of ferrous sulfate to rats having sulfur mustard applied to their skin was seen to induce the erythematous form of the lesions, with death in 3-4 days.

The similarity between the influence exerted by mustard burns and caloric burns with a sufficiently extensive first degree burn producing more rapid death than a third degree burn, was of interest.

Analysis of the body of an animal killed by a mustard burn reveals abnormal amounts of unsaturated fatty acids and reduced amounts of sterols. In some cases, where death occurred after more than three weeks, body sterols were found to be almost completely lacking. In these cases, almost no insaponifiable fraction could be found. The lesion itself, especially an erythematous and edematous one, was very rich in unsaturated fatty acids. Histological study of these skin lesions revealed changes similar to those obtained through the intradermic injection of concentrated solutions of body acid lipidic fractions. The study of these lesions further revealed that the lesions themselves were separated from the organism by a barrier of adipose cells, the result of an exaggeration in number of the cells of the subdermic fatty layer.

We studied these important changes from several points of view. We could show that an exaggeration of the adipose layer underneath the skin occurs when lipoids with negative character, such as polyunsaturated fatty

acids, thiolipoids, etc. act upon the skin. Thus, this subcutaneous adipous formation appears to be a defense weapon, designed to keep such lipoids from passing into the organism. The defense appears to be unequal for males and females, as shown in the following study.

In collaboration with the late Prof. R. Leroux of the Faculty of Medicine in Paris, we studied histological changes in the ears of rats after local application to the skin of a small amount of pure sulfur mustard. Normally there are no adipous cells in the pavillion itself except at its base. Twenty minutes after the application of sulfur mustard on the skin of the ear, 2 or 3 layers of adipous cells were seen in the connective tissue between skin and cartilage. (Chapter 6, Note 22)

Curiously enough, the rapid appearance of adipous cells 20 minutes after application occurred in females and not in males. The spaying of females or castration of males did not change this response even after a lapse of months. The administration of male sex hormones to female rats—spayed or not—or of female sex hormones to males—castrated or not—also produced no change. It was only by the administration to males, over a period of days, of a sufficiently large amount of the insaponifiable fraction obtained from the bodies of rats that this rapid response was induced. The administration to females of the acid lipid fraction obtained from rat bodies was seen to prevent the rapid adipous response. This difference in response between males and females, can be related to the differences in the amounts of members of the two groups of lipids ordinarily found in males and females, as mentioned above.

We studied sulfur mustard from the point of view of pharmacological activity. Doses of 100 mcgr./100 gr. of body weight (of a 0.1% solution of sulfur mustard in oil) were nontoxic in rats and mice. Except for an intensive local reaction at the injection site, no important immediate changes were obtained in humans when 1 to 3 cc. of the 0.2% solution was injected intramuscularly. The influence upon pain—a decrease in the intensity of acid pattern and an increase for the alkaline pattern—was only temporary. The influence on tumor evolution was not sufficient to warrant clinical use of this agent, especially in view of the persistent and intensive systemic changes toward an offbalance of the type D which appeared after a few days. Through its anti-A action, which is the most intensive of all agents tested, sulfur mustard remains one of the most interesting substances for experimental studies, especially for the effects exerted upon the anti-fatty acids.

Epichlorohydrine

The importance of the relationship between the energetic centers present in alkylating agents and their ability to produce type D offbalance, led us to study a group of these substances which, at once, have both short molecules and two polar groups in close proximity. The desire to have such an agent with lipoidic property as well led us to study epichlorohydrine which corresponds to propane and has an epoxy group binding C₂ and C₃, while C₁ binds a chlorine. Soluble in neutral solvents, epichlorohydrine becomes soluble in water only after hydrolysis. Its biological activity differs from other chlorohydries such as chloropropanediol or trichloropropane, both of which can be considered to be closely related to the substance produced by hydrolysis of epichlorohydrine. The acute toxic dose of epichlorohydrine was found to be 6 mgr./30 gr. for mice and 25 mgr./100 gr. for rats by intraperitoneal administration; 22 and 35 mg./100 for mice and rats by subcutaneous injection. In tests for chronic toxicity, it was apparent that doses of 5 and 1.5 mgr. injected daily were well tolerated respectively by rats and mice. With higher doses, the animals became rapidly emaciated before dying. Used orally in drinking water, a solution of 1/3000 was well tolerated by rats and mice even for months. With the use of solutions of 1/2000, only a few animals did not lose weight, while a solution of 1/1000 invariably induced weight loss.

There were no effects observed upon microbes or bacteriophage.

It appears that epichlorohydrine, acting below the morphological levels, induces changes similar to those seen for other alkylating agents. However, it is not upon the desoxyribo-nucleic acids present that an important action is seen but in the lipidic system at these lower levels. Epichlorohydrine seems to act also at other levels. The influence upon pain—an increase for alkaline pattern, a decrease for acid—was more noticeable than for sulfur compounds. Delayed effects, however, were more obvious than immediate ones. The influence upon wound healing was similar to that of polyunsaturated fatty acids. Cancerous cells, such as those from mouse ascites, were destroyed in vitro by a 0.5 solution of epichlorohydrine. The effect in vivo upon sarcoma 180 or Ehrlich ascites tumors was most interesting. Administered by subcutaneous or intramuscular injection, epichlorohydrine had no effect on the tumor even in doses as high as 2.5 mgr. daily. However, when administered in drinking water in a 1/1000 solution, it prevented the development of ascites in 19/20 animals. But the toxicity was too high. A 1/2000 solution, used as drinking water, controlled the condition in more than 50% of the animals, while a 1/3000 solution showed favorable re-

sults in only a few animals. Under the same conditions, there was no apparent effect upon solid tumors in mice, even those induced by subcutaneous injection of ascites tumor cells.

In humans, all effects upon the tumors were interesting and will be discussed below. The influence upon systemic patterns was relatively reduced except for a marked effect upon the elimination of calcium in urine, obtained even with small doses which produced no other changes. Repeated injections of organ cells treated *in vitro* with epichlorohydrine were able to induce severe degenerative changes in the respective organs. Experiments with tumor cells treated and administered in the same manner are still in progress.

THE ELEMENTS

We have discussed previously the method used to classify the elements in accordance with their predominant biological intervention. The place of the elements in the periodic chart, which establishes the relationship between their structure served as a further basis for this systematization. The capacity to induce changes towards an offbalance of the same type was found to be a common property for elements in the same series in the periodic table. The series could be separated into two groups, one Ht (from heterotropic) inducing an A and the other Hm (from homotropic) inducing a D offbalance. Elements systematized as different periods in the chart have been found to have predominant activity in various compartments or groups of levels which form the hierarchic organization of complex organisms. We have related each element to a compartment (or sometimes even to a level), which didactically is called the compartment (or the level) of the element. We have tried to utilize this systematization in the study of the pharmacological activity of elements.

From the beginning, several basic facts about biological activity became apparent. Often the elements, used as such, do not induce the changes which characterize their physiological activity. Basically elements act in normal physiology through specific compounds suited to the compartment or level to which they belong. In general, knowledge of the level of an element permits us to identify also the proper compound and its activity. The intervention of an element at levels of the organization other than its own can be understood only in terms of the relationship between the element with its characteristic compound the proper level and the compounds present at other levels.

Two factors are fundamental in determining the activity of an element:
a) its availability and b) the possibility to enter into its proper combination.

The nature of any abnormality in activity of an element can be recognized only by relating it to these two factors. The amount of the element available and the capacity of entities of the level to which the element belongs to manufacture the proper compound, ultimately governs the amount of the element at its own and at the other levels.

Under normal conditions, the entities of the proper level utilize only the amount of the element needed to maintain the normal constants. The rest of the element usually is eliminated. An excess of the element thus does not induce a permanent excess at the proper or higher levels under normal conditions. Such an excess normally is only temporary. A persistent excess of the element at the proper level and at the higher level indicates an abnormal general availability. A persistent excess at the higher level only corresponds to a qualitative deficiency at the proper level. The organism maintains an excess of the element at the higher level in an effort to compensate for the qualitative deficiency at the proper level. Thus, an excess of an element at the higher level indicates a qualitative deficiency at the proper level, only if the value of this level is found low.

When there are low values at higher levels, we also need to know the amount present at the proper level in order to interpret the abnormality. Low values of the element at higher levels with a low amount of the proper level indicates a quantitative general deficiency, while a high value at the proper level permits us to recognize a qualitative excessive utilization of the element at the proper level. For example, in cancer, copper is low in tumor cells and liver, but abnormally high in blood. When a high amount of copper appears in blood, the diagnosis of a qualitative deficiency in utilization in cells or an abnormal general amount can be made by investigating the amount in cells. A low amount of copper as found in tumors and liver cells in subjects with cancer make the qualitative nature of this deficiency evident. The trouble lies not in too little copper available in the body but in the lack of the capacity of the cancerous cells to manufacture the compound through which copper becomes active, in this case, catalase.

A normal amount of an element at its proper level reflects normal utilization. The pathological amount can result from a quantitative or qualitative abnormality. Any anomaly in an element not only means an inadequate amount of it at its own proper level but in other levels as well. A quantitative deficiency results in an insufficient amount at its proper level. A qualitative deficiency—that is, incapacity of the entities to manufacture the proper compound—also leads to a reduced amount of the element at its own level.

Thus, in both cases, quantitative and qualitative deficiency, the amount

of the element present at its own level is low. However, in quantitative deficiency there is also a low amount of the element in the hierarchically superior level, while an increased amount at this superior level occurs when there is a qualitative deficiency at the proper level.

The same opposite variations between the amounts at the two levels is seen in the case of an excessive utilization of the element at its proper level. The amount of the element at the superior level is also high, if a quantitative excess is present but this amount at the superior level is reduced as a means of controlling the excessive utilization at the proper level if a qualitative anomaly occurs. This makes it possible to recognize the quantitative or qualitative nature of the abnormality in utilization of an element at its proper level by determining its amount both at this level and at the next superior level. Too much of the element present at the proper level indicates either excessive amount present or excessive utilization, while too little at the proper level can be due either to a qualitative or to a quantitative deficiency. A low amount at the higher level indicates either a quantitative general deficiency or a qualitative excessive utilization at the proper level. An excess amount at the higher level indicates either a quantitative general excess or a qualitative deficiency at the proper level.

This relationship, which is also critical for the understanding of the pharmacology of the elements, can be summarized in the following table:

Amount of Element at the Proper Level	Amount of Element at the Higher Level	Interpretation: Occurrence at the Proper Level
high	high	quantitative excess
low	high	qualitative insufficiency
high	low	qualitative excessive utilization
low	low	quantitative deficiency

From a practical point of view, we must have information on the amount of the element both at the proper level and at a higher level. We found that for the elements proper to the cellular level, such information can be obtained by comparing the amounts in plasma and red cells (or total blood). It is not the ratio between these values—as often supposed—which is important, but rather the values themselves. For changes at the systemic level, the comparison can be made between blood and urine, the latter corresponding to the level above the systemic.

The importance of this concept can be seen in the following examples. Potassium is a cellular level element. In cancer, in offbalance type A, potassium is present in abnormally high quantity in proliferating cells. It

is also found in high amounts in blood red cells. In these cases, potassium is found in low values in the hierarchically higher level in the blood plasma or serum. The abnormality does not reside in a simple hypokaliemia, but in excessive utilization of potassium at the cellular level, a low amount of potassium in red cells also would indicate a potassium deficiency. A high amount of potassium in serum and red cells can be interpreted, as mentioned above, as corresponding to a quantitative excess. The reduction in the quantity of potassium in red cells, together with an excess in the serum, indicates a qualitative deficiency at the proper level.

We have the true picture of the situation if we consider that "qualitative" excess or deficiency is determined by the ability to form the proper compounds. While it is the element as such which has to be administered in order to correct a quantitative deficiency, other factors must be changed to overcome qualitatively deficient utilization, excessive utilization, and quantitative excess. (Fig. 127)

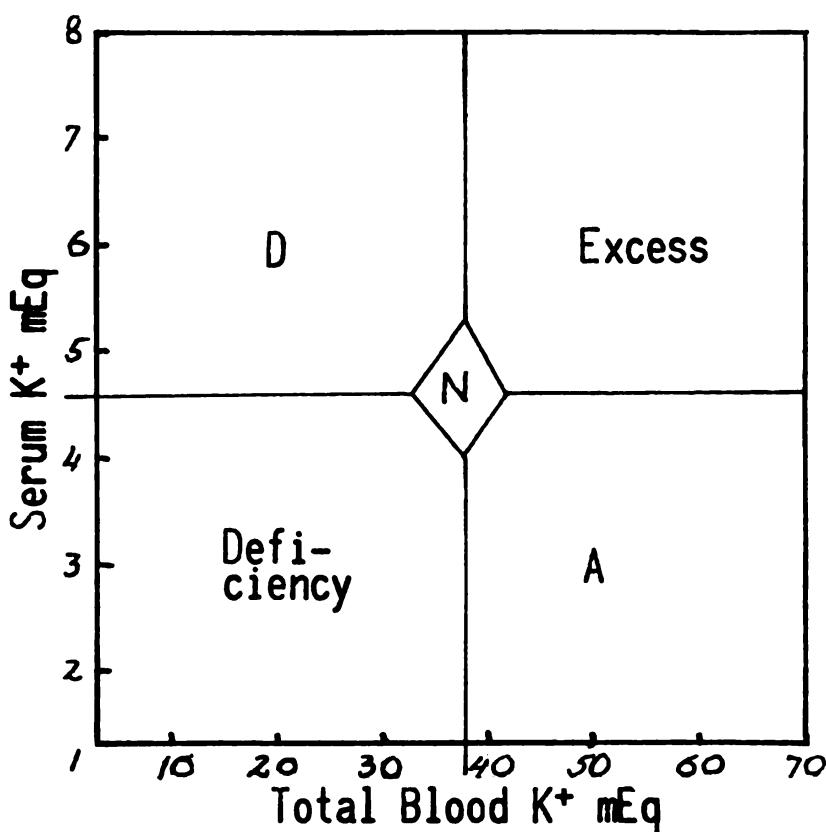


FIG. 127. The relationship between the amount of potassium in serum and in total blood permits to indicate the existing condition as being in normal limits, in quantitative deficiency or excess, or in an offbalance type A or D.

Although these relationships represent the most important aspect of the pharmacodynamics of the elements, still others must be considered. An excess or deficiency of an element at a superior level, even if it serves as a biological defense means to combat an abnormality at a lower level, represents a problem by itself for the superior level. The fact that the element does not belong to this level gives a noxious character to its influence. The influence exerted upon sensitive organs often induces important abnormal manifestations. Hyperkalemia, even originated by an abnormally low utilization of potassium at the cellular level, can lead to serious troubles in the function of the nervous system or of the heart.

Another important aspect of the reactivity of an element is its influence upon levels below its proper level. An element acting at a lower level usually has a biologically opposite effect to what it has at the proper level and therefore is a noxious influence. At the lower level, the A or D type of activity of the element is reversed.

Sodium, for instance, which is an agent of the A type of the metazoic compartment, produces an offbalance of type D at the cellular level, which is hierarchically inferior to its own. Similarly, Mg, which is a D agent at its own metazoic level, has an A inducing activity at the cellular level.

Analysis of the pharmacology of elements in terms of their A or D inducing activity, the level at which they belong and the compounds through which they act, is still only in its early stages although it represents a program of great promise. In the presentation which follows, we will try to interpret the data concerning the elements in terms of A and D inducing activity. We start with Hm elements having a D inducing activity. They parallel in their action the lipoids, with a negative polar group. TABLE XVII lists Hm elements or the D series and relates them through their periods to the organizational compartments.

TABLE XVII
HM ELEMENTS

<i>Compartments</i>	<i>Metals</i>							<i>Non-Metals</i>		
Organic	Be							C O		
Metazoic	Mg							Si S		
Cellular	Ca	Sc	V	Mn	Co	Cu	Ge			Se
Nuclear	Sr	Y	Nb	Tc	Rh	Ag	Sn			Te
Submorphol.	Ba		Ta	Re	Ir	Au	Pb			
Primary Biol.		Ce	Nd	Sm	Gd	Dy	Er	Yb		
Submolecular	Ra	Th	U	Pu	Cm	Cf				

We have already discussed the pharmacological activity of sulphur and selenium through the compounds in which they enter and will not discuss

them again here. Before analyzing the other elements, it is of interest to emphasize again a principal character of their activity. As most of the elements act through specific compounds, the factors which determine the entry of elements into specific biological combinations appear to be of capital importance. Availability of the element alone is only one factor in its pharmacodynamics. With this in mind, we have investigated some of the elements of this Hm group.

We know little about any influence of beryllium as a metal upon the organism as an entity. Its toxic effects are due to abnormal amounts active at lower levels. In the same period of the chart of elements, we have two nonmetals, C and O, for which the organism represents the proper level. The general pharmacological nature of oxygen is indicated by the role of oxidation in metabolic changes. Oxidation represents the first step toward catabolic, homotropic changes. The respiratory phase in the metabolism of carbohydrates, the oxidative fission of fatty acids, and the oxidative desamination of amino acids represent examples of the fundamental homotropic intervention of oxygen.

Acting at the systemic level, immediately inferior to its own level, oxygen has a different action. According to the rule mentioned above, oxygen, a D agent at its proper level, will have an A activity for the blood, which we will study below together with the other A agents. The homotropic relationship of oxygen to fatty acids was discussed above with the study of the biological role of these substances.

The relationship between CO_2 and fatty acids also is interesting. Large amounts of free fatty acids in the blood were seen to allow better fixation of CO_2 to hemoglobin, just as large amounts of sterols do for oxygen. We have investigated this correlation between CO_2 and fatty acids by keeping a fatty acid, such as linoleic acid, in an atmosphere of CO_2 connected to a manometer. A manifest negative pressure results. The venous blood, rich in CO_2 , which also shows a predominance of fatty acids, loses CO_2 and fatty acids during passage through the lungs.

Magnesium

Among offbalance D inducing elements of the II A series of the periodic chart, we first studied magnesium, which belongs to the metazoic compartment and thus, is related to the sea as original environment. Much of the activity of this element can be interpreted as D inducing activity. Mg in many respects is antagonistic to Na, the cation of the same compartment, a member of a series with an opposite A inducing character. High values of

Mg found in blood were related to adrenal insufficiency, (127) while low Mg levels usually occur when blood cholesterol is high. (128)

Excess of Mg was seen to induce adrenal deficiency. We explained this action through the antagonism between Mg and Na. We could thus counteract the salutary effect of NaCl in adrenalectomized rats through administration of magnesium sulfate parenterally. A similar effect was obtained even with oral use of magnesium thiosulfate. (*Note 9*)

The relationship of magnesium to the defense mechanism is of special interest. Mg seems to intervene in the lytic effect of sera upon ascites cancer cells (extensively studied in our laboratory by R. Willheim, P. Fluss and M. Auber) (129) which, as we have seen, represents a characteristic feature of D inducing activity. Similarly, magnesium prevents thrombosis, acting as an antithrombocytic agent. Not only does it prevent the appearance of fibrin, partly preventing the destruction of thrombocytes (130); but it also favors the lysis of already existing thrombi. (131) It appears, therefore, to be a valuable agent in the treatment of thrombosis. (132) Its concomitant action against cholesterol has led to its use in the prevention and treatment of coronary thrombosis.

Magnesium appears to be part of another defense mechanism, the non-specific one, represented by the properdin system. Properdin is active only in the presence of magnesium; neither Ca nor Na can replace it. (133) Higher amounts of magnesium increase properdin activity.

Magnesium sometimes is seen to parallel the action of Cu, another D inducing element. In animals fed milk too long, leading to a type A off-balance, the amount of magnesium falls along with the amount of Cu. The quantity of magnesium in the blood is low in humans with convulsion. (134) Mg appears to be especially effective in the prevention and treatment of "grass tetany" in animals, which often follows feeding on grass with high potassium content. (135)

Magnesium has a similar antagonism toward K and this can explain its activity in cancer. Lower than normal values of magnesium are found in cancer, as opposed to high amounts of potassium which is an A inducing element, and the low Mg seems to favor cancer growth. Moisture increases the amount of K and lowers the amount of Mg in plants, a fact related to cancer frequency in various geographic regions. (136) The difference between the preventive and curative actions of magnesium is of special interest. Administered after a carcinogen has been applied, magnesium reduces the percentage of cancers induced. (137, 138) It has minimal influence, however, once the tumor has appeared or upon trans-

planted or spontaneous tumors. We will discuss this occurrence below together with the effect of other elements.

It is necessary to bear in mind, when we have to choose nonspecific combinations in which to administer it, that the D inducing activity of magnesium is particularly manifested at the metazoic level. Magnesium sulfate appears to be suitable for parenteral use, while the thiosulfate appears to be suitable for oral administration. In these forms, Mg has been found to induce marked local alkalosis in the second day wound crust pH and to produce salutary effects upon pain of the acid pattern. A marked influence upon thiamine-induced convulsions in rats and mice has led us to use magnesium thiosulfate as a tool not only in tetany but in the treatment of convulsions. It appears to be effective in preventing epileptic seizures and valuable even in cases of status epilepticus. We have utilized the same preparation successfully in cancer when pain and preterminal conditions corresponding to an A offbalance were present. Less important effects are seen for magnesium sulfate and magnesium thiosulfate at lower levels.

Calcium

The biological activity of calcium, another member of the II A series and a D inducing element belonging to the cellular level, also is of interest.

In its absorption by grass from the soil, Ca parallels Mg, another D inducing element, but opposes K, an A inducing element. Grass tetany is thus induced by high K and low Ca and Mg values. Ca, which is another D inducing element like copper is antagonistic to zinc, an A inducing element. It has been observed that cancer is less frequent in the so-called calcerous clay regions where the soil is formed by limestone. (139) Together with other minerals, an optimum of calcium in soil may help to prevent cancer. While SiO_2 favors cancer, Ca appears to prevent it. Calcium also is an antagonist to zinc which, in high doses, seems to favor the development of cancer. (140) The relationship between Ca and K has permitted us to be more precise about the role of Ca in cancer pathogenesis. As opposed to K, which increases by as much as 60% in tumors, the content of calcium decreases by 44%. (141-147)

Confronted with K and Ca changes, it appeared interesting to see to which element we could directly attribute the increase in malignancy. In the regeneration of liver cells, where rapid growth without malignancy takes place, potassium is increased while the amount of calcium is unaltered. Similarly in other rapidly growing but normal cells, calcium is not diminished while K is increased. Potassium thus appears to be related to the process of cellular growth and multiplication which represents also an

added factor in transforming noninvasive into invasive cancer. However, potassium is not directly related to the cancerous character of the cells. On the other hand, reduced amounts of calcium appear to be peculiar to the cancerous process. (147) The reduced calcium in cancer is not due to a lack of the element in the organism since calcium is not only available but even apparently present in excess at the systemic level. As we have shown, a high urinary calcium index, indicating exaggerated excretion, is present in the type A offbalance. The anomaly resides in the low capacity of the cancerous cells to fix and properly utilize calcium. As calcium acts at the surface of the cell and its deficiency reduces cellular adhesiveness, (148, 149) lack of cellular calcium can be seen to increase the invasiveness of cancer cells and the tendency to metastases. Deficiency of calcium in cells appears related to the character of youth while excess seems to result in rapid aging.

The anomaly induced by the qualitative deficiency in calcium thus appears to be at the cellular surface. Related to it also are manifestations at the tissular level.

Administration of any calcium salt induces a manifest increase in local alkalosis (*Fig. 128*) of the second day wound crust pH. It appeared interesting that in bone lesions, especially in bone cancer metastases, the offbalance type A is characterized by an osteolytic process, the D type by an osteoplastic one. The local acidosis present in lesions with an A type of offbalance explains the mobilization of calcium in these osteolytic processes. Ca is deposited in important amounts in metastases with a type D offbalance, a fact which can be related to the local alkalosis resulting from the abnormal metabolism. This alkalosis represents a condition favoring the precipitation of calcium. Indirectly, the deposit of calcium in bone metastases appears to correspond to the D pattern of tissular abnormality. Calcium has a D inducing activity even in this case.

With calcium excreted in excess through the urine, the problem of calcium pharmacology in the A type of cancer is related to the form in which it acts at the cellular level, which appears qualitatively impaired. As the quantitative decrease must be considered to be a consequence of qualitative insufficiency and not a general quantitative deficiency, the problem is not to provide calcium but to find a way to insure better utilization at the cellular level. It is for this reason that administration of most calcium salts does not influence the evolution of experimental or clinical cancer, but has a preventive effect upon the induction of tumors through carcinogens. Administered after the injection of the carcinogen, calcium

appears to reduce the percentage of positive results. Administered after the tumors have appeared, the influence is minimal or nil.

As we have mentioned above, an excessive calcium excretion is, in itself, sufficient to indicate the existence of a deficiency in calcium utilization at the cellular level without a calcium deficiency in the organism. The therapeutic indication is for agents able to influence the fixation of calcium at the cellular level. Fatty acids which change the cellular metabolism so

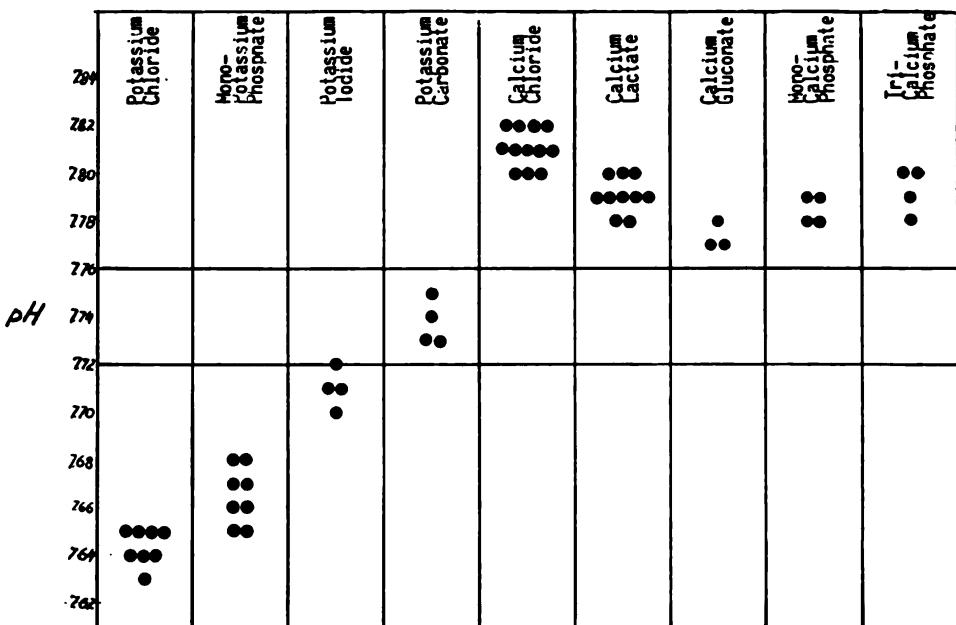


FIG. 128. The influence exerted by two elements, potassium and calcium, upon the second day wound crust pH shows a frank tendency toward acidification for potassium and alkalinization for calcium. For different salts, the differences result from the unequal influence exerted by the anion which works additively to that of the cation.

as to induce local alkalosis, have appeared to be the most active agents. Testosterone, and calciferol have appeared helpful but not nearly as active as fatty acids.

While high urinary excretion of Ca indicates a type A offbalance, low or no urinary excretion can result either from an excessive cellular utilization of calcium or from a type D offbalance at the cellular level. Other analyses can be used to indicate the probable occurrence. With calcium excretion low and other analyses indicating type D offbalance, all the chances are that the low excretion is part of the D offbalance. If, on the contrary, only the Ca excretion is low and the other values correspond to

A type of offbalance, the probability is a quantitative lack of calcium in the organism. This can be corrected by the administration of calcium parenterally or orally in any absorbable form. Low excretion as well as some symptoms can be overcome in a short time through the administration of sufficient calcium. In the opposite type of case, with metabolic calcium retention, administration of calcium will induce an increase in the intensity of symptoms.

Excess of calcium in the urine thus corresponds to lower values at the cellular level and need for fixation of calcium. This indicates again that the problem is not the amount of calcium present but the deficiency in its utilization.

Copper

Copper, from the IB series and another anti-A element of the cellular level, appears indispensable for the synthesis of heme of catalase. A deficiency of copper results in reduced activity of this enzyme. Similarly, the synthesis of hemoglobin is possible only in the presence of copper. Cytochrome oxidase contains Fe and Cu. Cu deficiency reduces liver cytochrome oxidase. (150, 151, 152) Cu is present in blood serum bound to a protein to form ceruloplasmin which also acts as an oxidase. (153) Cu, while favoring the synthesis of these substances, intervenes ultimately in the processes which lead to the active catabolic intervention of oxygen. In this way, Cu acts as a D inducing agent. Cu intervenes actively in the metabolism of sulfur. (154, 155) The transformation of sulfhydryl to disulphide is slow and incomplete when there is a copper deficiency. The same deficiency reduces the formation of phospholipids as seen in rat liver. (156) Indirectly, Cu favors anti-A activities.

The influence of Cu upon Ca metabolism also is indirect. We have seen that a local deposit of calcium in bones corresponds to a local D pattern. Deficiency in copper, a D inducing agent, permits the appearance of local A conditions, which in the case of bone, will result in a lack of calcium, the opposite of what is seen in local D offbalance. This correlation explains why, in spite of sufficient P and Ca, the lack of copper induces an osteomalacia with bone fracture and symptoms of rickets, as seen in animals with an indirect Cu deficiency caused by an excess of molybdenum, (157, 158, 159) an A inducing agent. The administration of copper helps to repair these fractures. (157) Parallel reductions in Cu and Mg occur in milk-fed calves. (160) A richness of zinc, like molybdenum, can provoke a deficiency in Cu and Ca. With copper deficiency and low catalase, resistance to infections is lowered. In brucellosis, a deficiency of Cu and Ca

coincides with reduced concentrations of Mn and Co in the blood and pituitary glands. (161, 162, 163) Cu, Ca, Mn and Co are all metals of the D inducing group.

In cancer, a qualitative deficiency of copper is found. A frank reduction of the catalase content is seen in cancerous cells as well as in the liver of cancerous subjects. (164, 165) On the other hand, Cu content is considerably increased in the blood of these subjects, with values even three times greater than normal, often encountered. (166, 167, 168) These values return to normal if the cancerous tumor disappears. (169)

In cancer, copper deficiency at the level of the cells is manifest, shown not only by reduced catalase but also by upsets in the cytochrome oxidase system, the heme system, the SH metabolites, and the phospholipids. The deficiency, however, is only local and consequently qualitative since an excess of Cu is found at the immediately superior level, the blood.

A local abnormality residing in inadequate capacity to utilize copper thus appears related to cancer. In normal animals, copper in excess can be utilized and is able to prevent the appearance of tumors. This explains why Cu, which protects rats against carcinogenetic azodyes, (170, 171, 172) does not influence the tumors once they have been induced, *i.e.*, once the qualitative insufficiency is present. The recognition of this difference between the form in which copper is utilized by the normal animal and the deficiency in the cancerous entities, has been the basis for a series of studies concerning this key problem in the pathogenesis of cancer. The therapeutic use of copper—and of other elements which we will discuss below—is not a quantitative but a qualitative problem.

Manganese and Cobalt

Two other elements of the cellular compartment have appeared interesting. Manganese by intervening as a catalyst in processes resulting in an activation of oxygen, indirectly manifests D inducing character. Its presence in smaller amounts in tumors or cancerous organs than in controls, has been considered. Just as with copper, no effects are seen in treating tumors with manganese compounds although a certain degree of preventive action is obtained in tumors induced by carcinogens. Similarly, with cobalt we have obtained a certain degree of prevention against tumor induction by carcinogens but no effect upon the evolution of tumors once they have appeared. No effects have been seen in transplanted tumors.

Heavier Elements

We have studied the activity of elements corresponding to the lower levels—such as strontium and tin for the nuclear; barium, gold and lead for the submorphologic; and cesium for the primary biological compartments. The lower the level of the element, the greater appears to be the preventive effect against induction of tumors by carcinogens. But the minimal, or complete absence of effect upon already existing tumor cells remains unchanged.

As mentioned above, we connected this paradoxical activity to the fact that, while normal cells are able to manufacture compounds through which the appearance of a cancerous entity can be prevented, these compounds are no longer formed if an entity is already cancerous.

These have been the considerations forming the basis for an entire series of studies of the role of various elements in the pathogenesis of cancer.

Elements in the Pathogenesis of Cancer

Investigations have been made of the amount and form of the element present in the normal animal as compared to the cancerous animal. The quantitative and especially qualitative differences have been seen to indicate the site of the abnormality largely responsible for the lack of influence exerted by the element. The results explain why the administration of the element alone is unable to influence the evolution of an already existing cancerous process. More interesting, they show what compound of the element could have an influence. This research, which is in progress, opens the door for possible therapeutic applications. It is through such compounds, present in the normal and lacking in the cancer-stricken animal, that attempts are being made to influence the evolution of cancer. An important step has been the finding that suitable compounds can be obtained by the treatment of fresh organs *in vitro* with some of the elements. Their study may make possible synthetic preparation of suitable compounds. The few results already obtained in experiments with animals confirm that D-inducing activity represents a factor which the promising elements share.

CHAPTER 13

PHARMACODYNAMIC ACTIVITY (PART TWO)

ANTI-FATTY ACID GROUP

PARALLEL TO INVESTIGATION OF AGENTS capable of correcting offbalances of type A, attention was directed to agents that might influence the opposite offbalance, type D. Since fatty acids are involved in the pathogenesis of type D offbalance, agents with anti-fatty acid properties had to be sought as correctives. Some of these are natural constituents used by the body to control normal and abnormal intervention of fatty acids. They were consequently isolated and studied. Synthetic agents also were obtained and studied, their choice largely inspired by the control mechanism used by the body.

Anti-Fatty Acids Constituents

We have seen that a free fatty acid loses most of its biological activity when its polar group is bound to another radical. This led us to investigate substances which naturally are bound to fatty acids. It could be shown further that each major group of fatty acids is bound in the organism to specific constituents. The saturated fatty acids are principally bound to glycerol, the low unsaturated acids to glycerophosphoric acids as lecithins, and the high unsaturated members to sterols. The conjugated fatty acids, found in abnormal conditions, appeared to be opposed by neoglucogenic corticoids. The constituents were conceived of as being naturally occurring anti-fatty acid substances and our first effort was to study how they intervene to balance the activity of fatty acids, especially when the latter act as pathogenic factors.

In this study, two types of influences were investigated: one, a relatively direct effect induced through a neutralization of the energetic centers resulting in a more or less advanced degree of inactivation of the fatty acid; the second, an indirect effect achieved through changes in the metabolic processes in which fatty acids intervene. In a different kind of intervention, the anti-fatty acid to which a fatty acid is bound governs its ultimate biological fate. For example, the bond to glycerol favors caloric metabolism. The bond to glycerophosphoric ion converts a fatty acid, saturated as well as unsaturated, into an organizational constituent. The bond to sterols favors a functional role, even for monoethenoids.

We started the study of the naturally occurring anti-fatty acids with those agents known to be bound to fatty acids in the organism. The simplest such agent is glycerol.

Glycerol

Glycerol is the most ubiquitous fatty acid-binding substance in nature. We attempt to explain this fact on the basis of glycerol's structure and the special biological role it confers. We have seen that fatty acid molecules take reciprocal parallel positions when they form monomolecular layers. In their bond to glycerol, these fatty acid molecules conserve this reciprocal relationship. (Fig. 129) This could explain why, in the body, the bond of fatty acid and glycerol always is a triglyceride, mono and diglycerides being

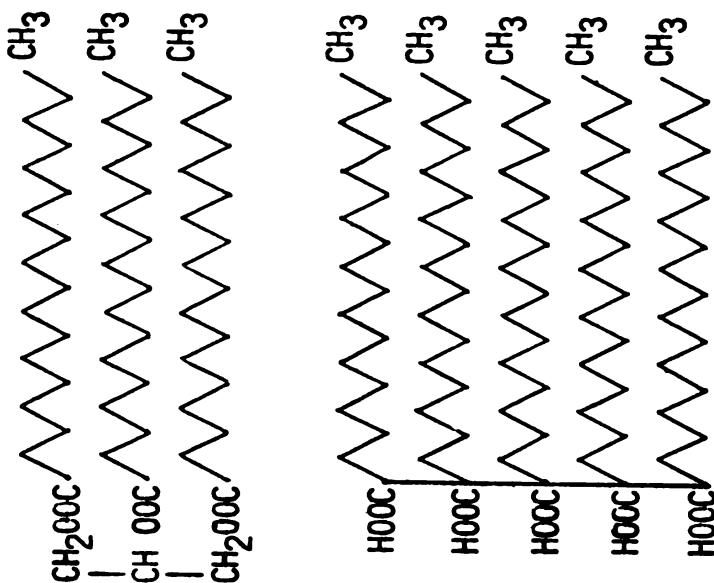


FIG. 129. The fatty acid radicals take in the triglyceride molecules a similar parallel position as when they form a monomolecular layer at the surface of water.

only intermediate steps. The same fatty acid has different biological activities if administered as free acid or as triglyceride. The combination seems to serve as an energy-furnishing metabolite. Bound to glycerol, fatty acids with long or short chains, saturated, monounsaturated, polyunsaturated, and even conjugated, seem to represent energetic reserves which are utilized as caloric metabolites, especially in those species which are able to store them.

With this relationship in mind, we administered glycerol with two objectives: 1) to obtain, as an immediate effect, the inactivation of the free fatty acids present in abnormal conditions through the neutralization of their polar groups, and 2) to eliminate these fatty acids by turning them into caloric metabolites.

Studying the activity of glycerol at different levels, we could see no influence upon phages. However, an indirect effect was observed upon viruses. Glycerol is widely used as a special medium for the preservation of viruses in tissues. Its preservative value can be correlated, at least in part, with its influence upon fatty acids. We have seen that fatty acids have a noxious effect upon viruses, leading to their disappearance in various organs. The treatment with pure glycerol reduces autolysis of organs through a dehydration effect. Curtailing the lytic activity of the enzymes active in autolysis, glycerol reduces the amount of fatty acids liberated through such autolysis, and thus prevents the destruction of the virus. Glycerol may also preserve viruses by acting antagonistically to any fatty acids which still manage to appear.

Glycerol has a bacteriostatic effect upon only a few species of microbes and only when applied in high concentrations.

A minimal influence upon cells was seen for glycerol in *Tetrahymena pyriformis* and ascites tumor cells. To study its action at higher levels, glycerol was administered orally or parenterally to animals or humans. Solutions of 20% glycerol were well tolerated when injected subcutaneously or intramuscularly. It should be noted that when glycerol was administered to complex organisms, it was largely absorbed and circulated without alteration, a fact which would explain the effectiveness of relatively small amounts. At the tissue level, glycerol induced a change of the local pH of a lesion toward the acid side, as seen in the second day wound crust. Figure 130 illustrates this. The change explains glycerol's action in increasing intensity of acid pattern pain and decreasing intensity of the alkaline. This influence upon pain was obtained constantly with very small amounts, permitting the use of glycerol even as a test for diagnosis of pain pattern. Intramuscular injection of $\frac{1}{2}$ cc. of a 20% solution or oral ad-

ministration of $\frac{1}{2}$ cc. of a 50% solution in water has been used for this purpose. However, later, when other agents were found to produce even more overt responses, we stopped using glycerol as a routine test.

Glycerol has almost no beneficial influence upon the healing of wounds or radiation lesions. Healing was even retarded in some experiments. Various changes in the evolution of tumors occur when host or transplant are treated with glycerol. In some, these changes are minimal; in others an obvious reduction in growth occurs. In a high proportion (12/20), a marked involution has been noted for Walker tumors in rats. Ascites Sa 180 in mice, after repeated intraperitoneal injections of a solution of 2%

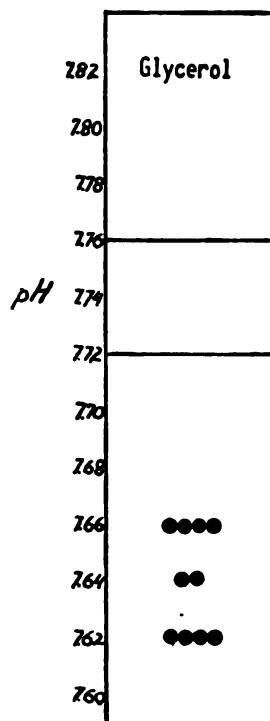


FIG. 130. Glycerol induces a lowering of the second day wound crust pH.

glycerol, disappeared in 70% of the cases. A lesser effect was seen in Ehrlich and Krebs ascites tumors and still less in the solid tumors obtained with these ascites cells. (Fig. 131) One of the most interesting effects of glycerol was that seen upon the tumors in humans where a manifest involution was obtained in cases in which an offbalance of type D was present. This important effect will be discussed below with the therapeutic use of glycerol. Glycerol administration had an interesting effect upon the amount of cholesterol in the blood in a few subjects. When ten drops of glycerol were given orally three times a day for a month or more, cholesterol values

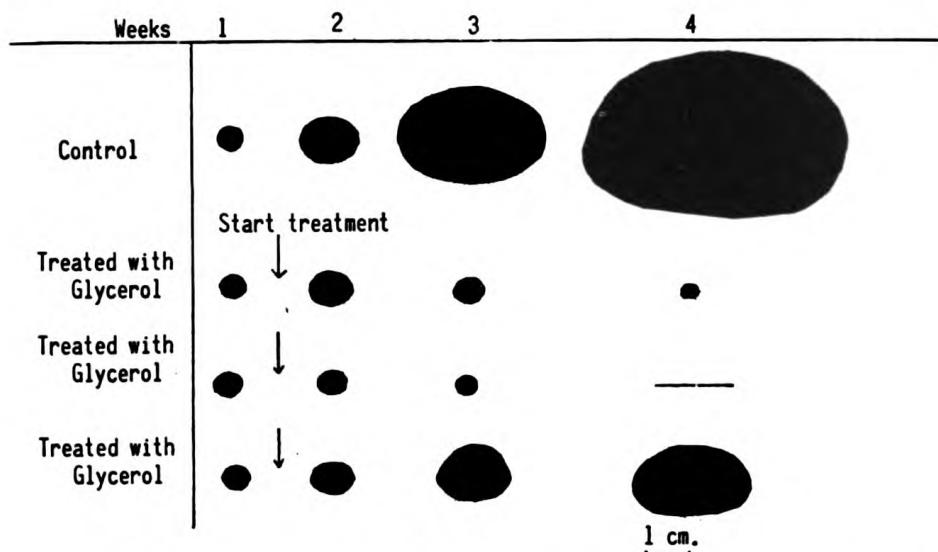


FIG. 131. Changes induced in Walker tumors in rats by the treatments of the animals with glycerol (daily subcutaneous injections with $\frac{1}{2}$ cc of 5% glycerol solution in saline isotonic solution).

decreased. In some patients, with no change in diet and no medication other than glycerol, values originally above 300 mgr./100 cc. serum fell to below 170 mgr. If these patients also were hypertensive, long-term administration of glycerol produced a reduction of blood pressure.

An impressive hemorrhagipar effect was noted, frequently ulcerated lesions starting to bleed shortly after administration of even a few drops of glycerol. The relationship of hemorrhaging to glycerol was clear when in the same subjects, repeated administration of this agent invariably was followed by bleeding. The bleeding usually was arterial; only occasionally was an oozing hemorrhage seen.

Many years ago we became interested in studying, in a group of severely burned subjects, the role of fatty acids in the pathogenesis of burn complications. Glycerol was administered to these patients with good effects upon pain. Before the use of antibiotics, one of the principal manifestations in burn patients with widely infected wounds was repeated chills. These chills also were influenced by glycerol. (*Note 1*) A direct action upon the parasympathetic system could be attributed to glycerol and could explain the effect upon chills. This view has been confirmed by studying the effects upon cardiac rhythm produced by intravenous administration of glycerol in rabbits. (*Note 2*)

Convulsions could be induced by glycerol in animals and also were seen to occur in humans. (*Note 3*) They could be induced, with much

smaller doses in animals when, along with glycerol, an otherwise harmless dose of deoxycorticosterone acetate was administered. Injection of 0.1 mg. of this hormone in mice weighing 25 to 30 grams, followed by an injection of $\frac{1}{4}$ cc. of a 5% solution of glycerol, induced convulsions which were usually lethal. In terminal cancer patients, too, concomitant administration of the cortical hormone and glycerol for a few days has produced convulsive seizures, which proved to be lethal in one subject in whom no

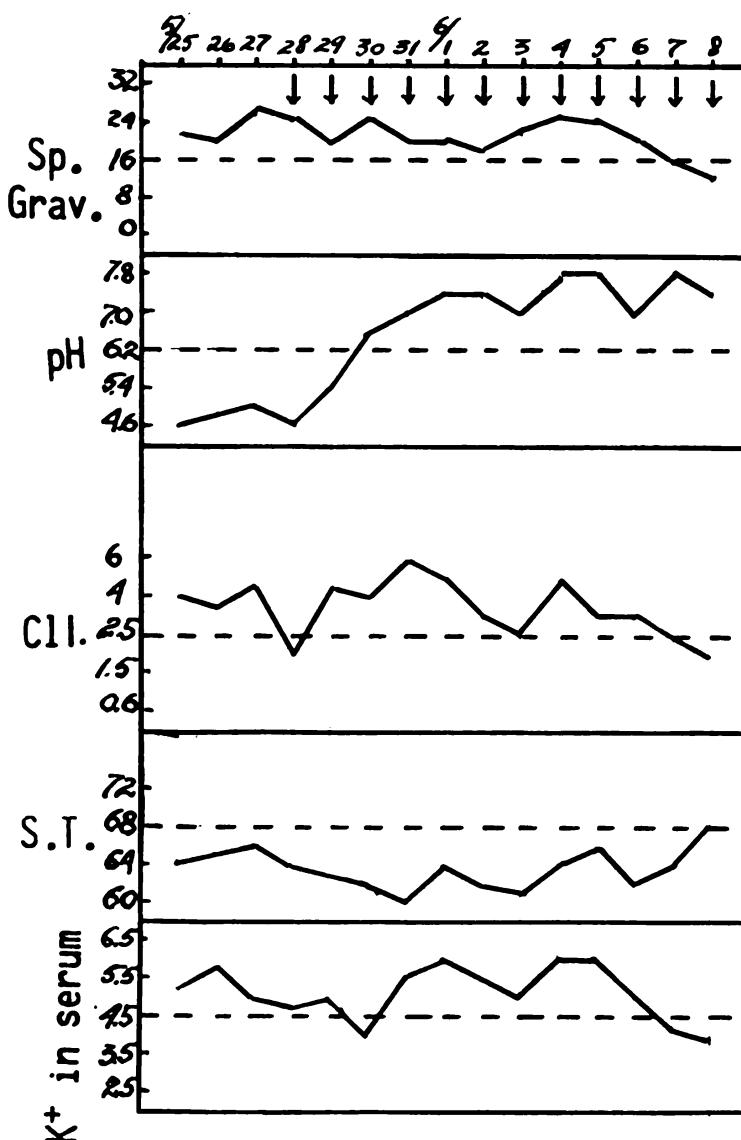


FIG. 132. The administration of 1 cc glycerol daily induces a change of the urinary pH toward the type A, before changes in the other analyses take place.

previous abnormal cerebral manifestations had been observed, and who had received these two medications for only a few days.

Of the different systemic manifestations influenced by glycerol, the effect upon acid-base balance is most striking. Even before any other effect upon systemic analyses becomes apparent, an immediate change of urinary pH from acid to alkaline values is induced by glycerol administered in sufficient quantity. (*Fig. 132*)

Glucose

The pharmacological activity of glycerol has raised the question of the relationship between this substance and glucose since some of the manifestations induced by glycerol could be obtained with glucose. The same effect is seen on local pH in the second day wound crust. Glucose administered for a few days prior to, and immediately following, wound induction shifts local pH toward more acid values. A similar effect is obtained on the pH of tumors. (173) Glucose decreases intensity of pain of the alkaline pattern and increases pain of acid pattern. We were able to induce convulsions in rats by injecting 20 to 25 cc. of an isotonic solution of glucose subcutaneously twice daily. After 4 to 6 days, convulsions appeared and often led to death. In some terminal cancer patients with brain metastases, who had had previous convulsions, intravenous administration of glucose as a therapeutic procedure produced convulsions. We have even seen lethal convulsions in a patient after a few days of intravenous administration of glucose in saline solution in conjunction with intramuscular doses of 1 mg. twice daily of deoxycorticosterone.

Although rarer than for glycerol, hemorrhages occurred after each glucose administration in some patients with previously bleeding ulcerated lesions. Bleeding stopped when glucose administration was discontinued. Renewed administration was followed each time by renewed bleeding. We want to emphasize this relationship of glucose to hemorrhage because of its clinical importance.

It seems possible that glyceric aldehyde and glyceric acid, which appear during glucose metabolism, play a role in the manifestations mentioned above.

Glycerophosphoric Acid

The ability to relate the pharmacological activity of glycerol to its bond with fatty acids, has led us to consider another substance able to bind fatty acids, glycerophosphoric acid. The bond of this acid with quaternary bases such as choline or ethanolamine, and the fatty acids results in phospho-

lipids which take part in the formation of boundaries and separating membranes. Although various fatty acids, both saturated and especially unsaturated, enter into these phospholipids, resulting in a variety of compounds, their general biological behavior appears to be the same. Certain fatty acids, the di- and tri-ethenic, however, are preferentially bound as phospholipids when they pass from the intestine into the circulation.

In investigating its influence as an anti-fatty acid agent, we administered glycerophosphoric acid, 50 cc. of a n/10 solution diluted in 1000 cc. of isotonic saline, glucose or other solutions, intravenously or subcutaneously. It had salutary effects upon pain of an alkaline pattern and upon corresponding lesions. A manifest influence was exerted upon the systemic acid-base balance, especially in cases with high urinary pH and with all other analyses showing an offbalance of type D. No influence was observed upon evolution of tumors in animals or humans.

The increased basal metabolism and a marked increase in work capacity, which are observed in subjects taking sodium glycerophosphate for long periods, has made us suspect a possible effect upon thyroid secretion. The appearance of thyrotoxicosis in a subject who had inadvertently taken a large dose of sodium glycerophosphate seemed to confirm this view.

Sterols

A third group of natural constituents, which act as anti-fatty acid agents is composed of sterols, which are absorbed and circulated bound to polyunsaturated fatty acids. Except in brain and red cells where only free sterols are encountered, the sterols are found both as esters and free substances in all cells, tissues and organs.

Cholesterol, phytosterol and a few sterols were utilized in pure form in our research. In addition, sterols were obtained and used as mixtures, as in the insaponifiable fractions of tissues, organs, organisms and biological products. In some studies, these fractions were further separated into constituents, largely through column chromatography. We used cholesterol in different preparations. (*Note 4*) Watery or gum cellulose suspensions were used for *in vitro* and *in vivo* studies.

Cholesterol was seen to induce a change in the shape of some bacilli, such as *B. subtilis*, *B. megatherium* and *B. anthracis*, turning them into irregular round formations. At the same time, their Gram positive staining became abnormally intense. The agar cultures had a creamy aspect. The influence upon Gram positivity explains the fact that Gram positive individuals could be obtained in cultures of various Gram negative microbes such as *Esch. coli* or *Eb. typhy* after repeated treatment with colloidal

cholesterol preparations added to broth. The Gram positive forms, however, could not be isolated.

Cholesterol was seen to influence red cells in form, shape, volume, sedimentation, velocity, and oxygen-combining capacity. A vermillion color, which persisted for a long time, was obtained through in vitro treatment of blood with cholesterol or through intravenous injections in animals. Although such injections were lethal, they induced the abnormal vermillion color. Cholesterol produced a manifest change toward less alkaline values in the second day wound crust pH. A favorable effect upon rabbit skin wounds was obtained, with abnormally intensive proliferation of the epithelium, this healing effect, however, was less manifest for irradiated wounds. In subjects with ulcerated lesions, prolonged administration of cholesterol was frequently observed to induce hemorrhages, especially of an arterial character. However, in patients with coronary occlusion or endarterial obliterations, the administration of cholesterol was followed by an increase of symptoms apparently related to exaggeration of the degree of occlusion. This effect upon blood vessels also could be seen in animal tumors. Administration of cholesterol induced zones of necrosis in the tumors which could be related to proliferation of the endarterial cells leading to thrombosis and ischemic infarct. The portions of tumor corresponding to these ischemic infarcts showed characteristic necrosis with unaltered structure but without the normal staining.

In animals injected with cholesterol and then submitted to trauma in the Noble-Collip drum, shock was prevented. Injection of cholesterol prior to the experiment reduced mortality to zero while in untreated controls mortality was high. A similar but less constant effect was obtained when cholesterol was administered immediately after trauma. It is noteworthy that in animals injected with cholesterol before being placed in the drum, the blood not only did not become abnormally black but the usual bleeding from the nose, mouth and paws (if not taped during the trauma) was abnormally bright red.

The effect upon experimental tumors was investigated through the dipping technique repeated in successive generations. Changes in the evolution of certain tumors, such as mammary adenocarcinoma in mice, were induced. The effect of cholesterol was similar to that of insaponifiable fraction but was less manifest and will be discussed in more detail later.

Cholesterol's effect upon the central nervous system was interesting. Often, immediately after administration, both in animals and humans, transitory somnolence was observed. However, repeated administration induced convulsions. (*Note 5*) Exophthalmia was seen in mice after injec-

tion of cholesterol and was most manifest 24 hours thereafter. It contrasted with normals and especially with animals injected with fatty acids or similar lipoids, who showed enophthalmia.

An ether-oil cholesterol solution induced paraplegia with early foot ulcerations in rats and rabbits. This occurred particularly in females and was related to a predominance of sterols in this sex. Castration or administration of sex hormones did not change this special susceptibility of the nervous system of females to cholesterol. However, the administration of fatty acids or of acid lipid preparations of organs or tissues did suppress it. (*Note 21, Chapter VI*) Changes in systemic analyses were generally not obvious and, when present, were slow in appearing. They corresponded to changes toward offbalance A.

Unsaponifiable Fractions (Insaponifiable or Non-saponifiable)

When insaponifiable fractions were prepared from various tissues and organs, big differences could be seen in the quantity and the number of sterol compounds naturally present. However, a certain specificity related to the origin of these insaponifiable fractions appeared most interesting. The insaponifiable fractions of various materials were prepared by the usual methods. Most of the fractions are soluble in oil in higher proportion than cholesterol, with some of them even miscible with oil. More concentrated solutions in sesame oil could be prepared than for cholesterol. In most of the experiments, 5 or 10% solutions were used. Colloidal suspensions also were prepared in the same manner as for cholesterol.

In spite of the extreme variations in sources of the insaponifiable fractions, almost all have some properties in common. Some are similar to cholesterol in their effects particularly at the lower levels. The marked differences appear at higher levels. They induce hemorrhages in the adrenals between the fascicular and reticular zones.

On the healing process, especially of radiation wounds, insaponifiable fractions of placenta, embryos and butter—materials related to growth—show impressively greater activity than cholesterol or preparations of insaponifiable fraction of other origin. They induce healing processes even in standardized radiation lesions where cholesterol has a weak effect.

In their influence upon tumors, the preparations of insaponifiable fractions of different organs differ markedly. No changes at all were obtained with some preparations such as from pig intestine, for example, while interesting results were obtained with others. The differences appeared especially evident in experiments in which a direct influence upon the tumor

was exerted. Transplants of Ehrlich mammary adenocarcinoma in mice were dipped in insaponifiable preparations and grafted. In general, no immediate visible effects were seen with this technique for the first transplant generation. By repeating the same procedure for following transplant generations, changes were obtained which varied with the preparations used. The insaponifiable fraction of human placenta, for instance, produced a marked increase in malignancy, together with morphological changes, the tumor changing from an adenocarcinoma to an encephaloid. Further treatment of the transplants led to still greater malignancy with a sarcomatoid transformation. Thereafter, negative results were obtained with new treatment of the transplants. (*Note 6*) With this procedure, placenta preparations showed a manifest influence even at the third transplant generation, and they were negative passages for the fifth to sixth transplant generations. With pig intestine preparations, even after ten successive passages, malignancy was unchanged.

The specificity according to origin was also seen in other experiments, such as in the influence exerted by these preparations upon the development of specific lesions produced by smallpox virus in low-reacting species such as mice or rats. Preparations from receptive animals, and especially from organs sensitive to the virus, were more capable of inducing local receptivity than were preparations from refractory animals. For instance, positive effects were obtained with vaccinia virus in mice and rats previously injected subcutaneously with the insaponifiable fraction of rabbit skin or brain, while no such effects were seen when the insaponifiable fractions of pig or hen intestines were used.

Differences were observed between the insaponifiable fractions of different organs for conditions principally manifested at the organ level. Conditions affecting mainly one organ were treated with the insaponifiable fraction corresponding to that organ. These preparations often appeared much more active than those from other organs. We investigated the effects of a heart insaponifiable fraction on patients with myocardial insufficiency, especially when responses to other therapeutic agents could no longer be obtained. In like manner, we used the insaponifiable fraction of liver for manifest liver insufficiency. The rate of liver regeneration in rats after subtotal resection was found most accelerated by liver insaponifiable fractions. The good effects obtained in the treatment of intractable diarrhea with insaponifiable fractions from pig and hen intestines will be discussed below. We investigated preparations obtained from lymph nodes and spleen for the treatment of shock, particularly in its acute form. Similarly we also used adrenal insaponifiable fraction to influence induced adrenal insuffi-

ciency, and brain insaponifiable fraction in an attempt to influence insomnia. The results of these studies will be discussed in the section dealing with therapy. The changes obtained with the respective preparations indicate that they have a specificity which represents an important factor in normal and abnormal physiology.

In a second group of researches, constituents of the rough preparations of insaponifiable fractions from various sources were separated by different methods. The ketonic and nonketonic constituents were obtained and, when tested in animals, showed several differences in biological properties.

Further research of specificity was made using separations through the chromatographic column method. Most of these preparations are still under laboratory investigation. Experiments are being conducted with different organ preparations, some fractions obtained being identified as common to all organs, while others are specific to one organ or to a group of organs. These experiments already have revealed a marked plurality of constituents for the insaponifiable fractions of organs which has to be related to the plurality of constituents found in the acid lipid fractions of the same organs and which was discussed above. The specificity seen for organs would thus greatly concern their lipidic constituents which form the acid and the insaponifiable fractions. It is especially in terms of specificity that the acid lipidic and insaponifiable fractions of various organs are being investigated in research now in progress. (*Note 7*)

Corticoids

The study of the defense of the organism against fatty acids focusses attention once again on the adrenals whose constituents appear to be part of the natural defense mechanism. To date, around 30 different crystallizable compounds have been isolated from less than a third of the total cortical extract. The amorphous part, biologically more active than the crystallized part, would contain other important compounds. Even if some of them are intermediary compounds or artefacts, adrenal intervention still is characterized by plurality of its active agents. Furthermore, several opposite tendencies are recognized between groups of adrenal compounds. While all the corticoids show a certain antagonistic action toward fatty acids, mineralocorticoids are, from several points of view, antagonistic to neoglucogenics.

With these considerations in mind, and recognizing the adrenals as one of the principal means for relatively rapid defense against noxious agents, we have investigated the relationship of the adrenals to lipids.

We have already noted the striking richness of the adrenals in arachi-