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 degradated 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

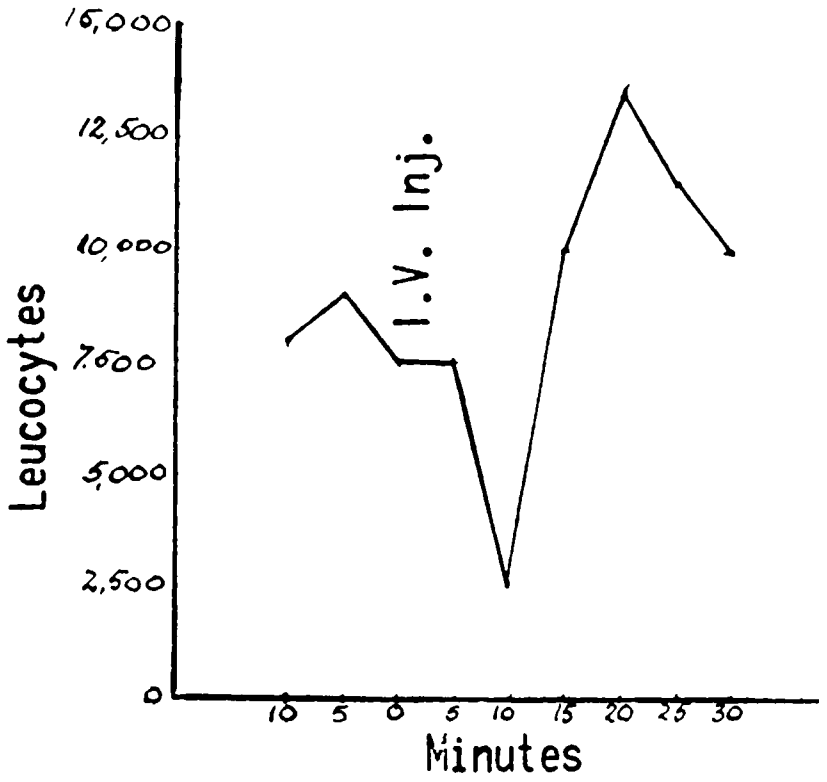


FIG. 75. *Diphasic response in the defense.* The intravenous injections to a normal individual of a foreign material such as of a suspension of killed microbes or of a colloidal metal induces a typical response which corresponds to the hemoshock. A *diphasic* curve seen in most of the analyses characterizes 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 antitryptic 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.

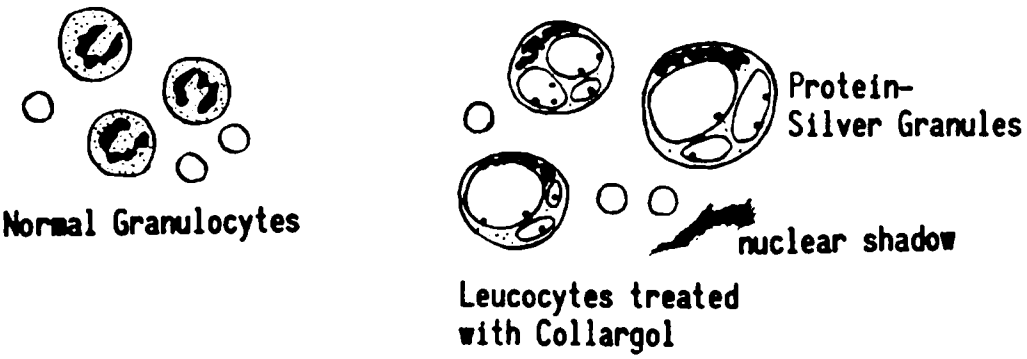


FIG. 76. Drawing of the changes induced by a *colloidal suspension of silver proteinate upon leucocytes.* The leucocytes were obtained by injecting broth intrapleurally to rabbits. Silver proteinate was added to the suspension of leucocytes and the changes observed in a microscope heated chamber maintained 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 didactically 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

Defense Stages	*Character*	*Intervening Factor Present*	*Nature Of the Processes*	*Means Used*	*Moment Of Appearance*	*Resulting Condition*	*Effect Of Immune Serum*
rimary mmediate	Non Specific	Antigen	Hydrolytic	Hydrolytic Enzymes	Immediate	Shock	Curative
rimary rolonged	Lipidic Defense	Antigen (probably in lipidic frac.)	Lipidic liberation	Lipids	From minutes to days	Toxic	Curative
llergic mmediate	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
llergic rolonged	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
mmune	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 consquently 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 alkalizing 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, $\frac{1}{20}$ 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 lipoacid 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 asymptomatically. 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. Conseqently, 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

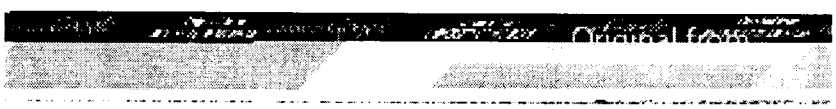
INFECTIOUS DISEASES

Incubation

Low	*Obligatory above 6 days*
Dyphtheria	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

Amount used	*Incubation time—average of 4 animals*
.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 thermostabile 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 nonheated 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 streptococcic 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 streptococcic 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 hypothecated 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.

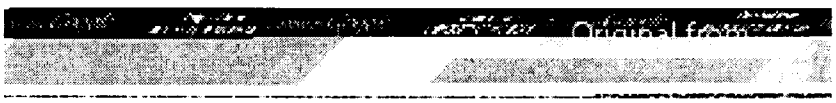
Even more interesting results were obtained when, in addition to these treatments of transplant and host, another treatment—that of the "bed" of the transplant—was added. The wound receiving the transplant was soaked with the mixture of insaponifiable fractions. Often after the graft, treatment with 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 ½ 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

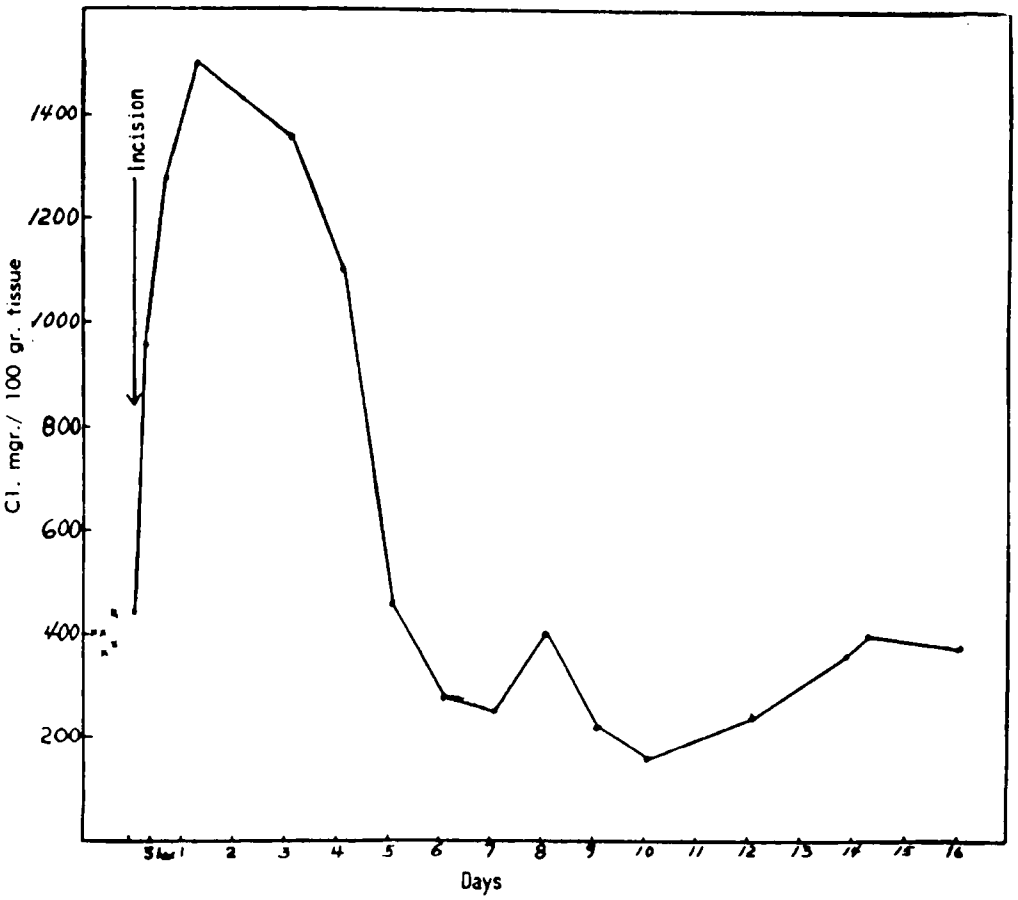


FIG. 78. *Wound chloride curve.* The curve of the amount of the chlorides present in skin wounds in rats corresponds to the average value obtained in 6 rats for each figure. A first phase, with high values corresponds to the offbalance D. This is followed by a second phase characterized by an offbalance 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

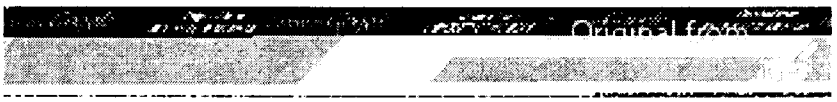
The grafting of cancerous cells in normal humans usually leads to the appearance of a growing tumor which (315) after a period of days, almost always suffers the same fate as a moderately heterogeneous transplant: death followed by resorption or expulsion. The time when this process occurs indicates 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.