STIMULANTS TO BACTERIAL VARIATION.

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Introduction.

In a previous article on the capillary endothelium in relation to antibodies[1], I suggested that the immune substances which are present in the living, actively immune animal may differ, both in their properties and in their range of action, from the antibodies demonstrable experimentally in an animal's serum, and that these differences may partly account for the greater efficacy of the former in the living body. I now wish to supplement the ideas which I there put forward.

There are many problems about the relations of parasitic bacteria to the animal body which cannot be satisfactorily explained by "antibodies," even if, as I proposed, this term be expanded so as to include new kinds and qualities of substances in addition to the properly authenticated serological antibodies. Various other factors have also to be considered. The one which I have made the subject of the present article is the influence of stimuli upon the vital capacities of bacteria.

When a cell grows in normal fashion, the "growth impulse" is taken for granted, as though it were simply part and parcel of the ordinary food supply. But growth may be accelerated, retarded, altered in character, or completely

[1] *Journ. Hyg.* XXII. p. 355, 1924.

inhibited without any evidence of deficiency in the quantity or quality of the material from which the cell obtains its appropriate "building-stones." Under such circumstances it is realised that something more is needed, something in the nature of a "stimulus," to start, regulate, and maintain the metabolic processes of hydrolysis and dehydration which are necessary for growth.

The reality of such stimuli is admitted, and it is recognised that they are equally of importance in accounting for normal growth and for deviations from the normal. They have not been isolated as chemical entities because the dynamics of living matter are too complicated. Still, they are chemical and physical agencies of one sort or another; they are not "vitalistic" in the sense of being something quite different from ordinary material forces.

Then what is a "stimulus"? The answer must be offered in a series of stages which involve the raising of other questions.

What are antigens, antibodies, and alexins? These are all highly respectable terms, the importance of which is taken for granted. But their definitions are vague and unsatisfactory. An antigen is something which causes the animal body to produce an antibody and it is then found that the antigen reacts specifically with this antibody; the definition of the latter is similarly expressed in terms of antigen; alexin is some property of fresh serum causing the completion, *in vitro*, of some reaction (generally of the antigen-antibody type) which would not be completed in its absence. One may elaborate these definitions with much detail, but one can do very little to clear up their obscurity; these postulated substances simply are what they do, and they have to be defined in terms of each other. Similarly with "stimuli." They are indispensable and highly respectable household words in all treatises on physiology and the kindred sciences, but it is often impossible to define them except in vague terms implying that they are the "something" to which an observed change is due.

Coming now to stimuli acting on the bacterial cell, how do these differ from other agencies which also act on the bacterium? In reply, one must first note that "other agencies" do not necessarily imply different and distinct substances, since a particular substance may initiate more than one kind of change in the cell. With this proviso, it may be said that a stimulus acts on the living cell and the result of the stimulus depends on the cell's own activities, any deviation from the normal (if such there be) being due to a change in the cell's metabolic processes; whereas other agencies, such as antibodies, enzymes, and non-specific toxins, act on some of the chemico-physical constituents of the cell, whether the cell be alive or dead, and produce results which are a direct consequence of this action and not a consequence of the cell's activities as a living organism. Thus substances which are antibodies or toxins are not, in these respects, cell stimulants, though they may also act as stimulants; *e.g.*, growth in immune serum may produce a bacterial variant and the presence of arsenic in a medium may induce the growth of a strain which is more resistant than the normal to this poison.

One naturally endeavours to form some sort of a mental picture which is a little more concrete than the bald statement that a stimulus is what it does.

When a bacterium is growing, there is a constant and systematic succession of changes in the loose attachment of chemical groups to the more complex and stable constituents of the bacterial protein; these changes, being associated with hydrolysis and synthesis (or dehydration), may be regarded as being, in one important aspect, changes in affinity for water on the part of the cell's component elements.

When a bacterium is in the resting stage but still alive, *i.e.*, capable of further growth, this succession of changes is not taking place.

But an alteration in the environment may convert the resting phase into the growing phase, *i.e.*, it may provide a growth stimulus, the explanation being that elements in the new environment produce, in the affinities of the bacterium for water, an unstable condition which leads to successive changes in a special order of sequence. This organised system of instability may be regarded as due to the normal growth stimulus.

But it is also possible for the environment to create a condition of instability which differs from the normal in some respect. Then the environment may act as a stimulant to bacterial variation, owing to a change in the attachment of particular chemical groups to the bacterium, and to a consequent change in the affinities of the bacterium for water.

This crude mental picture is an attempt to visualise and to reconcile the simplicity and the complexity of vital processes—simplicity as regards varying affinities for water, and complexity of the conditions determining these affinities.

TRANSMISSIBLE BACTERIAL AUTOLYSIS.

In a former report on bacterial variation and transmissible autolysis[1] I did not attempt to discuss the existence of "bacteriophage" as a living virus, since it appeared to me that the arguments against this view were so strong as to be practically conclusive. I therefore started with the assumption that "lytic principles" act as stimulants to bacterial cells but are not living organisms. I then proceeded to consider their mode of action. As the phenomena of transmissible autolysis are found only to occur with living and actually growing bacteria, I suggested that the lytic stimulus probably exerts its influence at the critical stage of cellular subdivision. My development of this view may be expressed briefly as follows.

It is desirable first to pay attention to the intimate way in which two essential processes of bacterial life are dependent on each other, *viz.*, the enzyme action which breaks up the food supply into particles suitable for assimilation and the synthesis of these particles to form living protoplasm. One feature of enzymes is that, after forming unstable combination with the material (substrate) undergoing digestion, they become dissociated from this

[1] Ministry of Health. *Reports on Public Health and Medical Subjects*, No. 18, 1923.

union and are left free to attack fresh substrate. But conditions may supervene, particularly in association with living matter, which tend to stabilise (instead of dissociating) the union between enzyme and digested food material, the result being that enzyme action ceases to be progressive and is replaced by a synthesis between substances formerly acting as enzymes and material upon which they acted.

Starting with this idea that the cellular enzymes of bacteria are not merely catalytic agents acting on a substrate, but are also the nucleus upon which bacterial protoplasm is built up, it would seem that, when a bacterium begins to grow, catalytic action upon its environment is at first predominant; then it passes through a critical phase which fixes or stabilises the constituents of bacterial individuality, a phase which is marked by the transition from catalytic action on the raw substrate to synthetic action on the elaborated product. When full development is followed by division, these processes are started afresh. Bacteria breed with that uniformity which is characteristic of species because both catalytic and synthetic action are attributes of the same protoplasmic substances.

Opportunities for variation (within the limits of species characteristics) are provided during the "critical phase" of transition from catalysis to synthesis, and the characters of the new generation will depend on the precise conditions which prevail when this phase is terminated by subdivision.

Subdivision may take place prematurely, before one or both of the segments have acquired sufficient stabilised protoplasm for independent growth. The result will be autolysis of those daughter-cells which lack this stability, and their dissolved products, by stimulating other bacteria to premature division and consequent autolysis, will act as a "lytic principle[1]."

It is of the greatest importance to note that lytic principles are not merely destructive agents causing the autolysis of bacteria; if they were, they would not be transmissible, since the survival of living and growing bacteria is necessary for their continued propagation. They are essentially stimulants to bacterial variation, giving rise to viable as well as to non-viable variants. Moreover, it has been shown, by experiments *in vitro* on successive generations of bacteria which have each been exposed to a lytic influence, that the surviving variants are not all alike. After the first action of the stimulant, some of them are (*a*) more resistant than the normal and some, though just able to survive, are (*b*) more sensitive than the normal. The process is repeated with the descendants of *a* and *b*; the weaker individuals die out and the stronger survive, perhaps including among the latter a few more hardy descendants of what were previously *b* forms. Carried to its conclusion, the process results in the production of a pure strain of highly resistant *a* forms, the other variant (*b*) having died out. Thereupon the pure *a* strain behaves like a normal culture, though more robust than the normal because the tendency to variation

[1] It is also possible that some lytic substance may escape from such cells before they arrive at the final stage of death from autolysis.

has been eliminated; it is no longer lysogenic, because the sensitive variants which, on disintegration, yielded the lytic substance, have disappeared. Perhaps the main interest of these experiments *in vitro* is that they raise the question whether similar stimuli may not be operative in the animal body, leading to the production of "resistant" and "sensitive" variants and thus helping to determine the animal's susceptibility or resistance to bacterial invasion. Is "epidemic virulence" evolved in this way?

The initial stimulus which produces transmissible lytic substance may be derived from many widely different sources, *e.g.*, faecal extracts from healthy or diseased persons or from healthy animals of various species, urine, diluted sewage, extracts of normal organs, peritoneal exudates of guinea-pigs inoculated with some bacterium, mixtures of bacterial culture and leucocytes, and so on. It may emerge "spontaneously" in an old culture; it has been obtained by treating bacteria with immune serum, by allowing the filtrate from a broth culture to act on fresh living culture, and by various other methods, such as shaking bacteria in distilled water or treatment with a small quantity of sublimate.

Hence it is extremely improbable that the stimulus is due to any one special substance. This is in accordance with the general fact that various physiological stimuli which produce the same effect are often of entirely different natures.

When a lytic principle has been obtained and is found to be transmissible, its precise range of action cannot be foretold; it is necessary to work it out by detailed experiment. For example, it may act only on the particular strain of the species from which it was derived, *e.g.*, a strain of *B. coli*, or it may attack the majority of strains of *B. coli* and also other species, such as dysentery and typhoid bacilli. The reason for these differences must, of course, depend on the special characters, chemical and colloidal, of the bacterial surface with which the lytic principle is brought into contact. But what these special characters are is unknown; one has simply to accept the fact that such differences exist. This, again, is in accordance with the general physiological experience that different cells may respond differently to the same stimulus.

OTHER FEATURES OF BACTERIAL VARIATION.

I do not think that transmissible autolysis is a phenomenon *sui generis*, but rather that it is to be associated with other manifestations of stimulation leading to bacterial variation.

This opinion is based on the following assumptions. (1) The influences which initiate and transmit autolytic change are not attributable to any sort of living viruses which prey on the bacteria, but are chemico-physical stimulants acting on the bacterial cell. (2) They are not simply "lytic principles"; besides causing growing bacteria to split off a number of non-viable forms which undergo autolysis, they affect the surviving daughter-cells in other ways.

(3) These modifications are not simply enhanced resistance or sensitiveness to a lytic influence but may affect other characters of bacterial growth. (4) As lytic changes may be initiated, in the same bacteria, by one or other of a great variety of causes, it is not likely that the original stimulus is some distinctive substance possessing chemico-physical properties specially associated with lytic action.

Apart from a tendency to autolysis, bacterial variants, whilst retaining the general characters of their species, may deviate from what is taken to be the normal in such respects as:—appearance of the colonies, morphology of individual bacteria, motility, enzyme action, capacity for growth, antigenic properties, and virulence.

What is likely to be the starting-point of such variations? In the life of an actively growing bacterium, the incidence of what I have termed the "critical phase" in the transition from catalysis to synthesis of food-material may be especially favourable for the disturbing action of a stimulus. When a young culture is growing under favourable conditions, without the production of any variants, it may be presumed that the stabilising influence which promotes synthesis acts exactly at the right time and in the right way. But, under other circumstances, a stimulus may act differently, causing the stabilising influence to operate (*a*) a little too soon, or (*b*) a little too late; and the consequences may be, respectively, (*a*) failure of some of the new generation of bacteria to develop their full biological properties, *e.g.*, loss of some function or of some antigenic constituent, or (*b*) the acquisition—after full development of biological potentialities—of some additional chemical groups which temporarily mask one or more of these potentialities, *e.g.*, disappearance and subsequent return of some biological property. And one might amplify these possible causes of variation by imagining that the intervening stimulus caused the stabilising influence to behave "not quite in the right way," thus producing some deviation from the normal molecular or colloidal arrangements for the synthesis of chemical groups into protein. Under this general view, autolysis is comprised as simply an extreme instance of variations in the daughter-cells, the disorganisation in the cellular mechanism being so great as to be incompatible with continued existence as an organised cell.

In relation to the causes of bacterial variation, probably much significance is to be attached to the fact that they commonly arise "spontaneously," *i.e.*, they arise *in vitro* without the introduction of any extraneous factor into the culture medium. This is frequently observed to be the case whether the variation be slight or profound. It may consist of no more than a slight irregularity in the synthesis of bacterial protoplasm, characterised by one feature only—irregularity in the disappearance and reappearance of a particular antigenic component; a condition, in fact, where it may be difficult or impossible to say which is really the "normal" bacterium and which is the "variant." Further changes supervene when there is opportunity for more prolonged action of the "spontaneous" modifying influences which may be

present *in vitro*. The colonies formed by the variants are distinctive; there is more profound alteration in agglutinability and agglutinogenic capacity; there is less tendency of the variants to revert to the parent form; then the variants, though still viable *in vitro*, are found to be non-viable *in vivo*, *i.e.*, they have lost their virulence; finally, the variant fails to survive even *in vitro* and succumbs to autolysis.

These facts suggest a comparison with the influences which are liable to produce bacterial variants *in vivo*. As the bacterium is placed in an entirely different environment, the influences now operative may be considered to be "extraneous" rather than "spontaneous." Still, when one considers results, there is a broad and very striking resemblance—though not a resemblance in every detail—between variants (including non-viable forms) produced *in vivo* and those produced "spontaneously" *in vitro*. This is not particularly surprising, since different agents, acting as physiological stimuli, may produce identical effects. It does, however, suggest an important point, *viz.*, that the new (animal) environment does not impose on the bacterium entirely new influences of a "foreign" or drastic nature but rather induces the bacterium to "modify itself" by employing very much the same mechanism which was operative in the test-tube.

It is quite probable that, alike in the animal body and in the test-tube, the commonest and most important cause of bacterial variation is neither an alteration in the food supply nor a change in the nature of the bacterial enzymes but some slight change in the environment which modifies the bacterial surface as regards its affinity for water.

THE INFLUENCE OF SYMBIOSIS.

Symbiosis amongst bacteria.

Bacterial growth may be initiated from a single cell, and the culture so obtained may, under suitable conditions, be propagated indefinitely; this is naturally to be expected with unicellular organisms.

But there is considerable evidence that the individual cells are not absolutely independent entities. Bacteria often help each other to grow. When a medium is seeded with a particular strain, growth may fail when the amount of inoculum is very small but succeed when it is larger; or, if the smaller seeding does not simply die out, rapidity and final abundance of growth may be greater when more bacteria are transplanted.

This evidence of co-operation between individuals of the same strain seems best explained by the assumption that each cell forms temporary union, by adsorption on its surface, with some substance secreted by the adjacent cells (or, in some cases, with the autolysate of such cells), and that this substance increases the cell's capacity for assimilating food and accelerates the events leading to its subdivision.

After a time, growth becomes less rapid and finally ceases. These changes

may mean that the food supply is becoming used up or is rendered useless by the accumulation of inhibitory waste products; but there is another influence which must also be considered. The continued adsorption of cellular products may have reached a stage of equilibrium, the successive phases being (1) a stimulus to metabolism and reproduction caused by these products, (2) a slowing down and, finally, (3) an inhibition of these processes, when the cellular products have been adsorbed in excess of the optimum required for stimulation. The suggestion, therefore, is that bacterial multiplication may, to some partial extent, be controlled by a self-regulatory mechanism, irrespective of changes in the nutritive characters of the medium.

If the bacteria in an undoubtedly pure culture (derived from a single cell or a single colony) were all exactly alike, it would suffice to form a quantitative conception of this influence of bacterial secretion upon bacterial growth, amounts increasing up to a maximum, x, being stimulative, and amounts exceeding x being unfavourable to growth. But it is now known that individuals in a pure culture may differ from each other; therefore the qualitative character of this influence has also to be considered.

Changes found on plating out a pure culture, particularly if it is old, may relate to the type of colony, antigenic properties, virulence, viability, or to other characteristics. Though such changes cannot be attributed to one factor alone, the influence of bacterial secretions or disintegration products, this factor cannot, as a rule, be excluded. For example, if some of the variants show the phenomenon of transmissible autolysis, it may be taken that dissolved bacterial substances were the starting-point of this change.

Similar considerations apply to mixed cultures. Species B may promote or retard the growth of species A. The action may be partly indirect, *e.g.*, B may convert the culture medium into one which is more favourable or less favourable for the growth of A; but direct action must also be included as part of the process, *viz.*, modification of A by the adsorption of substances derived from B. And the effect, as before, may be partly quantitative—on the output of new growth, and partly qualitative—on the characters of the new cells.

Leaving aside for the moment the question of distinction between a "stimulant" and a "building-stone," it may be noted that, when bacteria are growing together in pure culture, variants probably arise because the combining affinities of the surface of the bacterial cell for the materials in its environment are not absolutely fixed and immutable. For example, an affinity may be satisfied, or partially satisfied, by union with any one of three different kinds of material, a, b and c, which may all be present in the environment. It may be a matter of chance whether union is actually formed with a, or b, or c; but a bacterium which has united with a may be slightly different from one united with b, or c, and such differences may make a difference in the nature of the subsequent affinities of the bacterium and in the character of the materials which unite with it.

Or, again, in this "rivalry" between a and b, much will depend on the rate of combination; if it is the same for each, there will be a tendency for

the number of bacteria affected by the *a* influence to be about equal to the number affected by *b*, and this may be the starting-point for the differentiation of a culture into two varieties (A and B) each in about equal numbers; if *a* or *b* has the more rapid rate of combination, there will be a tendency for A or B to preponderate.

And there may be another important difference between *a* and *b*; the former may make a much firmer union than the latter with the bacterial surface. Here, again, the ultimate consequence may be some structural difference between the A and the B forms, perhaps a difference in the firmness of the synthetic combinations of assimilated food-material. Hence, perhaps, the one variety may be more resistant to hydrolysis than the other—a type of resistance which may be an attribute of virulence.

In discussing symbiosis as a factor in the production of variants, it must be remembered that this is only a one-sided view. Symbiosis is probably of equal importance in the repression of variants. It is now admitted that individual bacteria in a pure culture are never all exactly alike; and the statement no longer causes any surprise. One is rather inclined to wonder why such differences do not cause more confusion, why, for example, it is possible to obtain practical identity of all important biological properties by growing two subcultures of the same strain under identical conditions. The reason seems to be that, under appropriate conditions and with young and actively growing cultures, symbiosis tends to encourage the propagation of the dominant characteristics and to inhibit the development of the weaker variants.

Symbiosis between bacteria and the animal body.

Enquiries into bacterial infections are generally based on the assumption of antagonism between the bacterium and the animal host. Quite rightly—up to a certain point. But there is a danger of laying too much stress on this pictorial idea of "warfare," of a constant battle, ending in the survival of the fittest, between the parasite and the invaded body. It must not be forgotten that the animal body may not always be aware that bacteria should be treated as "enemies" but may, on the contrary, actually facilitate their growth.

In relation to bacterial growth the animal body may be considered as the source of two distinct kinds of material:—(*a*) stimuli (favourable or unfavourable) to such growth and (*b*) the supply of food as raw material which is convertible into bacterial protein.

The animal's stimulus to growth may be regarded as particularly favourable in the case of certain strictly parasitic micro-organisms. The leprosy bacillus, for example, seems unable to grow without the stimulus of something derived from the living cells of certain species of animals, something which there is no reason to postulate as being a special constituent of the protoplasm of the leprosy bacillus. Perhaps a similar animal stimulus is required for the growth of some of the invisible viruses which have not been cultivated *in vitro*.

Moreover, many of the ordinary parasitic bacteria which are capable of survival and growth in the saprophytic condition (their own bacterial secretions being a sufficient stimulus) often grow better in the body of a susceptible animal (*i.e.*, when aided by the stimulus derived from animal cells), *e.g.*, plague and anthrax bacilli and other organisms which produce an intense bacteriaemia in their host. Here, again, there seems to be a distinction between the bacterium's own growth impulse and the stimulus to growth which is derived from the host.

A distinction between the two kinds of stimuli may also be observed in cases where the one derived from the animal is relatively unfavourable to the bacteria. The tissues of cattle, for example, are relatively unfavourable to the growth of the "human" type of tubercle bacillus; but this inhibitory action may be overcome by artificial means, *e.g.*, by intravenous inoculation of large doses of the bacilli; and it is known that attempts to immunise cattle with living "human" tubercle bacilli are unsafe, since they may survive and multiply for lengthy periods and may be excreted in the milk. Similarly it has been shown experimentally that avian tubercle bacilli can be made to grow profusely in mammals. In these exceptional cases where natural resistance has been overcome, the bacteria have been able to supply their own stimulus to growth in excess of the adverse stimulus attributable to the fluids of the animal body.

But it is not always the case that the body fluids of a particular animal consistently act on the same type of bacterium in the same way; they may be favourable to its growth on one occasion and unfavourable on another. For example, a small dose of culture of tubercle bacilli is inoculated into a guinea-pig and produces a local lesion which leads to slowly progressive and eventually fatal disease; when the disease is established but before its termination, a small dose of the same culture is inoculated into a site of the body which has not yet become infected; this second site is found to be resistant and a tuberculous local lesion is not produced. Why this difference between the effects of the first and the second dose? As the bacilli were identical to begin with, it must be due to some difference in the action of the body fluids on the bacteria. On the occasion of the first inoculation, these fluids enabled the bacilli to acquire and retain invasive capacities; on the occasion of the second, the body fluids prevented the acquisition of invasive capacities though not interfering with the retention and transmission of such capacities which had already been acquired.

The feature of special importance is that this change in the properties of the animal fluids is the result of infection.

Comparison of bacterial with animal cells.

At this point it is interesting to consider possible resemblances between bacterial and animal cells in respect of some of the conditions which influence their growth.

(1) As regards symbiosis there is, of course, no comparison between the elaborate regulatory mechanism of the animal body and the growth of bacteria. But a suitable parallel may be found in the cultivation of tissues *in vitro*. Here it has often been noticed that growth takes place when the tissue cells are numerous and in close proximity to each other but fails in cells which are isolated.

(2) What causes the embryonic cell to change into the adult type? It has been suggested in the case of bacteria that adsorption of more than the optimum amount of bacterial secretion may help to account for the slowing down of bacterial growth; possibly, when embryonic cells have accumulated up to a certain amount, there is a similar excess of cellular secretion, which now has a retarding effect on growth and leads to some qualitative change in the new generations of cells. Observations on tissue cultures in a medium containing plasma derived from animals of different ages apparently indicate that, after the embryonic stage is terminated, the plasma may have a restraining influence on growth in the living body, and that this influence increases with the age of the animal.

(3) During the processes of recovery after trauma and in inflammatory reactions, there is again reversion to a more actively growing, though not actually embryonic, type of cell. Here the new cellular stimulus is probably attributable, at least in part, to adsorption of material from disintegrated cells, since it is known experimentally that growth may be stimulated by extracts of leucocytes or of various types of tissue cells. Recovery is accompanied by reversion to the normal adult type. These processes may present some analogy to the changes produced in a bacterial culture which, after becoming stabilised on artificial media, is transferred to a new environment, such as the body of a susceptible animal, which provides fresh stimuli to the bacterial cells. The effect may be a reversion to some of the characters which have been temporarily lost on the artificial medium; on return to the old environment, these characters may again disappear.

(4) Is there, as some authors have suggested, anything comparable between transmissible bacterial autolysis and the autolysis which is a constant feature of malignant growths? There are certainly very obvious differences. With bacteria the two most striking features are that the lytic principle (1) is usually propagated through successive cultures of normal cells, and (2) makes normal cells become abnormal, *i.e.*, lysogenic. In malignant disease, (1) autolysis is transmitted through abnormal (malignant) cells; and (2) the lytic principle does not convert normal into abnormal cells; it may stimulate the growth of normal adult tissue *in vitro*, but it does not cause such tissue to assume the malignant type. Still, it is just possible to find a parallel with respect to one feature which is observed as the result of continued exposure of bacteria to a lytic agent through many generations. I do not refer to the end result, which, in a successful experiment, is the production of a pure culture insusceptible to lytic action and non-lysogenic; I am thinking of that inter-

mediate stage where the bacteria are more resistant than the normal to lytic action but are still partially susceptible to lysis and still retain lysogenic power. This stage may present some resemblance to what one may imagine to be the development and propagation of the malignant cell. Owing to some stimulant which is slightly different from the normal, a normal cell divides into two daughter-cells, of which one (a) is normal and the other (b) non-viable. It may accidentally happen that the autolysed remains of b stimulate a to divide into a^1 (apparently normal and viable) and b^1 (non-viable). A similar action may be repeated in succession by b^1, b^2, b^3 etc. on the viable descendants of a^1. This chapter of accidents may come to an end without anything further happening; or it may terminate because the descendants of a^1 become more sensitive than the normal to lytic action and all fail to survive. Another possibility is that some of these descendants become more than normally resistant to lysis, though still partially susceptible and still lysogenic. Such cells would possess features specially associated with malignancy; in virtue of their lytic principle, they would exert and transmit to their offspring a powerful stimulus to premature proliferation and, in virtue of their relatively high degree of resistance to autolysis, the cells which survived would exceed, from generation to generation, the number which perished. Thus there would be a similarity between this (the final) phase of transmissible autolysis amongst malignant cells and that intermediate stage of the same process amongst bacteria to which I have called attention. The resemblance is masked because there is usually a marked difference in the end results. With bacteria, in the ordinary test for lytic action, autolysis generally takes place very rapidly and the lytic phenomena are then more conspicuous than the stimulus to new growth; with malignant cells in the animal body, autolysis is slower and cellular proliferation is the predominant feature.

(5) When either bacteria or animal cells have acquired virulence, *i.e.*, capacity for invading the tissues of the animal body, it is found in both cases that this capacity is inherent in the cells themselves and, given a favourable environment, may be transmitted *ad infinitum* to successive generations of cells. The interesting point is that, in order to retain this capacity, the bacteria must remain normal, *i.e.*, must be fully equipped with their ordinary mechanism for metabolism, whereas the animal cells must remain abnormal, *i.e.*, must retain a degenerate form of metabolism characterised by imperfect assimilation of food and premature subdivision.

(6) The same stimulus may be favourable to one kind of cell (promoting vigorous growth) and unfavourable to another (causing degeneration and autolysis). Thus the stimulant action of the animal body helps one species of bacterium to grow (natural susceptibility) and inhibits the growth of another (natural immunity). Similarly, treatment of an animal by various means (*e.g.*, inoculation of defibrinated blood or olive oil or application of x rays) may cause the animal to produce a stimulus which increases the growth of some cells (*e.g.*, lymphocytes) and inhibits others (*e.g.*, a cancer

graft). It is to be noted that an overdose of the agent, such as x rays or olive oil, does not cause the animal to produce a stimulus but is merely noxious and lowers the animal's general powers of resistance.

(7) For the distinction between (*a*) stimuli to bacterial growth and (*b*) material utilised by the bacteria as food a parallel may perhaps be found in observations on the cultivation of animal tissues *in vitro*. Drew, for example[1], has shown that for the growth of adult tissue two different substances are requisite, (*a*) one which acts as a stimulus and (*b*) one which furnishes the nutritive material. Extract of embryonic tissue acts as (*b*) and he has not been able to find any substitute for this. When the cells are implanted on the ordinary medium containing (*b*), there is a lag of many days before growth commences. During this period of delay some of the implanted cells undergo autolysis and it is the accumulation of this autolysate which acts as (*a*) the stimulus to growth. The lag may be abolished by planting the cells on a medium which already contains (*a*) in the form of an artificially prepared autolysate of adult tissue. When growth has started, it may be continued indefinitely by transplanting to media containing (*b*) alone; if, however, the transplants are made on media to which artificially prepared (*a*) has also been added, "early degeneration with ultimate death of the whole culture takes place." Thus there is a sharp distinction between the nutritive properties of (*b*) and the stimulative properties of (*a*); and the stimulus of (*a*) may be either favourable to the initiation of growth or, if cumulative, unfavourable to continued propagation.

IMMUNITY.

Natural immunity and natural susceptibility.

To begin with stimuli associated with natural immunity. It is reasonable to suppose that the property which confers immunity is associated—though not identical—with the property conferring those species characteristics which are demonstrable, serologically, by the precipitin test. One must, I take it, explain the individuality of an animal's serum by assuming that the different cells of the body split off certain by-products which find their way into the plasma and there form certain chemical and colloidal combinations; these combinations, though unstable and constantly changing *in vivo*, are characteristic of the species and become stabilised in the serum in certain characteristic ways. In the naturally immune animal some of these circulating substances are chemically equipped in such a way that they (1) are adsorbed by the bacterial surface and, thereupon (2) split off, or otherwise interfere with, components which are linked to the bacterial protoplasm and are necessary for its vitality, *i.e.*, they prevent the progress of the dehydration synthesis or, possibly, combine with a bacterial enzyme in such a way as to render it inert.

But the association, amongst animals, between natural immunity towards

[1] *Lancet*, I. p. 833, 1923.

particular bacteria and serological blood-relationship does not amount to a strict correlation of the two properties. Closely related animal species sometimes differ sharply in immunological respects. Moreover, the combining properties which confer natural immunity in the circulating plasma are generally labile and disappear in the serum, whilst the serum, though not antibacterial, retains its species characteristics. Hence it has to be admitted that these special combining properties seem to be a more or less accidental result of the animal's biological individuality.

Similarly, natural susceptibility can only be described, in the present imperfect state of knowledge, as being "accidental"; it means that those elements circulating in the plasma which are the expression of the animal's biological individuality do not possess the same sort of combining affinities for substances attached to the bacterial protoplasm; and no explanation for this circumstance is available.

It may seem disconcerting to confess that, in the present lack of knowledge, the property which makes one animal naturally immune and another naturally susceptible to a particular bacterium is simply an "accident" or "incident" which cannot be explained. Recognition of the difficulty leads, however, to some reflections as to the nature of the action exercised by the immune or the susceptible animal on the bacterium. The very fact that the character of the influence, in relation to different animal species, appears so irregular and haphazard suggests an important group of physiological actions in which this lack of demonstrable correlation between cause and effect is a conspicuous feature. I refer to the phenomena of cellular stimulation and cellular inhibition.

Perhaps I ought to elaborate this point a little more. There is no evidence that the naturally immune animal does not possess a practically unlimited supply of food material which is suitable for the nutrition of every known parasitic bacterium; in this respect there is no difference between the immune and the susceptible animal (*e.g.*, the difference between the fowl's and the rabbit's susceptibility to anthrax is not due to differences between fowl protein and rabbit protein). It has not been found possible, either by chemical or biological analysis, to obtain from the naturally immune animal some substance which has a specifically toxic or lytic action on the bacterium towards which it is immune; in fact the search for such specific substances has been abandoned as hopeless.

Still, there must be some unknown cause which would account for the difference between natural immunity and susceptibility. It seems to me that it probably depends on the extreme sensibility of the bacterial surface to very minute differences in the composition of the animal's plasma. These differences, I have suggested, are comparable to differences in physiological stimuli, being favourable to the cell's metabolism in one instance and inhibitory in another. And for the word "stimulus" one might substitute the rather more definite conception of an alteration in the cell's permeability, the idea being

that the immune animal modifies the selective ingress and egress of fluids in some way which is incompatible with the cell's internal processes of hydrolysis and dehydration, while the susceptible animal does not cause this interference in the normal permeability but may make the conditions of permeability actually more favourable to bacterial growth.

An unfavourable stimulus, as distinct from a toxin, may be thought of as a stimulus to continued catalytic action, unaccompanied by synthesis and resulting in bacterial death from autolysis. This may explain death of the anthrax bacillus, for example, in a naturally immune animal. Under exceptional circumstances, where the bacillus is excluded from the free action of the body fluids of such an animal, this stimulus is not operative and the bacilli may multiply, a fact which is additional evidence that inhibition of growth is not due to lack of suitable nutritive material.

The above considerations suggest that the problems of natural immunity and natural susceptibility do not necessarily await solution in terms of antigens and antibodies.

Acquired active immunity.

I propose first to consider certain features of immunity to the anthrax bacillus.

It is an old idea, and was suggested by Preisz in his work[1] on anthrax, that acquired immunity is in some way a reinforcement of natural immunity and that all animals, from the naturally immune to the most susceptible, possess some degree of natural resistance.

This view may be elaborated in various ways. To develop it in line with my suggestions in the preceding section, one would assume that in the circulation of the naturally immune animal there are substances which have combining affinities for the surface of the anthrax bacillus and alter its relations to its environment in such a way as to render it non-viable. These substances are highly labile; they disappear from the animal's serum and cannot be recovered from it; they cannot be "reactivated" by introducing the serum into the body of a susceptible animal.

The normal elements circulating in the plasma of a susceptible animal do not possess combining affinities which are strong enough to make firm union with the surface of the anthrax bacillus. When such an animal is actively immunised, the disintegrated protoplasm of the anthrax bacillus interacts with these normal elements circulating in the plasma in such a way as to increase their affinities for the surface of the anthrax bacillus. Perhaps, as I suggested previously[2], the interaction to which this modification of the plasma is due takes place at the surface of capillary endothelium, and is brought about by filtration of the plasma through endothelial cells which have adsorbed disintegrated bacterial protein.

[1] This work is worth consulting in its original form (*Centralbl. f. Bakt.* Orig. XLIX. p. 341, 1909), as it contains many interesting and valuable observations.

[2] *Journ. Hyg.* XXII. p. 355, 1924.

In the first stage of active immunity, these modified elements of the animal body are, as in natural immunity, highly labile and disappear from the serum; the animal acquires immunity, though the serum has no antibacterial properties. Substantial active immunity may be established without any advance beyond this stage.

Attention has often been called to this fact, the production of active immunity to anthrax without the presence of demonstrable antibodies in the animal's serum. When discussing antibodies, I suggested[1] that phenomena of this nature may perhaps be explained by the existence in the living body of effective antibodies which, owing to their labile nature, disappear from the serum. In the present connection I wish to call attention to another aspect of this kind of immunity. What is the mode of action of these labile substances *in vivo*?

There is a difference between (1) disintegrated bacterial protein and (2) the surface of the living bacterial cell. In active immunisation, the change produced in the body is due to the stimulus of (1), but the new substances which are produced act primarily on (2). Their reaction with (2) may be (*a*) simply a union with the bacterial protein, analogous to the first stage of an agglutination reaction *in vitro*, or it may be (*b*) a modification in the chemico-physical condition of (2), due to loose contact followed by prompt dissociation and therefore in the nature of a stimulus which is not associated with the agglutinin type of union; and, theoretically, the reaction might be (*c*) a relatively firm union of the agglutinin type accompanied by a chemico-physical modification. The main point of practical interest is that *b* may occur without *a* (antibacterial action without agglutinins), just as *a* may occur without *b* (adsorption of agglutinin not incompatible with the vital properties of the bacterium). In this connection, it seems to me, the probable existence of this *b* type of reaction is more important than the question whether it is due to something which, if it could be obtained *in vitro*, ought to be called an "antibody."

It does not follow that active immunity in other bacterial infections is due to the same principles as in anthrax. In other cases, serological antibodies may be demonstrable and may be of considerable importance in the mechanism of such active immunity; still, they do not suffice to explain it completely and here, also, one of the factors may be a stimulant action on the bacterial surface as distinct from an adsorption of antibody by bacterial protein.

Passive immunity.

To start again with anthrax, if an animal which is already actively immunised receives repeated large doses of anthrax bacilli, fresh substances are found in the circulation. These, it may be assumed, are due to interaction between disintegrated bacterial protein and elements of animal protein. They

[2] *Journ. Hyg.* XXII. p. 355, 1924.

do not persist indefinitely *in vivo*, but, if the animal is bled before they have disappeared, they form highly stable constituents of the serum. When such serum is introduced into a susceptible animal infected with anthrax, it confers passive immunity; as it is not antibacterial *in vitro*, it is inferred that it probably acts *in vivo* not by direct combination with the bacilli but by "activating" the animal's plasma, *i.e.*, by breaking up some constituents of the plasma in a new way, so that they acquire new affinities for the bacterial surface, the result being that the animal's "growth stimulus," originally favourable for the anthrax bacillus, has now become unfavourable. This idea of the mechanism of passive immunisation in anthrax infections may be of importance.

The problems of anthrax immunisation have always been of special attraction to bacteriologists, because it is thought that their solution would throw light on general principles of immunology. They have not yet been solved. What I have written above is only one way of attempting to explain some of the difficulties or, more correctly, merely an attempt to restate an unsolved problem in terms which may, in some degree, approximate to its real nature. Is it reasonable to suppose that in other infections, as well as in anthrax, the same kind of mechanism is operative in natural, active, and passive immunity?

In the case of anthrax, the idea that both active and passive immunity are simply a reinforcement of natural resistance (already inherent in the susceptible animal) has generally been arrived at by the method of exclusion; it has not been possible to account for the facts by any sort of recognisable antibody to the bacilli and therefore it has seemed necessary to postulate indirect action, *viz.*, action of something (? antibody) on the tissues or fluids of the body, in consequence of which the latter behave antagonistically towards the anthrax bacillus. I have attempted to regard this inherent property of the animal body not as inherent "resistance" but as a cellular stimulant which may vary in its action on the bacillus, being sometimes favourable to its growth, sometimes (perhaps) indifferent to it, and sometimes adverse; then the acquired immunity (active or passive) of a susceptible animal would mean the change of its stimulant action from one which was favourable (or indifferent) to one which is adverse.

Would it be justifiable to attempt a similar explanation in the case of other bacteria? Not when acquired immunity can be explained in other and simpler ways, as by the direct action of a demonstrable antibody on the bacterium. Unfortunately, this cannot always be done, sometimes because there is active immunity without known antibodies, and sometimes because antibodies, though demonstrable, will not confer passive immunity. In such cases it may not be inappropriate to raise questions as to the significance of cellular stimuli.

Cell stimuli in relation to other factors.

Leucocytic enzymes.

It has long been recognised that phagocytic cells, particularly leucocytes, may protect the animal body against invading bacteria in other ways than by ingesting them. Leucocytes contain digestive enzymes, which are released when the cells undergo death and autolysis; and these liberated substances form part of the non-specific defensive mechanism in both natural and acquired immunity.

I may refer briefly to some of the older work on this subject.

Pettersson has been an important member of the school which regarded the leucocytes as a source of bacteriolysins. These substances, according to Pettersson[1], were distinct from the serum bacteriolysins and differed from the latter in being more highly resistant to heat. Though they might act within the leucocyte after phagocytosis had occurred, they were not secreted from the living, uninjured leucocyte; hence he called them endolysins. He obtained them as extracts by repeated freezing and thawing of the leucocytes, and found that the leucocytes of different animals differed in their amounts of soluble bactericidal substances for particular bacteria.

Extracts from the leucocytes of an immunised animal were not very markedly more bactericidal than extracts from the leucocytes of a normal animal of the same species. This he considered natural, because leucocytes did not live for long and at the end of an animal's immunisation they were not likely to be very different in composition from what they were at the beginning.

The action of his leucocytic extracts was somewhat slow. He noted this particularly with pneumococci. At the end of 6 hours' incubation with the extract there was often a definite increase in the number of cocci; but this was followed by a rapid decline, which was well marked when plates were made 18 hours after the commencement of incubation.

In the course of further experiments, Pettersson also found some evidence *in vivo* that the injection of fresh leucocytes might afford some amount of protection against bacterial infection. The action of leucocytic extracts was weaker and more uncertain; but the addition of such extract to fresh leucocytes increased the protective action of the latter.

Important differences have frequently been noted between *in vitro* and *in vivo* experiments with leucocytes and leucocytic extracts. Weil and Nunokawa, for example[2], produced large accumulations of leucocytes in guinea-pigs by intraperitoneal inoculation with broth; 18 hours after this injection, they introduced into the peritoneal cavity a small number of anthrax bacilli (in some cases not more than 200) obtained from the exudate of an anthrax-infected guinea-pig. Since rapidly fatal infection ensued, the leucocytes were evidently unable to exercise any protection. On the other hand, they found that guinea-pigs' leucocytes contained substances which were markedly bactericidal *in vitro*, not merely against bacilli from cultures or against strains known to be avirulent but, in an equal degree, against the capsulated forms of highly virulent strains taken fresh from the body of an infected animal.

In order to exclude the possibility of phagocytosis or the vital secretion of bactericidal material, they used in their first experiments the extract and deposit of leucocytes which had been killed by alternate freezing and thawing twice repeated. Not only was this material potent against virulent strains, but no increase of resistance could be developed

[1] *Centralbl. f. Bakt.* Orig. XLII, p. 56 (1906) and XLV. pp. 160 and 235 (1908).

[2] *Centralbl. f. Bakt.* Orig. LIV. p. 262, 1910.

in such strains by repeated passage through susceptible animals (guinea-pigs or mice). They then compared these results with the action, *in vitro*, of living leucocytes. Here again they found a definite bactericidal action against virulent, encapsuled anthrax bacilli, although in half out of the eight experiments recorded the action of the frozen leucocytes was more or less definitely stronger.

As there was no phagocytosis, the nature of this bactericidal action remained to be explained. They made three preparations: (1) their usual suspension of living leucocytes in serum; a similar suspension was allowed to stand for 3½ hours; it was then centrifugalised and (2) the supernatant fluid was used; (3) the deposit of leucocytes was also used, after the addition of fresh serum. Eight samples of virulent "animal" bacilli were tested and uniform results were obtained. (1) and (3) were equally, and strongly, bactericidal; (2) showed little or no evidence of bactericidal action. Evidently, therefore, the leucocytes did not spontaneously excrete bactericidal substance into the surrounding fluid.

The authors concluded that anthrax bacilli, as they had previously found to be the case with the bacilli of swine erysipelas, had an affinity for the bactericidal substance within the leucocytes and removed it from these cells; then the bacilli were killed by "aphagocytic action."

This affinity was a property of several varieties of bacteria. They showed that killed bacteria of three sorts, typhoid, cholera, and "animalised" anthrax, on incubation with leucocytic extract for 1 hour at 37° C., were equally efficacious in destroying the capacity of the extract to kill anthrax bacilli. In this property of the bacteria there was no evidence of specificity; at all events bactericidal activity for a particular bacillus could be removed in a non-specific way. They also obtained evidence that dead bacteria had a similar, non-specific property of inhibiting the bactericidal action of living leucocytes.

In dealing with the bactericidal properties of leucocytic extracts in relation to their aggressin theory, Bail and Weil[1] observed that such extracts were highly destructive for "animal" anthrax bacilli, even more so than for culture bacilli. Moreover, they found that, when leucocytes were suspended in pure aggressin and frozen, this extract was highly bactericidal for animal bacilli; in fact, when the leucocytic endolysins (Pettersson) were once set free, the strongest concentration of an antibacteriolytic exudate could not inhibit their action.

They regarded the aggressin as an abnormal secretion elaborated by the anthrax bacillus during its growth in the animal body and not produced during growth under ordinary saprophytic conditions. This aggressin acted directly on the leucocytes, not by fixing bactericidal material given off by the cells but by preventing the leucocytes from releasing this material. Thus, when leucocytes were treated with anthrax aggressin and then centrifugalised and washed, they were less bactericidal than normal leucocytes or than leucocytes treated with an exudate not produced by anthrax bacilli. But when the cells which had been treated with anthrax exudate were frozen and thawed, it was found that the bactericidal property of the extract was equal to that obtained from normal cells. The paralysis of leucocytes by aggressin was specific, *e.g.*, cells treated by anthrax aggressin were still active for *B. subtilis*.

I think the above extracts, taken from a very voluminous literature, will suffice to show that the older writers attached a high degree of importance to the direct lytic action of leucocytic enzymes upon bacterial protein. The subject possesses an equal degree of importance at the present day, notwithstanding the changes which have taken place in ideas about immunological reactions.

To correlate the older ideas with the subject of the present article, one

[1] *Arch. f. Hyg.* LXXIII. p. 218, 1911.

must draw a distinction between (1) the action of leucocytic material as a stimulus to the vital properties of a cell (*e.g.*, properties concerned with capacity to perform those dehydration syntheses which are necessary for growth) and (2) its action as an enzyme upon the properties which living and dead protein possess in common. The older work was occupied with (2), though one may perhaps discover, in the light of recent knowledge, that it contains some links connecting it with (1).

The latent period in the action of Pettersson's leucocytic "endolysin" may possibly be attributable to the time required for the leucocytic extract to develop its properties as a stimulus to bacterial autolysis; and the partial protection which he found was afforded by the inoculation of leucocytes may also be associated with a "lytic stimulus."

The work of Weil and Nunokawa may be taken as indicating that the material which acts as a stimulus to the formation of lytic substance may be adsorbed by dead bacteria in a non-specific manner.

The attempt of Bail and Weil to reconcile their aggressin theory with the bactericidal properties of leucocytic extracts seems to me to be confusing and unsuccessful; their observations on the specific action of "aggressin" upon leucocytes may perhaps mean that the bacterial extract, present in their "aggressin," adsorbed lytic substances in a selective manner.

These are a few of the examples in which it may be found that the older writers called attention, perhaps unconsciously, to the importance of the fact that, when the body is invaded by bacteria, it has to deal with living protoplasm and not merely with foreign protein.

Interest in leucocytes as a source of cell stimuli is not confined to bacteriologists. One learns from recent work on the cultivation of tissues *in vitro* that, under certain experimental conditions, material derived from leucocytes appears to stimulate growth, and it also appears probable that such material is important, *in vivo*, as a stimulant to growth in processes of inflammation and the repair of tissues.

Perhaps these findings may be compared with the capacity of leucocytes to initiate transmissible bacterial autolysis, a capacity which, in my view, is in the nature of a bacterial stimulant. In this connection Bordet's opinion is worth quoting. Referring to his observations on intestinal bacteria and the phenomena of autolysis, he says[1]:—"External influences such as that of a leucocytic exudate modify the bacterium, inducing the latter to elaborate a lytic substance capable of diffusing itself and bringing about the same autolytic phenomenon through successive cultures."

It is thus possible that leucocytic material is equally a stimulus to cellular division with bacteria as with tissue cells, the difference being that, in the latter case, the new cells are presumably as normal as their ancestors, whereas, in the former, the majority of them are non-viable. Thus the same leucocytic material may be a destructive agent when it stimulates premature subdivision,

[1] *Brit. Med. Journ.* II. p. 296, 1922.

as with bacteria, and a helpful agent when it stimulates the normal growth of animal cells either *in vitro* or *in vivo*.

It must, of course, be remembered that leucocytes are by no means the only cells in the body from which stimuli to growth and to autolytic change can be derived.

Doubtful enzymes.

It is always a satisfaction to be able to deal with something which is more or less definitely concrete, something which can be put into a test-tube and bear a name descriptive of its essential property. This has been the case, at least to some considerable extent, with leucocytic enzymes.

Now I come to some more obscure factors about which I desire to raise the question:—May not the true explanation of some doubtful immunological principles be ultimately found in terms of enzymes rather than, as I have suggested above, in terms of cell stimuli?

There is the view that natural immunity against particular bacteria is due to normal secretions of the animal body which act as enzymes destructive to the bacteria, and that the partial resistance of the susceptible animal is due to the same enzymes, which are present, though not in sufficient concentration to be completely effective. Then acquired immunity (active or passive), in the obscure cases where it cannot be explained by antibodies, would be attributed to a stimulus (of bacterial protein or of immune serum) which causes the body to secrete these enzymes in greater quantity.

These ideas would coincide, in the main, with Preisz's way of explaining anthrax immunity. He came to the conclusion, as I have already mentioned (p. 331), that all normal animals, even the most susceptible, possessed in their tissue juices material, probably of leucocytic origin, which was antagonistic to the anthrax bacillus. But this material differed in quantity and concentration in different animal species. If there was enough to kill the bacilli promptly, the animal was immune; if there was less than this amount, some of the bacilli remained alive until they formed capsules; with this protective envelope, they were more resistant against the animal's bactericidal substances and produced a general infection. The essential property of an immune serum, in his opinion, was simply to stimulate in the animal body the production in greater quantity of those bactericidal substances which all normal animals possessed in greater or less degree.

He arrived at these opinions by the method of exclusion, first showing that there were no recognisable antibodies which would explain anthrax immunity. I need not recapitulate here his evidence upon these points, but I think it is interesting to describe his way of investigating the question whether immune serum had the property of increasing the production of bactericidal substances in the animal body.

For this purpose, he inoculated mice subcutaneously on three successive days with 0·5 c.c. of immune serum and, after the last injection, introduced a silk thread under the skin. After the threads had remained there for 24 hours, he soaked them in a broth culture

of anthrax and then inserted them into fresh mice. Control threads were kept for 24 hours in the subcutaneous tissue of normal mice and were then infected with culture and inserted into fresh mice. No difference was found microscopically between the cellular contents of the immune serum threads and the control threads. The controls produced fatal anthrax in the usual way. The mice which had received the threads from the immunised animals either survived completely or lived considerably longer than the controls.

What was the reason? Fresh threads which had been soaked in either immune or normal serum and then dipped into the culture were fully virulent; so he could exclude the possibility that the threads from the immunised mice had absorbed an effective quantity of the immune serum which those animals had received. He also showed that the blood serum of passively immunised mice did not possess bactericidal properties. Anthrax bacilli grew in it freely, and a thread, which had been soaked in it for 24 hours and then infected, was fully virulent.

Preisz completed this series of experiments with some tests *in vitro*. Threads which had been kept in the tissues of passively immunised mice were broken up and incubated in a broth suspension of young anthrax culture. In some of these tests the bacilli were killed, though in others no bactericidal effect was demonstrable. As the contents of the threads (before they were infected) consisted mainly of leucocytes, he thought that their bactericidal action might be explained on the hypothesis that these leucocytes had been stimulated by the immune serum to excrete bactericidal substances.

His general conclusion, as I have already indicated, was that the essential function of anti-anthrax serum in the animal body was to stimulate increased production of normal antibacterial substances.

I am not prepared to disagree with this line of thought. There are plenty of mysterious enzymes in the animal body and many of them may be antibacterial, though it is not necessary to suppose that those which possess this property are all derived from leucocytes. In these doubtful questions it is impossible to assert that a particular influence is or is not due to an enzyme as distinct from what I have called a "stimulus." I think the possibility of the latter kind of influence ought to be borne in mind, but I have no desire to claim for it an exaggerated importance.

If Preisz's experiments with silk threads had been done at the present day, they might perhaps have suggested to him that the process was similar to that described by Bordet in the initiation of a transmissible bacterial autolysis by means of leucocytic exudates. It is tempting to think that the threads taken from the immunised animals contained lytic substances, though it is not clear whether the leucocytes adherent to the threads or some product of interaction between serum and leucocytes provided the condition necessary for stimulating a bacterial autolysis.

Will enzymes explain, or partially explain, another obscure factor? I refer to certain experimental results which Morgenroth and Abraham[1] have attributed to what they call "depression" immunity. Working on antistreptococcal immunity, which they were unable to explain as being entirely due to bacteriotropic action, they made some observations on mice which are suggestive, though one would like to see them corroborated by a larger series of experiments.

The mice were inoculated intraperitoneally with 0·5 c.c. of undiluted antistreptococcal serum, a dose which was found to confer a considerable amount of protection. Three hours

[1] *Zeitschr. f. Hyg. u. Infektionskrankh.*, C., p. 323, 1923.

later, they received an intravenous injection of streptococcus culture in doses which were all rapidly fatal for the controls.

The interesting feature of the experiments was the repeated fluctuations in the bacteriaemic condition. At a period of from 2 to 5 days from the initial bacteriaemia, the cocci would almost, or entirely, disappear from the blood stream; on about the 5th or 6th day, there would again be well marked bacteriaemia, persisting for about a day and then again disappearing; on the 8th day, three of the animals showed a third bacteriaemic phase, which declined on the 9th day; and in one case a fourth bacteriaemic rise was found on the 12th day.

It was also noted that the original haemolytic and highly virulent streptococci became rapidly attenuated and that sometimes there was a reversion to the virulent type in the second bacteriaemic phase.

As the authors point out, direct action of the immune serum on the cocci (after the manner of an ordinary antibody) would not suffice to explain these data. In their view, the function of the serum is to retard the invasive properties of the cocci for a brief period, during which the animal is enabled to bring its own defensive mechanism into play. They regard the first disappearance of the bacteriaemic phase as a manifestation of a special kind of active immunity (*viz.*, "depression" immunity), superimposed upon the influence of the serum. The second bacteriaemic rise coincides with the disappearance of the primary action of the serum and, if no other factors were operative, would speedily be followed by the death of the animal. But what actually happens is a regular and prompt decline in the bacteriaemia, due to a new manifestation of active immunity. This condition is not attributable to a new formation of ordinary antibodies, because, if it were, it would persist. On the contrary, it is replaced on the following day by a renewed bacteriaemia, which may again disappear and afterwards reappear. All these periodic oscillations of resistive capacity they regard as characteristic of "depression" immunity.

Thus, in the authors' opinion, "depression" immunity and attenuation of virulence are factors of essential importance and must supplement the conception that immunity is due to phagocytosis promoted by the action of a bacteriotropic antibody.

I think it may be accepted that the data provided by Morgenroth and Abraham cannot be explained in terms of Ehrlich's antibodies. But the significance, in these experiments, of what they call "depression" immunity is an open question.

I do not think it has been proved that the leucocytes have nothing to do with the observed states of "depression." Even if it be conceded that immunity is not satisfactorily explained by phagocytosis of cocci which have been sensitised by a bacteriotropin, there are still other possible functions of leucocytes to consider.

One cannot afford to ignore the old work on the liberation of antibacterial enzymes by disintegrated leucocytes. Is it unreasonable to postulate that, in streptococcal infections such as these, this liberation may occur in successive waves, with a quiescent interval between each of them? On this view, there would be phagocytosis of sensitised or partially sensitised cocci during the first phase of bacteriaemia and many leucocytes would be killed and disintegrated during this process; the enzymes liberated from them would promptly reduce the bacteriaemia; when these enzymes were exhausted, the cocci would reappear; there would again be phagocytosis followed by release of enzymes from broken up leucocytes; and so the process might continue,

with a reproduction of the fluctuations said to be characteristic of "depression" immunity.

Thus, it seems to me, enzyme action might go a long way towards explaining one of the striking features of these experiments.

The other feature to which Morgenroth and Abraham call special attention —attenuation of the streptococci—I should be inclined to explain as due to a stimulant action. One knows that material from disintegrated leucocytes may initiate a transmissible bacterial autolysis; it may also exert a less profound action, causing the splitting off of variants which are still viable but attenuated. And oscillations in this attenuating influence may perhaps be correlated with fluctuations in the output of the leucocytic material.

But is that all the explanation, or have I been attributing too much to the leucocytes? These are not the only cells in the animal body with which bacteria are concerned; the products and secretions of other cellular constituents may also have an important influence on bacterial growth.

And in other infections where resistance to reinfection is notable, as in syphilis and tuberculosis, it seems quite impossible to attribute the observed phenomena to the activities of leucocytes or any other special type of cell. The same remark probably applies to the grafting of malignant disease, where a similar resistance to reinfection has been observed. The condition, in each case, may be thought to be due to some systemic change affecting the animal organism as a whole.

The operation of some sort of systemic change seems also to be indicated in the earlier work by Morgenroth and his colleagues on "depression" immunity which I have discussed in a previous report[1].

They observed that mice injected with streptococci of low virulence ("green") were more resistant than normal mice to infection with highly virulent haemolytic streptococci. The results seemed to agree, whether the inoculations were made subcutaneously, intraperitoneally, or intravenously; the prepared mice survived the dose of virulent streptococci (which it was not necessary to inject by the same method as in the preliminary inoculation) longer than the controls which had not received the less virulent organisms. It was noticeable that this "depression" immunity manifested itself very promptly. In some experiments it was definitely evident in 6 hours and was fully developed in 24 hours. The condition was not permanent, apparently owing to a remission in the course of the initial infection. It remained well developed up to the end of the 4th day, but, when the "superinfective" dose was administered on the 5th day, the greater number of the mice succumbed to acute infection, and, when the test was made on the 7th day, almost all the animals died, like the controls, in 24 hours. Cultures from the organs of mice which had exhibited "depression" immunity showed that the growth of the haemolytic streptococci had not been inhibited; it was often found that these organisms were present in very large numbers, greatly in excess of the number in the control mice which had died from acute infection. Another interesting fact was that these haemolytic streptococci recovered from the superinfected mice were fully virulent when tested on normal mice.

For the production of this systemic change with such rapidity, it seems necessary to suppose that the bacterial constituents or products act directly

[1] Ministry of Health. *Reports on Public Health and Medical Subjects*, No. 22, pp. 11–12, 1923.

on the normal elements of the animal's plasma and at once modify their "combining affinities" for the surface of streptococci. A similar explanation has been suggested for the equally prompt and more profound action of anti-anthrax serum. This, it appears, does not combine with the bacilli but acts indirectly, by producing a systemic change; *i.e.* the serum promptly interacts with normal elements in the plasma of the infected animal and causes them to assume "combining affinities" for the surface of anthrax bacilli.

Antibodies.

Certain properties of antibodies bear no resemblance to cell stimuli. The lysins lead to the direct destruction of bacteria by enzyme action; the bacteriotropins or opsonins prepare them for ingestion by the phagocytes; agglutinins may be adsorbed by the bacterial surface without exercising any influence on vital processes of the bacterium. In these instances, the bacterium is not stimulated; it is merely passive.

Here I am concerned with instances where antibodies are also stimulants and are associated with the production of bacterial variants.

I think the most useful example is the production of pneumococcal variants by growth in immune serum, as described by F. Griffith[1]. Here the influence which produces the variant can be identified without hesitation; it is the action of the specific "type" antibodies on the growth of the homologous strain. The variant is viable *in vitro* but not *in vivo*; and its loss of virulence in the animal body is shown to be due to loss of those elements in bacterial structure which are associated with capsule formation and production of "specific soluble substance." Hence the action of the serum in protection tests is readily explained; the serum produces a variant which, owing to these defects, is not viable in the animal body.

A feature of special interest in this work is that the stimulant action is found to be strictly confined to "type." Types I, II and III, being all pneumococci, must have many properties in common; but Type I serum will only act as a stimulant with Type I strains, and similarly with the sera and strains of Types II and III.

I called attention to what I regard as similar phenomena in a former report[2] in which I discussed immunological reactions with antigens artificially prepared by the introduction of a known chemical component. In some cases the new component has the effect of narrowing down the range of the antigen-antibody reactions. I quoted an example given by Pick.

> It was found that an antiserum prepared by immunising with diazobenzol-oxprotein would precipitate only diazobenzol-oxprotein, not normal oxprotein nor the protein of man, horse, or dog, which had been turned into the diazobenzol compound.

In general terms, when an antigen A (here the serum of a particular species of animal) is linked to a new antigenic character *b* (here a known

[1] Ministry of Health. *Reports on Public Health and Medical Subjects*, No. 18, 1923.
[2] *Ibid.* No. 13, p. 49, 1922.

chemical compound), the combination A*b* may retain its original specificity (due to A) whilst acquiring a new "constitutive" specificity (due to *b*); thus the antiserum produced by immunising with A*b* may react only to A*b* antigen, not to *b* coupled with an antigen other than A, nor to the normal A antigen, nor to A coupled with *c* or *d* or *e*, etc.

Similarly with pneumococci, A being their common species characteristics, and *b*, *c*, *d* etc. the characteristics peculiar to each type. The immune sera stimulate the production of variants in a highly specific and selective manner which is determined by "type" characteristics.

With other bacterial species quite different results might be found. The immune serum obtained by treatment with a single strain might act on all or several different "types" of the species and might also have some action on other species. For the existence of such differences there is no satisfactory explanation; they have to be accepted as facts, which, in connection with the present subject, coincide with the haphazard characteristics of cellular stimulants.

I now come to another curious point in relation to the specificity of stimuli (as demonstrated by antibody reactions) which it is equally difficult to explain.

Such stimuli may initiate transmissible bacterial autolysis. For example, a stimulus *a* (derived from bacillus A) may cause a normal culture of bacillus C (an organism different from A) to produce lytic substance c^1, which may be transmitted to a fresh normal culture of C, and so on, indefinitely. Another stimulus *b* (derived from B, an organism different from both C and A) may produce the same effect on C, yielding a supply of lytic substance c^2, which may be propagated *ad infinitum*. One might suppose that, after several transmissions through identical cultures of normal bacillus C, c^1 and c^2 would be completely identical with each other. But this may not be the case. If the lytic properties of the propagated c^1, and c^2 be tested on a culture of A, it may be found that c^1 produces lysis whilst c^2 fails to do so.

How does c^1 "remember" its affinity for A? Did *a* cause the protoplasm of C to break up in a special way, *viz.*, into particles containing affinities for both C and A? And was this special mechanism of autolysis perpetuated in the transmission of c^1 from culture to culture of C?

In a previous report[1] I quoted two examples in which this peculiar "remembrance" of a former state was demonstrated by immunising animals with a lytic substance and obtaining in their serum the corresponding specific antibody.

(1) Bruynoghe and Appelmans[2] prepared antisera with two typhoid "bacteriophages," the one obtained from Strasbourg and the other from Louvain. The latter was originally a coli "bacteriophage" but had been adapted to the typhoid bacillus and had lost all action on coli. It was found that its antiserum neutralised not only the Louvain "bacteriophage"; but also its predecessor, the coli "bacteriophage"; it had no action on the Strasbourg "bacteriophage." The Strasbourg antiserum neutralised only the Strasbourg "bacteriophage."

[1] Ministry of Health. *Reports on Public Health and Medical Subjects*, No. 18, pp. 31–2, 1923.

[2] *C.R. Soc. Biol.* LXXXVII. p. 96, 1922.

(2) Gratia and Namur[1] obtained somewhat similar results with antisera for substances which were lytic for staphylococci. The one substance (BH) was lytic primarily for *Staphylococcus aureus* (H) and the other (BV) for *Staphylococcus albus* (V); but, whereas BH acted on many strains of these organisms, including V, BV was strictly specific for V. The antiserum prepared with BH was specific for BH alone and the antiserum for BV was equally selective for BV. After the lytic principle BH had been allowed to act on V and had been transmitted ten times through V, the action of the two antisera was tested on the lytic substance finally obtained. It was found that no change had taken place; specific neutralisation was still obtained by the BH antiserum and not by the BV antiserum.

Is there any use in attempting to explain these apparent instances of a peculiar "remembrance" or "inheritance" of a particular affinity? In natural immunity it has to be admitted that it is useless to attempt impossibilities; the constituents of the plasma are too complex for analysis and one cannot single out any particular element, present in the naturally immune and absent in the naturally susceptible animal, which has a combining affinity for the bacterium in question; one can only assume that such differences exist.

But in the instances referred to above it may seem possible to go a little way towards the analysis of the experimental data, because it would appear to be a relatively simple question of the particular combining affinities possessed by certain lytic substances and their antisera. One method would be to postulate the presence of two constituents in the lytic substance, one (A) of which is "dominant" (*i.e.*, free to participate in chemical reactions), whilst the other (B) is "masked" (*i.e.*, is linked up with other chemical groups in such a way that it cannot directly participate in fresh chemical reactions). Thus a lytic principle, though containing both A and B, may be neutralised by an anti-A serum but not by an anti-B serum.

I am not prepared to agree that this is the right explanation. My main reason for mentioning it is to call attention to the dangers of adopting this method of explanation as a general principle. If one starts applying it first to one particular instance and then to others, one soon becomes involved in the general postulate of a multiplicity of antibodies, each selective for a corresponding substance in a multiplicity of antigenic constituents. Even as regards true antigen-antibody reactions, there are reasons, which I discussed in a previous article[2], for thinking that this postulate is untenable. And, in so far as the substances which act as antibodies also act as stimulants, it is a postulate which cannot possibly be maintained, since it would be absurd to assume that for each special kind of stimulative effect there is a special and chemically distinctive kind of stimulus. The examples from the idiosyncrasies of pneumococcal types are the exception, not the rule.

The above considerations make one pause before attempting to form some sort of simple mental picture which would help to explain why an antibody, *i.e.*, (1) a participant in an antigen-antibody reaction, can also act as (2) a stimulus.

The easiest conception would be that the same combining group in the

[1] *C.R. Soc. Biol.* LXXXVII. p. 364, 1922.

[2] *Journ. Hyg.* XXII. p. 355, 1924.

antibody is operative in each case and combines with the same specific material, the differences being due to the after-effects of the combination. In (1) it unites with some chemical group attached to the bacterial protein and either it remains united or, if union is followed by dissociation, this release leaves an intact bacterium and an intact antibody. In (2) there is union, as before, with a specific "building-stone" at the surface of the bacterium, but this union with the bacterium is promptly followed by dissociation; the "building-stone," however, being more firmly attached to the antibody than to the bacterium, is carried away from the latter; this interference with the normal process of bacterial metabolism leads to the production of a variant.

This kind of explanation might pass muster for variants amongst pneumococcal types, but I am afraid that, in reality, the action of immune bodies is often much more complicated, because each of them usually possesses a wide range of different combining affinities and these cannot be explained on the "mosaic pattern" postulate that each immune body is really a "mosaic" of different antibodies. I may quote in illustration some recent observations of Landsteiner and van der Scheer on precipitins[1]:—

"The results of precipitin reactions with antigen containing binding groups of known chemical constitution leave no doubt as to the fact that a single precipitin will regularly react with other substances if their chemical structure is sufficiently near to that of the homologous antigen. The alternative idea, so widely accepted,—that a given immune serum which reacts on the homologous antigen A and also on antigen B derived from another species consists of antibodies specific for a common group of A and B and contains other antibodies specific for a group peculiar to A,—is not tenable in this case."

Nutrition.

It is easy and frequently useful to draw a distinction between a stimulus and a food. The former causes the cell to function in a particular way but is not incorporated as part of the structure of the cell; the latter is utilised by the cell as a "building-stone."

At the same time it must be remembered that it is often impossible to draw a hard and fast line of demarcation between the two. Some chemical or physical agency may alter the characters of the available food supply, making the cell's task of assimilation either easier or more difficult; such an agency cannot be classed either as a stimulant or as a food, though it indirectly serves both purposes. In the symbiosis of bacteria, the products of bacterial action which help other bacteria to grow may be more than merely stimulative; some of them may become synthesised in bacterial cells. A bacterium may, to begin with, be unable to break up and utilise a particular substance, *e.g.*, lactose; but it may, by repeated subculture in a medium containing lactose, be "trained" to ferment this substance; the lactose, then, has acted first as a stimulant and afterwards as nutrient material.

I need not multiply instances. No confusion need arise if one remembers

[1] *Journ. Exp. Med.* XL. p. 91, 1924.

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that stimulants, enzymes, antibodies, and food material are not necessarily separate entities, and that one substance may exercise more than one function.

SUMMARY.

1. Immunity cannot be completely explained by antigen-antibody reactions, even if the term "antibodies" be made sufficiently elastic to include various obscure properties which are exhibited, *in vivo*, in the actively immune animal. Various other factors have to be considered. One of these is the influence of stimuli upon the vital capacities of bacteria.

2. Transmissible bacterial autolysis appears to be due to a stimulus acting upon the growing bacterial cell and leading to the splitting off of a certain number of daughter-cells which are non-viable, and consequently undergo autolysis.

3. Transmissible autolysis is not due to a stimulus *sui generis* but is no more than a particular incident in the general phenomena of bacterial variation.

4. The secretions of bacteria in pure culture may stimulate, control, or retard their growth and may lead to the production of variants.

5. When introduced into the animal body, bacteria encounter stimulants of animal origin which may be either favourable or unfavourable to their growth and are to be distinguished from the stimulants attributable to the bacteria themselves.

6. One aspect of the differences between natural immunity and natural susceptibility may be interpreted as due to differences in the stimuli inherent in the particular animal species and to consequent differences in their effects upon the particular bacterial species.

7. Similarly, when no better explanation is available, the acquired immunity (active or passive) of a susceptible animal may be interpreted as a change of the animal's stimulant action from one which was favourable (or indifferent) to the growth of a bacterium to one which is adverse, *i.e.*, a stimulant to the reproduction of daughter-cells which are non-viable in the animal body.

8. Leucocytes are one of the sources of material possessing two kinds of properties, *viz.*, (*a*) stimulant action on the growth capacities of cells (both bacterial and animal) and (*b*) enzyme action on the constituents which living and dead cells possess in common. The older researches on the characters of leucocytic extracts were occupied with (*b*), though they may occasionally be linked up with (*a*), since there are some indications that their leucocytic material was also acting as "lytic substance."

9. In some cases it must remain, for the present, an open question whether demonstrable antibacterial action is attributable to some more or less obscure enzymes or to what I have termed "stimuli."

10. A stimulus may be a substance which is also an antibody, and its stimulative properties may be highly specific. But it would be absurd to assume that for each special kind of stimulative effect there is a special and

chemically distinctive kind of stimulus. Both stimuli and antibodies usually possess a wide range of different combining affinities which cannot be explained on the "mosaic" theory that each different combination is due to the presence (in the stimulus or antibody) of a different chemical group.

11. A stimulus, as distinct from a food, causes the bacterial cell to function in a particular way but is not incorporated as part of the structure of the cell. This convenient distinction, however, does not imply that there is necessarily a sharp line of demarcation between a stimulus and a food.

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