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BACTERIAL VIRULENCE AND IMMUNITY.

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CONTENTS.

THE PHYSIOLOGICAL ASPECT.

IN all branches of physiological work experimental data are employed to explain vital processes, but it is recognised that the attainment of this object is very far from complete. The main events which occur in living matter are too complex for adequate expression in terms of chemistry and physics. They can, however, be co-ordinated under some general conceptions which may be accepted as physiological principles. The information acquired by experimental analysis is subordinated to these principles and serves to enlighten them. This may be taken as the physiological aspect.

The position is the same in the study of bacterial virulence, which is essentially a physiological subject. Many of the facts which have been ascertained about infection and immunity help to throw some light on virulence, but they fail to explain that mechanism of bacterial growth upon which

virulence depends. Hence, in the attempt to analyse virulence, one is constantly thrown back on principles which must be accepted as unexplained physiological facts, for example, the facts that the bacterial cell possesses a complex organisation and that this organisation is activated in particular ways by particular stimuli.

Such facts as these are admittedly mysterious but they cannot be ignored, because they are the data of primary importance to which immunological information is subsidiary.

Antigenic Complexity.

Functions of haptenes.

I think the following postulates are fairly well accepted by biochemists.

(1) Bacterial haptenes are chemical components which are capable of reacting specifically with antibodies. When they are united with bacterial protein, haptenes are fully antigenic, *i.e.* they can stimulate the production of antibodies *in vivo*. But they do not possess this latter antigenic capacity when they are dissociated from protein.

(2) In immunological reactions there are distinctions between the haptenes on the surface of the bacterial cell and those within the cell. The former, which may not be identical with the latter, are responsible for the ordinary reactions *in vitro* between intact bacteria and agglutinating sera. When the bacteria are broken up during immunisation, both the latter and the former may be effective in the production of antibodies, in so far as the haptenes retain union with protein.

(3) The specific properties of haptenes are sometimes masked, owing to the occurrence of racemic forms or to the union of their optically active element with some other chemical component.

(4) As there are varying degrees of affinity in antigen-antibody reactions, absolutely rigid specificity is not always observed. A haptene may unite with more than one antibody or an antibody may unite with more than one haptene.

These four postulates as regards the different ways in which a haptene may function, or fail to function, are of interest in relation to classifications of bacteria which are based on serological differences. Some of these classifications are highly complex, a fact which is often interpreted as meaning that the individual bacterium possesses a large number of different antigens. But differences in the antigenic and combining capacities of a relatively small number of haptenes might account for a relatively large number of different "types" or "subtypes" without the need for postulating a correspondingly large number of different chemical constituents. A useful economy will be effected if, without violation of laboratory facts, thinking in terms of "haptenes" will bring about some reduction in the multiplicity of "antigens."

Elementary and elaborated antigens.

In the building up of the bacterial cell from generation to generation there is reiterated formation of identical materials which are characteristic of the particular type of cell under propagation and acquire a degree of differentiation which forms part of the cell's individuality. Collectively, they form the elementary protein structure of the cell; individually, they may be described as "physiological units."

A mere accumulation of such "units" would not explain the life of a cell; they must be organised or arranged in a particular way in relation to each other, in order to set up that particular sequence of interactions which is the expression of the cell's functions and metabolism.

As regards the synthesised products of metabolism, a distinction must be drawn between those which are turned out of the cell (in addition to the waste products which are not synthesised) and those which are retained, either by the cell membrane or in the interior of the cell, where they may form either loose or firm union with what I have termed the "units."

Such union may modify the mechanism of the cell, and, consequently, the conception of the fully equipped bacterial cell as a "machine" consisting of organised units which turn out special products does not allow for the fact that the "machinery" is not altogether independent of its products. With this proviso, a distinction between the elementary structure of the cell and its elaborated products can often be maintained and is of significance in relation to antigenic characters. There can be no doubt that the elementary "units" of the cell may, to some extent, be reflected in serological antibodies; and it is equally true that bacterial products may assume antigenic importance. The former antigens may be termed "elementary" and the latter "elaborated."

The simplest example of the distinction between antigens attributable to the elementary units of a bacterium and elaborated antigens attributable to its products of metabolism is to be found in the diphtheria bacillus.

In major respects, diphtheria bacilli form a uniform and uncomplicated species. Their characteristic product of metabolism is their toxin, and this appears to be always the same, since no qualitative differences have been found between the toxins and antitoxins obtained from different strains. This implies a strongly marked uniformity in the cell's organisation. When one comes, however, to antigenic characters as exhibited by agglutination tests, uniformity is conspicuously absent. Strains with quite different antigens produce the same toxin. I interpret this as meaning that identity of organisation does not necessarily involve complete identity of the protein units of which the cell is composed. Two machines may turn out identical products, but the component parts of the one machine need not all be made of the same material as the component parts of the other. Different strains of diphtheria bacilli may be equally true to species though they may have slightly different methods of synthesising the protein units which form the component parts

of the machine for the production of toxin. I think this example serves to show that the distinction between the elementary units of a cell and their organisation is of importance.

With the diphtheria bacillus, then, the main analysis of the "antigenic complex" is straightforward. The toxin is the main antigen; the other antigens which may be demonstrable serologically are subsidiary and irregular. It is just possible that, when a culture is prepared for immunisation by heating to a temperature which will destroy any toxin present, the destroyed toxin may unite with other antigenic components and make them a little different from the antigenic components of an avirulent culture. But this possibility does not amount to a distinctive feature upon which much reliance can be placed.

I now come to pneumococci, which, I consider, provide an equally important example of the distinction between "elementary" and "elaborated" antigens, though the conditions are more complicated than with the diphtheria bacillus.

A characteristic, virulent pneumococcus produces an antiserum which not only agglutinates the homologous cocci but also forms a precipitate with material, called "specific soluble substance," which passes out of the bodies of the cocci. This secreted substance, however, is incapable of producing antibodies; it is only a haptene, though it is fully antigenic when it is united with the protein of the bacterial cell.

Whilst retaining capacity for vigorous growth *in vitro*, the pneumococcus may lose its virulence. Under these circumstances, it no longer secretes its characteristic haptene and the antigenic property associated with this haptene tends to disappear.

The natural interpretation of these facts is that the change in antigenic properties depends on the distinction between the elementary constituents of the cell and their products of metabolism. When these elementary units are fully organised in a suitable environment, they produce a substance which, in combination with bacterial protein, becomes the predominant antigen; if they are less well equipped, this substance is not elaborated and the elementary antigens of the protein units then emerge.

In these respects the pneumococcus resembles the diphtheria bacillus, though the special product of the former differs from the toxin of the latter in its firmer association with the bacterial cell and in its lack of immunising properties when liberated from the cell. There is another interesting difference. Different strains of diphtheria bacilli may be shown by serological analysis to possess different elementary units, but the toxins which they produce are identical. Amongst pneumococci there are sharp differences in their special products—hence their classification into Types I, II, and III and the heterogeneous Group IV; but, when the pneumococcal types lose the capacity to produce their special substances, their antigenic differences from each other tend to disappear. It seems that the same elementary units when differently

organised, may elaborate different products, in contrast with diphtheria bacilli where different units may be organised so as to elaborate the same product.

Stages in the elaboration of antigens.

These elaborated antigens, which I regard as constituted by the union of bacterial protein with special products of bacterial metabolism, are worth further consideration.

The changes which may occur within a given type of pneumococci are suggestive. For laboratory data on this subject I have relied mainly on F. Griffith's work[1].

It appears that a degenerate pneumococcus may be in one of two conditions: (1) it may retain the capacity to recover, in full, the original type characters; (2) loss of virulence may be associated with complete loss of type characters.

A temporary modifying influence—growth in homologous serum overnight—produces, on plating, some "rough" colonies which do not secrete the soluble substance and are not virulent. But a "rough" culture (*R*), produced under these conditions, may, if placed in a specially favourable environment (as in the body of a susceptible animal), revert to the characteristic of the original culture, viz. growth in "smooth" colonies (*S*), and then it again yields the soluble substance and regains its virulence.

This is a good example of condition (1). As the loss of a characteristic is only temporary, it appears to be due merely to some temporary incapacity of the cell's organisation, just as a diphtheria bacillus may cease to turn out toxin on an unfavourable medium, but will continue to do so when transferred to a suitable medium.

In the degenerate condition there is still some retention of type characteristics, as shown by the following specific relations which have been noted between *S* and *R* cultures and antisera.

(*a*) *S* serum forms a precipitate with a filtrate of *S* culture but not with a filtrate of *R* culture, whilst *R* serum gives no precipitate with a filtrate of either *R* or *S* culture. Hence soluble substance is neither secreted by *R* culture nor combined antigenically with the bodies of *R* cocci.

(*b*) When *S* culture reacts with *S* serum, a combination of precipitation and agglutination is produced (floccular agglutination), but *R* cultures with *R* serum gives agglutination without precipitation (granular agglutination). Thus precipitation indicates that soluble substance is associated with the reacting cocci and has been combined with the cocci used to prepare the serum.

(*c*) *S* serum agglutinates *R* (granular) as well as *S*, but *R* serum does not agglutinate *S*. *S* culture, therefore, appears to contain the *R* antigen, which is masked by combined soluble substance in the intact cocci but is liberated when the cocci are broken up during the process of immunisation.

[1] *Reports to the Ministry of Health*, No. 13, 1922, No. 18, 1923; and personal communications.

(*d*) Repeated absorption of *R* serum with *S* culture effects some reduction of agglutinin for *R* culture, and vaccination with *R* culture confers some protection against *S* culture, though *R* serum does not protect against *S* culture. Here is some further evidence that *S* and *R* cultures possess a common antigen.

The readiest way of explaining the resemblances between *S* and *R* which are indicated above would be to attribute them to identity of the protein units of which the cocci are composed. The differences between the two would then be due primarily to differences in the organisation of these units, which is effective for the production of soluble substance in the case of *S* but not in the case of *R*.

The difficulty about such an explanation is that it would apply rather to stage (2), where degradation has proceeded so far that the *R* strains of one type are indistinguishable from those of another. Their relationship to each other and to the *S* strains certainly does appear, under these circumstances, to depend on the antigenic properties common to all pneumococcal protein.

But, in comparing *S* and *R* forms within the same type and when the degradation of the latter has only arrived as far as stage (1), some different explanation seems to be required. The *R* form, with which no soluble substance appears to be associated, may still be identified serologically with its original type, even when it has lost the capacity to revert to *S*, with occasional exceptions after prolonged passage. How is one to reconcile this partial retention of type characters with the generally accepted belief that type is determined by the presence of a particular kind of soluble substance?

Perhaps the organisation of the *R* form, in this condition of stage (1), produces something which is on the way to become soluble substance but remains incompletely elaborated. This hypothetical antecedent would not be secreted as the soluble substance which seems to be associated with virulence; but it might unite with the protein units and this combination might serve to differentiate the antigen from the antigens of other types. The antecedent, as distinct from the fully formed soluble substance, may be associated only with the "granular" kind of agglutination and not with the "floccular" kind of combined precipitation and agglutination. And why should not the production of two kinds of agglutinin by *S* antigen ("floccular" for *S* cocci, "granular" for *R* cocci) be explained by the presence in *S* cocci of both antecedent and fully elaborated soluble substance, the former being mainly in the endoplasm and the latter in the highly developed ectoplasm?

If there may be a distinction between (*a*) the antecedent, and (*b*) the fully developed phase of those products of metabolism which are characteristic of a bacterium, differences in immunological reactions may emerge which depend on whether the effective antigen consists of bacterial protein united with both (*a*) and (*b*) or with (*b*) alone, or with (*a*) alone.

Such differences may occur with bacteria other than pneumococci, though their products of metabolism may not be always so stable or so sharply

distinctive as the pneumococcal "soluble substance." This idea is worth considering in relation to what has been termed the "double receptor apparatus," in laboratory investigations of certain intestinal bacteria.

E. Weil and A. Felix[1] have shown that the class of bacteria to which I refer possess (*a*) stable receptors (resistant to heat at 100° C.) and also (*b*) labile receptors (much more readily destroyed by heat). The former produce antibodies with which they give fine or granular agglutination; the latter produce different antibodies with which they give a different kind of agglutination (coarse or floccular). A typical strain possesses both (*a*) and (*b*) antigens and produces both (*a*) and (*b*) agglutinins. In some strains (*b*) is masked, as shown by failure, *in vitro*, to agglutinate with or to absorb (*b*) agglutinin; but the presence of (*b*) antigen in such strains may be revealed by their capacity to produce (*b*) agglutinin *in vivo*. It is sometimes possible, however, to destroy (*b*) antigen completely by heating at 100° C. or by growth on phenol agar; and such material will produce *in vivo* a pure (*a*) serum.

May not strains of these bacteria produce two forms of one and the same substance, a stable form (endoplasmic) and a more highly elaborated, labile form (ectoplasmic), which differ antigenically when united with the protein constituents of the cell? And may not these two forms be produced in different proportions, the more elaborate one being sometimes too scanty to be antigenically effective as a constituent of the surface of the intact bacterial cell? The distribution of antigens on the outer membrane may be different from their distribution within the cell.

Like pneumococci, these bacteria exhibit changes from "smooth" to "rough," though it would not be safe to assume that the analogy is complete in every respect.

The following observations on the Salmonella group are taken from Bruce White's work[2]. (1) *R* forms tend to give unstable suspensions in normal saline. (2) Flocculating antigens and agglutinins are identical in quality in *S* and *R*, but in *R* they "tend to become reduced or even obliterated." But *R* forms which fail to flocculate "may still absorb and stimulate flocculating agglutinins when the dosage employed is massive." *R* forms, unless the flocculating antigens are weakly developed, "are liable to exhibit the specific and non-specific phases of Andrewes." As regards protection, "the roughest of rough strains, those which show no flocculation and no absorptive action on flocculation, effect perfect immunisation against smooth strains." (3) In many cases the granular antigens of *R* closely resemble those of *S*; but in other instances, "particularly where the flocculating factors are markedly reduced, new granulating antigens and agglutinins absent in the smooth parent strains and sera respectively come into evidence."

My suggestion that bacteria may elaborate two forms—(*a*) an antecedent form, and (*b*) a fully developed form—of some substance which acquires

[1] *Zeitschr. f. Immunitätsforsch.*, Orig. **29**, p. 24, 1920.

[2] *Medical Research Council*, Reports **91** (1925) and 103 (1926).

special antigenic characters when united with the cell's protein may perhaps be applicable to these *S* and *R* forms of intestinal bacteria.

It appears that the changes involved in the transition from *S* to *R*, presumably due to the influence of environment, may be less abrupt and less profound than with pneumococci. Apart from some physical change in the surface of the cell, with which the conformation of the colonies and the tendency to auto-agglutination are associated, the internal mechanism of the cell may be very little altered, and the output of the antigenic factors in forms (*a*) and (*b*) may remain much as before.

Even when the change is more advanced, the output of (*b*), though diminished, does not cease, since *R* forms which fail to flocculate still retain some capacity for absorbing and producing flocculating agglutinin; and they may still exhibit the "diphasic" phenomena associated with the (*b*) antigen. In this retention of (*b*) they differ from completely degenerate *R* pneumococci.

With intestinal bacteria in an advanced stage of *R*, why do the granular antigens tend to differ from those of *S*? In such cases the output of (*b*) is much diminished; so (*a*) has additional opportunities of combining with the protein units of the cell. It is just possible that this fact may alter its antigenic character, without the need of postulating that a qualitative change has taken place in (*a*). Or it may be suggested that the apparently new thermostable antigens manifested by the *R* forms were actually present in the *S* forms but were then masked by the more highly elaborated and "specific" antigens; in contrast to the latter, the former might be regarded as "generic."

What is the significance of the "diphasic" condition of this more elaborate and labile antigenic substance?

Andrewes[1] has shown that in certain types of *Salmonella* the antigens responsible for floccular agglutination exhibit two alternate phases which are sharply distinguishable by their behaviour in agglutination, though the bacilli are alike in other respects. "In the one phase the specific properties of the type predominate, with only a scanty group element; in the other there is a predominance of the properties common to the group, the specific element, though present, being feebly developed; intermediate phases were not found. It was further shown that these peculiarities of phase are not stable, mutation occurring on subculture."

This "diphasic" condition may indicate that there are two stages in the elaboration of that material which, when united with the protein units of the cell, constitutes the thermolabile antigen. The first stage corresponds to the "group phase." Synthesis may stop at this point, being immediately followed by the critical condition which causes the cell to subdivide. Or elaboration of this material may proceed a little further; in its changed condition, it forms, when united with the protein units, the "specific" antigen.

On this conception, the Salmonella group may exhibit three successive stages in the elaboration of this antigenic material by the cell, leading, in

[1] *Journ. Path. and Bact.* **25**, p. 505 (1922), and **28**, p. 345 (1925).

order, to the production of (1) thermostable antigen, (2) thermolabile "group" antigen, and (3) thermolabile "specific" antigen. In a fully equipped bacterium (3) predominates, and there may be very little evidence of (2); if division takes place at a somewhat earlier stage, (2) is greatly in excess of (3).

Comment.

In any attempt to correlate virulence with immunological reactions one is immediately confronted with the increasing complexity of serological data. These, as laboratory workers admit, are not all of equal significance in relation to virulence; many of them are more concerned with questions of technique in the identification of strains by serological methods. This latter problem is one which the investigators are naturally expected to settle amongst themselves; and I have no desire to intervene. But one cannot discuss the relations of antigens to virulence without making some reference to that antigenic complexity which the serologist generally describes as a "mosaic pattern."

I think that variations in the functions of haptenes are partly responsible for the intricacies of serological analysis and that differences in the avidity with which a particular haptene combines with reagents *A*, *B*, and *C* may sometimes be represented in the "mosaic" as a complex of antigens *a*, *b*, and *c*.

The "mosaic" idea postulates a greater or less quantity of one antigen associated with different quantities of other antigens. This is in accordance with the results of serological titrations; but, for the purpose which I have in view, it would not satisfactorily represent the qualitative distinction I wish to draw between elementary and elaborated antigens, a distinction which is based on the functions of these antigenic substances in the economy of the cell.

Owing to puzzling variations in the antigenic behaviour of "diphasic" strains of bacteria, the "mosaic" pattern has sometimes been described as "kaleidoscopic." The differences which I have suggested in the degree of elaboration of one and the same antigenic substance may partly account for this "kaleidoscopic" phenomenon. But, though I have illustrated my meaning by brief reference to some of the laboratory findings, I feel that it rests with the serologists who have made these analyses to deal with the question of antigenic phases in their own way. I am not attempting to propose a new method of serological classification.

Though I think that some effort ought to be made to reduce the number of different antigens which it is necessary to postulate, I do not dispute the fact, to which I refer in the next section, that definitely new antigens may emerge in the course of bacterial growth.

Antigenic Structure and Physiological Properties.

Structure and function.

When the bacterium builds up the elementary protein units which are necessary for its growth, each unit is a complex of chemical groups. In the process of synthesising these, they may not always be put together in precisely the same way, either as regards the firmness of their union or as regards resultant stereo-chemical configuration of the unit as a whole. Such differences may be immaterial physiologically, but the immunologist may find that they are conspicuous in serological reactions. For example, two strains of diphtheria bacilli may be identical in toxicity but may differ more or less widely in antigenic properties attributable to their protein structure.

Perhaps my meaning needs a little expansion and illustration. Two of the necessary components in the structure of a unit may be *a* and *b*, which combine to form *ab*. Their union, though sufficiently firm to persist in the living cell, may or may not be firm enough to survive when the cell is broken up. If it is stable, there will be the antigenic influence of *ab*; if it is broken up, this antigenic character will disappear and may be replaced by another. Or, again, there may be a group *ab* which can link up with the unit either on the *a* side or on the *b* side but not on both sides; union with *b* would leave *a* active (ready to form fresh combinations) and render *b* inert; union with *a* would have the reverse effect, the difference being symbolised by *Ab* as contrasted with *aB*. In the organisation of the cell, the behaviour of *Ab* and *aB* may be identical; but the two may produce different effects when used as antigenic components.

Further differences between the biological and the analytical aspect arise in relation to the elaborated antigens formed by union of (*a*) products of bacterial metabolism with (*b*) the elementary structural units of the cell.

There may be some slight variation in the mode of union between (*a*) and (*b*), perhaps in the stability of union, or perhaps in the resultant stereo-chemical configuration; and this may lead to antigenic differences, though causing no change in the internal economy of the cell.

Originally, the output of (*a*) may have been abundant, appearing as an external secretion as well as in union with (*b*). Owing to some modification of the cell's organisation, it may diminish and may then be entirely bound up within the cell. This change may leave the combined (*a*) and (*b*) antigen unaltered, though the loss of the external secretion may profoundly alter important biological functions of the cell.

The output of (*a*) may diminish still further and may finally cease, thus reducing and finally eliminating the combined antigen and allowing a pure (*b*) antigen to emerge.

There may be elaboration of (*a*) in different phases; antigenic differences may be determined by the particular phases with which (*b*) makes effective union. But these differences will not necessarily correspond with biological differences.

Variation.

It has been found convenient to draw a distinction between the development of the protein units of the bacterial cell and their organisation in relation to each other. Antigenic changes may occur in the former factor without altering the latter; and changes (reflected in changed antigenic properties of products of metabolism) may take place in the latter without altering the former factor. But, as mentioned earlier in this article, this apparent independence of the two factors may not always be maintained, since they are closely related to each other in the processes of bacterial growth.

Hence more complex situations have to be considered. A change in the cell's organisation, acting during the progressive synthesis of the protein units, may alter the construction of the latter, *e.g.* by causing them to select a new component *c* in preference to a former component *a*. And this change in the units may be followed by a change in their relationships to each other, thus leading to a fresh change in the cell's organisation.

This is not necessarily the limit of possible modification. The new organisation may produce a further change in the new units; and so, theoretically the process of variation may continue *ad infinitum*. But there is, in fact, a very potent restricting influence. Only those changes are possible which are compatible with the viability of the cell; and viability is impossible without retention of some degree of physiological specificity.

I now propose to take as an example the possible variation of pneumococcal types.

The pneumococcal species does not seem to be absolutely fixed, since the conversion of pneumococci into organisms indistinguishable from streptococci has been recorded not infrequently. If this wider change is possible, it may be thought that there is room for the less drastic alterations which would be involved in the transition from one type to another.

But it has not been shown experimentally that a typical strain, *e.g.* a fully virulent Type I, can be transformed into an equally typical strain of another type; and there is no direct evidence that such transitions occur in natural infection. On the other hand, it is not easy to accept the opposite doctrine that a type (including the virulent types of Group IV) never arises *de novo*. If this were true, it would be difficult to understand how the supply is maintained, since it is the rule that the virulent type either perishes completely or at least loses its identity during convalescence; and it is generally believed that such virulent types do not occur in nature apart from association with an animal host.

Virulence, however, can be acquired; and, as this property is closely connected with type characteristics, particularly with specific "soluble substance," it seems that there ought to be some way of explaining acquirement of these latter properties in the animal body.

Perhaps the postulated change does not occur abruptly or directly but is

accomplished in two stages, the first being degeneration and the second reacquirement of virulence.

For example, with a highly virulent Type I pneumococcus the first step in degradation appears to be loss of specific soluble substance and is associated with loss of virulence but with retention of some type characteristics, *e.g.* those associated with agglutination. When recovery of virulence takes place at this stage after animal passage, as is sometimes the case, there is complete reversion to the original characters of the type. If degradation has proceeded further, so that the strain can no longer be identified as Type I even by agglutination tests, it may still be viable not only *in vitro* but also on the free surface of a mucous membrane, though it would rapidly perish within animal tissues. Virulence may now, to all appearances, have been lost irrevocably, but it cannot be asserted with confidence that this is necessarily the case. It is still conceivable that a pneumococcus in this condition may again become virulent under favourable circumstances which modify the permeability of the cell membrane, allowing passage into the cell of material which can be synthesised into "virulence substances" and excluding entrance of material which would lead to hydrolysis before this synthesis can be accomplished This new organisation for the production of "soluble substance" need not be the Type I organisation which has been completely lost; perhaps it is more frequently of that irregular nature which is conspicuous in Group IV. And, indeed, there may be some influence which tends to make the new type different from the original one. The survival of an animal from infection with a particular type is usually associated with the presence of antibodies to that type. These antibodies may act as a stimulus to variation upon the degenerate but still viable pneumococci.

Finally, it is interesting to note that virulence, though closely associated with "soluble substance," does not depend on the precise chemical nature of this substance. Strains belonging to different types may be equally virulent, though their "soluble substances" differ in constitution and combining powers.

Specific antigens and virulence.

When the serologist has analysed an "antigenic complex" by means of agglutinins, he distinguishes between the importance of various protein elements present in each strain of bacteria. Some of them are "specific," *i.e.* represent the type characteristics of the individual bacterium; others are "not specific," *i.e.* do not possess this property, though they form part of the serological individuality of the bacterial cell. Cultures are described as possessing either "specific" or "group" antigens, or a certain amount of the one sort together with a certain amount of the other. And, apparently, two or more "specific" antigens may be present in the same bacterium; these may differ from each other quantitatively as well as in quality, the one present in largest amount being more "dominant" than the others in serological reactions.

Or a distinction may be drawn between ectoplasmic and endoplasmic

antigens. In the ectoplasm, there may be only one which is "specific" but several which are "group" and never "specific." In the endoplasm, an antigen may be "specific" in one strain but identical with an antigen which is "group" in another strain, the only differences between the two being in respect of quantity and, perhaps, avidity.

These complications are of obvious importance, but the serologist does not claim, as a rule, that his antigens are the direct representatives of different biological functions; and it must be recognised that the term "specific" is used in a very limited sense, since the full specificity or individuality of a bacterium consists not in a particular aggregate of antigenic constituents but in a particular sequence of vital processes, which makes one type of cell different from another.

There is a large amount of laboratory data which must be accepted as showing that many of the agglutinogens which are employed in the serologists classification cannot be correlated with either presence or absence of virulence.

Sometimes, perhaps, the "virulence substance" is too labile to act as an antigen, with the consequence that no antibodies to it are formed. Or the antibodies to it may be produced in the animal body and may confer active immunity, but they may not be sufficiently stable to survive in the serum and hence cannot be demonstrated either *in vitro* or in tests for passive immunity.

Then is virulence related in any way to those antigens which have been found important in the serological comparison and identification of strains? Sometimes a frankly negative answer is returned. It is stated that a classification based on cross-immunisation experiments would be quite different from one based on agglutination and absorption of agglutinin; and it is inferred that the antigens which produce the protective antibodies are independent of those which produce agglutinins.

I do not think that such a sharp demarcation is tenable as a general proposition. It may often happen that the bacterial structure on which virulence depends forms agglutinins as well as protective substances; though they are not always demonstrable simultaneously, since there is sometimes active immunity without agglutinins in the serum, there is no reason to postulate two independent antigens. Conversely, there is certainly no basis of laboratory facts which would warrant the inference that every bacterial structure which is known to produce agglutinins is, *ipso facto*, independent of the structure on which virulence depends.

The position seems to be that particular antigens may be a secondary attribute of virulence, but they are not the cause of virulence. Confusion need not arise if one discriminates between the biological aspect of bacteria as living organisms and their analytical aspect as a composite of various chemical structures.

I do not think that this view is inconsistent with recent chemical work which tends to show that, amongst many bacterial species, a special haptene

can be extracted which is characteristic either of a whole species or of a particular type within a species. This work, of course, is highly interesting and valuable; and it may be true that certain strains of bacteria are not virulent unless the chemist can extract a special haptene from them. But the haptene is not the biological organisation which is the cause of virulence.

In illustration of this distinction, reference may be made to pharmacological experiments which show that trypanosomes, bacteria, and other micro-organisms may acquire resistance to the action of certain drugs. When an infected animal is treated with the drug in doses which are not sufficiently germicidal to kill all the parasites present, the survivors often acquire resistance to its action and this capacity for resistance may be transmitted from generation to generation. Such resistance may be compared with that natural or acquired resistance to the action of an animal environment which is an important attribute of bacterial virulence. In both cases it may be conceded that the resistance of the micro-organism is associated with some particular chemical or chemico-physical condition of its outer membrane; but this circumstance alone does not provide an adequate explanation.

In the case of the drug, it has been found necessary to abandon the simple chemical hypothesis that the parasite, when sensitive, had direct combining affinities for the drug and that resistance is due to a loss of these affinities. The process is much more complex and appears to involve active co-operation of the tissues and fluids of the animal body. Probably there is first an interaction between the drug and some elements in the animal body and then the products of this interaction produce the change in the parasites. Hence the distinction between parasites which are sensitive to a drug and those which are resistant needs some correction. The distinction should rather be between resistance and sensitiveness to their animal environment. When the susceptible animal is not treated with any drug, the parasite is resistant to that environment; when the environment is changed in character owing to the action of the drug, the parasite is at first sensitive and its growth is inhibited, but, if it retains capacity to reproduce itself, its descendants may acquire resistance. The result is not determined by the mere occurrence or absence of a particular chemical reaction but by a biological process affecting the internal organisation of the daughter-cells which are produced under the influence of the changed environment. This qualitative difference in organisation is the essential change; its consequence is that the cell produces an outer membrane which is resistant to its new environment.

There is a similar difficulty in the attempt to correlate differences between the virulent and the avirulent condition with the absence or presence of combining affinities between particular constituents of bacterial protein and particular elements in the animal's plasma. Sometimes, it is true, it may appear sufficient to say that a bacterium is killed *in vivo* by direct combination with a "bacteriolysin" in the plasma, or that the bacterium survives because it has no side-chains which will "fit" with any lysin present in the circulation.

But it would be quite impossible to elaborate these simple chemical conceptions into a satisfactory theory of virulence. The conditions which determine whether a bacterium is viable in the animal body are much too complicated, and the use of biological conceptions is indispensable, just as it is in dealing with the action of drugs. The virulent bacterium must possess an organisation which enables it to produce a resistant outer membrane. This organisation cannot be expressed in terms of antigens or other combining affinities. Its products may be antigenic, but they are no more than collateral attributes of virulence.

The inadequacy of a purely chemical explanation is also illustrated by another interesting resemblance between resistance to drugs and virulence. Changes in virulence brought about by residence *in vivo* are not always specific in relation to the host. Residence in one species of animal may either exalt or lower virulence for a different species. Similarly, treatment of an infected animal with a particular drug may either increase or diminish the resistance of the parasites towards another drug of quite different chemical composition. In both cases it would be quite arbitrary and unconvincing to postulate an indefinitely large number of different chemical affinities. One has to resort again to less concrete biological conceptions. The change is due to an alteration in the organisation of the cell which leads to different products of metabolism, with consequent differences in the resisting powers of the cell membrane.

Such changes in organisation are not necessarily identical with changes in specificity.

One may also note that serological "relationship," as indicated by partial identity of antigens, though much used as an aid to classification, does not help to decide whether a particular bacterium will share the property of virulence which its serological "relative" is known to possess. As regards this property, the ancestry of a bacterium is of less interest than its future potentialities, viz. its possible range of variation.

Comment.

After allowing for the possibility that some part of the complexity in antigen-antibody reactions within a bacterial species or sub-species may be due to different stages in the elaboration of the same antigenic substance, there remains abundant evidence that analysis of these reactions is complicated by the presence of different elements which participate in the construction of the bacterial cell.

On comparing two strains, identical in all other respects, it may be proved serologically, as a fact which must be accepted, that one contains a particular haptene which is not present in the other. In another case, the serological results may be best explained by quantitative differences in the amount of a particular haptene which is present in both strains but is antigenically less effective in the one than in the other. Again, serological variations of a

particular strain may be due to the loss of a haptene formerly present or to the acquisition of a new haptene. Or one may imagine a more elaborate explanation of some of these variations; the haptene may be composed of two linked elements, *a* and *b*, which may unite with bacterial protein sometimes on the *a* side and sometimes on the *b* side, thus causing the actively antigenic element to be *b* in the former case and *a* in the latter.

The above examples will suffice to illustrate the fact that an explanation which postulates an insufficient number of antigens is quite as unsatisfactory as one which demands too many.

I do not think it would be useful to adopt a method of exclusion in attempting to determine the relations of antigens to virulence, *e.g.* to discard antigens which are also found in avirulent strains and to select those which are only observed when the bacteria are virulent. Superficially, it might meet with some measure of success; when a strain which is virulent produces a protective serum but fails to do so when it has lost its virulence, the property of virulence seems to be associated with a special antigen; in pneumococci, for example, it appears closely related to the antigen formed by union of "soluble substance" with bacterial protein, and in diphtheria the toxin is a good example of an antigen closely identified with virulence. Yes; it must be conceded that bacteria elaborate material which is both antigenic and closely associated with virulence, but that is all. A bacterium depends for its virulence not on these substances alone but on its entire structure, and many elements which compose this structure are also demonstrable antigenically in avirulent strains.

Moreover, apart from antibodies to exotoxins, the opinion is gaining ground that it is not permissible to separate antibodies into distinct substances, some of which produce lysis, others agglutination, and so on. This is an additional reason against postulating a causal relationship between a particular antigen and virulence.

It is further to be noted that immunity and virulence do not depend merely on the presence or absence of union between a particular antigen and antibody. It is also a question of conditions determining bacterial metabolism.

The above remarks are not intended to disparage the significance of antigen-antibody reactions in relation to vital processes. Such reactions, as demonstrated *in vitro*, are often characterised by extremely delicate selective activity and may be taken as examples of similar selective action which occurs in the living cell. Reactions of this nature are not always dependent on some substance which is produced by immunisation; they are sometimes attributable to what the serologist calls "natural immune bodies," which means that substances *a* and *b* may react like antigen and antibody though they are not specifically related to each other, since *b* was not produced by *a* (foreign protein) nor by any substance similar to *a*. The important point is that, apart from the production of antibodies, vital processes are characterised by

non-specific reactions which may be as highly selective as typical reactions between antigen and antibody.

Selective chemical union is only the primary event in an antigen-antibody reaction; the consequences of this union may assume a variety of forms, such as neutralisation of a toxin, agglutination of bacteria, precipitation of a serum, bacteriolysis, and so forth. Similarly, selective union *in vivo* is only the beginning of a variety of different processes.

THE BACTERIAL SURFACE.

Influence on internal organisation.

What is the nature of the alteration which occurs when a virulent strain becomes avirulent after repeated passage on culture media?

As hydrolysis and dehydration are constantly associated with catabolism and anabolism in all forms of growth, one naturally thinks of some change in the cell's affinities for water, accompanied by changes in the hydrogen ion equilibrium and resulting in changed capacity for synthesis.

The virulent and the avirulent strain may grow equally well *in vitro*; so they must both be capable of synthesising what I have called the elementary protein units of the cell. But they may not be equally capable of elaborating the residual material which finds its way into the cell, *i.e.* the material which is not immediately incorporated with protein. With the virulent bacterium the processes of dehydration may be carried a little further than with the avirulent, involving some greater elaboration of products of metabolism. Thus the difference between the two bacteria in that internal mechanism which I have termed "organisation" may resolve itself into a simple difference of affinities for water. Whatever the exact nature of the change, its cause must be ascribed primarily to the bacterial surface, perhaps because the cell membrane tends to become more permeable *in vitro* than it was *in vivo*.

With some bacterial species, virulence is unaffected by prolonged growth on ordinary artificial media and it is reasonable to assume that permeability of the surface is not altered by this mode of life. With other species, virulence is retained during a short period of artificial growth but is lost if such growth is prolonged. Under these circumstances, the tendency of the environment to modify the outer membrane may be slow but gradually accumulative. The slighter derangements do not involve interference with the internal mechanism for synthesis and therefore virulence is acquired, either at once or more or less readily, on transfer to the animal body. When it is found that virulence has been lost permanently, the change has not been confined to the surface but has led to some loss in the internal mechanism which no change in the surface can restore.

It thus appears that persistence of virulence in culture depends on whether the necessary "organisation" can be transmitted from generation to generation—and sometimes for a long period—without residence *in vivo*. Continuity of this equipment depends on the bacterial surface.

Perhaps there is a reversal of this process of degradation when virulence is acquired *in vivo*. To begin with, the bacterium may be living as a saprophyte on the surface of a mucous membrane and the bacterial envelope may be insufficient as a protection for life within the animal tissues. This outer covering may be modified by its environment, and it may happen that the altered membrane permits material to enter the bacterium under the same selective conditions which would regulate its entry into a virulent cell. Then a changed condition, perhaps more favourable to processes involving dehydration, would be produced within the cell and the cell's organisation would then become capable of effecting those more elaborate syntheses requisite for virulence.

This conception of the acquirement of virulence must be supplemented by the consideration already mentioned in the discussion of pneumococcal variation. If the avirulent condition was a degradation from a previously virulent state, it does not follow that the newly acquired virulence will be of precisely the same characters as the former. One must allow for possible qualitative differences in that organisation of the cell which is responsible for virulence, differences due to the particular nature of the environment and to the idiosyncrasies of its modifying action on the cell-membrane.

Further questions as to qualitative differences in virulence arise from the curious fact that residence of bacteria in one species of animal may preserve or even enhance their virulence for that species and simultaneously lower their virulence for another species.

In explanation of this change one must again give first consideration to the bacterial surface, though it need not be necessary to assume that this membrane retains any chemical elements which are specific for the animal in which the bacteria are growing. But the animal's plasma has characters peculiar to the species, and their influence on the bacterial membrane may be different from the influence of a plasma belonging to another species of animal. One thinks of some influence causing minor changes in the permeability or selective action of the membrane and resulting in some slight qualitative difference in the material passing into the cell. The protein units of the cell are built up as before, but the residual material, being somewhat different, is elaborated rather differently before being disposed of as products of metabolism. This involves some change in the mechanism or "organisation" of the cell, a change which is handed on from generation to generation in the new environment.

Thus one arrives again at the conception of qualitative differences in the bacterial surface, leading to changes in that organisation of the cell which is responsible for virulence. A bacterial membrane specially adapted for one kind of animal host may be less suitable for bacterial growth in a different species of host. The conditions are probably similar to those which I have already discussed with reference to the pharmacological action of drugs.

Protective functions.

The condition of the bacterial surface obviously affects virulence not only as a selective filter facilitating the admission of appropriate food but also as a protective mechanism for the exclusion of injurious influences.

Such influences may be due to substances quite alien to the bacteria or they may be caused by the selective action of an antibody.

"Sensitisation" of the bacterial cell by antibodies is of particular interest and involves some discussion of the relationship between virulence and susceptibility or insusceptibility to phagocytosis.

There can be no question about the importance of phagocytosis as a mechanism of defence for the disposal of dead or foreign material. But the teleological ideas of purposive "warfare" with which it is often invested are somewhat embarrassing. The simple, prosaic facts, so far as they concern virulence, may be outlined as follows.

A phagocyte ingests a bacterium much in the same way as it ingests inert particles of inorganic and organic material. At the point of contact, the surface tension of the animal cell is lowered and ingestion proceeds as a purely physical reaction. There is no fermentative or other activity on the part of the bacterium and there is nothing purposive about the behaviour of the phagocyte.

In the absence of a precise scientific explanation, the bacterial quality which allows of ingestion may be described as "stickiness." Some bacteria, *e.g.* tubercle bacilli, are always sticky, whether virulent or not. Some species are only sticky in the avirulent condition; when virulent, their lack of stickiness is probably due to some special product of metabolism, associated with virulence, which becomes combined with the bacterial surface; interaction between this surface and an appropriate antiserum will induce stickiness.

The animal cells may digest the ingested bacteria or they may be unable to do so. In the latter event, the bacteria may be able to grow within endothelial phagocytes, or, if taken up by multinuclear leucocytes, they may find these useful vehicles for their dissemination to sites favourable for bacterial invasion.

When, as a result of being "sensitised" with some natural or acquired immune body, bacteria are ingested and subsequently destroyed by any phagocytes which they encounter, what is the extent of the damage attributable to the immune substance? The bacterial surface is altered in such a way that it lowers the surface tension of the phagocyte; that is agreed. But is there not also alteration in the permeability of the bacterial surface, thereby allowing penetration of the phagocytic enzymes? Suppose the sensitised bacterium did not meet with a phagocyte; would it still be viable in the animal body or would it perish owing to altered permeability and damaged organisation incompatible with virulence, just as a pneumococcus loses its virulence when grown in immune serum? These questions are intended to lead to one of a

more general nature. Are there two readily distinguishable kinds of defence in the animal body, one attributable to phagocytes, aided by opsonins or bacteriotropins, and the other of a different and purely humoral nature?

Whilst recognising the importance of phagocytosis, I do not think that this distinction can be maintained, nor that the functions of what are called "bacteriotropins" (including "opsonins") can be sharply demarcated from the functions of other immune bodies.

A bacterium is virulent and resists phagocytosis. Is that resistance the explanation of its virulence? Under the influence of the appropriate immune serum, the bacterium becomes amenable to phagocytosis. Is the therapeutic or protective property of the immune serum due to the production of this change? I do not see any obligation to answer either of these questions by a straightforward Yes or No. I think it would be preferable to short-circuit them, on the ground that they raise false dilemmas. Resistance to phagocytosis may be a secondary attribute of virulence but it is not the primary quality which enables a bacterium to grow *in vivo*. The immune serum is effective because it damages the surface of the bacterial cell and thereby leads to disturbance of the cell's internal organisation upon which virulence depends; then ingestion by a phagocyte serves a useful purpose, but it is not the primary act in the impairment of virulence.

This view is in accordance with the "unitarian" conception of antibodies. The sensitisation of bacteria which makes them amenable to phagocytosis is admittedly of importance; but it is only an incident in the process of resistance to infection and should not be treated *per se* as a special mechanism of defence employed by the animal host.

An alternative view, which seems to me less probable than the above, would be that opsonins or bacteriotropins produce no change in the surface of bacteria except as regards amenability to phagocytosis, just as adsorption of agglutinin by bacteria may cause no interference with the internal structure of the bacterial cell. Then the actual damage to the bacterium would be attributed to the internal secretions of the phagocyte. This view raises an old controversy which, in my opinion, is hardly worth reviving.

Catalysis and Synthesis.

Bacterial nutrition.

The first requisite is obviously the provision of suitable food.

If a strain of diphtheria bacilli is grown on certain media, it may produce relatively little toxin. If it is then transferred to a favourable medium, it may produce toxin in large amount. Anthrax bacilli, when subcultured on ordinary media, generally show no more than traces of capsules. But transfer to serum or to the tissues of a susceptible animal promptly results in development of the typical capsules.

In these two instances, the characteristics of virulence—the toxin and

the capsules—are evidently dependent on nutritive conditions. Incidentally it may be noted, as a fact which is perhaps of greater importance, that residence on the unfavourable medium has not impaired that organisation of the bacterial cell which enables it to produce the toxin or the capsules.

It seems easy to give examples of the simple proposition that bacterial virulence requires the provision of suitable food. But in reality this requisite is often very difficult to analyse because one has to distinguish between "foods" and "accessory factors," *i.e.* between material which is actually incorporated as an essential ingredient in the protoplasm of the fully equipped bacterium and "factors," either chemical or physical, which are necessary to stimulate appropriate bacterial synthesis but are not themselves assimilated as "building stones." I propose to discuss this question in a later section.

The more immediate subject of interest is the fact that bacterial nutrition involves two kinds of selective action, catalytic—for the preparation of food—and synthetic—for bacterial construction. How is virulence related to these two functions?

One may begin by considering to what extent virulence is dependent on the possession of special enzymes.

Taking, first, natural immunity towards bacteria which are virulent for other animal species, it may be asked whether such immunity is due to bacterial starvation arising from lack of appropriate bacterial enzyme. It is doubtful, for two reasons. Differences between natural immunity and susceptibility imply a very high degree of specificity which is attributable to the animal body, not to the bacterial enzymes; one cannot reasonably assume that the latter are endowed with a delicately selective mechanism which enables them to obtain food from one species of animal but not from another. Further, under special circumstances such as overwhelming dosage or exclusion from the general circulation, bacteria may live and multiply in a naturally immune animal.

It is more probable, therefore, that natural immunity is due, directly, to the vulnerability of the bacterial cell-membrane in relation to its animal host and, indirectly, to consequent inability on the part of the bacterial organisation to synthesise material which would form a protective surface.

If a strain belongs to the class of saprophytes, either owing to permanent degradation or because it never possessed any virulence, are its enzymes necessarily different from those of a bacterium which is virulent when introduced into a suitable host? Such a non-virulent strain might very frequently grow as a saprophyte on the surface of a mucous membrane; and its outer membrane would have opportunities, as suggested in my discussion on the acquirement of virulence, of receiving adventitious protection. But virulence would not be acquired. Perhaps this is due to lack of synthetic selective ability. It is also possible that catalysis is at fault; the enzymes of such permanently saprophytic bacteria may not be capable of splitting up animal material, *in vivo*, into suitable food. I am not here dealing with the possibility

that the saprophyte might secrete a toxin which would convert living into dead animal material and thereby make it amenable to the action of the saprophytic enzymes.

But the most important class of bacteria to consider is those which may change both from the virulent to the avirulent condition and from the latter condition to the former. Is this change attributable to a change in the character of the bacterial enzymes?

In many cases there seems no particular reason to suppose that its enzymes, whilst the bacterium is avirulent, are not capable of breaking up animal food in much the same way as the enzymes of the virulent strain. There does, however, sometimes appear to be a direct association between virulence and a particular enzyme, as, for example, when a virulent haemolytic streptococcus loses its virulence and its haemolytic property simultaneously.

I think that this question should be considered in conjunction with the many attempts which have been made to classify bacteria in accordance with their capacities to break up sugars and allied compounds, salts of organic acids, or other carbohydrates. These tests sometimes bring out useful differences between particular strains and, though it is impossible to identify a particular antigen with a particular enzyme or group of enzymes, the distinctions brought out by the "sugar" tests may, in biological respects, be as important as some of the distinctions between antigenic components. Certain of these enzymes, like certain antigens, may be regarded as secondary attributes of virulence, but they are not the primary attributes upon which virulence depends. Synthetic selective action is more important for virulence than catalytic selective action.

Probably the enzymes referred to are not antigenic but are readily destroyed *in vivo*, the normal cycle of events being—secretion of enzyme which acts upon external substrate and then is broken up, ingestion of digested substrate and conversion of some of this material into fresh enzyme, secretion of this enzyme and renewed action on fresh substrate.

Whilst admitting that there are differences between the enzymes of different bacteria, *e.g.* between parasites and strict saprophytes and also between different bacterial species, irrespective of virulence, one is reluctant to suppose that each bacterial type has its special enzyme which breaks up the supply of food into products specially adapted for synthesis with the protein characteristic of that type. These enzymes are not likely to possess the peculiarly differentiated kind of catalytic action which this assumption would imply; it is more probable that they break up their substrate into material which is too elementary to possess any bacterial specificity. It seems safer to ascribe differences in type to differences in protein synthesis, without differences in enzyme action. Specificity would thus depend on the highly selective affinities of side-chains in the growing protein molecules, not on a special equipment for enzyme activity.

A similar view may be taken with regard to the catalytic action of those

permanent constituents of the bacterial cell which are not secreted as enzymes. The special feature of this material is that it acts in two stages. First there is reconstruction (involving catalytic action) of the material presented to it as substrate, and then this action is terminated from time to time by union of the elaborated substrate with the permanent elements of the cell. In this process of building up protoplasm, the second stage, the act of synthesis, must be regarded as specific; but there is no need to attribute a similar specificity to the earlier, or catalytic, part of the reaction.

Antagonistic enzymes.

The struggle of bacteria for existence in an animal host is sometimes regarded as involving a conflict of enzymes. The bacterial enzymes and the animal enzymes are supposed to be matched against each other in their attacks, respectively, on the animal tissues and on the invading germs; the forces on each side are effective in greater or less degree, and the outcome of the infection depends on whether bacterial proliferation is allowed to outstrip the destruction of bacteria or the reverse.

These ideas of "warfare" may be of some interest, provided that they are not interpreted in a literal sense. The interactions are automatic, not purposive, on the parts of both bacterium and host.

As regards merely nutritive conditions, it is obvious that the bacteria cannot thrive unless they can prepare their food by means of their appropriate enzymes; and, if animal enzymes break up the bacteria, this task becomes impossible. There seems no need to elaborate this theme.

A little more may be said about the idea of a "conflict" of enzymes in relation to immunological processes, if one uses the term "enzyme" in the more general sense of a substance which may exhibit catalytic action but does not necessarily resemble the ordinary digestive enzymes in other respects.

Though there is no fully accepted explanation of the way in which antibodies are formed, it appears possible that it may involve a sort of "conflict" between bacterial and animal catalytic action. When bacterial protein is broken up in the animal body, it does not always give rise to antibodies. For example, no demonstrable antibodies may be found after parenteral injection of bacteria into a naturally immune animal. Perhaps the reason is that catalytic agents circulating in the animal's plasma rapidly carry on the disintegration of bacterial protein to a stage where all specificity is lost; hence no specific elements survive to make that firm union with the surface of animal cells which is necessary for the production of antibodies. In other animals, such survival does occur and antibodies are formed with greater or less readiness, varying according to the individual as well as according to the species. Perhaps these variations depend upon differences in the potency of the animal's enzymes, resulting in differences in the quality and quantity of the specific bacterial elements which survive, with the retention of certain catalytic properties, and become attached to animal cells.

These cells are probably endothelial. But I do not think it necessary to postulate that the endothelial cell, owing to the stimulus of the adsorbed bacterial antigen, proceeds to manufacture and secrete the appropriate antibody. That, I think, would be demanding rather too much from the internal equipment and organisation of endothelium[1]. It seems to me simpler, as I have suggested elsewhere[2], to adopt a filtration theory. The adsorbed antigen acts as a selective catalytic agent upon the fluids which pass through the endothelial filter and changes their chemical configuration in such a way that they become antibodies.

Here, then, the "conflict" results in the survival of more or less effective bacterial "enzymes," which, in this blind sort of "warfare," proceed to manufacture antibodies as "weapons" against their own bacterial protein. The end result, when immunity is attained, is the establishment, in the constituents of the animal's plasma, of a new equilibrium which is unfavourable to the vitality of the bacteria in question.

But perhaps the main centre of interest is not so much a "conflict" but turns rather on the variable relationships between enzyme and substrate.

Enzyme and substrate.

In the formation of antibodies it appears probable that the antigen, on becoming attached to animal cells, behaves as a catalyst. When antibody is brought into contact with free antigen, chemical union is only the primary event. What follows? It is usually taught that antibodies are enzymes, produced by the stimulus of foreign protein introduced parenterally and equipped with the special function of digesting such protein. Such enzyme activity certainly seems suggested by the bacteriolytic antibodies. Thus the former function of the antigen is reversed, it is now substrate instead of being catalyst.

But antibodies do not always resemble enzymes. In precipitation, for example, the antiserum appears to behave as substrate and the antigen as catalyst. In specific precipitation of pneumococcal antiserum this is the relationship which is observed; but, when the growth of pneumococci is modified by the specific action of antiserum, the antiserum is the catalyst and the pneumococci are the substrate. So the same antiserum may exhibit sometimes the one function and sometimes the other.

In other instances, as in neutralisation of toxin by antitoxin or in bacterial adsorption of agglutinin, the relationship of enzyme to substrate does not appear to hold good for either antigen or antibody.

These variable relationships between enzyme and substrate are certainly of interest in the "conflict" between bacterium and host, but, as I suggested

[1] For the view that antibodies are internal secretions of the reticulo-endothelium which pass directly into the blood-stream the reader may be referred to Bieling, *Zeitschr. f. Immunitätsforsch. Orig.* **38**, p. 193, 1923.

[2] *J. Hygiene*, **22**, p. 355, 1924.

above, this "conflict" is better expressed in terms of catalysis and synthesis. There is catalytic action of the bacterium on the host which may be divisible into the breaking up of material which it can synthesise as food, destructive action on the tissues of the host, and the formation of antibodies. There is catalytic action of the host on the bacterium which consists of changes commencing at the bacterial surface. The sequel to these changes is "conflict" within the bacterium between synthesis and catalysis.

The above considerations suggest that the resistance of the host depends not so much on antibodies as on the possession of effective catalytic activity, whilst the resistance of invasive bacteria depends on effective synthesis rather than on destructive action.

Bacteriophage.

There is another possible sort of antagonism, which has nothing to do with animal enzymes but suggests that bacteria are liable to be attacked by autogenous enzymes.

I refer to the agent which is commonly called "bacteriophage." I agree with the majority of authorities that this substance is not a living parasite, and I think it is unnecessary to be detained by the controversy which is still carried on about this issue. The subject of immediate interest, in discussing bacterial capacity to act sometimes as a catalytic agent and sometimes as a substrate, is that, in the phenomena of transmissible autolysis, bacteria both produce "lytic substance" and are lysed by this substance.

This lysis differs in its origin from the ordinary autolysis of dead bacteria. The latter event may be ascribed to the action of the enzymes which are normally prepared and secreted by the bacteria and break up material in the environment into suitable food. Like other enzymes, they do not attack the cells which produced them until these cells are dead. But "lytic substance" only attacks living and growing cells and is thus sharply distinguished from the ordinary enzymes, though, after the onset of death, the latter may assist in the final act of lysis.

"Lytic substance" is also much more highly selective in its action than an ordinary enzyme. The range of specificity varies. Sometimes it is confined to the bacterial strain from which it was derived; sometimes it acts on several types of the same species; and, not uncommonly, it may select one or more strains of bacteria belonging to different species. One cannot well attribute an equally high degree of selective capacity to the enzymes which are secreted by the bacterium and act on its environment.

Bacterial growth involves the replacement of intracellular enzyme activity by intracellular synthesis at the appropriate time. "Lytic substance" disorganises this sequence of events. It interferes with the internal organisation of the nascent daughter-cells at an early stage of their synthesis and before they have become viable, probably by creating in the interior of the cell a condition more favourable to hydrolysis than to dehydration and thus

reversing the intracellular transition from enzyme activity to synthesis. It thus differs from an ordinary enzyme in its mode of action as well as in its greater specificity. It is probably not a secretion, in the sense of something specially elaborated by the cell, but an abnormal product of disintegration.

Agents which cause interference with synthesis during bacterial growth may be regarded as stimulants to bacterial variation. Slight interference may prevent full development of the equipment for virulence; somewhat greater or more prolonged interference may completely inhibit this equipment, though not interfering with purely vegetative capacities; still more drastic interference, as when an appropriate "lytic substance" acts upon non-resistant cells, completely paralyses synthesis and allows enzyme action to run on to autolysis.

On the other hand, stimulants to variation may have the opposite effect upon bacterial synthesis. They may enable the bacterium to elaborate products which confer resistance to its environment. This is one of the properties of the agent to which the somewhat misleading name of "lytic" substance has been given. It may stimulate the growth of bacterial variants which are particularly resistant to lysis.

Comment.

In simple experiments *in vitro*, an enzyme is a catalyst which accelerates a reaction between two known substances but remains unchanged, both in quantity and quality, at the end of this reaction.

Catalytic action is essential to all vital processes, but the conditions in the living body are extremely complicated. There is an indefinitely large number of different catalysts. The reactions which they accelerate take place between a large variety of substances which are generally complex and often of unknown chemical constitution. The catalyst operates in a highly complex medium and its activity may be affected by imperfectly known, or by quite unknown, changes in the physical condition of this medium. The catalysts are labile, some more labile than others; so the amount of substrate which they can break up is not indefinitely great (until a state of equilibrium is reached) but depends on their degree of lability and on the rapidity of their renewal. The catalyst is also liable to qualitative change, one of the most important of such changes being its permanent union with substrate and consequent cessation of its former catalytic activity. Another difficulty is that it is often impossible to decide whether a substance ought to be called a catalyst or not. It may produce a chemical change in its supposed substrate and this change may give rise to further changes; but such a sequence of events may be different from the catalytic acceleration of a particular reaction. In other cases, the questionable property may be rather of a physical nature. If it sets in motion some cellular activity which would not take place otherwise, one would call the agent a stimulus rather than a catalyst. But suppose such activity is already in progress at a slow rate and then some influence supervenes which

accelerates it; it is often difficult to decide whether one should lay emphasis on the catalytic action or on the stimulation. The phenomena of "bacteriophage" provide special examples of the difficulty in distinguishing between a catalyst and a stimulus.

As regards the conception of "antagonistic" enzymes, it seems that the fate of a bacterium depends not so much on a conflict of enzymes as on the state of equilibrium which determines whether the bacterium can complete its synthetic activities before being acted upon as substrate.

It is evidently impossible to effect any simple correlation between enzymes and virulence. Still, the subject demands analysis because virulence is concerned with vital processes which are intimately dependent on catalytic action.

But catalysis and synthesis do not explain everything. A merely chemical conception of the material which passes into the bacterial cell, as being either suitable for synthesis or inadequate or actually injurious, does not seem sufficient to account for the puzzling qualitative changes which may occur in virulence. Here it seems necessary to fall back upon the vaguer physiological conceptions of qualitative differences in the stimulants which gain access to the interior of the cell and produce modifications in its organisation.

ACCESSORY FACTORS.

Adjuvants derived from the bacteria.

The usual distinction between a food and an adjuvant is that the former provides material which is incorporated within the bacterial cell, whereas an adjuvant is not utilised in this way but is helpful, or necessary, to bacterial growth as an agent which acts as a stimulus or removes an inhibitory influence.

But it is not always easy to decide which is the more appropriate term. Growth may fail when a culture medium is seeded with a very small number of bacteria but it may take place without difficulty when a larger number of organisms is introduced. It is probably correct to say that such growth requires a stimulus, provided as a bacterial secretion, which is not effective unless there are enough bacteria to produce it in sufficient concentration. But "cannibalism" has also to be considered. At first the bacteria are unable to grow in their strange environment; some of them die; the survivors obtain suitable food from the bodies of their dead companions and, if given enough of this nutrition, produce more vigorous offspring which are able to assimilate their artificial environment.

To take another example, F. Griffith has found that avirulent pneumococci may regain virulence *in vivo* if the animal also receives an inoculum of dead, virulent pneumococci. It is possible that the soluble substance associated with the dead cocci may behave as a sort of adjuvant. Being adsorbed by the surface of the avirulent cocci, it may alter the selective permeability of that surface and, in consequence of this change, the internal organisation of

the cell may now be enabled to effect the syntheses requisite for virulence. Or, as F. Griffith has suggested to me, the influence of the dead material may be more direct; the avirulent cocci may use it as food which is easily synthesised into that soluble substance requisite for their virulence.

There is a further distinction between a food and an adjuvant. The latter, when it is a chemical substance, is effective in much smaller amount than the former and is less readily used up. But here, again, discrimination between the two is sometimes impossible. It may be necessary for every fully equipped cell to assimilate a particular substance and to retain it for the exercise of certain internal functions; in such cases, though a very minute quantity may be sufficient, the substance must be regarded as a food and not merely an adjuvant.

Though one cannot always be sure whether a substance is merely an adjuvant and nothing more, the principal fact remains that there is an important, but very imperfectly defined, class of substances which act mainly as adjuvants, *i.e.* as accessory factors not incorporated in the structure of the bacterial cell.

Culture of certain bacteria or spores is injected into an animal and readily produces infection. In a control experiment with the same culture washed free from the extra-bacterial products of its former growth, the inoculation fails. The material which promotes growth *in vivo* in the former experiment is an example of an adjuvant derived from the bacteria.

But it is sometimes impossible to decide whether adjuvants of bacterial origin promote growth by direct action on the bacteria or whether they assist the bacteria in an indirect way. Exotoxins and liberated endotoxins are obvious examples of the latter kind. Such material favours the bacteria by lowering the host's resistance and also by causing damage to the tissues which makes them available as bacterial food or as accessory factors for the promotion of bacterial growth, the direct adjuvant then being of animal origin.

To return to direct adjuvants of bacterial origin, is promotion of bacterial growth their only function? Probably not; they may also function as haptenes and, in this way play a part of considerable importance in immunological processes. Though free haptenes do not produce antibodies, it does not follow that union with bacterial substance is the only condition which makes them fully antigenic. It is possible that they may acquire this property by union with substances derived from the animal body. Such antigens, produced by a combination of bacterial and animal products, may produce antibodies which unite *in vivo* not only with the completed antigens but also with the free haptenes, thereby interfering with the latter's function as adjuvants to bacterial growth.

Interplay between such haptenes and these hypothetical antibodies may thus be of importance as causing disturbance of the balance between the synthetic capacities of the bacteria and the catalytic capacities of the animal

host—a disturbance associated with changes in hydrolysis, dehydration, and the hydrogen ion equilibrium.

How does this view stand in relation to the "aggressin" theory? A virulent bacterium must be able to grow and to maintain its appropriate structure *in vivo*; is it also requisite for the virulence of invasive parasites that they must be able to secrete material which has an antagonistic action on the cells or fluids of its host?

Those who support the "aggressin" theory or some modification thereof would return an affirmative answer. According to this theory, invasive bacteria manufacture in the animal body a special product ("aggressin") which opposes the resistance of the animal host. The substance is antigenic and may be neutralised by its corresponding antibody. Virulence depends upon "aggressin," acquired immunity upon "anti-aggressin."

A few years ago I discussed this theory in detail[1]. My conclusion was that its value was still an open question; it had neither been proved nor refuted, but still remained of interest as an attempt to explain some obscure problems of immunity which could not be accounted for in terms of the better accredited antigens and antibodies.

The term "aggressin" is not attractive, because it is a reminder of old and tedious controversies which nobody wishes to revive. It has another disadvantage. Those who retain some belief in the main principle of the theory still maintain that "aggressins" are neither exotoxins nor endotoxins but special products, of a different nature, elaborated by the bacteria.

But, if "aggressins" are not toxic agents, why not abandon this bellicose word and discard the idea that they are special instruments for "attacking" the animal host and that they are, in turn, "counter-attacked" by a specially manufactured antibody? Why not regard their "hostility" as meaning no more than that they are adjuvants to bacterial growth *in vivo*? "Hostility" towards the host would then be a property of the bacteria but not of these adjuvants.

Then does this mean that the theory is quite devoid of value? No. One may retain the conception of special substances and special antibodies which are immunologically important. My suggestion is that the special substances are bacterial adjuvants which are also haptenes and may become fully antigenic, and that their special antibodies protect the animal host by reacting with these adjuvants.

Adjuvants derived from the animal host.

A study of virulence must not overlook the fact, which is not sufficiently appreciated in the "aggressin" theory, that the animal body, instead of being antagonistic, is the ideal environment for some kinds of bacteria. For some species, growth *in vitro* seems to be impossible or can only be accomplished by the addition of some food or adjuvant of animal origin. Owing to its animal

[1] *Ministry of Health Reports on Public Health, etc.* No. 22, 1923.

origin, such material cannot be antigenic in the infected host; so conceptions of "aggressins" as antigens and "anti-aggressins" as their antibodies (or any modifications of such ideas) are inapplicable, though the material does, in fact, make the bacteria "aggressive" towards their host.

There is again a difficulty similar to one mentioned in the last section. It is sometimes impossible to decide whether an accessory factor of animal origin is incorporated within the bacterium as food or serves merely as a stimulus; it may exercise both functions. To revert to the experiment showing that washed spores fail to grow *in vivo* without some assistance, the requisite help may be provided by applying some chemical irritant or stimulus to the animal tissues. Then it is the damaged tissue which initiates growth and its action may be nutritive as well as stimulative. Still, the fact remains that stimulation, apart from nutrition, is an important property of animal adjuvants.

Adjuvants, peculiar to the animal body and necessary for the growth of certain parasites, appear to be of two kinds. Some are only dynamic in the living animal, probably because their properties depend on the physico-chemical activities of living matter. This may be the reason why some parasites will only grow in living tissues. Other stimulants can be extracted from animal tissues as dynamic chemical substances and will activate bacterial growth *in vitro*, *e.g.* derivatives of red blood corpuscles.

It must be assumed that there are many different varieties of these accessory factors, that they act differently on different bacterial species, and that they may behave in different ways towards the same species. Their influence on the bacteria may sometimes be indirect, as when they remove an inhibitory influence such as an accumulation of peroxide. When their action is direct, it may affect sometimes one bacterial structure or function and sometimes another. Hence their relations to virulence are not always the same.

Can their influence on virulence be distinguished from their action as stimulants to growth? When the infective organisms are exclusively parasitic in the animal body, it may be impossible to discriminate between the two kinds of influence. But there may be a subtle difference. As I have already pointed out, virulence may undergo a qualitative change *in vivo*, owing to the specific influence of the animal environment or as a consequence of the introduction of a drug which acts indirectly by producing a change in the animal elements. In such cases there may be a distinction between the merely nutritive stimulus of the accessory factors and their effects upon virulence.

With bacteria which grow on artificial media as well as in the animal body, the influence of the accessory factor upon virulence may be more clearly defined. Without this factor, growth may still be abundant but it may be a defective growth, involving loss of one or more functions, of which virulence is the most important. Such loss may be permanent or the virulence may be

restored on adding to the medium an animal extract containing the requisite accessory factor. The bacterial organisation is sufficiently fixed to carry on the saprophytic processes of continued existence, but it is labile in its capacity for the more elaborate processes of synthesis requisite for virulence. In other cases, the accessory factor is necessary for all the functions of the bacterial organisation and growth is impossible without it, even *in vitro*.

At the other extreme there are the bacteria which, without losing their pathogenicity, grow vigorously on ordinary culture media and sometimes on simple synthetic media when given an abundant supply of oxygen. Here there is no proof that either growth or virulence requires special accessory factors derived from the animal; absence of effective antagonistic influences may seem sufficient to explain bacterial capacity for invasion, together with retention by the bacterium, when living *in vitro*, of a fully equipped internal organisation and a protective outer membrane. But perhaps some further explanation is needed for the remarkable fact that bacteria can adapt themselves so readily to the differences between the saprophytic and the parasitic methods of metabolism. It may be that, when a bacterium is suddenly called upon to live as a parasite, its own resources are insufficient, to begin with, and assistance is obtained from accessory factors provided by the animal. These factors, by furnishing food or by acting as stimulants, would accelerate bacterial growth at the critical stage where the fate of the bacterium depends on the time factor. If growth of the individual bacterium is too slow, it succumbs to its strange environment before it has completed the syntheses requisite for virulence.

As regards their origin, there is an important distinction between adjuvants derived from the bacterium and those derived from its host. But one must remember that each class of material is subjected to a succession of interactions in the animal body and one cannot assume that it must remain completely unchanged in the process. The former class may acquire the impress of their animal environment and the latter may be modified by interaction with the bacteria. So it may not always be possible to maintain a sharp differentiation between the two on the ground that the former, being foreign to the host, may be antigenic, whilst the latter cannot be antigenic because it is a normal product of the host. Absence or loss of such antigenic properties would be favourable to virulence; retention or acquisition of them would tend to create an antibacterial influence.

SUMMARY.

Bacteriologists are not in a position to disregard the limitations recognised by the physiologists. Precise data which are available from experiments are far from sufficient to explain the properties of living matter; they must be supplemented by vaguer conceptions about the functions of a cell, its internal organisation and its susceptibility to stimulative or inhibitory agencies—conceptions which cannot yet be translated into recognised chemical substances and physical properties. One must therefore start by recognising

that bacterial virulence, being dependent on vital processes, cannot be fully explained in chemico-physical terms.

The most valuable laboratory data of an exact nature are the extremely delicate selective reactions between bacterial antigens and antibodies. But the serologist's work has become highly complicated and many of the antigens which find a place in his analytical classifications are not likely to indicate features of bacterial structure which are directly concerned with virulence.

In the first place, the special combining affinities of antigens are attributable to what Landsteiner has termed "haptenes," the behaviour of which depends on their chemical environment. Differences in antigenic activity which are revealed in the serologist's "antigenic complex" do not necessarily denote structural differences due to haptenes of different chemical constitution.

There is another respect in which antigenic substances are of unequal value as an index of the constitution of the bacterial cell. The serologist is concerned with the presence or absence of demonstrable antigens but not with their mode of origin. The question of origin is, however, of physiological importance. Individual bacteria are organised structures which elaborate certain products, some of which are haptenes. Such products may be retained by the bacterium and become fully equipped antigens after union with bacterial protein. Antigens formed in this way I have called "elaborated" antigens. But the protein which constitutes the elementary structure or machinery of the cell possesses antigenic properties of its own, when not united with the products which it may elaborate. Antigens attributable to this protein structure alone I have termed "elementary." This distinction is of importance in relation to virulence and immunity, though it does not emerge in the serologist's "mosaic," which presents all antigens as being on the same plane.

Elaboration of a product by the bacterial cell is not a single act but a succession of processes which may not always be carried on to the final stage. When such products are haptenes, there is therefore a possible distinction between their characters in the earlier stages of their development and in their completed form. Thus the same haptene may differ markedly in its antigenic behaviour in different developmental stages. Such differences perhaps help to account for some of those puzzling facts about the emergence and disappearance of antigens which have been observed by serologists in cultures of the same bacterial strain.

Sometimes, then, antigenic differences do not connote participation of a new chemical element in bacterial structure. But this is not always the case. In building up its protoplasm, the bacterium does not always synthesise its material in exactly the same way; and, as the material presented for synthesis is not invariably the same in every respect, the elements which are utilised may sometimes differ in chemical character. Antigenic differences may be attributable to these circumstances. But there is no strict parallelism between the differences in structure which they reflect and differences in function.

Incorporation of new elements within the bacterial cell, with consequent alteration in the cell's internal organisation, is one way of accounting for variation. The range of variation is limited by the fact that only those changes are possible which are compatible with the viability of the cell; and viability is impossible without retention of some degree of physiological specificity.

It is easy to understand changes which merely involve degradation, as when the influence of environment causes a bacterium to lose its virulence and its antigenic "type" characters. But bacteria, after degradation to the non-pathogenic stage, sometimes reacquire virulence, together with antigenic characters associated with virulence. It does not seem necessary to assume that these fresh antigenic properties must be the same as those which the bacteria possessed before degradation; therefore transition from one type to another seems theoretically possible, not *per saltum*, but through an intermediate stage of degradation.

Particular antigens may be a secondary attribute of virulence, but they are not the cause of virulence. Pneumococci may be identical in virulence though possessing different specific antigens. Chemical extraction of a particular haptene does not explain virulence; nor does serological "relationship," as indicated by partial identity of antigens, help to decide whether a particular bacterium will share the property of virulence which its serological "relative" is known to possess.

Confusion as to the relation of specific antigens to virulence need not arise if one discriminates between the biological aspect of bacteria as living organisms and their analytical aspect as a composite of various chemical structures. In illustration of this distinction I have referred to experiments on the therapeutic action of drugs in certain parasitic infections. The drug does not effect direct combination with some chemical structure in the parasite; *i.e.* chemical structure is not the key to virulence. The drug acts indirectly, probably by effecting a change in the fluids or tissues of the animal host, with the result that there is a modification in the vital processes of the infective agents; *i.e.* virulence depends on a biological factor, the internal organisation and metabolic activity of the micro-organisms.

The difficulty of correlating virulence with particular antigens does not detract from the high importance of reactions of the antigen-antibody type in infection and resistance. In such reactions, selective chemical union is only the primary event. Though this may be the matter of main interest to the serologist, the succession of changes which follow this union is of chief importance in relation to bacterial growth and virulence. How is the selective chemical combination going to affect bacterial metabolism?

Metabolism depends, in the first place, on the condition of the bacterial surface, which functions as a selective filter, facilitating the admission of appropriate food and excluding injurious influences. Efficiency in both respects is requisite for virulence. With some species, provision of material for synthesising a capsule seems to be the more important; other species

depend less on the protection of their outer membrane and mainly on ability to produce and secrete a toxin.

When the bacterial surface is injured by an antibody, one result may be lowered resistance to phagocytosis. But probably other changes also occur which are detrimental to virulence and are independent of the acquired capacity to lower the surface tension of a phagocyte. Resistance to phagocytosis is a secondary attribute of some species of virulent bacteria but is not the primary quality on which their virulence depends.

Passing now from the surface to the interior of the bacterium, it is to be noted that bacterial nutrition involves two kinds of selective action, catalytic—for the preparation of food—and synthetic—for bacterial construction. Virulence is related to both these functions but appears to be more closely associated with the latter. There is no good reason to suppose that natural immunity means that the bacteria die of starvation because their enzymes are incapable of breaking up the animal material into food; loss of vitality is better explained by damage to their mechanism for synthesis. And so with susceptible animals; when they recover, it is not because the bacterial nutritive enzymes have been destroyed or inhibited but because there has been interference with the construction of bacterial protoplasm. Virulence depends on retention of physiological specificity, which is manifested in synthetic rather than in catalytic selective activity.

Apart from enzymes needed for bacterial nutrition, there is the question of "antagonism" between bacterial enzymes which damage the host and animal enzymes which are harmful to the bacteria. Ideas suggestive of purposive "warfare" are misleading, but there may be said to be a sort of "conflict" between the catalytic capacities of bacteria and host. If the bacteria are destroyed rapidly by catalytic agents derived from the animal, they are unable to behave as antigens; if antigenic capacity is retained *in vivo*, then, by catalytic action on the plasma or tissues, the bacterial elements proceed to manufacture antibodies as "weapons" against their own bacterial protein.

In catalytic reactions between host and bacteria, sometimes the former is the substrate and sometimes the latter. The sort of "conflict" which is here suggested is better expressed in terms of catalysis and synthesis. Catalytic action of bacterium on host includes preparation of food, destruction of tissue, and formation of antibodies. Catalytic action of host on bacterium alters the bacterial surface; and the sequel to this change is a "conflict" within the bacterium between synthesis and catalysis. The resistance of the host depends mainly on effective catalytic activity, that of the bacteria on effective synthesis.

An interesting example of intrabacterial oscillations in the equilibrium between synthesis and catalysis is afforded by "bacteriophage," which, to some extent at least, behaves as an autogenous enzyme. But "bacteriophage" does not affect exclusively the catalytic side of the balance; instead

of causing bacteriolysis, it may enhance the synthetic capacities of the bacterium and so lead to the production of a race which is highly resistant to lytic influence.

There are other respects in which "bacteriophage" does not resemble an enzyme. It is much more highly selective in its action and it differs from enzymes in that it operates only on living material. It acts as a stimulus to variation.

Stimulants cannot be fully explained in physico-chemical terms as agents affecting synthesis or catalysis. They may produce qualitative changes in the internal organisation of the cell which cannot be analysed but must be accepted as physiological facts.

Stimulants to bacterial growth *in vivo* are roughly divisible into those derived from the bacteria and those provided by the host, though this distinction is not always valid, since some of them may be products of interaction between bacterial and animal elements.

Some of the stimuli derived from the bacteria may be present *in vivo*, first as free haptenes and then as antigens formed by their union with animal protein. The antibodies produced by these antigens would combine with free haptenes and thereby rob the bacteria of their adjuvants to growth. This suggestion may serve as a substitute for the old theory of "aggressins." The "aggressins" are not aggressive towards the host but are adjuvants to bacterial growth and are also haptenes; the "anti-aggressins" are antibodies to these adjuvants.

With some bacterial species, adjuvants of animal origin are essential for growth. Animal adjuvants may also be useful to initiate and accelerate growth when bacteria which grow well on ordinary media are transferred from culture to the animal body.

What are the main features which emerge from this review of virulence?

Virulence is complex because it depends not only on the chemical structure of the bacterium and its products but also on the mechanism of its growth and the development of its synthetic and catalytic activities.

So far as chemical structure is concerned, a considerable amount of information can be obtained by direct chemical analysis of bacterial constituents and by application of the more delicate serological tests, though these data do not suffice to explain biological processes.

Antigen-antibody reactions are also useful indirectly, as providing concrete examples of a much wider range of selective reactions which probably occur *in vivo*. I refer to reactions in which the bacterial element need not be an antigen, according to the immunologist's definition; the substance which reacts with it need not be an antibody, in the sense of something produced by a bacterial antigen; and the "anti" conception is inappropriate, because the animal element is not necessarily injurious to the bacterium. But the main fact remains that many of these reactions must be attributed to highly selective affinities which are similar to those demonstrable in ordinary serological work.

These selective reactions are only the commencement of a series of events which then follow in a particular sequence, determining bacterial growth and equipment. Virulence depends on the nature of this sequence, not merely on the initial chemical or physico-chemical reaction which has served as a stimulus.

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