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COMBINING AFFINITIES IN BACTERIAL VARIATION AND CARCINOGENESIS.

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I. BACTERIAL VARIATION.

MY purpose is to discuss "combining affinities" as expressions of the principles which are gradually emerging out of the ever increasing accumulation of laboratory data about variation. At the present stage these principles cannot be expressed with any air of finality; they will need frequent revision and reconstruction in the light of new experience. But the study of bacterial variation has already arrived at the interesting point where the appearance of a variant is to be regarded not merely as a fact which must be recorded but as a

fact which ought to be explained. Where possible, one would like to express the vague postulate of "stimulants to variation" in more concrete terms of "combining affinities."

The "base line" to start with is recognition that not variation but homogeneity of growth is, and will always remain, the more significant and generally the predominant feature of bacterial propagation. If one could understand the laws which make bacteria "breed true," variation would be a much simpler problem. These laws, being concerned with vital phenomena, are still obscure to a large extent; but it is at least possible to say something about the mechanism of living matter in relation to homogeneity of growth.

The next step is to consider some of the simpler kinds of variation, (*a*) where the change is directly attributable to an intrinsic capacity of the bacterium, and (*b*) where it may be directly ascribed to the influence of some recognisable external agent.

Then come the more complex conditions, *e.g.* in variations of invasive virulence and in bacteriophage phenomena, where there is an interplay of internal and external factors.

Some recent views on combining affinities.

Synthesis.

Homogeneity of growth implies that the bacterial cells must be uniform in their synthetic capacities, so that the "building stones" which are presented to them are always elaborated into the same kind of material.

The postulate of a very large number of specifically selective enzymes, existing as fixed constituents within the cell and each choosing independently its own material for assimilation, is not enough, because it does not explain the mechanism of the controlled sequence of events requisite for normal growth. And, after allowing for the extreme chemical complexity and multiplicity of cellular constituents, it seems arbitrary, and perhaps unreasonable, to assume that a separate chemical entity exists for each of the diverse activities exercised by the growing cell. There is the danger that unlimited coinage of enzymes will lead to debasement of the currency.

The requisite hypothesis must involve the vitalistic factor in some form or other. Expressed in general terms, it implies the conception of a regular cycle of changes in the substance of the growing bacterium. In one phase of growth, one particular "building stone" is selected for synthesis, with the rejection of all others; in the next phase, another constituent is chosen, and so on. On this view, periodic or rhythmic change is a property of living protein and is the essential and primary attribute which regulates growth.

This conception may be developed in a variety of ways. First I select from Raper's address[1] on "The Synthetic Activities of the Animal Cell" certain remarks which are also applicable to bacterial growth:

[1] *Lancet*, September 6th, 1930, p. 509.

"The extreme specificity of the reaction which necessitates that at a given phase of the synthesis one particular amino-acid and that one alone can be added to the next link in the molecule, requires such a multiplicity of enzymes and such a remarkable degree of control of their action as to be almost outside the range of probability.... The experiments of Willstätter and others have shown that to some extent the specificity of enzymes is accounted for by the 'carrier' with which they are associated. It is not inconceivable that a catalyst capable of bringing about the union of amino-acids in the living cell and ultimately fashioning its protoplasm may be attached to or associated with a 'carrier' which, instead of having a fixed configuration, as with the enzymes that we can extract from the dead cell, has one which is continually varying, this dynamic state being characteristic of the living material of the cell. If, further, we could assume that the variations in the configuration of the 'carrier' were cyclic, always going through a definite series of phases, it might be possible to account for the fact that at any particular phase of the cycle the configuration would be such as to favour the synthetic union of one particular amino-acid rather than any other because of its spatial arrangement."

For other views on the structure of living matter I call attention to the valuable discussion by the Faraday Society[1], in which the opinions of many authorities were expounded and criticised. Here I must limit myself to mention of a few salient points.

It seemed to be the prevalent opinion that the vitalistic property which regulates growth must reside in protein. Peters, for example, suggested that, as the true stamp of cellular individuality is borne by the proteins, "it must be the proteins which are the responsible directive agencies"; and Lloyd expressed the same view.

As to the mechanism of control, there were sharp differences of opinion, which appeared to depend mainly on the relative importance attached to (1) "the normal architecture of the cell" and to (2) the significance of "dynamic equilibrium." Peters constructed his hypothesis almost entirely upon (1) and elaborated a highly complex conception of a regulative "chemical architecture" permeating the entire cell. Woolf took the opposite view (2). He denied the need "to invoke structure in protoplasm in order to explain the course of chemical events" and maintained that their control "is effected by the enzymes, mutually influencing one another by means of their several reaction products." In this way he explained the possibilities of self-regulation. "When the totality of chemical events in the cell is considered... it seems that there is enough possibility of mutual control to account for the observed orderly nature of affairs, without recourse to further hypothesis."

Hopkins said: "We have come to believe that the living cell, considered from its most general aspects, is a system in which surface catalysis controls many and diverse chemical events, while the high degree of co-ordination and organisation to which these events attain may be due in some way to the nature and architecture of the colloidal apparatus in which they progress."

The above quotations will suffice to show that the synthesis of living matter is now a subject which is definitely within the sphere of serious scientific consideration.

Surface activities.

Enzymes. Homogeneous growth implies that there is uniformity of the enzymes on the bacterial surface which catalyse the material to be utilised as food. Recent research has thrown new light on the probable nature of these catalytic activities and has provided a conception of combining affinities which, as in the

[1] *Colloid Science applied to Biology*, London: The Faraday Society, 1931.

case of synthesis, seems preferable to the older idea that there is an extremely large number of chemical entities acting as surface enzymes. Brief reference must here be made to two authorities, Quastel and Rideal.

In a growing culture it is often difficult to observe or analyse catalytic action because catalysis is promptly followed by synthesis and hence the products of catalytic action are not identifiable. Quastel has therefore devised methods for studying bacterial enzymes in the absence of growth[1], *i.e.* when the bacteria are placed under such conditions that, in the time required for the experiment, little or no growth takes place. Such bacteria, which he calls "resting," in a special sense of the word, "are simply bacteria in a state of non-proliferation and may be investigated in a manner similar to enzymes or catalytic systems." Working by this method, Quastel finds that the number of specific enzymes apparently possessed by bacteria is very large. For example, at least 56 are demonstrable for *B. coli* as specific "hydrogen transportases." Quastel remarks that "the actual existence in one cell of such a large number of specific enzymes dealing with but one type of phenomenon seems very doubtful." This and other considerations have led him to abandon the view that each enzyme is a special chemical entity. In its place, he substitutes a chemico-physical conception of "activating centres" on the surface of the cell. "If the enzymic activity of the cell be considered as due to the active centres which form part of the colloidal aggregates of the cell, there is no necessity to regard the cell as elaborating numerous specific molecules each possessing a specific activating action on a particular substrate....The centres are simply a property of the surface structures of the colloidal materials which make up the cell as a whole."

A somewhat similar view is expressed by Rideal[2]. He postulates a "mosaic" of different combining groups on the bacterial surface. "Whenever on the surface of the complex mosaic containing both protein and carbohydrate there appear a certain number of reactive groups, *e.g.* $-CHO$, $-COOH$ and $-NH_2$, in a particular configuration, these form the 'enzyme' surface, capable of causing reaction in adsorbed substances of suitable stereo-chemical configuration and containing likewise suitable and suitably spaced reactive groups." This view that each "enzyme" is "merely a special grouping of active groups in the mosaic" dispenses with "the necessity of elaboration by the micro-organisms of a great variety of distinct and complex chemical compounds." It will be noted that "mosaic" here means an arrangement of physiological units, which is quite different from the more familiar "mosaic of antigens" postulated in serological analysis.

Rhythmic change. When a bacterium is growing it is to be expected that the chemico-physical structure of its surface will vary in different stages of development. May some of these changes be of an orderly and rhythmic nature, like those suggested in discussing synthesis? If they are, they must be regarded as normal and not as an expression of variation. Opinions differ on this point, which raises a question of frequent occurrence in the interpretation of variation. Is a particular bacterial phase a transitional feature of normal development or is it a fixed attribute of stabilised growth? A good example of the debate on this subject is provided by the controversy about peculiarities observed in the rate of disinfection *in vitro*. Are these peculiarities due to transitional phases of homogeneous growth or are they caused by the emergence of variants?

This interesting problem has been reviewed recently by Miss Chick[3]. To raise the main question in its simplest form, when a culture of bacteria, obtained under conditions which

[1] *J. Hyg.* **28**, 139, 1928.
[2] *Med. Res. Counc. Bacteriology*, **1**, 138, 1930.
[3] *Ibid.* **1**, 179, 1930.

ought to promote uniformity, is exposed to the action of a disinfectant, how is it that the germs are not all killed in the same time?

The readiest answer would be that it is the normal tendency of a growing culture to produce variants, some of which are more resistant than others. As Miss Chick points out, this explanation raises difficulties. In particular, it is not easy to reconcile with the logarithmic character of the death-rate which is so frequently observed; *i.e.* if the death-rate is taken at equal and successive intervals of time, the number which die in any one interval tends to be a constant proportion of those alive at the beginning of that interval. On the hypothesis of variable resistance, as due to definite bacterial variants (a few germs very sensitive, a few very resistant and the majority in an intermediate condition), the death-rate would be likely to increase after the first interval and it would not be likely to give a logarithmic curve.

The alternative view rejects the postulate of variation and regards the individual bacteria as being essentially similar to each other, particularly when the logarithmic survivor-curve is clearly exhibited. The explanation offered is that the bacterial protein undergoes an orderly succession of rhythmic changes, being susceptible to the disinfectant in one phase but not in another. The condition of the proteins is thought to be analogous to that of cane sugar when subjected to hydrolysis; only a proportion of the sugar molecules are, at a given time, in the condition which enables them to unite with water and this proportion bears a constant relation to the concentration of unchanged molecules. "One must assume," says Miss Chick, "that protein molecules in a living organism are not free protein molecules as in a solution of egg albumin, but are less independent one of another, and undergo their rhythmic changes not separately but in some way as a whole, so that at any moment of time all or none are open to attack. This hypothesis as to the differences between 'dead' protein and 'live' protoplasm is, however, without experimental foundation."

There are a good many hypotheses about the condition of growing bacteria where the support of experimental evidence does not amount to rigid proof. But it does not seem permissible to reject this particular one on that account; it appears to be compatible with the facts, whereas the alternative (variation), though it cannot be easily dismissed, seems more difficult to reconcile with accepted laboratory data. The former has also the advantage of being in harmony with the postulate of rhythmic changes in bacterial synthesis which has already been discussed.

Cyclogeny.

Perhaps I ought to conclude this section with brief mention of the hypothesis usually termed "cyclogeny." This conception, though attractively enterprising, is not in accordance with my views as to the conditions regulating homogeneous growth which are to be taken as the "base line" in the study of bacterial variation.

"Cyclogeny" may be defined as the assumption that it is usual for a bacterial species to pass through a series of morphological and biological changes which constitute its life-cycle, the tendency being to return to the starting-point. This is supposed to be the normal course of events, in the light of which variation is to be interpreted. It is obvious that this postulate involves a "base line" which is entirely different from mine.

About this conception I am content to express my agreement with Arkwright's criticism in his article on Variation[1]. "The view that bacteria are

[1] *Med. Res. Counc. Bacteriology*, **1**, 369, 1930.

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very similar in structure and life-history to the higher fungi, and that a life-cycle exists, has gained some fresh supporters recently, but the argument in favour of cyclogeny seems to the present writer to rest chiefly on doubtful theoretical grounds and forced analogies."

I need only add that the conception of a "growth cycle" which I have supported is radically different from "cyclogeny."

Combining affinities and variation.

Starting with these newer ideas about combining affinities, one may attempt to formulate a "base line" for the study of variation.

In orderly growth the cell must be controlled and it is natural to assume that this directive force resides in the protein; it is the property, due to the unstable energy of living matter, which distinguishes living from dead material. The mechanism of control involves a high degree of selective activity and this is best explained by supposing that the protein passes through a rhythmic cycle of change, so that selection, in the process of synthesis, is determined by each particular phase in the cycle. Thus the individuality of the bacterial cell resides in this cycle of change and is not a static component recognisable by chemical or biochemical analysis of the dead material.

This orderly instability of living protein provides a reason why combining groups attached to the protein molecule vary in their activity. Their activity is determined not only by the chemical structure of their protein carrier but also by the particular phases through which that carrier passes. Such elasticity of combining power is in accordance with, and must be supplemented by, the newer conception of enzymes which regards them not as fixed chemical entities but as "centres," the activity of which depends on stereochemical configuration and other physical conditions.

The importance of this view, it seems to me, is not confined to the interpretation of enzyme reactions *in vitro*, into the technical details of which I am not competent to enter. It suggests principles of general applicability to the combining capacities of the living cell, irrespective of the more limited question as to the nature of enzymes. Thus, in general terms, when an interaction takes place between the cell surface and something in the environment, it is not necessary to assume in every case that there is a specifically selective molecule on that surface; the response of the surface may be attributable to a "centre of activity" which, as Quastel says, is "simply a property of the surface structures of the colloidal materials which make up the cell as a whole."

A further point of importance is that there may be overlapping of constituents forming potential "centres of activity," so that an individual component of one centre may, under other circumstances (change in the colloidal stability of the cell surface), participate in the activity of another centre. Thus, if the units (side-chains attached to protein) on a particular surface area be called *a*, *b*, *c*, *d*, etc., and if the "centres of activity" consist of groups of these

units possessing the appropriate chemico-physical configuration, there may be three centres, *ade*, *bfg* and *chi*, each possessing special combining affinities; then a chemico-physical change may cause these three centres to disappear, as such, with the emergence of a new centre, *abc*, possessing combining affinities which differ from those of the three former centres. This conception of an elastic array of "centres of activity," which are capable of readjustment, will be utilised freely in the following pages on variation.

SPONTANEOUS VARIATION.

It is now common knowledge that, under identical conditions of environment, variants may appear in a culture derived from a single cell or from a colony of homogeneous individuals, *i.e.* under precautions which exclude the suggestion that the variant was really present to begin with, though undetected. From this it follows that some of the daughter cells are not exact duplicates of the parent cell and, as external conditions are uniform, such variations may legitimately be described as spontaneous. Inequality of subdivision or its occurrence at different phases of development may help to account for these irregularities.

Vigour of growth.

The activity of the living protein, upon which growth depends, may not always be of the same degree of intensity. Not only do different bacterial species differ very considerably in their vigour of growth, but individuals of the same strain may also differ from one another in this respect. This difference, which is often observed in plating out and transplanting a culture, is not necessarily associated with any difference in synthesis; the "poor" growth may retain, in its individual members, the full cycle of development, without any evidence of degenerative change. This intrinsic difference in vitality, which cannot be explained by external chemical or physical influences or by "lag," seems to me an important type of spontaneous variation: It is variation in the vigour of synthesis without any concomitant change in bacterial equipment.

Natural instability of bacterial equipment.

Completion of the growth cycle. The readiest instance of spontaneous variation, due to change in the stabilisation point marking the termination of development, is provided by the "diphasic" condition first described by F. W. Andrewes. Why should a pure culture, growing under conditions which seem in every respect favourable for the retention of its original characters, produce in irregular succession a mixture of "group" and "specific" forms? It seems highly improbable that individuals of a pure strain should have two different mechanisms of protein synthesis, and it is still more unlikely that one of the mechanisms changes itself, at random, into the other. The more reasonable explanation is that the diphasic condition is related to the rhythmic

or cyclical factor in synthesis. The mechanism of synthesis, though primarily the same for each individual, is liable to be stabilised either before or after the elaboration of the "specific," or fully equipped, form. If before, construction is terminated at the more rudimentary or "group" phase. As no extrinsic factors can here be invoked in explanation, one must assume that intrinsic inequalities in bacterial vigour here manifest themselves not by variation in the time required for complete development but by variation in the stabilisation point which marks the termination of development. This type of variation is naturally associated with a change in surface attributes. The "group" and the "specific" phases are strongly contrasted in their antigenic properties.

Sometimes, though not always, the change from the S to the R form may also be regarded as spontaneous, *e.g.* when R forms make their appearance in an S culture without any particular reason why they should. There is simply stoppage of the cycle of development at the intermediate or R stage, with a consequent difference in surface attributes.

Interruption of the growth cycle makes it less certain that, when growth is resumed in subculture, the cycle will be carried on to the same terminal point as before. Thus, variation after a culture has been allowed to age may not always be attributable to an external influence (accumulated products of metabolism or other changed condition in the medium); it may be due to a manifestation, in subculture, of a spontaneous change of the protein which activates growth. For example, an old S strain may have lost some of its vigour and may then tend to revert to R on subculture; or rapid subculture may bring about increased protein vigour, accompanied by change from R to S.

Intermediate stages in the growth cycle. It is known that bacteria may thrive on a large variety of different media and therefore, along with the preservation of species characters which are necessary for vitality, there must be a considerable degree of elasticity in the mechanism of synthesis. Hence it hardly seems probable that on one and the same medium, particularly if this is highly complex, there is no elasticity of this mechanism or that absolute homogeneity of synthesis is an inviolable law. It is more likely that, at some stages of growth, combining affinities are not always rigidly fixed. Instead of synthesising material *a* as a matter of course and rejecting *b* and *c* which are simultaneously presented to it, the bacterium may find that *b* or *c* is also quite suitable as alternative material; one of the latter may be accepted by some of the cells and *a* may be rejected; and this alternative choice may make a difference in the next stage of synthesis. Again, there may be differences in the rate of combination between individual growing bacteria and *a*, *b* or *c*, or possibly in the firmness of union. Thus, the resultant growth may present a more or less marked degree of spontaneous variation owing to lack of precisely rigid uniformity in the rhythmic cycle of protein synthesis.

Surface instability. Spontaneous variation of the surface may also arise from causes not attributable to any deviation from homogeneity of synthesis. In general, one may apply here what has been said above about surface "centres

of activity." These are not fixed and immutable but, irrespective of external selective influences, are liable to changes due to the colloidal instability of the bacterial surface. In so far as these changes in the surface "mosaic" may pass through an orderly cycle in the growing cell, they stand for the homogeneity of normal growth; but if they are irregular, as they often may be, they are attributes of spontaneous variation. This factor of variability might be used in support of the argument that peculiarities in the rate of disinfection are due not to different phases in the growth cycle of homogeneous bacteria but to the emergence of variants.

DIRECT INFLUENCE OF AN EXTERNAL AGENT.

In this section I confine myself to the simpler conditions where the appearance of a variant is directly associated with a known external influence.

There at once arises a question which recurs throughout the study of variation. Is the external influence merely selective action on a spontaneous variant or does it convert a previously normal cell into a variant? This question is often difficult to decide, because it is necessary to concede considerable elasticity to the conception of spontaneity in variation, particularly in view of the possibilities mentioned above, of (*a*) alternative opportunities for synthesis, and (*b*) natural variations in the combining affinities of a growing bacterial surface.

Selection.

A useful example for discussion is the production of a new enzyme by "sugar training."

Here the newer conception of enzymes as "combining centres," in the sense outlined above, seems distinctly helpful. It removes the difficult assumption that, if enzymes are definite chemical entities, there must either be a creation *de novo* of such a complex substance or it must really have been latent in a few of the bacteria to begin with. The idea that there is simply emergence of a new "combining centre" is much easier.

Adopting the latter view, one may start with a cell which is not necessarily abnormal but is a growing cell, the surface of which is not completely elaborated. Here there may occur, temporarily and occasionally during surface changes associated with growth, a combining "centre of activity" of the right configuration to "fit" the sugar, a "centre" which would have ceased to be active if the cell had completed its development undisturbed. Thus, the starting-point for the change would be not the selection of a definitely spontaneous variant but the abnormal selection of a normal combining affinity not previously utilised. Alternatively, one might suppose that the cell with the new combining affinity actually was a spontaneous variant arising by slightly abnormal subdivision of a normal cell. In either case, as the process of training may take a long time, it must be assumed that at first the sugar can only find a few responsive bacteria; these derive energy from the reaction and hence their

growth is encouraged, the end result being that all or most of the surviving germs possess this new combining activity.

Here the idea of "selection," on the part of the sugar, of something already to be found on the bacterial surface seems reasonable and compatible with the facts. "Modification," in the sense of the creation of a new combining centre on the bacterial surface by the activity of the sugar, is less probable and would lead one to expect a more rapid conversion, without the need for frequent passage in culture.

Modification.

Change in stabilisation point. Common instances are the conversion of the S into the R form by growth in anti-S serum, the change from R to S by the influence of anti-R serum, and the reduction of the H to the O form by growth on phenol-agar. The variant is produced by a definite external influence which causes a change in the stabilisation point. As it is evident that the S and the H are the fully developed forms, whilst R and O are the minus variants, the reasonable assumption is that the former are complete and the latter incomplete elaborations of the same mechanism of bacterial synthesis.

The anti-S serum prevents the formation of S substance on the bacterial surface but allows the bacterium to become stabilised in the earlier phase of development (R). The anti-R serum prevents the stabilisation of an R surface and so allows growth to proceed until the S stage is reached. The phenol inhibits growth beyond the O stage.

Here it would be impossible, or at least unreasonable, to explain modification as a selective action on pre-existing variants which had originated spontaneously. As the modification is not completed immediately, one might concede that there are differences in the surface susceptibility of individual bacteria; but it would be unwarrantable to suggest that, when a careful bacteriologist describes a culture as pure S or pure R, he is always, in reality, dealing with mixtures of the two forms. Nor, under careful experimental conditions, can the change be explained as due, or partly due, to an intrinsic factor, increase or diminution in the vigour of growth which leads to a change in the stabilisation point. A *minus* variant may grow as vigorously *in vitro* as the *plus* form and still remain *minus*; and a *plus* may be changed to *minus* under conditions which do not involve retardation of growth.

Nutrition. I have already mentioned the contingency, as a possible cause of spontaneous variation, that a bacterium in a complex environment may deviate from the homogeneous standard because it finds alternative opportunities in the synthesis of its nutritive material. Here I am referring to a definitely new nutritive influence; it is one of the causes of modification which is so well known that I need not provide detailed examples.

I have only one comment to make. It has often been remarked that variation produced by a special nutritive influence is usually of a temporary nature, reversion occurring when this special influence is withdrawn. The temporary nature of the change seems to me less important than the fact that the

change from a lower to a more elaborate mode of synthesis is possible. In the former state, the bacterium continues to reproduce a mechanism for potential synthesis which is not used, but it responds at once when the opportunity for utilisation is presented. This elasticity of synthetic capacities is remarkable and may be interpreted as a readjustment of combining affinities.

Surface activities. Apart from any possible interference with synthesis, an external influence may modify surface activities. The chemical type of modification is of particular importance and is well exemplified by the ordinary antigen-antibody reactions. On the bacterial surface there is some stable and definite chemical complex; this unites with some equally definite combining property in the plasma or serum and modification of the bacterial surface is the result. But, as I have indicated in discussing active and potential combining affinities of the normal cell, there are other types of reaction which cannot be explained in this way. The chemico-physical action of an external influence may cause a change and readjustment of surface "centres of activity." This is different from the simple union of one chemical substance with another, but it is of at least equal importance for the living cell and therefore is a factor in variation. It involves a dynamic conception of surface activities, which are not reducible to an array of "antigenic components." There are many properties of the living cell which are not revealed when its components are utilised as "foreign protein" for the production of antibodies.

VARIATION OF INVASIVE VIRULENCE.

Coming now to the more complex causes of bacterial variation, I select invasive virulence as being the subject of greatest medical interest. I propose to deal with it in general terms, illustrated by concrete examples. Many of the latter are obtainable from the intensive study of pneumococci, both in this country and abroad, and particularly from the work of F. Griffith[1], together with the confirmation and amplification of his results by Dawson[2].

Equipment for virulence.

Apart from the possible secretion of toxic, irritant, or "aggressive" substances which may assist invasion, the bacterium must be prepared to resist the chemical, physico-chemical and vitalistic activities of the host. And, in addition to the general qualities of the circulating plasma in relation to the invasive bacteria, the local conditions of the tissue fluids are of importance; a particular bacterial species may establish itself in a particular site but may find other regions unfavourable, whilst other species may differ in their local selective action. These bacterial idiosyncrasies emphasise the subtle complexity of the conditions on which growth *in vivo* depends.

[1] *J. Hyg.* **27**, 113, 1928.

[2] *J. Exp. Med.* **51**, 99 and 123, 1930, and *Proc. Soc. Exp. Biol. and Med.* **27**, 989, June, 1930.

The bacterial outer membrane. As resistance of the host usually implies the existence of some property in the plasma which "fits" centres on the bacterial surface and thereby causes loss of bacterial integrity, bacterial virulence, in its negative aspect, may be ascribed to the absence of such vulnerable groups, a condition generally attributable to a progressive synthesis (beyond the rudimentary or avirulent stage of growth) which invests the surface with protective material. This material, to take the simplest explanation first, is of such a chemical nature that it resists disintegration by any of the chemical combining groups present in the body fluids; the host does not possess any natural or acquired "antibody" to it. A further consideration is that this protective material stabilises the surface, so that the changing conditions of its living environment are not able to produce on it any new "combining centres" which would be vulnerable to attack.

Bacterial adaptation. But the protected surface is only part of the explanation of virulence; the other requisite is capacity for growth, which, as already intimated, depends on highly complex local and systemic conditions. Here the internal mechanism of the bacterium is involved. It must pass through its orderly cycle of synthesis; if its living environment interferes with this cycle, growth is impossible and the protective surface cannot be formed. Animal resistance is an interference with bacterial metabolism and this interference may be effected in a variety of ways; action, similar to that of a specific antibody, upon a bacterial surface is not the only way. Adaptation to growth *in vivo* depends on the complex of conditions facilitating or hindering synthesis; it is not simply equipment with a chemical substance resistive to the animal's chemical activities. There must not only be absence of detrimental combining affinities but also possession of the combining affinities requisite for adaptation.

The most conspicuous feature of a virulent strain of pneumococci is the possession of a "specific soluble substance" which, as well as being excreted, enters largely into the composition of the protective capsule. One could not have a better example of the association of virulence with a definite chemical entity (here a polysaccharide). If this substance is not formed, the coccus is not virulent; if the substance is damaged by the action of its specific antibody, virulence is lost.

The other requirement for virulence—adaptability—is equally well illustrated by pneumococci. The naturally susceptible and the naturally immune animal are alike in this respect that neither of them is provided with any of the specific antibodies to the various forms of specific pneumococcal carbohydrate. So here the presence or absence of such specific antibodies does not explain the difference between immunity and susceptibility; it must be due to other differences in the animals' systemic influences. The explanation is that bacterial synthesis is not adaptable to the one environment but is to the other. To some extent, there is a similar factor in acquired immunity, where increased animal resistance does not always run parallel with development of a demonstrable antibody to the specific carbohydrate; bacterial synthesis is unable to adapt itself to some subtle change which has taken place in the plasma's activities.

Enhancement of virulence by selection.

On plating out and testing the colonies of a culture which is pure according to the strictest bacteriological criteria as regards its origin, it is sometimes found that individual colonies differ in virulence—a difference which must be ascribed to spontaneous variation. So it is conceivable that a culture which is supposed to be avirulent and homogeneous may really contain a few variants which possess some degree of virulence; these may be selected by animal passage for survival and multiplication, with the end result of a definite enhancement of virulence. One might entertain a further possibility. The same result of enhanced virulence by selection and passage might occur if a few variants arose spontaneously not *in vitro* but immediately after introduction into the animal body.

It may readily be admitted that selection of spontaneous variants is sometimes the means whereby virulence is increased *in vivo*; but I think there is at present a tendency to utilise this hypothesis too freely. It is insufficient and unsatisfactory if it is put forward in general terms as expressing the main principle which determines enhancement of virulence. One must not forget the other side of laboratory experience—the innumerable occasions where, on plating out a culture and testing colonies (or single bacteria), there is found to be strict uniformity as regards possession or lack of virulence. One cannot accept the general thesis that all cultures are naturally heterogeneous in this respect. It cannot replace the more important principle that the individual avirulent bacterium may be, and often is, converted into a virulent form not by a spontaneous change but by modification due to external influences.

As an example of "selection," one can readily imagine that the process of converting an S culture of pneumococci to the R form is sometimes not quite complete; if such an R culture is changed into S in the animal body, the obvious explanation is that there has been selection of the latent S forms.

Recent research on pneumococci also provides good instances in which it is impossible to appeal to selection of a pre-existing variant as the cause of exaltation in virulence. It has been shown by F. Griffith, and confirmed by Dawson and others, that pneumococci can be experimentally changed from one type to another, after preliminary reduction from the virulent S to the avirulent R form. As the change can be made from the R form of Type I into the S form of either Type II or Type III or any of the various S types of Group IV, it would be absurd to suggest that in the original culture of R Type I there were lurking a few S forms of each of these numerous types, with emergence of the particular one which was "converted." The fact that the acquirement of virulence (as manifested by the S character) was due to an actual change in the constitution of individual cocci cannot be disputed.

Enhancement of virulence by modification.

How is such conversion brought about? All that is pre-existent in the bacterium is the capacity for making the change, a property which may be regarded as a capacity for extending its cycle of synthetic activity, with resultant synthesis of the material which is requisite for virulence.

The change must be due to the environment and the simplest example is the introduction of some new material which is at once utilised as food for the construction of virulent equipment. Next comes the question of "accessory substances" as a means of producing the virulent variant. With some bacterial species it is known that minute quantities of such substances are necessary for growth, not as forming building stones for the major constituents of the bacterial substance but as vitamins or catalysts which stimulate bacterial construction. Are there also stimulants of this nature which are responsible for virulence? For example, when the virulence of a bacterium is raised by animal passage, does this mean that some minute constituent of the animal body has been ingested and is responsible for the change?

Here reference may again be made to Arkwright's article on Variation. About virulence acquired by passage through a susceptible animal he says: "If the virulence is then truly hereditary, this must imply that a working mechanism capable of forming anew the required component is set up in the bacteria under the influence of the material taken up from the medium." In other cases, he suggests, the requisite ingredient is probably present in great excess and so may be utilised by many generations of bacteria, "without true biological inheritance occurring."

On the kindred subject of adaptation, he writes: "In the case of adaptation to a new host a change in the bacterium may take place similar to that associated with increase of virulence. Some special constituent of the animal body in question may be taken up by the bacterium and act as a special means of promoting contact between parasite and host, and may thus assist the attack on the animal cells. Such a hypothetical adjuvant may be compared to a flux which is needed in bringing about a simpler physical union." The validity of this conception I must leave as an open question. It may be taken as an interesting way of expounding the old idea (Bail) of the difference between "animalised" bacteria and bacteria grown in artificial culture.

Though acquired virulence may be partly explained as due to the ingestion of special nutritive material, the processes involved must often be more complex than the simple incorporation of a definite chemical entity. The plasma of the animal to which the bacterium is to become "adapted" possesses a complex of chemico-physical "combining activities," some of which interact with and modify the "mosaic" (in the chemico-physical sense of the word) on the bacterial surface. The resultant change in the mosaic, involving a change in its stabilisation point, causes a difference in the material which is catalysed and absorbed into the interior of the cell, where it is synthesised into the new material requisite for virulence. Perhaps one may form a simple mental picture of the process by supposing that the surface of the bacterium, prior to modification, contains each of the three units, *a*, *b* and *c*, but not the active centre *abc*; the stimulus provided by a particular combining centre in the plasma "activates" the bacterial centre *abc*, which is requisite for preparing material to be synthesised into the equipment for virulence.

It is also possible that the modifying influence of the plasma may be partly attributable to its acquirement of a new property. Some of the original bacteria may have produced an antibody and this may be the factor which changes the stabilisation point of those remaining in the tissues—a change similar to that

which may be effected by growth *in vitro* in the presence of specific antiserum. Antibodies due to a bacterial stimulus are not always "antibacterial" in an antagonistic sense. Such assistance of an acquired antibody in the enhancement of virulence by adaptation is one way of explaining the emergence of epidemic virulence. Here the clinical facts indicate that there has been a special bacterial adaptation to the human species—a change which is not necessarily reflected in virulence tests on laboratory animals.

There is a further point of interest in the virulence gained by adaptation to a new species of host. A bacterium is virulent for host A and its condition may be symbolised by S^a (*i.e.* the bacterium which is fully equipped for dealing with A). But S^a is not virulent for host B; its condition, relative to B, may be called R^b (*i.e.* incomplete equipment for dealing with B). It is possible, however, that R^b may become virulent by modification in its new host B; it may there acquire the virulence S^b, by a mechanism similar to that suggested in the last paragraphs. Obviously, S^a and S^b differ from each other in their equipment. So the "working mechanism" of a bacterium is such that it can change its methods of synthesis, a property which is to be distinguished from mere change in stabilisation point (as when imperfect development stops at the R stage, whilst completion of the full cycle goes on to S). The change is a qualitative alteration in the periodicity of synthesis.

Change by modification may be illustrated from recent experimental work on pneumococci.

Living R culture is made, by the action of killed heterologous S culture, to develop into the same heterologous S. Here is a good example of nutritive influence. I suppose that, on contact between the surface of R and the killed S, a reaction takes place in which R is the catalyst and S the substrate, a condition of the reaction being that S must be assimilable (a property which is destroyed by overheating). The next step, presumably, is that catalysed material of the dead S cocci is absorbed into the interior of R and built up into the specific soluble substance characteristic of S. Then the offspring of the new cocci have acquired the property of making the same new S substance out of animal material.

An important feature of the pneumococcal change is that there must be a stimulus to variation (a disturbance in the stabilisation point) acting on the R cocci. When the transformation is accomplished in experiments on mice, the animal body provides this; ordinarily it would be too strong, as it would turn the R cocci into non-viable variants; but here they are protected from this fate by adsorbing the killed S material. Recently, Dawson has produced the change *in vitro*; probably the presence of anti-R serum in his medium would provide the requisite stimulus to variation.

This experimental variation of pneumococci ($S^a \rightleftharpoons R \rightleftharpoons S^b$), by adaptation to an artificial change in the environment, does not exactly bring to light a new principle but rather illustrates and extends a principle already recognised, *viz.* that adaptation to a different animal environment (involving different nutritive conditions) may change a bacterium from S^a to S^b.

Loss of virulence in the animal body.

I propose to discuss here some of the reasons why the susceptible animal (A) does not always succumb when the bacterial organisation is at first in the favourable condition which I have symbolised as S^a.

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There are often definitely specific causes. In the progress of infection, antibodies which interfere with bacterial growth or adaptation are likely to be formed and, in addition to such as are demonstrable serologically, more subtle changes in the combining properties of the plasma may also be produced by the stimulus of bacterial constituents and may become antibacterial. There are other possible changes which may arise spontaneously but their discussion had better be postponed until something has been said about the intervention of known extraneous influences.

It is known that substances introduced parenterally may produce a changed reactivity in the plasma which is selective for material other than that which caused its production. Some of these substances produce accidentally specific results, which are interesting though they cannot be satisfactorily explained. For example, substance *a* (non-bacterial) may produce a new combining affinity in the plasma which is selective not for *a* but for certain bacteria, not for bacteria in general but only for certain combining groups peculiar to the surface of particular bacterial species.

"Accidental" specificity may be illustrated by some of Walbum's work on the selective therapeutic action of simple metallic salts[1]. Here there seem to be peculiar examples of acquired immunity which, though specific, is not attributable to the stimulus of specific antigens.

He injected rabbits intravenously with staphylococci known to be of high virulence and then treated them (in the opposite ear) with intravenous doses of a metallic salt, the first dose being given an hour after the introduction of the staphylococci; a total of eleven therapeutic doses was administered during the course of three weeks. Forty-seven different metals were tried; two, tin and circonium, were curative, whilst the others had no effect. For comparison, he gives the results of treatment with an equally large number of metals on mice infected with rat virus and on rabbits infected with tubercle bacilli. Caesium and iridium were curative against the rat virus, the other metals being ineffective. Against tubercle bacilli, cadmium and manganese were effective, whilst the others were useless, with the exception of a few which showed a slight action.

On the supposition that this work can be confirmed and extended, how is the apparently specific action of the metals to be explained? It seems highly improbable that they can have a direct action on the bacteria. Nor is it likely that, on the reticulo-endothelial theory, they can stimulate particular cells to secrete specific antibodies. My suggestion is that they may attach themselves to the capillary endothelial filter and modify the plasma which passes through it, the action of different metals being different in this respect. The fact that a few of the changes in the plasma exhibit new combining capacities selective for particular bacteria is a matter of chance for which no explanation is forthcoming.

Somewhat similar conditions seem to be involved in "non-specific protein therapy," apart from its effects as a mere irritant which stimulates tissue resistance. Foreign material, here a complex not definable as a particular chemical substance, produces a change—the precise nature of which is more or less a matter of chance—in the combining affinities of the plasma. In my view, this change is due to filtration through endothelium which has adsorbed this foreign material. The result is that the plasma acquires new weapons which happen to "fit" sundry bacterial surfaces.

[1] *Communications de l'Institut Sérothérapique de l'État Danois*, Tome xx, 1930, p. 477.

Reverting now to loss of virulence in an animal host which may arise spontaneously, without the intervention of extraneous influences, one can understand that metabolic changes associated with disease are likely to cause changes in the plasma constituents and that some of the latter may happen to be antibacterial, the process being a sort of self-made non-specific therapy. The vulnerable point on the bacterial surface is not necessarily an antigen capable of producing an antibody; it is simply a "combining centre" which may be "activated" by non-specific changes in the plasma.

SIGNIFICANCE OF BACTERIOPHAGE IN VARIATION.

The interpretation of variants due to this cause will naturally depend on the view which is taken as to the nature of bacteriophage.

For my part, I am in agreement with those who reject the virus theory and regard phage as a bacterial product, the origin of which may be due to a variety of non-specific influences. Its transmission means that it is taken up by a susceptible bacterium and causes this cell to produce a fresh supply by some perversion of metabolism. The nature of this "perversion" I have discussed in a previous article[1].

Here I am mainly interested in two aspects of phage activity, the way in which it affects bacterial synthesis and the possibility of explaining the peculiarities of its selective action by the conception of variable "centres of activity."

Synthesis. I have already dealt with variants which may arise from two modes of interference with bacterial synthesis: (1) the synthetic cycle may be terminated, or stabilised, before complete development has been reached; or (2) the cycle may be altered by the introduction of something new which is synthesised.

(3) Phage activity behaves differently. It draws attention to another aspect of growth, phases of transition as distinct from phases of stabilisation. In the changes which are occurring at every stage of development there are bacterial constituents which will disappear, as such, when the next stage is reached, because they will be merged in a higher process of bacterial construction. Such labile constituents may be regarded as scaffolding, as intermediary substances which are replaced when the edifice is completed. The special property of phage is that it produces abnormal stability in some of these naturally labile cellular constituents.

The more usual result of this abnormal stabilisation, by phage, is the production of non-viable variants; the growing bacteria break up prematurely and the stabilised elements which are released constitute the newly formed phage. Less frequently, this abnormal stabilisation is not so drastic and does not lead to autolysis; then phage is responsible for the production of living variants characterised not only by resistance to this particular phage but also by retention of lysogenic power, due to the presence of some abnormally

[1] *J. Hyg.* **29**, 124–7, 1929.

stabilised material. In this particularly interesting example of a bacterial variant one may say that there is completion of the edifice in the form of a living cell without removal of all the scaffolding or intermediary substances utilised in the process of construction. In this respect there is qualitative change in the periodicity of synthesis.

Living variants produced by phage may manifest other abnormalities besides the one mentioned here (possession of lysogenic power); but I need not discuss them in detail. In explanation of their origin, the old dilemma frequently arises. Was the action of a phage simply selective or was it a creation *de novo*? For example, when, after the action of a phage, a culture changes from S to R, perhaps all that really happened was that a few R forms were present to begin with and the phage, being unable to attack them, allowed them to multiply, whilst eliminating all the vulnerable S forms. The alternative would be that phage modified synthesis and thus led to the production of an R surface which was unsuitable for adsorbing it. But in the production of the lysogenic variant direct modification by phage is unquestionable.

Centres of combining activity. Reverting to what I have said in previous sections about newer conceptions of a surface "mosaic" in which different side-chains may be grouped into a variety of "centres of activity," one may imagine that the surface of the phage particle possesses several such units or side-chains (*a*, *b*, *c*, etc.) and that these behave in a special manner when two or more of them can act in conjunction as a group. The action of a phage need not be confined to one strain of bacteria, because there are several possible ways in which the units may be combined into different groups, each with its special sphere of activity. Under some conditions of environment, some groups are active (*e.g. abc*) but not others (*e.g. adf*), though all the units persist and may present a different arrangement of group activity when the environment is altered. Here there is a wide potentiality for variation or adaptation for which it is not necessary to postulate any "vitalistic" factor but merely variation in chemico-physical influences.

For example, a large number of phages may lyse the Shiga organism (because they all possess the group *abc*), but they may differ from each other in their action on heterologous bacteria (because other groups, *bcd*, *dfg*, etc. may be active in one phage but not in another). Again there is the phenomenon of "remembrance." Take three different types of bacteria, *A*, *B* and *C*, of which *C* is at first normal. A lytic filtrate from *A* causes *C* to produce a lysin c^1 and a filtrate from *B* causes it to produce an apparently identical lysin c^2. The two lysins c^1 and c^2 are propagated for a long period by passage through *C* and then are tested on *A*, when it is found that c^1 produces lysis but c^2 fails to do so. The reason why c^1 "remembers" *A* presumably is that c^1 contains an arrangement of surface units in which there is the potentially active group x (not required for action on *C*); c^2 may contain the same surface units but they are not spaced in such a way as to allow the formation of group x (which is requisite for action on *A*). The same phenomenon may be reflected by antilytic sera; a serum antilytic to a phage derived from *A* may prevent a heterologous phage from acting on *A* without preventing its action on other bacteria, because it is selective for the group x which the heterologous phage happens to carry.

SUMMARY

Although knowledge of principles determining variation is imperfect, some progress has been made and further help may be obtained from some of the newer conceptions about combining affinities regulating normal growth. There are reasons to believe that growth depends on a rhythmic cycle of synthesis, peculiar to living protein, whereby at each successive stage one particular "building stone" is selected for synthesis, with rejection of all others. There is also some evidence that the surface of a growing bacterium passes through periodic phases. Additional light on the activities of the bacterial surface is thrown by the new conception of enzymes which regards them not as fixed chemical entities but as chemico-physical "centres of activity."

These ideas are all useful in the study of variation, because they suggest features in the mechanism of growth where deviation from the normal is possible. In particular, the conception of "centres of activity" need not be confined to enzyme reactions *in vitro* but may be applied generally to the combining affinities of the living cell. The emergence of variants may often be explained by the liability of these "centres" to change and reconstitution under chemico-physical influences.

In "spontaneous" variation (without change in the environment) the reason may be that the synthetic cycle of growth has diminished or increased in vigour or that growth has terminated before completion of the full cycle of change. And spontaneous variation may occur because absolute homogeneity of synthesis is not an inviolable law; at a particular phase of growth in a complex medium, *a*, which ought to be the next material selected for synthesis, may sometimes be replaced by *b* or *c*. It is also probable that the regulatory mechanism is not always sufficiently precise to secure absolute uniformity in the surface pattern of potential "combining affinities."

When a variant is apparently produced by the direct action of an external agent, it is sometimes difficult to decide between instances of true modification and cases where there has been simply selection of a spontaneous variant. In the production of a new enzyme by "sugar training," the new conception of combining affinities is helpful; according to this, there is simply emergence of a new "combining centre," not the appearance (or creation) *de novo* of a new and complex chemical entity. Here the activity of the sugar may be attributed to "selection" of something occasionally present on the bacterial surface rather than to "modification." On the other hand, when reversion between the S and the R forms is produced by growth in the appropriate antiserum, there must be direct modification of the bacterial surface, not selection of a pre-existing variant.

Modifications of virulence, produced *in vivo*, are more complex, as they involve interactions between bacterium and host. The former's equipment for virulence involves capacity for adaptation, which is not simply equipment with a chemical substance capable of neutralising or resisting something in the

plasma. When virulence is raised by animal passage, the explanation sometimes may be that there has been simple selection of pre-existing virulent variants. But enhancement by true modification undoubtedly occurs. This cannot always be explained as due to the introduction of a new food or "accessory substance." There must often be reactions between bacterium and host which modify the bacterial surface activities, leading to change of the material passed into the cell and changed methods of synthesis (or selection of alternative material for synthesis). The result is adaptation to a new environment, *e.g.* acquirement of virulence for a new species of host or change of type character by a pneumococcus owing to an artificial change of environment. When there is loss of virulence for a naturally susceptible host, the plasma may have been altered either by the acquirement of specific antibodies to bacterial antigens or by non-specific means (*e.g.* by administering metallic salts or by "non-specific protein therapy"). The main point is that the plasma unites not necessarily with an "antigen" but with a combining centre which has been activated on the bacterial surface.

As regards variations due to bacteriophage, in my view phage is a bacterial product, normally labile, which is stabilised and, on release from the cell, produces a similar stabilisation in other susceptible cells. This abnormal stabilisation is another mode of producing variants by interference with synthesis. It may be assumed that the surface of a phage possesses variable centres of combining activity and that these explain its peculiar selective properties and adaptability, without resort to the postulate of "vitalistic capacity."

II. CARCINOGENESIS.

In bacteriology one can see the emergence of principles underlying variation, though patient and repeated reconstruction will be needed before they are established on a broad and substantial basis. The origin of a malignant variant from a normal mammalian cell is much more nebulous; but there must be some explanation and therefore the search for it must be continued until it is found.

The line of attack which I have chosen may be called immunological, in the broad sense of the term which considers non-specific as well as specific types of interaction between particular cells and the animal body in which they reside. There is an obvious difference from bacterial immunology, where the cells in question are composed of protein which is naturally foreign to their host; but there remain features of interest which the two subjects possess in common. There is the mechanism of cellular growth, governed by principles which are to some extent the same for bacterial and animal cells; both kinds of cells possess a certain capacity for variation, the range and limitations of which must conform to these principles regulating intrinsic capacity for growth; and, as regards environment, both invasive bacteria and the cells of the host

are exposed to the same controlling factor, the animal's systemic influences, which, though liable to modification, persist as an expression of the animal's individuality. A further point in common, according to my view, is that neither a bacterial variant nor a malignant variant is produced by a mysterious "virus"; in both cases the change is due to a "perversion of metabolism," in explanation of which one must search for new light on the intimate mechanism of cellular growth.

Following the ideas outlined in the preceding part of this article, I propose to pay special attention to "combining affinities" in relation to carcinogenesis.

NORMAL REGULATION OF GROWTH.

Autonomous regulation.

Mammalian cells can be cultivated *in vitro*, on a medium which is alien to the plasma of the animal from which they were obtained. The autonomous regulation of growth here exhibited by a group of cells must also be a factor in the normal growth which occurs in the normal animal. It implies that individual cells possess a considerable amount of independent capacity for self-regulation.

As regards synthesis, I consider that much of what I have said about bacteria applies to the mammalian cell. The vitality of the latter depends upon the specificity of its protein; and its normal growth, in its autonomous aspect, may be regarded as due to a rhythmic cycle of synthesis controlled by that protein, which must retain its individuality, including some degree of type specificity, as long as the cell preserves its integrity. This intrinsic controlling influence implies that the true specificity of the cell does not depend primarily on the characters of the surface "mosaic" but upon the individuality of the living protein which carries active or potential "combining groups" constituting this "mosaic." The surface activities of the cell, which exercise their usual functions in the preparation and acceptance of nutritive material, are subject to this control. Thus cellular individuality, as with bacteria, involves not only a static conception of a particular protein pattern but also a dynamic conception of a particular cycle of synthetic activity.

Apart from the retention of elementary species characters, which is necessary for continued vitality, there is another feature of the autonomous aspect of growth in which mammalian cells may bear a certain resemblance to bacteria. In a given environment there may be continued reproduction of potential combining affinities which are not utilised by the cell so long as its environment remains unchanged.

As a familiar example in bacteriology, one is aware that bacterial equipment for invasive virulence may be retained during subculture *in vitro*, though this equipment was not required for saprophytic growth. Similarly, a culture of cancer may continue its malignant growth if transferred to the correct species of host, though its invasive equipment was not

utilised *in vitro*. Conversely, a culture of normal cells does not become malignant by propagation; presumably it retains the normal surface pattern which would respond to systemic control of growth, though this response was not stimulated *in vitro*.

Hence one may reasonably suppose that there may also be retention of some unused cellular capacities during many conditions of growth in the animal body, and that these are liable to be brought into activity by a local change in the environment of particular cells. Thus, on the surface of a cell there may be the units *a*, *b* and *c*, which are not used as a combining centre *abc*; but a change in the environment may "activate" this centre. In the former condition there was retention of chemical units which were susceptible to chemico-physical readjustment; in the latter state this readjustment has taken place, without the creation of any new chemical unit.

Such autonomous retention of unused capacities is a possibility which may assume importance as a predisposing cause of variation.

Systemic control.

On the surface of the normal animal cell there must be combining affinities of various sorts and I think it is advantageous to regard these, as in the case of bacteria, not as separate chemical entities but as chemico-physical "centres of activity" which are not all active at the same time but overlap, so that an individual component of one centre may also be a potential component of another centre. I suggested as an example of bacterial surface activities that there might be three centres, *ade*, *bfg* and *chi*, each of which served some special purpose, and that on the same surface area there might be a regrouping of centres; the centre *abc*, possessing different activities, might be formed, with consequent disruption of the three former groups.

Applying this conception to systemic influences regulating animal growth, one may imagine that some constituents of the plasma activate and then combine with *abc*, thereby interfering with *ade*, *bfg* and *chi*, which may have acted as enzymes; growth stops because fresh "building stones" are not made. The reverse process of stimulating growth might be regarded as action on the cell surface which abolishes *abc* as a centre and releases the three enzymes, thus leading to renewed activity. Such action is probably much more complex in reality; these imaginary examples are only intended to suggest the sort of mechanism which may serve for readjustment of surface activities.

Another important aspect of systemic control over animal cells is the requirement of specificity. This property might be overlooked or taken for granted in normal animal growth, because a cell which had lost its species specificity would be intrinsically defective and would not be expected to survive; but it comes into prominence in grafting experiments. A graft into a homologous host may "take"; but it will fail if introduced into a heterologous animal, owing to incompatibility between the protein of the plasma and that of the grafted cells. And so in the natural growth of the animal body there is constant observance of this law of protein compatibility. The con-

trolling factor must depend in some way on the vitality of the plasma, because animal cells will grow *in vitro* on protein derived from alien hosts.

It is interesting to compare the conditions in natural immunity towards bacteria. It is sometimes observed that a bacterial species will perish *in vivo* but will thrive on the animal's serum, which therefore cannot be antibacterial. The reason, one may say, is that the dead protein is relatively inactive and is easily acted upon as substrate, whereas the living plasma is not a passive substrate but is a complex of systemic influences, possessing phases of enzyme activity which will disintegrate any vulnerable groups on a cell surface. But, if this is taken to imply that the living plasma possesses "natural antibodies" which have disappeared from the serum, the nature of such antibodies needs explanation. For example, an animal may be naturally immune towards all kinds of pneumococci, irrespective of their types; this cannot be due to possession of specific antibodies towards all the polysaccharides which may furnish type characteristics. It is more probable that the activities of the plasma prevent the pneumococcus from completing the rhythmic cycle of protein synthesis which is necessary for growth, the action being, in this sense, "anti-protein," though not due to an ordinary specific anti-protein antibody. The plasma is "anti-" not necessarily to the dead chemical pattern of a foreign protein but to the living mechanism of synthesis peculiar to the pneumococcus. Similarly, the immunity towards animal cells which do not conform to species specificity is probably not due to the creation (or original possession) of a specific antibody to a particular foreign protein but is due to incompatibility between the activities of the plasma and the synthetic activities of the cell, the result being that the cell's protein cannot execute its rhythmic cycle of growth.

Thus these two aspects of systemic control over animal cells, viz. (1) control of growth and (2) the requirement of species specificity, do not mean that there are two independent kinds of "mosaic" on the surface of the cell, but that the cell's equipment consists of both (1) an array of surface combining affinities and (2) dynamic activities peculiar to the protein of which the cell is composed.

The interest of this second kind of control, in relation to the present subject, lies in the fact that the cancer cell conforms to it; it is viable because it has retained the mechanism characteristic of species specificity.

SPONTANEOUS APPEARANCE OF A CANCER VARIANT.

The "mixture" hypothesis. First a preliminary point must be dealt with. When a bacteriologist describes the emergence of a variant A, he is required to show that he has not been working with a mixed culture in which A was present to begin with, though not at first detected. This "mixture" hypothesis is sometimes employed in explanation of carcinogenesis. It is supposed that a tissue which subsequently becomes malignant never consisted entirely of

homogeneous, normal cells; some of them were always in condition A (the malignant variant) and manifested their character when "freedom of control" gave opportunity for growth. Though this easy way of solving the problem ought to be mentioned as a survival of the "embryonic theory," I agree with those who think it is not a fruitful line of speculation.

A "natural tendency" to malignancy. The above "easy solution" is not infrequently presented under a rather different guise. The tissue in question is at first supposed to be normal and homogeneous; but, since spontaneous variation is possible with mammalian cells as it is with bacteria, it is assumed that (*a*) some of the daughter cells deviate from the normal and become A. This view is supplemented by supposing that (*b*) some normal controlling force usually inhibits this "natural tendency," a tendency which has free play whenever this force is in abeyance. I do not think this view has been substantiated either for (*a*) the "spontaneity" of malignancy or for (*b*) the "controlling force."

Spontaneous change in susceptibility. Here one abandons the "easy solution." Carcinogenesis is a complex process, requiring the participation of some influence, x, external to the cell. But it is known that some tissues are more liable to become cancerous than others; and the reason, apart from the influence of local environment, may be that different types of cells differ in their liability to undergo such spontaneous changes as make them susceptible to x. One may suppose that susceptibility to x depends on the capacity of the cell, in the course of its growth and metabolism, to present on its surface some particular combining centre, *abc*, which may be activated by x. Some cells may never produce it; others may form it occasionally, as a transitional phase in the normal cycle of growth; and others may present it more frequently, perhaps as a stabilised product of completed development. Liability to produce it may vary with differences in the vigour of growth or with differences in the stability of the cell surface, either during the process of growth or at its completion. This view is compatible with what has been said above about autonomous retention of unused capacities as a predisposing cause of variation. The cell may automatically reproduce the inactive units, *a*, *b* and *c*, which are serving no special purpose in the economy of the cell; but a change of environment (the introduction of x) may activate them into the new combining centre *abc*. To this limited extent, spontaneity may be accredited as a factor in carcinogenesis, viz. as a spontaneous change of certain cells into a state of susceptibility towards an external influence which may initiate the precancerous condition. The next step is to consider the nature of x.

Direct influence of an external agent.

In bacteriology it is often possible to say that the emergence of a variant is definitely due to the action of a particular substance possessing a particular property; and the action may either be selective (upon a combining affinity

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already present in the bacterial cell) or reconstructive (by a modification of the cell's combining affinities). Do analogous considerations apply to carcinogenesis?

On the part of the cells which are to become variants, one may agree that there is selection followed by modification; selection, because only particular cells (or cells in a particular condition) are susceptible to the influence of the mysterious x; modification, because the effect of this influence is to produce a definitely precancerous change. But is there direct action of a particular substance upon these cells? For example, when there is a known external agent such as tar, does it, in addition to producing a state of "chronic irritation," act directly upon susceptible cells so as to make them either "precancerous" or definitely cancerous? Whilst such action has not been proved to be impossible, there are several reasons why it is improbable. As the change requires a long time, prolonged action seems to be necessary, and it is known that this action may be continued long after the carcinogenic substance has ceased to be administered. During all this period the cells concerned have not remained stationary but have probably passed through many generations. These conditions make it difficult to postulate a continuance of direct and frequently repeated union between the carcinogenic substance and the affected cells. Indirect action is therefore the preferable explanation, the assumption being that the tar produces in the local environment a change which persists, irrespective of its original cause, and that it is this change which is in some way responsible for the altered condition of the affected cells. It may be said that the disadvantage of this view is that x still remains mysterious, being no longer directly attributable to tar. The compensating advantage is that it links up these cancers which follow upon a known irritant with the much greater number where there is no known external agent; in both classes alike, causation must be found in a changed condition of local environment consequent upon chronic irritation. The next difficulty is to form some idea of the nature of these local changes which are likely to predispose to cancer.

MORE COMPLEX CONDITIONS IN CARCINOGENESIS.

The latent period.

The difficulty about this important stage in the origin of cancer is that no precise information is available as to what actually happens; it is only possible to draw inferences, based on subsequent events, about the probable course of the latent or preparatory phase.

Carcinogenesis is not merely a degenerative process involving simply a loss of equipment, because degeneration of a cell's activities does not make it a cancer cell. But it seems likely that there is some degeneration from the normal, forming the precancerous condition, which prepares the way for reconstitution into definite malignancy. There may be some analogy, though perhaps not a close parallel, with the mechanism of bacterial variation, where S^1 must

be reduced to R before it can be changed to S^2. Here S^n (the fully equipped normal cell) must become the degenerate R (the precancerous cell) before it can be converted into S^c (the fully equipped cancer cell). If this view be accepted, the latent period is obviously concerned with the stage of degeneration; whether it is also occupied with reconstruction is more doubtful.

The time factor of the latent period is usually very long and its duration often seems to be, roughly, in direct proportion to the total span of life of the animal species concerned. Perhaps it is worth remembering that cancer is not unique in this respect. In other diseases the pathological condition may be due to a degeneration of particular cells, *e.g.* nerve cells, in a particular area and this may be a slow and insidious change in the mechanism of nutrition which continues for years before clinical manifestation. Moreover, the cause, particularly in affections not due to micro-organisms, may be as obscure as in cancer.

A degenerative change, which presumably occurs in the latent period and is subsequently conspicuous in the cancer cell, is loss of capacity to respond to systemic control of growth, though species specificity is still retained. This is definitely a cellular loss; there is no loss on the part of the body's general systemic influences. And this loss may be associated with other changes; there may be emergence of new combining affinities as well as loss of old ones, all going to make up the precancerous condition. The change is of a biological nature which cannot be traced step by step with the aid of observation and experiment. One can only attempt some hypothesis which is likely to fit in with the known facts. (1) The change is a modification, which is produced very slowly, in the combining affinities of particular cells in a localised area; it is a sequel of chronic irritation, or perhaps rather of some failure in normal recuperation after the actual irritation has subsided. (2) Its production is not an invariable sequel to irritation but occurs irregularly, owing to the variability of two factors, (*a*) cellular susceptibility to modification and (*b*) the particular kind of local change in the environment of these cells.

In explanation of (1) I suggest the following hypothesis. Products of chronic irritation have been adsorbed by the endothelial filter of the local capillary channels, with consequent modification of this filter during the latent period. Hence there is modification of the plasma circulating in this area; it possesses local characters different from the normal systemic influences in the general circulation. This implies a different method in the control of local growth, because the altered plasma selects and neutralises different combining affinities on the surface of the cell. Under these conditions of readjustment, there is emergence of (*a*) new combining affinities and (*b*) loss of some of the old ones. This change would not be likely to take place *in vitro* because it requires the activity of living plasma to stimulate the emergence of (*a*). When the latent period comes to an end, (*a*) are no longer of use as a mechanism of response to local influences, because these have disappeared, but they may be utilised in a different way, by presenting surface groupings which possess

new and abnormal combining capacities; and (*b*) manifests itself as loss of the mechanism which enables normal systemic influences to regulate growth.

As regards (2), the reason why some forms of irritation produce the precancerous change while others fail to do so, one has to admit ignorance. It is impossible to single out any one factor which produces the requisite change in the environment of susceptible cells. The influence, whatever it may be, must therefore be regarded as non-specific. I refer primarily to the great majority of cases where cancer is autogenous and is not attributable to any known chemical or physical agent. Where there is a known extrinsic and non-specific factor, such as tar, the effective agent may be adsorbed by the endothelium of the local capillary channels, with resultant changes in the plasma similar to those already suggested. Or it may simply assist the tissue in forming products of irritation which, as in "spontaneous" cases, prove effective when adsorbed on local endothelium. If there is direct action of tar on endothelium, a parallel might be found in the experiments of Walbum, quoted earlier in this article. His results may be interpreted as meaning that there was a selective action of certain metals upon the general capillary system, with the result that a new and selective anti-bacterial complex was produced in the plasma. Here the effect is strictly local; but the irregularly selective action of Walbum's metals may perhaps be compared with similar irregularities of different chemicals as regards carcinogenic potency. There is again non-specific action which is highly selective.

One can therefore form some sort of a mental picture of the way in which the latent period is occupied with a slow degenerative change, leading on the part of the affected cells to loss of response to systemic influences regulating growth, a loss which may be associated with other changes, constituting equipment with new potential combining affinities.

Is the latent period also occupied with the conversion of the precancerous into the malignant cell? This will depend on what one conceives to be the nature of this more intimate change.

The emergence of the malignant cell.

Cancer may arise under a great diversity of conditions and its origin is very slow and apparently complicated; it is therefore unlikely that the whole process can be explained by the direct operation of some single factor (a chemical or chemico-physical agent) which has the specific property of converting a normal cell into a cancer cell. In the earlier stages of the change, so far as the course of events in the latent period can be surmised, the degeneration of particular cells into a precancerous condition appears to be due to non-specific influences, just as many authorities consider that various non-specific causes may initiate "lytic principle" in a bacterium. But one is reluctant to think that the process of carcinogenesis is entirely non-specific from beginning to end. In their essential properties, all malignant cells behave very much in the same way and it is therefore natural to suppose that the same kind of influence

participated in their genesis, *i.e.* that there is some specific factor. Hence one is led to consider whether the complexity of the process may not be attributable to two kinds of influence, the one non-specific and the other specific, the former being responsible for the degenerative phase and the latter for conversion into the fully equipped cancer cell.

What, then, is this supposed specific factor? It must be frankly admitted that no categorical reply can be given; but the question is constantly being raised and some endeavour to answer it must be made. As this factor must be something which acts on the degenerate or precancerous cells themselves and on no others, it is tempting to think that, after the analogy of specific antibody production, it must owe its origin to cells in the precancerous condition.

The suggestion that the precancerous cell may be antigenic in its own animal host is not capable of actual proof but I think it is worth considering. Such cells retain their species specificity and therefore cannot be antigenic in the same way as foreign protein. But their protein carries on its surface combining affinities (potential haptens) which are different from those of the normal cell and may possess new and abnormal properties in addition to lack of response to systemic control; it is possible that these differences, being "foreign" to the host, may find antigenic expression. The emergence of a new antigen is a common experience in bacterial variation and it is not unreasonable to suppose that it is also possible with mammalian cells. If this be conceded, there will be a definite reason for the difference between the forms of irritation which predispose to cancer and those which do not; the former are effective because they produce a particular antigenic change in susceptible cells.

If, then, disintegrated material from such antigenic precancerous cells finds its way to the site of antibody production (in my view, if it is adsorbed by capillary endothelium), the result will be a change in the plasma which makes it specifically selective for precancerous cells. To borrow an analogy from bacteriology, if the precancerous condition is equivalent to the R form, the plasma will now contain an anti-R antibody which, on union with the growing R cells remaining at the local focus of change, will prevent their stabilisation in the R form. Under this influence the cells are not destroyed but assume another kind of stabilisation which is characteristic of the true cancer cell. Here the typical bacteriological effect of anti-R serum (stabilisation of R cells into the S form) is not analogous, because the cancer cell is not a progressive development from R to the original and normal S. But one might perhaps compare the transmission of a specific bacteriophage in those instances where the selective action of phage produces not lysis but a lysogenic and viable bacterium. The phage, in my view[1], penetrates the specifically susceptible bacterium and produces abnormal stabilisation of intermediate products of metabolism which would have disappeared if normal growth had gone on to completion. Similarly, the specific anti-substance to the precancerous cell may stabilise protein structure at a stage when it is carrying an intermediate and

[1] *J. Hyg.* **29**, 117–31, 1929.

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more rudimentary equipment of combining affinities which would have disappeared on completion of the R form. On this view of the emergence of the cancer cell, the change effected in the R cell is not reconstruction to a stage of development higher than R but a degradation to a form more rudimentary than R, though still viable owing to the retention of homologous protein. Thus, if the normal cell be S^n and the cancer cell S^c, the process $S^n \rightarrow R \rightarrow S^c$ is a progressive dedifferentiation.

When and where does the formation of this hypothetical anti-R substance occur? It is not easy to imagine that it is produced locally, during the latent period. This would imply that the two stages in carcinogenesis were going on concurrently ($S^n \rightarrow R$ and $R \rightarrow S^c$) and that the local endothelium was responsible not only for the changes in the plasma requisite for R but also for those needed to produce S^c. Perhaps the better alternative is that it occurs when the latent period has been brought to an end by some fresh inflammatory reaction. Then some R cells are disintegrated and carried into the general circulation, where they produce their antibody. When this is formed, the circulation carries it to the local focus of surviving R cells, which are converted by its specific action into S^c. Thus anti-R antibody will be an acquired systemic influence due to an antigen of local origin. I wonder if this hypothesis is likely to satisfy any of the numerous writers on cancer who think that the disease is not entirely local in its origin but postulate a special systemic influence acting on a locally prepared tissue.

For my part, whilst putting forward this hypothesis for consideration, I admit that it has not been proved. In this obscure subject all that can be done at present is to attempt a slow advance beyond the view that cancer is caused by an x which is entirely unknown, and to hope that, out of many possible constructive hypotheses, the most useful will eventually survive. With all cells, both bacterial and mammalian, the range of possible variation is strictly limited and must be compatible with conditions about which some knowledge has already been gained. With bacteria, it is known that acquirement of a new antigen is a common example of variation, and that the antibody produced by this antigen may cause modification (not cytolysis) in the homologous bacterium. Adoption of these two ideas may be offered as a partial substitute for the unknown x of carcinogenesis. The precancerous cell possesses equipment which is alien to its host, but it has not yet acquired invasive power; it may be compared to the saprophytic condition of a bacterium which is tolerated by its animal host, but only in secluded situations. The transition to the true cancer cell involves a further change of equipment which may be compared with adaptation for virulence on the part of a bacterium previously living as a saprophyte.

SUMMARY.

Some of the principles which are gradually coming to light in bacterial variation may ultimately be of assistance in the much more obscure problem of explaining the change from the normal mammalian cell to its malignant variant. I refer in particular to conceptions, which I have discussed in the preceding part of this article, about the mechanism of cellular growth and the nature of a cell's combining affinities.

Mammalian cells possess considerable capacity for self-regulation and, in this respect, may be compared with bacteria as regards both their synthetic activities and their surface "mosaic" of combining "centres," some of which are capable of readjustment and thus constitute a predisposing cause of variation.

Systemic control, in so far as it stimulates or restrains growth, may be explained as a readjustment of combining affinities on the surface of the cell. Another important aspect of systemic control over animal cells is the requirement that these must retain the characters peculiar to their species. This influence of the plasma may be compared to natural immunity towards bacteria; the plasma is not equipped with specific antibodies, but its activities seem to be incompatible with the synthetic activities of the heterologous cell, the result being that the cell's protein cannot execute its rhythmic cycle of growth.

The cancer variant does not appear "spontaneously"; some extrinsic influence, x, is necessary. But "spontaneity" may be conceded to this extent that there may be spontaneous change of certain cells into a state of susceptibility towards x, which may initiate the precancerous condition.

Taking a broad view of the various circumstances which may induce the precancerous state, it does not seem possible to regard x as a special substance which acts directly upon the cells in question. More probably x is a changed condition of local environment consequent upon chronic irritation. On this view, a known carcinogenic chemical compound does not act directly upon the cells but indirectly, by bringing about a change in environment.

Carcinogenesis may be divided into two stages. (*a*) There is a degenerative change, involving loss of some surface combining affinities; this, according to my hypothesis, is not merely a loss but involves a rearrangement of surface groupings, with consequent appearance of new and abnormal combining centres. These changes constitute the precancerous condition. (*b*) There is a further change which invests these cells with the invasive equipment of malignancy.

The latent period is occupied with (*a*). About what actually happens one can only offer surmises. My suggestion is that localised chronic irritation leads to a change in the adjacent endothelium, with the result that the plasma filtered into this area is abnormal and gradually produces the precancerous

change in certain susceptible cells. These cells lose the combining affinities which, on termination of the latent period, would make them susceptible to systemic control of growth, and acquire different and abnormal combining centres. The influences which may produce these changes in the plasma cannot be attributed to any one substance but must be regarded as non-specific.

But it is not necessary to assume that the whole of the carcinogenic process is non-specific. A specific factor may be operative in (*b*), the change from the precancerous to the definitely malignant cell. My suggestion, which I have discussed in the light of certain bacteriological analogies, is that some of the abnormal combining centres of the precancerous cell are antigenic and, on the termination of the latent period, produce an antibody which is specific for surviving precancerous cells and changes them into the malignant condition.

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