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CHEMICO-PHYSICAL STABILITY AND CANCER[1].

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CONTENTS.

CONFLICTING HYPOTHESES ABOUT MALIGNANCY.

What is the significance of the Rous sarcoma?

UNTIL about four years ago the Rous sarcoma did not appear to the majority of cancer investigators to be a disease of any great importance. It was regarded rather as a curiosity, peculiar to the fowl, about which interesting questions might be raised. The agent which transmitted the disease was evidently a filter-passer; but was it a living virus or not? Was the tumour histologically a true sarcoma or an infective granuloma? Questions such as these provided material for discussion; but there appeared no urgent need for their settlement, as the disease was not usually thought to possess any high significance in relation to mammalian malignancy.

During the last few years the position has changed; it may be said that the remarkable prominence now given to fowl sarcoma has become the most conspicuous feature of experimental work on malignancy.

One result has been that fresh complications and confusion have been introduced into the general problems of cancer. The main cause of the trouble is the free transition of thought, on the part of many investigators, from the avian to the mammalian disease. Observations and hypotheses derived from the former have been taken as valid for the latter without due verification. And, apart from an unjustifiable tendency to explain the mammalian disease in terms of fowl sarcoma, there are innumerable differences of opinion as to the real nature of the two diseases; consequently the results and inferences of one investigator cannot easily be compared with those of another.

[1] In continuation of the paper in *J. Hygiene*, **28**, 9–32 (8. VIII. 1928).

As these difficulties are a serious obstacle to progress, an attempt should be made to clear the ground by clarifying one's ideas about the real significance of the Rous sarcoma in relation to the general cancer problem. What are the main issues which should be disentangled from elements of confusion? Answers to this question ought to be helpful, though they are not at all likely to be unanimous; it must be recognised that observers are entitled to view the subject from different angles and that it is impossible to eliminate the personal equation.

Three definite hypotheses.

(1) *The living virus.* First, there is the straightforward, unequivocal view that all forms of true malignant disease are caused by invisible, living viruses. By "unequivocal" I mean that there is no compromise about the need for a mysterious "second factor." In the simple and obvious sense, a second factor is always requisite to produce infection; it may be defined as "opportunity." For example, if a pathogenic bacterium is to produce disease, it must gain access to the body and must find susceptible tissues; the tissues may be rendered susceptible by one or other of a variety of irritants; and so forth. But that is all; the bacterium must be given its "opportunity." So with the invisible cancer virus; when given its "opportunity," this virus, and nothing else, is the actual cause of cancer.

One can readily understand that a pathologist who wishes to support this view might set before himself the following programme. (*a*) He might start with the Rous sarcoma, in the belief that this is likely to be the easiest material in which to find the causal virus. (*b*) Having discovered, to his own satisfaction, how to isolate or at least to demonstrate this virus, fowl sarcoma would be relegated to the background; the experience gained would be utilised merely for guidance, in the hope of making the independent discovery, irrespective of what was known about fowls, that mammalian cancer is also caused by a filter-passing virus. After succeeding with both (*a*) and (*b*), the remaining part of his task would be (*c*) to explain how his viruses cause the peculiar cellular changes which are characteristic of malignancy.

The above programme is entitled to respect from all persons, whatever may be their particular creeds. The plan is definite, thoroughly intelligible, and comprehensive; it ought still to be pursued by those who believe in it. It is no disparagement to say that it has not yet attained any great measure of success. Other ways of trying to penetrate the mysteries of malignancy have also failed to accomplish any brilliant achievement.

Of the three items in the programme, (*c*) is still in abeyance, because (*a*) and (*b*) have not yet been settled. As regards (*a*), it is agreed on all sides that the disease, the Rous sarcoma, is transmissible by a filter-passer, but it is not yet decided whether this agent is a true, living virus or a non-living substance, something in the nature of an enzyme, which is created *de novo*; the balance of evidence seems to be in favour of the latter view. There is still

less support for the virus hypothesis as regards (*b*); indeed the balance of evidence is very strongly against transmissibility of either a living or a non-living extracellular agent in the mammalian disease.

(2) *The autogenous enzyme.* It has often been suggested that some of the "invisible viruses" are not true living, unicellular organisms, which multiply by subdivision and are not created *de novo*, but are chemical substances, not endowed with life, which, under favourable circumstances, are produced *de novo* by living cells. This view has been applied to the cause of cancer. Put in the form of a definite, uncomplicated, and readily understood hypothesis, it may be outlined as follows:

(*a*) Fowl sarcoma is due to a complex chemical substance which is produced *de novo* within certain cells; it is released from these cells and is then capable of "infecting" normal cells of similar type; these latter cells propagate it and again release it; and so the cycle is continued. This substance is closely comparable with "bacteriophage," according to the view, which is held by many though not universally accepted, that "bacteriophage" is not a true virus but a chemical complex which is produced and propagated by the agency of living bacteria.

(*b*) Mammalian malignancy is due to a similar transmissible agent which is unequivocally the true cause of the disease, as with fowl sarcoma and bacteriophage. It is created *de novo* by certain cells but is not itself a living organism; and it requires no "second factor" beyond "opportunity," as defined above.

(*c*) Further work must be done before the change of cellular mechanism caused by these transmissible agents can be explained.

This hypothesis, when compared with (1), does not show any greater progress as regards (*b*) and (*c*). As to (*a*), though it must be conceded that opinions still differ, I agree with those who think it is in a much stronger position than hypothesis (1).

(3) *Chronic irritation.* The third hypothesis which I regard as being of major importance agrees with the second about the explanation of (*a*). But it considers mammalian malignancy to be essentially different from fowl sarcoma. It therefore rejects the explanations given under (*b*) in the first and second hypotheses and substitutes for them the "chronic irritation" hypothesis. Hence it starts at once with (*c*), *i.e.* it attempts to explain the mechanism of the change from the normal to the malignant cell (in mammals) without postulating any filter-passer or other transmissible agent which might be termed an *ens malignitatis*.

About this third hypothesis it may be said that it contains an element of common sense and an element of weakness.

It is only common sense to insist on those obvious differences between fowl sarcoma and mammalian malignancy which have been demonstrated repeatedly by the vast majority of workers. They have shown that the former contains a filter-passer which reproduces the disease readily and almost

invariably, whereas the latter does not; the former converts normal into malignant cells; the latter does not. Surely these are reasonable grounds for insisting that the causes of the two diseases are different.

The weakness of the hypothesis is its vagueness. It is not yet able to provide a really tangible explanation of those changes in the internal life of the cell which lead to the malignant state.

Complications and confusion.

But many persons would refuse to give unqualified acceptance to any one of the three simple, cut-and-dried hypotheses which I have formulated above. That is only to be expected, when one realises how complicated the hypotheses about malignancy have become. Just as the 20 amino-acids used as "building stones" for protein provide a practically infinite number of possible combinations, so it would be easy to find 20 different ideas about malignancy which might be constructed into an unlimited variety of hypotheses.

To a certain extent, this liberty of choice must be accepted as inevitable. The three hypotheses I have selected are intended to serve as landmarks, emerging above the many subsidiary complexities which undoubtedly exist. Other persons are entitled to choose different landmarks and to fill in the intervening territory in different ways. But there is a limit to this freedom. There are always some general principles which ought to be observed in the construction of theories.

In the first place, there is a difference between what the physicists would term a "law," *i.e.* a general proposition which may be accepted as proved, and a working hypothesis, which, though more or less plausible, is not necessarily valid. I think that neglect of this distinction has caused a good deal of confusion which might be avoided.

For example, the transmissibility of fowl sarcoma by a filter-passing agent has been proved and amounts to a law. The law is not in the least invalidated by the observation that occasionally, and contrary to expectation, a filtrate is inactive. This interesting discovery merely means that the exact conditions under which the law operates have not yet been fully worked out. But this law is not valid for mammalian cancer. Any person who chooses to do so can say that he thinks a similar agent is the cause of the mammalian disease. But that statement is not a law; it is merely a working hypothesis and, in this instance, one for which there is very little experimental support. Neglect of this distinction is partly responsible for misleading attempts to explain mammalian malignancy in terms of fowl sarcoma.

And in dealing with hypotheses, as distinct from laws, reasonable care should be taken in adducing evidence which is supposed to support a particular theory. The most obvious precaution is to see if one can exclude simpler and more readily acceptable explanations of the facts brought forward.

I may quote as an example an instance where neglect of this point has been the cause of considerable confusion in Germany. I refer to Heidenhain's

experiments which, he claimed, demonstrated the infective nature of cancer. He inoculated a large series of mice with human malignant material and found that a small percentage of his animals subsequently developed malignant disease. As the fallacy of his argument has been exposed in detail by Cramer[1], it will suffice here to quote a sentence from the summary of Cramer's article. "Since the frequency of malignant new growth in Heidenhain's series is not greater than the number of spontaneous tumours recorded in the observations of Murray and Miss Slye, Heidenhain's experiments cease to have the significance which he attaches to them."

Other attempts have been made to show that a transmissible *ens malignitatis* may be derived from human beings or other mammals. Here, again, one has to remember the possibility of alternative explanations. The disease may develop not because the inoculated material was malignant but simply because it was an irritant; or the animals inoculated may have been taken from a batch particularly susceptible to cancer; and so on.

All I wish to urge is that neglect of such precautions has often led to confusion. I am not prepared to maintain that every alleged demonstration of a transmissible *ens malignitatis* derived from mammals can be explained as an experimental error or an illogical inference. Malignancy is too obscure to justify such a confident statement. But it is permissible to say that further confirmation on an extensive scale is required before this hypothesis can be regarded as plausible; and to support it by analogies from fowl sarcoma is unsound and misleading.

Whilst adverse criticism is often necessary in order to clear the ground, one feels that it ought to be followed by something of a more helpful and constructive nature.

In the following sections I accept as my starting-point the view which I have outlined as hypothesis (3). As I have admitted, its disadvantage in relation to mammalian malignancy is that present knowledge of life within the cell is insufficient and "chronic irritation" is not a very tangible explanation of the changes in the cellular mechanism which produce malignancy.

I think that perhaps some advance may be made by treating the subject as part of a more general question concerning factors determining cellular variation. From this aspect the problems of transmissible bacteriolysins, fowl sarcoma, and mammalian cancer may be usefully correlated with each other.

CHEMICO-PHYSICAL STABILISATION AS A GENERAL PRINCIPLE IN VARIATION.

In discussing any particular principle or hypothesis in relation to cellular variation there are usually two dangers to be avoided.

Repeated insistence on one idea may be necessary, but it ought not to convey an impression of exaggerated importance. So I wish to make it clear that the stabilisation which I propose to discuss is not intended to explain

[1] *Lancet*, 30. VI. 1928, p. 1347.

everything. It is a mechanism by which the appearance of variants is made possible; but there are many other factors in variation which are of equal significance, though consideration of them does not enter into the present paper.

In the second place, if a word or a phrase amounts to no more than a bare restatement of an accepted fact, its reiteration explains nothing. Thus any normal or abnormal condition of a cell which persists long enough to be recognisable may be said to be "stabilised"; but the word is not particularly helpful if it is merely equivalent to "demonstrable" or "obvious." I hope I shall not be accused of using the term in this superficial sense.

Variation implies the production of a new condition in the growing cell; and growth involves a succession of changes in which one chemico-physical phase of some of its constituents is followed by another. These changes are interfered with if one particular phase is stabilised instead of being transformed into the next phase; such interference disorders the cellular mechanism and is one of the ways in which variants are produced. With this interpretation, I think, stabilisation is a useful conception and its consideration is of importance. There is nothing new or ingenious about it; everyone knows that, in the production of highly complex organic compounds, the chemical constitution of the combination is at first labile and may assume one or other of a variety of different forms, until the presence or absence of a disturbing influence on the conditions of equilibrium makes it "settle down" into the one form in preference to any of the alternatives. This simple conception is usually taken for granted and perhaps that is why the significance of stabilisation is often neglected. All that is needed is recognition; no proof is required.

As an example in bacteriology, I may first mention Andrewes' "diphasic condition" in certain types of *Salmonella*, where the change of a culture from the "specific" to the "group" phase or *vice versa* appears to be spontaneous and uncontrollable. Here, it seems, there are equal facilities for the building up of protoplasm in two different ways; or, as I have suggested[1], the "specific" phase may be a more elaborated form of the "group" phase and growth may be terminated by subdivision either before or after this elaboration is attained. Whether this opinion be right or not, the interesting feature in the present connection is that one or other of these two phases has been temporarily stabilised in the individual cell. As both types of cell are of equally normal occurrence, either may happen to be the variant, *i.e.* the culture which has changed from one phase to the other. A further point of interest has been brought out by W. M. Scott[2]. In some bacteria in which the "specific" and "group" phases have been observed, transition from one to the other is not always random and uncontrollable. A strain may more or less definitely settle down to one phase, which it retains in subculture.

If, as seems reasonable, the diphasic condition is to be brought into line with other forms of variation, it may be regarded as exemplifying alternative

[1] *J. Hygiene*, **26**, 242 (1927). [2] *J. Hygiene*, **25**, 405 (1926).

loss and recovery of an attribute (here a specific antigen), the non-specific or "group" phase being the *minus* variant. It may thus be compared with (*a*) change from the *S* to the *R* form, followed by reversion of *R* to *S*, or with (*b*) loss followed by recovery of virulence, the *minus* variant being the *R* or the non-virulent condition. The difference is that the diphasic change very often seems to occur spontaneously, whereas a definite influence, generally an unfavourable environment, is usually responsible for the commonly occurring *minus* variant of the *R* or the avirulent type; and still more special conditions, such as animal passage, are needed to effect the more difficult and rarer recovery from the *minus* to the fully equipped bacterium. Also the change effected is more likely to be perpetuated, given a suitable environment, from generation to generation than is the "specific" or the "group" phase. But the main feature of interest, as with the diphasic phenomena, is that the condition of the bacterium depends on whether stabilisation, *i.e.* arrest of the normal cycle of chemical changes, occurs at an incomplete or a complete stage of development.

Stabilisation is also a factor, usually taken for granted without explicit mention, in predisposition to bacterial modification. It is well known that some species are much more liable than others to the different forms of variation which have been observed in bacteria. And strains belonging to the same species may also differ in this respect. This means that in the bacteria more liable to change it is more frequently possible for stabilisation of particular cellular constituents to take place in different ways, *i.e.* for arrest of development to occur at different junctures.

To take another example, it is a common observation that, when a bacterial variant is produced by a change of environment, the variant may retain its new characters after return to the old environment. The bacterial constituent which is responsible for the variant has been stabilised. This change of the bacterium varies in its durability according as the persistence of the new attribute is brief, long, or permanent.

Finally, there is the change from living to dead bacterial protoplasm, which is the most drastic effect of stabilisation, *i.e.* the production of a non-viable variant by the fixation of chemical constituents in such a way that continuity of the vital mechanism is impossible.

These examples will suffice for the present purpose. Bacterial variants are produced by stimulants which modify the activity of living protoplasm by altering the reactive capacity of some cellular constituent, with subsequent stabilisation of this alteration.

The delicate serological tests which are used to demonstrate minor variations in bacteria are not always applicable to animal tissues, though it is known that the antigenic qualities of some animal cells are highly complex and individualistic. So one cannot always say with confidence that the latter vary in the same ways as bacteria. For example, it is not proved that animal cells may be "diphasic."

But one cannot assume that the normal daughter cells of a growing animal tissue are always identical with the parent cells in every minute respect. Whilst remaining viable and fully equipped for their normal functions, there is still the possibility that minor changes may occur; and one of these may be of a "diphasic" nature which, being reversible, does not directly result in the production of a permanent variant. But liability to such "diphasic" growth may be an attribute of that predisposition which makes some tissues more susceptible than others to influences which produce gross changes, *i.e.* one phase (perhaps the phase of less complete development) may be more sensitive to extraneous influences than the other; whereas other tissues may always be in a relatively insensitive condition.

This application of the "diphasic" idea is merely speculative. One is on firmer ground in pointing to the fact that, whatever may be the explanation, animal tissues differ from each other in predisposition to modification just as much as bacteria. This predisposition differs in different tissues and it may also differ in the same tissues of individuals of the same species. Further, the latter differences may be inherited, as is also the case with bacteria.

Of the more profound cellular changes the commonest is the *minus* variant, which is due to some definite external influence and may or may not revert to the original form; reversion, when it does take place, is not spontaneous but due to some fresh change of environment. Examples of such changes are frequent in the ordinary processes of degeneration and recovery which occur in animal cells.

With regard to the *plus* variant in bacteria, a parallel may be found in the acquired capacity of animal cells (probably endothelial) to produce antibodies after the adsorbed antigen, which acted as the original stimulus, has disappeared. The change in the cells which produces antibodies has been stabilised. Its duration is variable and may be roughly measured by the time, varying from a few weeks up to the duration of life, during which the host resists reinfection.

Perhaps this is enough to illustrate the general principle that, in both bacterial and animal cells, stabilisation is a factor in variation.

Chemico-physical stabilisation as an explanation of some invisible agents.

Transmissible bacteriolysins.

First, there is something to say about the normal stabilisation of the normally growing cell. This I have always considered, as indicated in a previous paper[1], to be the first matter on which attention should be fixed if one wishes to arrive at a proper understanding of bacteriophage phenomena.

Two features of intracellular life are intimately connected with each other, the enzyme action which breaks up the ingested food into components suitable

[1] *J. Hygiene*, **23**, 319–20 (1924).

for assimilation and the synthesis of these units to form living protoplasm. When behaving as catalysts, the enzymes first form unstable union with the substrate to be digested and then, becoming dissociated therefrom, are left free to deal with fresh substrate. This is followed by the stage of synthesis. A critical point is reached when the union between enzyme and prepared substrate is stabilised; catalytic action ceases to be progressive and is replaced by a synthesis in which the substances formerly acting as catalysts form permanent union with the material upon which they acted. This is normal stabilisation; and the cellular components which are stabilised are the substances which acted successively as catalysts and as synthesising agents.

In normal progressive growth this stabilisation is only temporary. When full development of the cell is followed by subdivision, the cycle of changes begins afresh; catalytic action is again predominant in the daughter cells until temporary stabilisation again supervenes, to be succeeded by fresh subdivision. Bacteria breed with that uniformity which is characteristic of species because catalytic and synthetic action within the cell are attributes of the same protoplasmic substances.

Coming now to deviations from the normal, it can easily be understood that, if temporary stabilisation and subsequent subdivision do not take place exactly at the right phase of development and exactly in the right way, the daughter cells, or some of them, are likely to be variants. One kind of variant, which is very much in evidence in bacteriophage phenomena, is the non-viable variant which promptly succumbs to autolysis.

Here I arrive at a further point, which is of special importance in relation to the subject of the present article. What is the difference between (1) ordinary bacterial autolysis which leaves no lytic principle behind, and (2) transmissible autolysis?

First, emphasis must be laid on the important fact that in (2) a new substance appears. It is new because it is not demonstrable in (1) and also because it is antigenically different from the antigens found in the normal bacterium.

For the interpretation of this fact it is usual to appeal to a "perversion of metabolism." What sort of a "perversion"? If it means a change which causes the bacterium to elaborate something which it never elaborated before, it seems to me that one is expecting too much from the cell's capacity, in supposing that it can adopt a new mechanism just before it succumbs to autolysis. A large variety of non-specific influences may initiate a lytic principle; it is hardly reasonable to assume that the cell promptly responds to each of them by manufacturing a new product. An easier explanation, which is in simpler accordance with the facts, is to draw a distinction between labile and stable disintegration products. In (1), simple autolysis of the normal cell, certain constituents of the cell, which were engaged in the processes of catalysis and synthesis, are highly labile and at once break up, leaving no evidence of their previous existence; in (2), transmissible autolysis, these same constituents are stabilised. Abnormal fixation of a chemical complex is

all that need be postulated; the creation *de novo* is simply a stabilisation *de novo*. This is a more definite conception than "perversion of metabolism," because it is chemico-physical and not merely "biological." It is an instance of the kind of change which is constantly occurring in the realm of organic chemistry, the change of a complex compound from a labile to a stable constitution.

The high stability of the substance termed "bacteriophage" or "lytic principle" is shown by its general behaviour. In sealed tubes a lytic filtrate may retain its activity for years. The active substance is also resistant to drying and it withstands the action of many chemicals to a relatively high degree.

Other properties of this substance which are of interest here may be briefly related. It is only produced in living and growing bacteria and it has the attributes both of an enzyme and of a stimulus to variation. It is a filter-passer which exhibits the usual characters of a small aggregation of protein molecules. Bacteriophages of different origin often differ to some extent in their physical properties, *e.g.* degree of resistance to heat, and in their susceptibility to the action of chemicals; but their main differences are bio-chemical, as shown by their selective action on bacteria and their specific antigenic properties.

These lytic principles provide good examples of the relationship between chemico-physical stabilisation and specificity. At the outset, when they are initiated by a non-specific agent, specificity is entirely derived from the bacterium acted upon, though it is not always exactly the same, *i.e.* stabilisation does not always occur exactly in the same way. When once produced, it is the rule for the lytic principle to act in the same way upon cells of similar strains and in these new cells it stabilises material similar to itself. If the new cell is different but still susceptible (*i.e.* equipped with some combining affinities for the lytic agent), stabilisation may be different, being the resultant of two factors, the specificity of the acting bacteriophage and the partially different specificity of the material acted upon. Hence the bacteriophage derived from such cells may exhibit change in its range of specificity. It may show new characters together with a "remembrance" of characters derived from its original source.

The mechanism of its production affords a strong indication as to the nature of the particular material which is stabilised to become bacteriophage. I have already called attention to the importance, for a proper understanding of bacteriophage, of those intracellular constituents which are concerned with the catalytic and synthetic activities of the normal cell. The pathological change evidently consists in an abnormal stabilisation of these same constituents, so that, while retaining their affinity for the bacterial protoplasm and some of their properties as enzymes, they resist the influences which normally bring their activity to a close, at the critical stage of cellular subdivision, and remain unaffected by their environment.

As the stabilisation takes place within the cell, it interferes with the cell's vital activities, the usual result being death of the cell, autolysis, and liberation of these stabilised constituents. It is interesting to note, however, the existence of experimental evidence showing that there may be some release of these abnormal constituents prior to and not necessarily followed by the death of the cell.

The released substance is transmissible because it has a specific selective action on normal cells of similar type or possessing some similar combining affinities; and the cycle of events is carried on by stabilising the same kind of material in the cells upon which it acts. This view, I think, is preferable to the idea that the agent is merely an enzyme which propagates itself out of the substrate provided by normal cells, though I agree that bacteriophage does resemble an enzyme in some of its properties.

Transmissible autolysis, then, implies the production of a variant (a bacterium sensitive to lysis) and the mechanism of its production is the abnormal stabilisation of a bacterial constituent.

Finally, there is another property of "bacteriophage" which is of particular interest in relation to fowl sarcoma. It may produce a variant which is both resistant and lysogenic; instead of becoming autolysed, the bacterium grows and transmits lytic principle to its offspring. Here, I take it, there is again an abnormal stabilisation of the intracellular material which builds up protoplasm; but the change is not incompatible with cellular life and the changed constituent is propagated within the daughter cells.

Fowl sarcoma.

It is not my purpose here to discuss rival hypotheses. I start with the view that both bacteriophage phenomena and fowl sarcoma are caused by agents or influences which can be produced *de novo* and are not living viruses.

One knows from recent work that this sarcomatous condition can be initiated experimentally by treatment of living cells with various non-specific chemical agents, including chemical extracts from some normal tissues. This non-specific influence produces a specific change. From the affected cells an agent can be liberated which is transmissible through normal cells of the same type, *i.e.* the agent has become highly selective. This agent is a filter-passer and towards physical and chemical reagents it behaves as a small aggregation of protein molecules with stimulative and catalytic activities rather than as a living virus.

In explanation of these facts I think that resemblance to bacteriophage is helpful, provided that it is appreciated in what I consider to be the right way. I may repeat that, to understand bacteriophage, one must begin with that normal stabilisation of the normally growing cell which is concerned with the catalysing and synthesising intracellular elements responsible for ordered growth. The production of bacteriophage is concerned with these same elements, which are now stabilised in an abnormal way, with consequent

perversion of their functions. Similarly, in the production of fowl sarcoma the intracellular elements concerned are those which are normally responsible for catalysis and synthesis in the growing cell; they are stabilised in an abnormal way, with the result that they remain stable when released from the cell and then act, like bacteriophage, as a specific transmissible agent.

The initial production of the agent must be distinguished from its continued propagation. For reasons given when discussing bacteriophage, I do not think it is altogether satisfactory to say that the original non-specific stimulus to fowl sarcoma causes a "perversion of metabolism," or that the abnormal product of this metabolism is a specific agent which, like an enzyme creating itself out of substrate, can produce a similar perversion in kindred normal cells. I think it is simpler and approximates more closely to the facts to regard the initial change as a stabilisation of intracellular elements which, when liberated from the cell, produce a similar stabilisation of corresponding elements in normal cells.

Now one comes to certain obvious differences from transmissible bacteriolysis. The "infected" sarcomatous cells, though very liable to degenerative change and necrosis, are viable and grow into a tumour, whereas the "infected" bacteria, though they may secrete bacteriophage before they die, usually perish and autolyse promptly. In fowl sarcoma, the stabilised element which may be extracted from the cells acts, when within the cell, as an agent which modifies but does not destroy living species' characters; bacteriophage usually destroys the organisation on which living species' characters depend and leaves only elements which, though still specific, are disintegrated and non-viable.

The contrast, however, is less marked when one remembers that bacteriophage, apart from its transmissibility, may modify without destruction; it may produce viable variants which are both lysogenic and resistant to lysis. This condition is more than a mere laboratory curiosity; it furnishes a useful parallel to the behaviour of the cells in fowl sarcoma. Abnormal stabilisation of a particular cellular constituent leads to the production of variants. These are not necessarily non-viable; sometimes bacteria survive after the action of bacteriophage and temporary survival is the general rule in fowl sarcoma.

Reconciliation between this diversity in behaviour is complete when one considers the difference between living protoplasm and dead protein which I discussed in a preceding paper[1]. The former possesses in a high degree that unstable energy which is necessary for vital processes; death means the loss of this special form of energy, though there is retention of ordinary chemical activity, as is exemplified in a high degree by these transmissible agents. Lytic principle in its extracellular form is dead; it is also dead when it is produced within a cell which is about to undergo lysis; when within a lysogenic but resistant cell, its chemico-physical constitution is different, because it is then a component part of living protoplasm. Similarly, the extracellular

[1] *J. Hygiene*, **28**, 13 (1928).

transmissible agent of fowl sarcoma is dead; when this principle is contained in a growing sarcomatous cell, its characters are those of living matter.

Mammalian malignant disease.

In comparing the mammalian disease with fowl sarcoma and bacteriophage, care is necessary to distinguish between laws and tentative hypotheses.

About the two latter conditions a substantial array of facts has accumulated concerning the production and transmission of a filter-passing causal agent. These facts must be amenable to definite laws and such laws are already emerging, though there are still differences of opinion about their detailed interpretation. For example, it is not yet agreed whether the agents are living cells or not and my explanation of their production may not be correct; there are plenty of alternatives. But these controversial matters do not alter the fact that investigators are well within sight of underlying principles.

The position in regard to the mammalian disease is very different. The simple fact that transmissibility by a filter-passer has not been proved must force recognition that ideas borrowed from bacteriophage and fowl sarcoma serve here not as laws but merely as tentative hypotheses.

In attempting to formulate the hypothesis that there is an underlying principle which mammalian malignancy shares in common with the two other diseases, I should begin by accentuating differences rather than resemblances between the postulated common factor or agent. In bacteriophage phenomena, the characteristic condition under which the agent retains its stability is extracellular; in fowl sarcoma it is equally stable both within and outside the cells; in mammalian cancer it is strictly intracellular.

Why not accept these differences as granted? Certain constituents of three different cells may be very much alike in essentials, as being in each case a perverted form of the material which builds up protoplasm; and this resemblance will still hold good, irrespective of the fact that the conditions of their stability differ, the first being stable only outside the cell, the second both outside and within the cell, the third only within the living cell. It is upon this resemblance, I take it, that the common underlying principle should be based.

Having propounded this as the essential hypothesis, one might then supplement it with minor considerations. For example, the stability of bacteriophage is not necessarily extracellular, since it can sometimes be maintained and propagated within the cell; and sometimes the agent of fowl sarcoma is devoid of extracellular stability, with the consequence that, as in mammalian cancer, transmission is only possible by grafting with intact cells. These matters are both interesting and apposite, as illustrating different kinds of stability for one and the same substance; but it would be a dangerous mistake to make them the main argument for resemblance to mammalian malignancy.

Thus, by renewed consideration of the fact that in the mammalian disease there is a profound intracellular change which is handed on to the daughter cells but perishes outside its living cellular envelope, one arrives once more at the conception that there is production of a variant by chemico-physical stabilisation of a cellular constituent.

Then something may be said about the resemblance between fowl sarcoma and the mammalian disease as regards the special characters of malignant growth. Work on the cultivation of tissues *in vitro* has shown that normal cells can be made to continue growth for an unlimited period, provided that the medium is constantly maintained in a favourable condition. So unlimited capacity for growth cannot be said to be the special property of malignant cells. But in the living body there is this difference that the latter maintain their growth under conditions which inhibit the growth of normal cells. This indicates a profound difference in the mechanism of growth. In the normal cell, selection and synthesis of food material is readily checked by slight changes in the environment, usually termed growth-inhibitory influences. In the malignant cell, the growth activities have become insensitive to such influences. The change probably means a reduction of a more complex and more unstable system of equilibrium between cell and environment to one which is less complex and more stable. This appears to be effected by a greater stability of the constructive enzymes of the cell and consequently there is a readier adjustment to environment without loss of their activities.

Here is a way in which, I think, upholders of the "chronic irritation" theory may offer something a little more helpful than a purely negative and adverse attitude towards certain other views which have come into prominence. The "ubiquitous virus" and the mysterious *ens malignitatis* may be replaced by a common principle determining cellular variation; and, whilst rejecting attempts which have been made to interpret mammalian malignancy in terms of fowl sarcoma, it may be conceded that the latter does serve a useful purpose in exemplifying a principle which is also dominant in the former.

This view, as I have indicated in a preceding section, may be harmonised with one of the general principles of cellular variation.

Summary.

The prominence recently given to the Rous sarcoma has increased the confusion of hypotheses about malignancy. With a view to clarification, one may say that there are three simple hypotheses of outstanding importance, viz.: (1) the living virus hypothesis, which regards living viruses as the actual and effective cause of both the avian and the mammalian disease; (2) the autogenous enzyme hypothesis, which ascribes both diseases to the development within living cells of an *ens malignitatis* resembling an enzyme rather than a virus; and (3) the "chronic irritation" hypothesis, which explains mammalian malignancy on this principle and considers fowl sarcoma to be of a different nature.

In the present paper I start with acceptance of the third hypothesis and proceed to treat the subject as part of a more general problem in cellular variation, confining myself to the influence of chemico-physical stabilisation as a factor in the production of variants.

After illustrating the importance of this factor as a general principle in variation, I discuss in more detail its significance in "bacteriophage" phenomena, fowl sarcoma, and mammalian malignant disease.

From this aspect, the "ubiquitous virus" and the mysterious *ens malignitatis* may be replaced by a common principle determining cellular variation.

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