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CANCER—A BIOLOGICAL APPROACH

I. THE PROCESSES OF CONTROL

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The understanding and control of cancer is the most urgent problem of medicine to-day. The volume of research on the subject, particularly in America, is vast and heavily biased in two " practical " directions. There is a strong tendency to concentrate on investigations that may provide leads (*a*) to the elaboration of tests for the early diagnosis of cancer, and (*b*) to its cure by non-surgical means. This has led perhaps to an undue interest in the biochemical aspects and especially the enzymic activities of tumour cells. Clearly if a chemotherapeutic substance is to be found it will be something which can differentially inhibit some processes by which tumour cells differ from normal cells. Such a bias is both inevitable and desirable, but it should have no influence on any attempts to gain a clear picture of the process in terms of general biological concepts.

Scientists may be divided into three groups. There are those, like myself, who believe that at every stage in scientific development it is necessary to provide the best available generalizations as a guide to effective work, both in the application of knowledge to human needs and in the planning of future research. At the other extreme are those who feel that the only valuable scientific activity is to concentrate on some significant facet of knowledge until the facts are incontrovertible and are expressible in some general statement, preferably mathematical in form, that is acceptable to all competent workers. Then we have available a defined unit of knowledge which can be used *reliably* when the time comes to apply the knowledge in any field where it is required. The great majority of scientists take an intermediate position, usually finding their immediate interest in the detailed study of a chosen field, but interested in learning of the emergence of general pictures in the various wider fields.

The present approach to a discussion of the general problems of cancer springs directly from an interest in the nature of antibody production and the ideas of macromolecular pattern developed in a recent essay (Burnet, 1956). It is necessarily based not on personal work in the field or on a comprehensive knowledge of the original literature, but on recent general treatises and compilations, reference to the original papers being made only when these provide essential contributions to the discussion.

Requirements for any General Theory of Cancer

It will be universally admitted that the essence of the group of phenomena we know as cancer or neoplastic growth is *growth* of cells free from the normal *control* exercised by the organism as a whole. Experience of tissue culture suggests that most or all mammalian cells have a capacity for growth when provided with appropriate nutrients. The real problem of cancer is then to understand the processes of control by which normal cells from the fertilized ovum to the end of life are maintained in morphological and functional condition appropriate to the needs of the organism at the time. Perhaps it should be stressed how much more complex and difficult to understand are the processes by which a finger retains its character than what is happening in a carcinoma of the lung. Cancer is a negative condition—a manifestation of the breakdown in one or more aspects of the positive control that welds the cells of the body into a single functioning unit—the organism as a whole.

The failure in cancer is due not to any weakness of the organism but to a change in the character of the cells rendering them in one way or another insusceptible to the normal control.

This statement is self-evident when we consider the phenomena of metastasis and experimental transplantation. When cells from a gastric carcinoma produce metastases in the liver there is nothing to suggest any weakness in the control of liver cells. The secondary nodules are clearly due to cells from the primary tumour. This is even more clearly seen in the case of experimental transplants to animals homozygous with the original host of the tumour. The same two sets of phenomena indicate also that the change in the cells is something handed on from one generation of cells to the next. In the broad sense of the term the change is a genetic one. It is immaterial at the moment whether investigation will in any given case eventually give a more precise label to the genetic change, whether it represents a true somatic mutation, transfer of plasmagenes, incorporation of a virus into a " provirus " state, or some new concept yet to be formulated.

Any hypothesis of a general basis for neoplastic growth must be related directly to this function of control—any other type of associated change can only be regarded as secondary. It is therefore from our point of view impossible to accept Warburg's hypothesis that a change from aerobic to anaerobic glycolysis is the essential feature of cancer cells. If cancer is, as indicated above, essentially a negative biological phenomenon like starvation, scurvy, agammaglobulinaemia, or phenyl-pyruvic oligophrenia, the only legitimate way

to discuss its relation to general biology is *to use the phenomena of cancer* as seen clinically and experimentally *to throw light on the nature of normal controls in the organism.* Basically this is the theme of the present essay.

Process of Control

It is in line with popular present-day thought to apply concepts from mechanical or electronic control and communication systems to biological phenomena. This has been specially evident in discussions of neural mechanisms, but the general principles would be equally applicable to any other type of biological control. Since there is no evidence of more than very minor influences of nervous action in the control of morphology, this will not be discussed. Forestalling subsequent discussion slightly, we may say that there is very much to suggest that control is a function of the movement of complex molecules carrying patterns equivalent to coded instructions from one part of the organism to another. Further, there is no substantial alternative to such a view; "fields of morphogenesis" may be a necessary provisional concept, but there is no serious suggestion that a physical field in any normal sense is concerned.

Any type of control of a collection of living cells subject to trauma and to variable demands on their function will have to incorporate a number of "components." (1) There will be needed a built-in "plan of action." The whole mechanism must function to favour survival of the individual, and we shall probably be correct in regarding the general plan of action in any developed organism as being to maintain or regain the *status quo.* (2) Information must be provided in some form about the state of the cells in question, particularly as it is relevant to the need of more or less cells or of greater or less functional activity. This is the "feed-back" needed by all mechanisms for any type of homoeostatic control. (3) A central control mechanism is needed which will respond to this information by the despatch of appropriate "messengers" to the cells under control. (4) In the cells there must be a reception mechanism to accept instructions so delivered and to induce the appropriate cellular reactions.

This is obviously a generalized statement made to cover such an example as the interaction of pituitary and thyroid, which will be discussed later. Probably all biological control mechanisms need these four components, but one can conceive situations where they may be difficult to recognize. One has in mind the work of Weiss and others on the capacity of cells in appropriate conditions of tissue culture to come together into patterns significantly similar to the structures found in corresponding normal tissues. Here we seem likely to be concerned with something that can be looked on as an interchange of information and instructions between surfaces coming into contact. The third form of control to be discussed, which is referred to rather loosely as "immunological," will probably be found to fit rather clearly into the framework outlined above.

The general hypothesis of control by the interaction of complementary macromolecular patterns has been outlined in what was primarily a discussion of antibody production (Burnet. 1956). This is directly derivative from Ehrlich's hypotheses of lock-and-key action in immunology and chemotherapy. Most of the discussion in *Enzyme, Antigen and Virus* was concerned with protein pattern and the ways in which complementary patterns, not necessarily in the same medium, could arise. It was concluded that for the functioning of such systems there must be means of which (*a*) patterns can be replicated; (*b*) patterns can be used as blueprints from which complementary patterns can be fabricated; (*c*) information encoded in pattern, say on protein or DNA, can be expressed in another medium—for example, RNA or polysaccharide; (*d*) specific union of pattern and complementary pattern can initiate an appropriate reaction —for example, enzymic breakdown of substrate, liberation of histamine, initiation of oestrus, and the like.

The mammalian body contains a wide variety of differentiated cells which differ greatly in the way they are produced, in their potentialities of multiplication, and in their average life as cellular individuals. At the one extreme we have the erythrocytes and a wide variety of what appear to be highly expendable mesenchymal cells, including lymphocytes, histiocytes, granular leucocytes, and fibroblasts. These are produced in permanent and temporary centres of proliferation, spleen, bone marrow, lymph nodes, and a variety of submucosal collections throughout the body. After a life measurable in days or weeks, these expendable cells are got rid of largely by phagocytosis and intracellular digestion, while another large proportion is shed into the intestinal lumen or liberated on some other mucous membrane.

Next we have the epithelial surfaces of tissues exposed to external or internal environments—skin, and alimentary and respiratory mucous membranes. Here attrition, trauma, and infection are everyday occurrences, and there is a steady replacement of older cells by the reproduction of basal cells or their equivalent. Worn out or damaged cells are shed into the environment. These cells in a sense are always growing, and it is significant that the incidence of cancer in the corresponding situations is high. Carcinoma of the skin, larynx and bronchi, stomach, large intestine, and rectum are all common.

Rather closely related are the organs whose lining is modified sharply in relation to sexual periodicities of menstruation, pregnancy, and lactation. In ovary, uterus, and breast there are hormonally determined changes of cellular proliferation and regression. In the uterus excess cells are shed to the exterior. In the breast the process by which the lactating organ regresses to the quiescent stage probably involves a quiet autolysis of superfluous cells and absorption of their substance into the general pool of amino-acids, nucleotides, and other building blocks.

The endocrine organs have a special part to play in the control of bodily function, and provide equally important examples of the way in which cellular proliferation and function is controlled. In such an organ as the thyroid the amount of specifically functioning cells depends in part at least on the concentration of thyrotropic hormone reaching it from the pituitary. With regression the number of cells diminishes, presumably again by autolysis without the intervention of phagocytic processes. It is likely that similar situations hold for the other endocrine organs.

Most of the major tissues of the body seem to belong to the next class in which the average cell life is long, perhaps indefinitely long, but in which, when demand arises, proliferative and reparative processes take place readily and efficiently. Compensatory hypertrophy of remaining portions of tissue is regular after removal of any considerable proportion, such as one member of a paired organ. Liver, kidney, heart, lungs, and skeletal muscle belong here.

Finally we have the permanent cells of the nervous system which once maturity is reached are not expendable.

This provides a basis for the general consideration of the processes concerned in the development and evolution of cancer. For any given type of cell we have two questions—what are the ways in which control may be lost, and, if one or more such controls are lost, is there any way by which they can be reinstated? Since every cell of the mammalian body is derived from the fertilized ovum, it is reasonable to believe that to some extent all cells are subject to all methods of control, just as all cells have certain common metabolic functions. In practice, however, we find that the effective controls vary greatly from one cell type to another, and it will be convenient to divide them into three major types which can be discussed separately: (1) Hormonal control of cell growth and functional activity of certain organs. (2) Local control (*a*) by mechanical pressures, tensions, etc., and (*b*) by mutual interaction of cell surfaces. (3) "Immunological" control associated with the "self-markers" of the body.

Hormonal Control of Growth

It is a striking fact that the most effective chemotherapy of any type of cancer has been the use of oestrogens in prostatic carcinoma. This underlines the significance of hormonal control of growth.

The special quality of hormonal control can perhaps best be seen in the effect on the epidermis of the tadpole of premature exposure to thyroid hormone (Lindeman, 1929). This results in metamorphosis, and as part of the process we find cytolysis and atrophy of epidermal cells on the caudal side of a well-defined line, and proliferative changes on the cranial side. Changing hormone concentrations can in this fashion trigger biologically appropriate changes of different types in various target organs or tissues.

In higher vertebrates the effects are seen largely in endocrine tissues, but there is much to suggest that to some extent all cells are potentially subject to hormonal control. A point that may be of special importance is that control takes the form either of proliferation (and increased functional activity) or cytolysis and atrophy in which the cell substance is quietly broken down and its components are added to the general metabolic pool of the body. They are not "mopped up" by the scavenging cells in the way that most expendable mesenchymal cells are, and they appear to have little or no individual immunological specificity. There is some direct experimental work to support this view. Woodruff and Woodruff (1950), for instance, carried out extensive experiments on the transfer of thyroid tissue from one guinea-pig to the anterior chamber of the eye of another. He found that the tissue became established in the new host if there was a need for it—that is, if the host had been thyroidectomized. The evidence was highly suggestive that in the process by which the foreign tissue became established the stroma of the donor was destroyed and replaced by that of the host, only the specific thyroid cells being genetically still those of the donor.

The general basis of endocrine control has already been briefly discussed, and it has been pointed out how the pituitary control of thyroid function provides one of the most clearly manifested feed-back mechanisms in biological control.

There are a relatively large number of instances where controls of this general type can be distorted by accidental or experimental circumstances to produce hyperplastic or neoplastic growth. Thiouracil and other goitrogenic substances which decrease thyroxine output will induce the formation of thyroid tumours as a result of stimulation by thyrotropic hormone. These tumours are transplantable to other rats (presumably not genetically homozygous) provided the recipients have been rendered thyroxine-deficient either by thyroidectomy or by administration of antithyroid substances (Bielschowsky *et al.*, 1949). A relatively slight change in the situation can give a different picture, involving neoplastic change in the pituitary. Large doses of radio-iodine ^{131}I given to mice will destroy the ability of the thyroid to produce thyroxine, and there is therefore a continuing call for thyrotropic hormone, and the pituitary cells responsible for its production give rise to tumours (Gorbman, 1949). These tumours can be transplanted to mice treated with ^{131}I but not to normal mice. On transfer their autonomy increases and they can eventually be transplanted to untreated mice. Even in this autonomous state, however, they continue to secrete thyrotropic hormone.

Oestrogens will induce testicular tumours in mice by a basically similar mechanism, and, as might be expected, when such tumours are transplanted they will grow to tumours only in recipient mice treated with stilboestrol or other oestrogens.

A different type of experimental artifice to induce endocrine overactivity is to transplant a rat's ovary into the substance of the spleen, the other ovary being removed. A high proportion of such transplanted ovarian tissues give rise to granulosa cell tumours (Biskind and Biskind, 1944). This is interpreted as resulting from the destruction of oestrogens produced by the ovary during their passage through the liver (in the portal circulation). There is thus a continuous stimulus to pituitary liberation of gonadotrophic hormone without the normal feed-back control. The same type of result is found in mice.

Local Controls

It is quite certain that the actual controls playing on all morphologically significant cells are more numerous and subtle than anything that could be suggested at the present time. All that can be attempted is to outline the simplest mechanisms that might account for some of the more easily recognized features of the phenomena of local repair, regeneration, and growth.

In the first instance there is the response to pressures and tensions which mould skin and skeletal tissues into the form needed by simple mechanical considerations. Even a fairly rapidly growing benign tumour of the subcutaneous tissues allows the skin to keep pace in covering it. There are hosts of similar mechanical controls which play their part in reshaping a functional limb after severe trauma or reconstructive surgery. The detail of the processes by which pressures and tensions lead to modification of growth and shape of cells is outside the field of this discussion, but it will have to be elucidated in full if a complete account of the mechanisms of control is to be provided. Indications of more direct influences associated with the cells themselves can be obtained from a variety of sources. A striking example is to be seen in the classical experiment in which an emulsion of disaggregated sponge cells of two species will come together into aggregates, each composed only of the one species.

Recent work, particularly from Weiss's laboratory, has shown that chick embryo cells, dissociated into single units by treatment with trypsin, can under appropriate conditions of tissue culture give rise to a tissue-like association (Moscona, 1953, 1956). An analysis of the process indicates that the first step is for the cells to become grouped according to kind, aggregating into clusters by a variety of processes which seem to include random encounter, directional migration, and surface interactions. The next process is one of organization within the aggregates and their interaction with clusters of cells of other types to develop the tissue-like appearance.

Essentially similar results are described by Leighton (1951, 1954), who used a type of three-dimensional tissue culture in cellulose sponge for embryonic and tumour cells. In this situation cells developed histological patterns very similar to those of tumours growing in the body. From both types of experiment it is clear that a significant proportion of the control of cellular relations is intrinsic to the cells themselves.

If attention is directed particularly on the mutual interaction of contiguous cells, the simplest working hypothesis is that when two adjacent cells are in their appropriate position in the organism the *status quo* is maintained by an interchange of information—in the form of patterned macromolecules—across the interface. Where a surface is free this particular control must be replaced by something of appropriately different character. If cells are removed by trauma or operatively, the gap will be filled by the proliferation of those adjacent to the gap. If this is a simple reaction, we could deduce from it that, in addition to the disappearance of the adjacent cell, either a failure of that cell to provide information of its existence or an inability of the cell itself to "recognize" the information might lead to the same result—that is, proliferation until in one way or another the remaining controls brought it to a halt.

It would seem that the first requirement for malignant growth is a loss of the control mediated by physiological contact with adjacent cells. The locally invasive character of a cancer, the feature which makes its histological diagnosis possible, is in itself the clearest indication of this. Proliferative reactions in general must eventually be seen against

the background of normal controls, a specially important type being the proliferative response to certain virus infections. This is the basis for the great amount of work and speculation that is associated with the virus theory of cancer, and it is best discussed in relation to that theme.

The various mechanisms which are concerned in embryonic morphogenesis must undoubtedly be relevant, but it would be hopeless for anyone not personally familiar with the field to attempt to draw general principles from their complexities.

One field, however, may be mentioned because it indicates very clearly the concept of control by mutual interactions of cells with which we are primarily concerned. In all vertebrates it appears that cells concerned with pigmentation are derived from a special group of ectodermal cells, the neural crest. At appropriate periods in embryonic life these cells pass to the various sites where melanoblasts will eventually be functionally active.

This migration provides extensive opportunities for experimental study of the process. Weiss and Andres (1952), for instance, injected embryonic cells, including melanoblasts, intravenously in chick embryos of a non-pigmented strain. They found that donor melanoblasts proliferated and produced melanin granules of the sort corresponding to their genotype only in locations in the host where they would normally have produced pigment in a bird of the donor strain.

By appropriate experimental action the normal supply of melanoblasts can be prevented from reaching an area of chick embryo skin. If this skin is now transplanted to replace an area of skin on a normal embryo it is rapidly and freely invaded by adjacent melanoblasts. This does not occur if the graft is of normal skin (Rawles, 1944).

There can be little doubt that migration of the primary melanoblasts is directed by processes involving mutual cell contact and that the sites of their functional proliferation are also determined in this fashion. The extent of their multiplication is also clearly related to processes involving the cell community and not by any self-limitation in their capacity to multiply.

All that need be attempted here is to apply some of the ideas drawn by Weiss from such studies to some aspects of tissue interactions at the immunological level that seem likely to be specially relevant to neoplastic phenomena. In a recent discussion (Burnet, 1956), I have tried to make use of Weiss's concept of complementary interaction between cell surfaces to broaden the idea of immunological "self-markers."

The Immunological Approach

The essence of the self-marker hypothesis (Burnet and Fenner, 1949) is that at a late stage in embryonic life the scavenging cell system of the body (reticulo-endothelial cells, etc.) is conditioned to recognize as self-components certain potentially antigenic constituents of the expendable cells of the body. A variety of foreign potential antigens which by accident or experimental manipulation may be present at the relevant time will also be recognized as self. They will then become part of that class of substances which are incapable of acting as antigens in the conditioned animal.

The observational and experimental basis for this concept is now extensive. The clearest evidence is in regard to tolerance of cells which are antigenically distinct but derived from another individual of the same species. Here complete tolerance can readily be demonstrated from twin studies in cattle, sheep, chickens, and one human case. In all these instances red-cell chimeras have been shown to persist indefinitely. In addition, Anderson *et al.* (1951) showed that non-identical bovine twins (with a common placental circulation during foetal life) were receptive to mutual interchange of skin grafts. Billingham *et al.* (1956) showed experimentally that inoculation of cells from a homozygous strain of mice B into late foetuses of strain A resulted in tolerance of the grown mice for skin transplants from a B mouse. This tolerance was specific, being confined to skin grafts from strain B.

In all these examples we have the probability that the tolerant condition is associated with the continuing survival of the genetically heterologous cells in the body. Just as accelerated homograft immunity is produced only by experience of living cells, it may also be claimed that immunological tolerance of this type is dependent on the persisting foreign cells which are capable of "mopping up" any antibody that may be produced.

A variety of evidence, however, is strongly against this view. The most cogent experimental result is also due to Medawar's group. If a tolerant white mouse of strain A carrying a healthy black patch of skin of strain B is left untreated, the patch of foreign skin will remain healthy for an indefinite time. If, however, lymph-node cells from a normal A mouse are inoculated into the tolerant mouse they are accepted and can function normally. Within three to four weeks an immune response results in the breakdown and rejection of the black graft. This observation has two very important implications.

In the first place it shows that something has happened to condition the scavenging cells of the tolerant host which does not apply to similar cells reaching the body of the grown mouse. There can be no question of antibody against B cells being produced and absorbed or diverted by persisting B cells from the modifying prenatal injection.

In the second place it is clear that B cells in the tolerant mouse are liberating something which can stimulate unconditioned cells to produce a specific and destructive immune response. Everything suggests that what is liberated is the same antigen as is responsible for homograft immunity. Since purely epithelial skin grafts can provoke homograft immunity there is reasonable certainty that both the conditioning cells and the tolerated graft are contributing amounts of the antigen. In the tolerant animal this antigen is accepted as self—there is no immune response—but if normal lymph node tissue is made available the standard immune response occurs. Medawar *et al.* have found that a labile non-cellular antigen can be extracted from nuclei which will provoke homograft immunity. It seems highly probable that the same antigen, provisionally characterized as a DNA-protein complex, is the one concerned in provoking immunological tolerance.

From the point of view of cellular control in its relation to cancer the important aspect of these results is the indication that cells of many types are constantly liberating "self-markers" into the lymph and blood circulations. A hint of the way in which these markers may act is contained in the finding by Ebert (1954) that, when an animal is grafted with a tissue from a donor treated with an appropriate radio-isotope, labelled molecules "larger than amino-acids" pass selectively to the corresponding organ of the host. At the present time we have no more than hints that these specific agents have a control function, but those hints are insistent, and Green (1954) has built an interesting immunological theory of cancer by elaborating them. This will be discussed in a later section.

II. THE SIGNIFICANCE OF SOMATIC MUTATION

There is every reason to believe that mutation is at least as frequent in somatic cells as in the germ cells. Somatic mutation has been studied particularly in plants, where the phenomena of continuous growth make its effects more readily demonstrated than in the higher animals. In insects body colour mosaics due to somatic mutation at early stages of differentiation are not infrequent. In mammals most of the evidence for somatic mutation comes from studies in tumour pathology.

The essential difference between a mutation occurring in a germ cell and a similar process in a somatic cell depends simply on the extent and distribution of the cells descendant

from that in which the mutation occurred. A germ-cell mutation can potentially influence all the body cells of all those individuals who receive the modified allele in question. A somatic mutation can influence only those body cells which directly descend from the mutant cell. A mutation of any sort in a nerve cell can have no influence on any other cells but itself; a mutation in a cell giving rise to highly expendable descendants may have wider influence. Any mutation is, according to current opinion, an individual event occurring at a limited region of the genetic mechanism of a single cell. The potential effect of the mutation may be almost anything, although the chance of one type of specific damage will be more or less frequent than that of another.

From the practical point of view, however, we have to remember that every tissue is made up of many thousands or millions of cells. A modification of one cell or of any small proportion of the order characteristic of mutation—that is, $1:10^5$ per cell life—will have no detectable effect on the function of the tissue unless one condition is fulfilled. This is that the mutation results in a differential survival advantage for descendants of the mutant cell as compared with the descendants of normal cells. In other words, somatic mutation in higher animals is of no significance whatever unless it results in the loss or change of some quality which frees it in part from the normal control on its capacity to reproduce. Only mutations along the road toward cancer will be of any significance—they will also be almost the only ones detectable even in principle.

From a wide variety of sources it becomes evident that the change from a normal cell to a cancer cell is rarely if ever a single step. This is perhaps equivalent to saying that every normal cell in the body is played on by multiple controls, and that more than one of these controls must be abrogated to allow unrestrained or excessive growth.

There is first the clinical evidence of pre-cancerous changes in organs like breast, prostate, or skin. Benign hyperkeratoses are very common in the human skin. It is usual, too, to find that recurrences are more malignant than the tumour removed and that tumours held temporarily in check by hormonal treatment or irradiation always eventually break away from control. Later we shall mention the interesting relationship between age and incidence of cancer in various organs which points strongly in this direction.

At the experimental level the work of Berenblum and Shubik (1949) on the process of carcinogenesis in mice is relevant. They found that a brief treatment of the skin with a standard carcinogenic hydrocarbon would produce no tumours. If, however, the areas were subsequently treated for a prolonged period with croton oil, itself non-carcinogenic, tumours arose in numbers approximately proportional to the concentration of the carcinogen used. One essential step was induced by the carcinogen, but the so-called co-carcinogenic action of the croton oil was necessary to allow any manifest expression—that is, to induce or provide conditions for the appearance of some further change or changes.

Once a cancer has reached the stage of being transplantable to other individual hosts, the essential changes have taken place and any subsequent changes are, strictly speaking, irrelevant to the problem of the genesis of cancer. Nevertheless many changes do take place in the course of transplantation experiments, and it is logical to believe that, with due reservation, these changes are likely to be of the same general quality as those associated with the change from normal cells to cells recognizable as cancerous.

Perhaps the outstanding recent feature of work on transplantation has been the recognition that all populations of cancer cells are heterogenous and subject to the processes of mutation and selective survival. There are two main lines of evidence. The first is in regard to the process by which a tumour strain can extend its virulence to other hosts. The second is concerned with the changes observed in response to agents being tested for therapeutic potentialities.

Koprowski's experiments may be mentioned as typical of a number of situations where selective survival of a fraction of a tumour cell population occurs. He tested mice of one strain (ICR) which had been inoculated *in utero* with blood from another strain (C3H) for their susceptibility to an ascites tumour grown in C3H and to which ICR were normally resistant. Tolerance was found to be present, but after a few passages in tolerant hosts the prior manipulation became unnecessary. The ascites tumour strain now grew in all mouse strains tested as well as in ICR and C3H. It appears that tolerance was only partial but allowed sufficient proliferation to let a process of selective survival come into play by which a substrain that had lost further antigenic markers came into dominance.

Almost identical types of result are obtainable by experiments in which tumours are enabled to grow in foreign hosts by making use of some other artifice to weaken the normal immunological resistance. Stewart (1954) used inoculation of newborn mice to adapt a strain of leukaemia to foreign hosts. Earlier Krebs (1943) had adapted a lymphosarcoma by inoculating mice whose resistance had been diminished by *x*-radiation. In each instance once the strain had been persuaded to grow in the immunologically weakened host it became capable of transplantation to the normal grown animal of the previously resistant genotype.

Hauschka (1953) has elaborated this point of view from his studies of ascites tumours. These often show in association with an extension in range of host a change in the distribution of chromosome numbers in the cells from diploid to heteroploid. He showed that a small fraction of heteroploid cells in a tumour could be concentrated by propagation in a partially incompatible host. In his view loss of transplantation specificity is a result of immunological selection of cell types with least antigenicity amongst the available chromosomal variants. The possibility that the selection is rather for cell types which are unduly resistant to the destructive action of immunological processes must also be considered.

When transplantable tumours are exposed to therapeutic concentrations of radiations or antimetabolites it is the rule to find the eventual appearance of resistant tumour strains. Law (1952), working with mouse leukaemias, found that the tumour cell behaved in a fashion amazingly similar to what is observed in regard to drug fastness in bacteria. It could be shown in classical fashion that resistance was not induced by the agent but that its appearance was due to the selective survival of small numbers of mutants present in the pre-existent population.

A similar situation obtains when pituitary tumours are induced by radiothyroidectomy. Furth (1954) transplanted 13 such tumours, finding that primary passage was possible only in thyroidectomized recipients. On continued transfer, however, most of them became autonomous and capable of growth in normal mice. Here again we have the selection of an appropriate mutant by the conditions in which the population is compelled to grow.

From many directions, therefore, we find support for the working hypothesis that the development of malignancy is the result of a biological process in which successive mutations occur in cells capable of continued proliferation, by which a certain advantage is gained. If cell A mutates to A^1, and A^1 has a slight advantage over A, there will be a gradual conversion of at least some regions from a population of A to one of A^1. If mutation A^2 is much more likely to occur in A^1 than in A and A^3 more likely in A^2 than in the others, then we develop a situation that may be very relevant to the behaviour of spontaneous cancer in men and mice.

There is some reason for guessing that on the average about six controls would need to be abrogated before a cell with the full characteristics of malignancy would emerge. The process is shown in the accompanying Diagram, which represents an epithelial layer derived from constantly proliferating basal cells. While these are normal there is no reason to postulate any deviation from the rule that each

basal cell produces only the pillar of cells reaching from it to the surface, where desquamation occurs. A mutation A^1 may, however, result in a progressively wider column of cells with the character of mutant A^1 developing. When these cells become sufficiently numerous, the possibility of a second mutation becomes steadily greater and eventually new columns of cells lacking two controls will appear. The process will continue indefinitely until perhaps when the sixth type of mutation occurs we have the initiation of a focus of frank cancer. As Armitage and Doll (1954) have pointed out, this is the type of process which would give rise to the very constant and significant mathematical relationship that governs the age incidence of death from cancer in human beings.

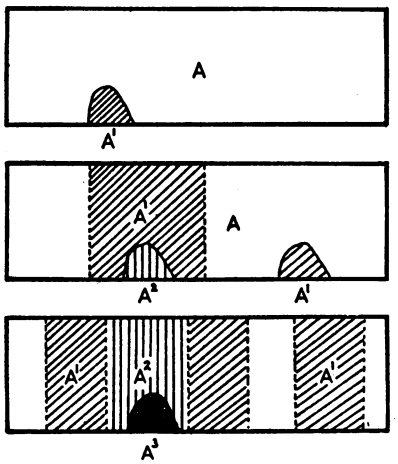

Diagram to illustrate the possible way in which successive mutation to more actively growing cell types could change the constitution of an epithelial cell layer derived from constantly proliferating basal cells (see text).

The frequency of cancer in general and of cancer of most important sites increases rapidly with age, and it is convenient to use a logarithmic scale to show the change in a compact and convenient form. With the usual time scale such a graph shows a gentle concavity downwards. If, however, the age scale is arranged logarithmically so that the intervals 10–20, 20–40, 40–80 have equal value, then the curves fall on straight lines. Exceptions have been discussed by Metcalfe (1955), notably cancer of the prostate, which shows a much steeper slope, and cancer of the ovary, which flattens after the age of 50. Where the incidence of a type of cancer has increased rapidly in the last 50 years, as is the case with bronchial carcinoma, a more complex relation holds. With these exceptions the curves are very uniform in slope and very close approximations to straight lines in America, Britain, and Australia.

The first explanation offered by Fisher and Holloman (1951) was based on the hypothesis that only one mutational step was necessary but that cancer did not develop unless a number of mutated cells found themselves in juxtaposition. If one mutational change occurs in random fashion throughout life and overt malignancy develops only when seven potentially cancerous cells are contiguous, then the observed age incidence would be expected. The interpretation offered by Armitage and Doll (1954), however, appears to be more in line with the clinical and experimental character of malignant change. They suggested that the observed log-log relationship would obtain if for the development of cancer a sequence of six to seven changes in the initiating cell are required. If a cancer cell is the end-result of a series of six or seven successive mutations, then, provided that the probability of the occurrence of each mutation remains constant throughout life and that each mutation is a relatively rare event, the age incidence of cancer would be as observed.

To express the situation in non-mathematical terms we can say that with increasing age more and more patches of cells in expendable tissues are the offspring of cells which have undergone more than one mutation in the line of progression to cancer. Perhaps a very clear indication of this is to look at the skin on the back of the hand of an elderly man and note its mosaic of colour and texture. Cowdry (1955) has treated these changes of skin epidermis with age in some detail, and though he does not specifically state that he regards the changes as mutational in origin, this is virtually implicit in his summary of the main changes, which include: increased thickness of epidermis in some areas, lessened thickness in others, diminution in hairs, sweat and sebaceous glands, patchy areas of hypo- and hypermineralization, occasional marked hypertrophy of single hairs. There are probably invisible mosaics of similar character in many other parts of the ageing body.

There is an interesting clinical implication of the somatic mutation theory of neoplastic change, particularly in the form envisaged by Armitage and Doll. It is a cardinal point of the theory that only if a mutation results in the loss of some structure or function by which general control over growth is exercised will the descendants of the mutated cell become sufficiently numerous to produce clinically or chemically detectable effects. The commonest effects will, of course, be those resulting directly from malignant growth of the cell descendants concerned. There should, however, be occasional opportunities for mutations not themselves leading to or toward malignancy to become manifest because they have occurred in a cell line which has also become malignant.

Macroglobulinaemia (see Mackay, 1956) is a relatively rare condition found in elderly patients in which the blood contains an abnormal globulin of very high molecular weight. It is always associated with a gross replacement of the bone marrow by lymphocyte-like cells which are clearly neoplastic and which in a small proportion of cases give a blood picture hardly distinguishable from chronic lymphatic leukaemia. Whenever the abnormal proteins from different cases of macroglobulinaemia have been compared they have shown sharp individual antigenic differences (Habich, 1953; Kanzow, 1954). Immunologically, and therefore genetically, the appearance of a capacity to produce macroglobulins in excess cannot be directly related to the changes resulting in gross proliferation of the pathological lymphocytes. The only reasonable interpretation is that in each case an independent mutation has occurred in the cell line from which the neoplastic cells have sprung. No other explanation seems capable of covering the facts, notably the rarity of the condition, its age incidence, and the heterogenous character of the abnormal protein.

Possibility of Directed Mutation

Mutation, whether occurring spontaneously or under the influence of mutagenic radiation, is regarded as a random occurrence, striking any given cell at random (a zero order reaction) and producing mutations of varied type, although for our present purposes only those associated with selective growth are observable. There is now, however, a fairly long list of chemical agents which can be called mutagenic on the basis of experiments on one or other form of organism, *Drosophila* and *Bact. coli* being the favourite test objects. These include a number of carcinogens, mustard gas and nitrogen mustard being the best documented. It is a necessary weakness of experimental work on mutagens that in general only rather gross mutations are recognizable, and, as might be expected, most of the known mutagens are toxic to cells at the stage of division and show a capacity to produce visible abnormalities in the chromosomes. More subtle changes may well be missed by current methods.

The observations and deductions of Bird and Fahmy (1952), who studied changes produced in the salivary chromosomes of *Drosophila*, suggest that carcinogens, like the diepoxybutane they used, may act to produce minor degrees of chromosome damage. They found a high proportion of minor deficiencies in the band pattern of the chromosomes of treated larvae; these varied from defects in staining properties to complete absence of a band. They suggested that the findings were best interpreted as due to chemical damage of the molecular pattern of a gene which prevents its normal replication.

As in every other natural situation, the effect of a mutagen is observable only under a limited range of conditions.

If we have a somatic mutagen acting on a mammalian body cell a mutant character can be recognized only under one of two conditions: (1) it may itself provide an opportunity for the cell concerned to produce more descendants than its unmutated congeners; or (2) it is associated either randomly or necessarily with another mutational change allowing selective overgrowth of descendants. Examples of the second class may be found in the variety of rare pathological conditions in which antigenically atypical proteins appear in the serum in association with a variety of neoplastic changes, including myeloma and lymphatic leukaemia.

The problem of the aetiology of cancer or of the mechanism of experimental carcinogenesis may represent essentially a choice between three alternatives. In each it is accepted that the malignant cancer cell is genetically different from the normal cell from which it is derived.

1. Is "mutation" produced by direct damage to chromosomal material altering the genetic code in random fashion? Those mutations which "release" a control against excessive growth will prosper, others will have unobservable results.

2. Are mutations always occurring and the effect of the carcinogen merely to provide, by its combined stimulation of destruction and regeneration, large numbers of cells in which mutations can occur and a specially suitable environment for any cell with a mutation appropriate for differential overgrowth in the special local environment provided by the carcinogen?

3. Does the carcinogen, acting on some key cellular component x, render x non-functional by combining with it, and, by one or other causal mechanism, induce the cell or its descendants to undergo a genetic change by which the component x is no longer produced? One can imagine that a persistent demand for x, owing to its inactivation as soon as it is produced, might in some way lead to damage of the gene controlling the specific pattern of x.

Unfortunately it appears to be extremely difficult to find an experimental choice between the three alternatives, the main difficulty being that by the nature of the material we are precluded from being able to assess what other types of mutations are occurring. The type of experiment which might be expected to throw some light on the matter is that used by Berenblum and Shubik (1949) to demonstrate the co-carcinogenic action of croton oil on mice. Here only a single application of a carcinogenic hydrocarbon is needed to allow the eventual development under prolonged croton oil treatment of foci of neoplasia in numbers approximately proportional to the concentration used of the primary carcinogen. Here it becomes almost impossible to assume that the initiating carcinogen did no more than make use of spontaneously occurring mutations. Somehow or other the primary agent rendered a cell and/or its descendants susceptible to the co-carcinogenic action of the croton oil.

Of the suggestions that have been made to account for the action of carcinogens in producing what is at least an equivalent of somatic mutation, one of the most interesting is Green's (1954) immunological theory of cancer. This makes some use of the self-marker concept of antibody production and immunological tolerance described earlier. Prior to Green's work, immunological ideas were called in, in cancer research, only in discussions of the phenomena of transplantable tumours. It was well known that on first appearance a spontaneous tumour will be transplantable only to mice homozygous with that in which it appeared. After it has been thoroughly adapted to transplantation within the pure strain, it will often be found possible, by one artifice or another, to obtain growth in another strain of mice. Eventually it may be possible to obtain a tumour which is transplantable to and grows freely in any strain of mice.

It is the orthodox opinion, which is backed by a variety of immunological evidence, that the basic reason for failure to grow in a heterologous strain is the immune response of the new host to the foreign marker antigens in the graft. If these marker antigens are lost the tumour develops a progressively wider range of transplantability. As this change is also associated in general with an increase in the other aspects which measure intensity of malignancy, it is a natural extrapolation to think whether loss of markers is not the primary determinant of malignancy.

Green developed his approach from the finding that certain non-carcinogenic hydrocarbons could render mice non-receptive to transfer of tumours which they would otherwise accept. He believed that a carcinogenic hydrocarbon acted by combining with one of the characteristic antigens of the cell type involved and so producing a new antigenic pattern and provoking an immune response against the modified cells. If a somatic mutation occurred by which a cell lost the marker with which the carcinogen could unite, this would give the cell an advantage, since it would not now be damaged by the immune response. If in addition the loss of the marker represented the loss of one of the points necessary for the organism's control the cell would be on the road to the development of malignancy. On this view the wide variation in the nature of carcinogenic molecules, and the equally wide disparity in the species and tissues susceptible to their action, depends essentially on the accident of specific capacity to combine with the pattern characteristic of a marker molecule.

Observations which may be highly relevant to this hypothesis concern the production of hepatomas in rats by administration of butter yellow and similar azo dyes. Here it has been shown that the dye, or derivatives thereof, is bound firmly to a liver protein which is found in the soluble fraction of homogenates and migrates slowly in electrophoresis. A single type of protein seems to be responsible for holding about 80% of the bound dye. When hepatomas develop they contain no protein-bound dye, and their content of the group of liver proteins concerned is much lower than in normal liver tissue. Dyes of this type have a variety of damaging effects on the liver, and small areas of regenerating cells arise, from some of which hepatomas eventually develop.

The suggestion that a specific protein is lost in the process is strongly supported by the immunological work of Weiler (1956). He showed that hepatoma cells contained very little or no specific liver antigen, and, by using Coons's fluorescent antibody technique, detected a series of changes in livers of rats fed the dye. In the earlier stages islets of cells developed which fluoresced less brightly than the normal liver cells surrounding them when stained with a specific anti-rat-liver serum. We thus have evidence of a series of changes involving the progressive loss of protein capable of binding dye products and, concomitantly, a series of changes in immunological character. As Green points out, these findings recall the fact that carcinogenic hydrocarbons are also bound early to proteins in epithelial cells of treated skin, but the fluorescence which evidences this binding is not seen in developed cancer cells.

It seems unlikely that we are dealing here only with the selection of random mutations. Even with associated damage and destruction with much regeneration of cells the speed of mutation would need to be greatly accelerated to account for the results. The possible interpretations are:

1. Spontaneous undirected mutation in cells rendered highly susceptible to mutation by the agent used.

2. Heightened selection against cells carrying the component with which the carcinogenic agent reacts. The only likely suggestion is Green's, that the action of the carcinogen creates a new (and foreign) antigen which provokes an antibody response. This will thus provide a specific selective agent acting against cells capable of binding the carcinogen. When such a mutation occurs it will have a high probability of giving descendants which will replace or overgrow the normal form.

3. By a mechanism perhaps related to what must be assumed to occur in the process of differentiation during development, specific mutations by loss occur when a functioning entity in the cell is unable to exercise that function. These result in the inhibition or disappearance of the genetic mechanism concerned with that function.

Broadly speaking, the choice is between interpretations 2 and 3, although it must be recognized that these are by no means mutually exclusive. Since 2 is concerned with a specific selective process and 3 with a hypothetical type of induced mutation, the two could well be combined.

Alternative 2, the immunological theory of cancer, has the great advantage of being susceptible to experimental attack along the lines discussed by Green (1954). I have not been able to find any account of experiments in which the action of standard carcinogens was tested in mice treated with *x* rays and cortisone to eliminate or greatly diminish the antibody response. A strong anticarcinogenic effect under these circumstances might speak strongly for the correctness of Green's hypothesis.

Alternative 3 is virtually what has been suggested by Haddow and others largely on the basis of the work by the Millers, Weiler, etc., on the production of hepatomas in the rat. It is wholly speculative at the present time, the difficulty of establishing it being the familiar biological one of differentiating between spontaneous mutations with selective survival and a direct environmental effect. Past experience should make us lean toward a Darwinian rather than a Lamarckian interpretation, but, on the other hand, we cannot ignore the possible analogies from the process of differentiation.

Differentiation can be legitimately regarded as a process by which the potentialities of a cell are progressively reduced with its taking on of a specialized function. Tissue culture studies indicate that to a considerable extent the differentiated cell is so in virtue of its inheritance. Even in the absence of normal body controls, its descendants differ from the descendants of other types of specialized cells. If this is so the differentiation must also involve the genetic mechanism. Various aspects of this must be "closed down" either by some form of inhibition or by actual irreversible loss of the genetic unit in question. This closing down cannot be of the nature of a random mutation; it is causally determined in the process of development. The sequence of cause and effect is unknown, but speculation would follow the lines adopted by Pollock (1953) in his interesting discussion of the way enzymes may be developed in the process of biochemical ontogeny. An enzyme is built when a substrate for it appears: a gene will be inhibited, destroyed, or lost when its final product ceases to be of functional significance. To convert that teleological statement into mechanism is a task for the future. At the very least it seems to demand that there is a "feed-back" of information from the cytoplasm to every functionally active component of the genetic mechanism. Most cytologists nowadays would probably be willing to allow this.

There may therefore be some justification for going beyond random somatic mutation to account for the high intensity of carcinogenic action of certain chemical agents.

[*Parts III and IV, with a list of references, will appear in our next issue.*]

The Casualties Union has published a booklet describing its history and work. In 1942 Mr. E. C. CLAXTON, then commandant of the Surrey County Civil Defence Rescue School at Leatherhead, founded the Casualties Union to provide skilled "casualties" for use in Civil Defence training schemes. So that they could play their part with realism these "casualties" were given a medical briefing and a theatrical make-up. The scheme proved so successful that by 1944 the School was turned over wholly to training members of the Allied Armies. When in 1945 Civil Defence was stood down the Casualties Union decided to continue its work, and still provides skilled "casualties" for use in rescue training of all types, first-aid lectures, and rescue competitions. The Union, with a membership of about 1,500, is run on voluntary subscription, and has extended overseas. Inquiries about the Union will be welcomed at its headquarters at 316, Vauxhall Bridge Road, London, S.W.1.

CLINICAL AND SOCIAL PROBLEMS OF PEPTIC ULCER*

BY

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Diagnosis

Establishment of the diagnosis of peptic ulceration can be difficult. The clinical picture may be atypical and there are fallacies about the various investigations.

Clinical Picture

Typically there is epigastric pain associated with food, eased by alkalis and coming on in attacks, but there are many variations. The pain may occur high up in the chest over the sternum, or lower down around the umbilicus or even in the iliac fossae. Sometimes the pain is predominantly in the loin under the costal margin, spreading into the back, simulating a renal pain, especially on the right side. It may be felt only in the back and thought to be muscular in origin—"dyspeptic backache." Apart from the position being unusual, the pain may range from a minimal discomfort to one of considerable severity. It is not uncommon for ulcer pain to come on unrelated to meals, particularly towards the end of the day; relief with alkalis, although the rule, may be lost, particularly with chronic perforation of the ulcer from the stomach or duodenum into the surrounding viscera. Sometimes no pain at all is felt and the ulcer reveals itself by bleeding or perforating. Heartburn, anorexia, periodic nausea, bouts of vomiting, waterbrash, all without pain, may be the only symptoms.

These unusual clinical pictures cause serious difficulties, particularly with patients in whom the radiologist has difficulty in demonstrating an ulcer. This occurs especially in ulcers in the roof of the pyloric antrum and with post-bulbar duodenal ulcer; in such cases a series of negative reports may be recorded before the presence of the ulcer is finally proved, perhaps by clinical complications, gastroscopy, or laparotomy. These patients tend to be diagnosed as having an abdominal neurosis, chronic pancreatitis, or lumbago. They may well develop a secondary neurosis from the uncertainty of their lives and from the failure of their doctors to establish a physical diagnosis. In practice these patients have a consistent story, clear-cut and unembellished by the flights of fancy so common with the true neurotic. The ulcer subject may have pain "like knives sticking in me," which is true, but he will not say he is being "burned alive with wind."

Why do some patients have atypical symptoms? The ulcer with a thick slough may itself be insensitive to pain while pylorospasm is producing oesophageal reflux and heartburn. Enterospasm may occur and the patient may complain of flatulence, trying to bring up wind unsuccessfully, as it may be locked in the splenic flexure of the colon. The ulcer may penetrate surrounding structures, giving rise to parietal pain in the back. A stomal ulcer after partial gastrectomy may become adherent to the diaphragm and chest wall, causing severe

*The second of two Lumleian Lectures delivered to the Royal College of Physicians of London on April 10 and 12, 1956. The first lecture was printed in last week's issue (p. 719).