ROLE OF GENETIC CHANGE IN NEOPLASIA*

BY

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In discussing the role of genetic change in neoplasia I shall confine myself to dealing with some aspects of the problem which appear particularly relevant to the proceedings of this Conference. Furthermore, I should say that this paper is based on the assumption that genetic change is an essential feature of the neoplastic process, and that I am using the word "genetic" in the broad sense to include not just changes at the gene level but also chromosomal rearrangements and changes in chromosome number.

To begin with, we must inquire into the meaning of the term "neoplasia." I am sure it is realized that the clinical manifestations of cancer represent no more than the terminal or near-terminal phase of a process the duration of which may well cover many years and the earliest stages of which may be unrecognizable by conventional techniques of cytological examination. The term "neoplasia" really covers this whole process, right from the time when changes are in some fashion or other initiated within a cell or a number of cells, whereby, among these and their progeny, there appears to be a breakdown of those factors which maintain structural differentiation within normal tissues. The nature of these factors, which govern intercellular relationships, is largely unknown.

The role of genetic change in the neoplastic process may be considered in at least two very general ways. Firstly, we have to think of genetic change in terms of the inception of neoplasia. Linked to this problem is the question of whether these initial changes themselves may not occur with greater frequency in cells the possessors of certain abnormalities of the genome. Such abnormalities may take the form of changes at the gene level, or of structural rearrangements of the chromosomes, or of the possession of an aneuploid chromosome content. In terms of the origin of these abnormalities we may be concerned with genic inheritance, or with the results of errors in gametogenesis of one or other or both parents, or with the results of abnormal cell division in embryogenesis. In the second place, and once inception has occurred, we have to consider the possible role of further genetic changes in the evolution of the neoplastic process, from the initial state, through a precancerous stage, to the final and often apparently completely autonomous state as seen in the florid clinical state of advanced cancer.

Even this is only an incomplete representation of the problem, because in relation to the mechanism of the initial damage we ought to consider the possibility of

the introduction of foreign genetic material—as, for example, through viral infection—or the loss of immunological specificity—for example, through the loss of histocompatibility genes. Furthermore, we ought to try to fit into this pattern the possible sites and modes of action of the known great classes of carcinogens, the many organic chemical agents, the metallic agents, the endocrine carcinogens, and nuclear and allied radiations.

I shall try to deal in greater detail with just some of these facets, and largely by reference to human tumours. Firstly, however, it will be of some value to discuss the two-stage hypothesis of cancer induction.

The Two-stage Hypothesis

Studies, particularly of chemical carcinogenesis in the skin of rabbits and mice, have led to the postulation of the two-stage theory of carcinogenesis, according to which two distinct processes have to be considered—initiation and promotion. Initiation, it is believed, brings about a "subthreshold" neoplastic state, characterized by the presence of cells which proliferate only during a subsequent and prolonged period of promotion. To recapitulate briefly some of the classical findings of chemical carcinogenesis, some substances have been found—for example, urethane—which are effective initiating agents but poor or ineffective promoting agents. Other substances have been found, however, which are effective promoting agents—for example, croton oil—but are weak or ineffective as initiating agents.

As Foulds (1958) has pointed out, the separation of the initiating and promoting phases opens up new approaches to the histological analysis of carcinogenesis, and, one would also add in view of recent developments, to the cytogenetical analysis of this process. A notable finding was that urethane, as an initiating agent, produced no recognizable histological change in the treated skin, but that the subsequent application of croton oil as a promoting agent produced a field of histologically recognizable change in the form of hyperplasia. Furthermore, it was found that if the action of the promoting agent was maintained for long periods of time, further but initially potentially reversible changes occurred in the form of lesions, some which regressed, some of which remained, some which continued to grow as apparently benign lesions. Some lesions, however, went a stage further in adopting the characteristics of invasive tumours.

This model of carcinogenesis has value in the consideration of the human situation, for one may postulate the initiating agent, whatever it may be, to act through the

*A lecture given to the Conference on Diseases of Genetic Origin at the Royal Faculty of Physicians and Surgeons of Glasgow on November 15, 1961.

inception of genetic damage. One may further postulate that, under the influence of promoting agents, cells carrying the initial damage will proliferate, and that during this stage of proliferation the initial damage itself may lead to an increased liability to the establishment of further genetic errors—some of these may become obvious in the appearance of cell lines with visible chromosomal rearrangements or with abnormal chromosome numbers. The eventual appearance of lines which are no longer responsive to those factors maintaining structural differentiation may well herald the development of the invasive malignant process. Thus we may obtain a situation in which, beginning with a single genetical error, we end with a tumour characterized by a high degree of genetic variability, and which through this variability is capable of adapting itself to changes in its environment.

Lung Cancer and Chronic Myeloid Leukaemia

I would like to instance two human tumours, one common and the other rare—the latter, however, is considered as we are now beginning to obtain an almost intimate knowledge of certain aspects of its evolution. The common tumour is lung cancer, the association of which with cigarette smoking is clearly recognized. An important feature of this association is that the probability of developing the tumour is sharply and quickly reduced by the cessation of smoking (Doll, 1959). Such an effect argues against the action of smoking as an initiating agent and in favour of its action as a promoting agent, presumably through the prolonged state of bronchial irritation. This, of course, leaves quite unexplained the problem of initiation, but I shall return to this later.

The second tumour is chronic myeloid leukaemia. The position at present about this tumour is that enough cases have now been studied, some 70–80 to my knowledge, to lead us to believe that we really are looking at a basic change in terms of the loss of material presumed to be from autosome 21, as expressed through the presence of the characteristic Philadelphia chromosome, and that we are close to, if we have not already arrived at, an appreciation of the significant initial damage in this form of tumour (see Nowell and Hungerford, 1960a, 1960b; Baikié *et al.*, 1960; Tough *et al.*, 1961). However, it is not known what is the origin of this damage; it may be the result of a random event affecting the chromosome, or it may in general terms be the result of the action of some external agent on the chromosome.

From our own experience in studying this disease it may be possible to discern much of the evolutionary process of the lesion, starting from a single line of abnormal cells in the chronic stage, and proceeding to the development of multiple lines of cells in the terminal acute leukaemic phase, lines which may be cytogenetically distinguishable one from another. In fact, I would like to suggest that we may regard the clinical stage of this disease in its chronic phase perhaps as analogous to a precancerous state. The widespread dissemination of the cells within the marrow system, and the fact that a proportion of patients die in the chronic phase, may be ascribed to the particular role and properties of the myeloid system within the economy of the body. On this postulate the autonomous malignant state is the acute transformation that takes place in the majority of cases.

We must now return, however, to the problems of the inception of neoplasia, and here we may confine ourselves to two very general questions, which cannot be entirely dissociated although they are so for the convenience of discussion. Firstly, there is the question of genetic susceptibility, and, secondly, we have to give some consideration to the process of ageing.

Genetic Susceptibility

It is generally agreed that the ability to breed out cancer-susceptible strains of animals does indicate that we must consider the existence of, for want at present of a better term, cancer-susceptibility genes. The demonstration of such susceptibility has been and continues to be of some preoccupation in human cancer. Many human pedigrees have been published in which there would appear to be a significantly high incidence of either a particular form of cancer or of several different forms of cancer—for example, Warthin (1913, 1925). Besides such pedigrees, particular attention has been paid to the incidence of breast cancer in the relatives of patients with breast cancer. From the work of Jacobsen (1946), Penrose *et al.* (1948), and Smithers *et al.* (1952) it is difficult not to conclude that in the field of breast cancer there is evidence of genetic susceptibility. Other surveys have been done on other tumours. One such tumour was leukaemia, investigated by Videbaeck (1947), who claimed to have found evidence that the frequency of the disease is increased among the relatives of leukaemics by comparison with the relatives of controls. However, a number of objections to the compilation of these data have been raised, and it is not now held that Videbaeck's findings are acceptable. Nevertheless, I shall in a moment return to this problem of leukaemia in relation to interesting preliminary data relating to the association within families of leukaemia and a number of other conditions.

There is no doubt, however, that a number of conditions of genetic origin carry a high predisposition to the development of tumours. I shall but mention tylosis and oesophageal cancer, and the intestinal polyposis and cancer. We have, also, to consider xeroderma pigmentosum, characterized by dryness, pigmentation, and ulceration of the skin, which is followed by the development of epitheliomata or basal-cell carcinomata. There is also neurofibromatosis, inherited as a dominant, and characterized by multiple pigmented areas of skin and mucous membrane and by the development of numerous fibromata in association with the central and peripheral nervous systems, some of which may undergo a sarcomatous transformation. Yet another condition is diaphysal aclasis, a dominantly inherited trait, characterized by the development of multiple osteochondromata arising usually between the ages of 5 and 10 years, and most often being found at the ends of the long bones. It has been suggested that as many as 10% of affected persons develop a chondrosarcoma at the site of one of these tumours. Another condition is that of epiloia with an irregular dominant inheritance, one important feature of which is the presence of multiple gliomata in the central nervous system. Finally, another outstanding member of this group of lesions is retinoblastoma due to the presence of a single dominant gene.

We may say, therefore, that there exists evidence, firstly, for some form of inheritance of cancer-susceptibility genes, at least in the case of some human tumours ;

and, secondly, that there are a number of instances of inheritance of genetic defect, such as I have just mentioned, which lead inevitably either to the direct appearance of a frankly malignant tumour, as in retinoblastoma—that is, no precancerous phase has been recognized—or to the development of benign lesions, some of which may be transformed into frankly invasive tumours, as, for example, in diaphysal aclasis, or to a remarkably heightened predisposition to environmental effects such as xeroderma pigmentosum. Certainly so far as these latter tumours are concerned the process of initiation would appear to be directly linked with the inheritance of abnormal genes.

Abnormal Chromosome Constitution

We come now to another group of conditions which are just beginning to emerge as an entity in relation to the problem of carcinogenesis, and consideration of which may serve as a bridge between the problem of genetic constitution and susceptibility and that of ageing. The presenting characteristic of this group of conditions is a developmentally abnormal chromosome constitution, which may be found to have as one of its important features an undue susceptibility to the development of cancers of one or more types. The classic example, and so far the only firmly established example, is mongolism. It seems now clear from the work of a number of people, particularly that of Stewart *et al.* (1958), that the risk of a mongol child developing leukaemia is increased about fifteenfold over that of his or her normally constituted counterpart in the general childhood population. It has recently been suggested, moreover, by Wald *et al.* (1961) that this predisposition is extended into adult life.

It will, however, be a long time before the situation is clear in respect of the other major group of chromosomal aberrations, those in which the sex chromosomes are concerned. However, already some possibly interesting leads are coming to light. Two cases of leukaemia have been found in males who had a sex-chromosome abnormality (Tough *et al.*, 1961; Mamunes *et al.*, 1961); these on their own, however, may not be of any particular significance, as males with sex-chromosome abnormalities are common at birth, the estimate at present being of the order of 2.65 per 1,000 live births (Macleane *et al.*, 1961). What is interesting, however, is the finding of a number of families, some published, others unpublished, in which either sex-chromosome abnormalities and leukaemia are associated, or sex-chromosome abnormalities and mongolism, or leukaemia and mongolism, or all three conditions—for example, Buckton *et al.* (1961), Miller *et al.* (1961), Baikie *et al.* (1961). Clearly it is too early to place any absolute value on the significance of these findings, but perhaps we ought to start thinking in terms of the inheritance of some predisposition towards abnormal cell division.

Another problem we should consider at this stage—and really an extension of that which I have just been developing—is that of the frequency with which we find developmental karyotype abnormalities in cancer patients by comparison with the frequency with which such changes are to be found in the general population. There are very few data on this at present, but in our own studies we have found two, and possibly three or four, such individuals among 40 cases of chronic myeloid leukaemia, and one among 38 cases of cancer other

than primary reticulo-endothelial tumours. Judging from our own experience of what may be termed a general population, this frequency is suggestively high.

Carcinogenesis and Ageing

A feature which has been of great interest to us has been the study of the distribution of chromosome counts in cells from leucocyte cultures, and we have done these in a fairly large group of individuals regarded as having a normal karyotype, and in smaller groups of individuals who had an XXY constitution, or who were trisomic for autosome 21, or who had presented with a variety of tumours excluding primary reticulo-endothelial tumours (Jacobs *et al.*, 1961; Buckton *et al.*, 1962). To recapitulate the findings, it was discovered that if the proportions of hypermodal and hypomodal cells found in preparations from leucocyte cultures were related to age, then there was a significant linear relationship, the proportions of both types of cells increasing with age.

The data are based on the study of 97 subjects thought to have a normal karyotype and ranging in age from less than 1 year to over 75 years; they are also based on a study of 42 examples of mongolism and 24 subjects with an XXY sex-chromosome complement. None was known to have a tumour. Similar studies were carried out on 38 subjects with a primary tumour, excluding primary reticulo-endothelial tumours, none of whom had had any treatment apart from two who had had fulguration of bladder papillomata. The observed numbers of aneuploid cells in this group of cancer patients did not differ significantly from those expected as calculated from the regression equations derived from the data on subjects with a normal karyotype. We believe from an analysis of the hypermodal cells that we are observing two effects, the production of artifacts during the preparation of cells for microscopy, but also the occurrence of real errors in division. Whether the latter are a feature fostered by the culture conditions, and the more easily so in the cells of older subjects, is, of course, unknown. It seems reasonable, however, to adopt the working hypothesis that the products of errors in division accumulate in the body with age.

There are one or two interesting facets of the problem of age and carcinogenesis which are relevant to this topic. Nordling (1953) studied the frequency distribution of cancer in adults in relation to age, and found that the log of the death rate increased in direct proportion to the log of age, but about six times more rapidly. He suggested that this relationship could be explained if the cancer cell is the result of seven successive mutations. Subsequently, Armitage and Doll (1954) took up this statistical approach in more detail, and studied various types of cancer, showing that this relationship held for tumours of the oesophagus, stomach, colon, rectum, and pancreas in women. For cancer of the lung, bladder, and prostate in men, and for lung, ovarian, breast, and cervical cancer in women, the observed frequencies diverged either wholly or in part from the theoretical curves, presumably owing to influence of a variety of environmental factors. Thus in breast cancer the relationship corresponds to the predicted relationship up to the time of the menopause, but thereafter the yield falls below that theoretically predicted. More recently Armitage and Doll (1957) have re-examined their approach to this study, and have constructed a model to explain these data in terms of a two-stage hypothesis.

Data are accumulating which suggest that age at exposure to a carcinogen affects the probability of tumour development. This was noted in man by Court Brown and Doll (1957) in their study of the incidence of leukaemia in persons treated with x rays for ankylosing spondylitis. Their data strongly suggested that for a given dose of x rays the probability of developing leukaemia increased with age at exposure. Further unpublished work in this particular study would appear to bear out these findings, and Doll (1962) has expanded this approach to the study of lung cancer in asbestos-miners and in gas-workers, and has re-examined from this aspect the data of Case *et al.* (1954) on the excretion of aniline dyes and bladder cancer. These further studies lend support to the idea that age at exposure to a carcinogen can be an important influence in determining the probability of developing a cancer.

It is difficult to get away from the problem of age and carcinogenesis, as it is clearly so fundamental to our understanding of the induction of neoplasia. We know nothing of the mechanism of ageing, a state of ignorance which has been thrown into sharp relief through the study of radiation effects.

Repeated observations on animals exposed to ionizing radiations have shown them to die prematurely—that is, animals which, if exposed to large doses of radiation, escape death from the acute phenomena of the radiation syndrome. These observations have stimulated widespread interest in the possibility that radiation exposure affects the ageing process. There is, however, considerable controversy among radiobiologists on whether radiation exposure exerts a specific or a non-specific ageing effect. That is to say, whether the expectation of life of the members of an irradiated group is reduced through the increased frequency of certain particular causes of death—for example, leukaemia or nephrosclerosis—or whether the members of the group die at an earlier age from any of the various causes of death which would normally operate in the group. A number of general theories have been put forward concerning the mechanism of life-shortening. Thus one such hypothesis ascribes the degenerative processes of ageing to the random loss of biochemical specificity consequent upon the loss of cellular information, presumably to a considerable extent genetic in nature (Yockey, 1956). Yet another general hypothesis proposes that senescence results from the accumulation of harmful mutations by cells throughout the body, and the effects of such mutations in dividing cells may be amplified through the establishment of clones (Failla, 1957; Henshaw, 1957).

The adherents of such theories to account for ageing naturally postulate that radiation-induced non-specific ageing, if in fact it is a reality, is due to the effect of exposure in increasing the rate of accumulation of harmful genetic changes. We must be guarded, however, in our approach to this vital problem; virtually all these concepts of ageing are conjectural, and one fact which must be taken into account is that so far no evidence of a non-specific ageing effect appears to have been found among the Japanese survivors in relation to leukaemia, and this some 16 years after exposure and following some 10 years of intensive study. The number of cases of leukaemia seen in the heavily exposed survivors is already three times greater than that expected in the entire anticipated lifespan of a comparable non-exposed population (Hollingsworth, 1960). This is not to say, however, that radiation cannot produce somatic

genetic change in man which is detectable many years after exposure. In our own work we have found convincing evidence of such change, using the blood-culture technique, no less than 13 years after exposure to a heavy dose of therapeutic x rays.

However, short of equating the process of ageing with the accumulation of genetic change, it is not unreasonable to believe that harmful genetic changes can accumulate with age, and that in some instances these changes may be such as to initiate the neoplastic state. Alternatively such changes may lead to the provision of cells sensitive to the action of a carcinogen which itself may initiate the neoplastic change or promote the emergence of cell lines, some of which may ultimately acquire the actively invasive and autonomous features of clinical cancer.

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On December 5 President Kennedy said that to get two soldiers the United States Army had to call up seven men. Of the five rejected, three were turned down for physical reasons and two for mental disabilities. The rejection rate was increasing every year. Speaking at a banquet given by the National Football Foundation, the President said the remedy lay in developing programmes for broad participation in exercise by young men and women. "We are under-exercised as a nation. We look instead of play, ride instead of walk." (*The Times*, December 6.)