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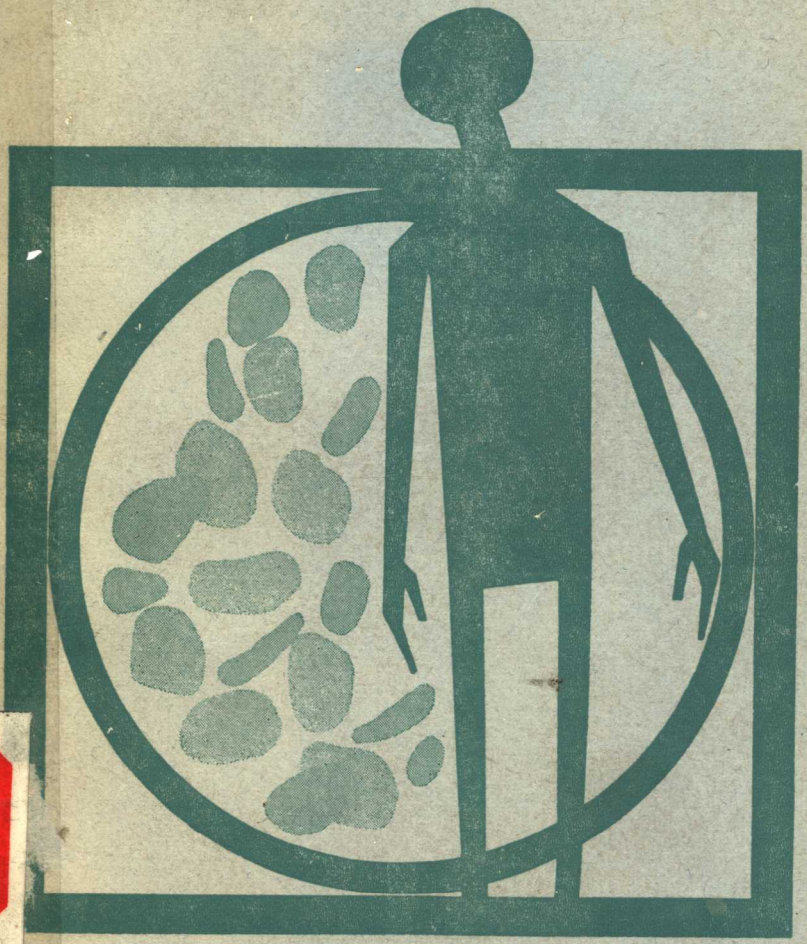

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PREFACE

This book can be regarded as an attempt to provide a reasonably up-to-date account of the interaction of immune processes with malignant disease in man. It is written in almost semi-popular form in the hope of bringing concepts of somatic mutation in relation both to immunology and to many aspects of human pathology to the notice of those physicians and medical students who have an interest in general biology. The work on the book was completed at the end of 1968 but I have added a few notes of more recent work bearing directly on my approach in an addendum, p.243.

I am again deeply indebted to my secretary, Mrs Lorna Nillson, for her work in all aspects of the preparation of the book for publication.

F.M. BURNET
December 1969

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CHAPTER I

INTRODUCTORY CONSPECTUS

The concept of immunological surveillance is something which has evolved rather inconspicuously in the last ten years. In my mind it takes the form of a broad hypothesis, which may soon have the status of a valid generalization, that an important and possibly primary function of immunological mechanisms is to eliminate cells which as a result of somatic mutation or some other inheritable change represent potential dangers to life. The only fully recognized example of such danger is the initiation of malignant disease, of cancer. From the human and medical angles the essence of the hypothesis is that, without immunological surveillance, cancer would be more frequent and occur at younger ages than it does. There may also be other lethal conditions related less directly to weakness of the surveillance function and the theme must be highly relevant to any discussion of the ageing process which ascribes importance to somatic mutation as a factor in senescence. An optimist might hint that a full understanding of the surveillance function might lead in one way or another to a reduction in the incidence of malignant disease and a significant prolongation of life span. As yet there is no real justification for such dreams.

This book is being written not as a part of the search for an elixir of youth or the cure for cancer, but because of the fascination of the theme for a speculative biologist.

Darwinism amongst somatic cells

For at least ten years my chief intellectual interest has been in immunological and pathological aspects of the interplay between somatic cells within the mammalian body. Over that

period there has gradually emerged a conviction that such interactions can be usefully considered from a Darwinian viewpoint. The mobile cells of the body including red cells, granulocytes and lymphocytes, are being produced and destroyed in large numbers all the time. At least in relation to lymphocytes it is known that there are wide functional differences within the population and, in all somatic cells, mutation and probably other inheritable changes in the genome can occur. Under such circumstances it is inevitable that something equivalent to Darwinian selection and evolution is going on within those populations.

This approach at the immunological level took the form of what I called the clonal selection theory of immunity. There is now a substantial body of support amongst immunologists for this general approach but a general hesitation in regard to the nature of the processes which at the somatic level are equivalent to mutation, recombination, etc., in providing the material on which selection can act. Put in the terms more commonly used in immunology, we are still in the dark as to the process by which diversity of immune pattern is generated. I have tended to speak of it as somatic mutation; others perhaps more correctly would express the process as differentiation by random choice of a large range of potentialities.

When one looks at the general process of differentiation in the light of what has emerged from immunology, there are a number of possibilities which arise. The essence of the clonal selection theory of immunity is that the particular form of differentiation which produces the diversity of immunoglobulins is stochastic rather than determinative. Those patterns which arise are then sorted out by the internal environment for either elimination, retention or proliferation probably with, in addition, more subtle distributions to particular function of those not eliminated. For the interpretation of the complexities of immunology there are clear virtues to this approach as against a determinative one in which each cell's function is fully determined by information present in the original zygote. Adaptive immunity is a highly specialized set of functions and it would be not unreasonable to regard the necessary production of

diversity in somatic cells as a unique mode of differentiation. There are, however, several other areas of differentiation in which processes of stochastic character bearing some similarity may be involved.

The development of the central nervous system immediately comes to mind. The general structure of the CNS must be determinative but there are hundreds of situations in which large numbers of similar cells arise and move to distribute themselves or their axons more or less uniformly over a defined region. Further, there is a great deal to suggest that the functional structure of a working brain is built up by something which may represent essentially a form of natural selection of circuits arising by random processes. There is another system in which something similar is likely to emerge, the diversity of olfactory receptors needed to cover a wide range of potentially odorous molecular structures. When one watches the behaviour of a dog, it is immediately evident that every other dog is of olfactory interest to the smeller, and this must surely imply that the dog's own odours must in some way be eliminated from the sensory situation. Only what is foreign is significant and there is a curious resemblance to the phenomenon of immunological tolerance. As in immune reactions there is a need for specific recognition of molecular structure and there could be some real resemblances at molecular and cytological levels in addition to the former analogy.

The important new field opened up by Mintz with her technique of fusing early mouse embryos to produce a single "allophenic individual" seems likely to provide other examples. If, for instance, animals of two coat colours are used, some of the allophenes show "zebra" mice with stripes of equal intensity but in any substantial series of such composites there is a wide spectrum from complete dominance of one colour to dominance of the other. In this and in many other comparable situations there is a process of competition and selection between the two types of cell at various stages of differentiation and morphogenesis.

These three areas are mentioned only to suggest that the two sorts of generation of diversity which I shall discuss are not unique in vertebrate physiology.

The general character of malignant disease

Cancer is apparently limited to vertebrates; there are cellular proliferations with some of the characteristics of malignant change in plants and insects but the conditions seem remote from anything observed in man or other vertebrates. It is highly significant that in man and in those animals for which there is adequate data, i.e., the domesticated mammals and the laboratory rodents, spontaneous malignant disease is predominantly a disease of old age. It will therefore have no bearing at all on the evolution of species most of whose individuals die from the activities of predators after a short period of reproductive life. For the study of the biology of malignant disease, man is by far the most suitable species. The essential features of malignant disease in man which bear directly on the understanding of the biological character of cancer are as follows:

(1) The characteristic pattern of age-specific incidence, the well known log.-log. relationship.
(2) The established monoclonal character of 98% of the cases of multiple myelomatosis and the patchy evidence for monoclonal character in a variety of other malignant conditions.
(3) The frequency of secondary inheritable change to greater malignancy (progression) where this is experimentally demonstrable.
(4) The basic concordance of the histocompatibility characteristics of a tumour with that of normal tissue in the animal from which it arises.
(5) The frequent superposition of a new antigen in the tumour cell clone allowing a limited

immune response to it in the autochthonous or a syngeneic animal.

(6) The evidence that a malignant clone may on occasion produce a demonstrable protein, hormone or antigen not produced by normal cells of the type from which it has arisen.

For ten or twelve years I have been labouring the obvious about the origin of a malignant clone without having the least effect on the pattern of cancer research. Put more or less in the form of a syllogism, it runs as follows:

(1) Somatic cells are as liable, or more liable to mutation than germinal cells, i.e. to suffer error in replication within the limits allowing a viable cell.

(2) Mutation is a stochastic process both in regard to the cell involved and the portion of the genome where the error occurs.

(3) The great majority of viable somatic mutations will affect some function of one cell among millions of normal cells. The effect will be completely undemonstrable unless with age there is a gross accumulation of functionally inadequate cells in the tissue concerned.

(4) The only mutant somatic cells which can produce a detectable effect are those for which the functional change can be magnified by the production of a disproportionately large clone from the mutant cell.

(5) There are many conceivable ways by which mutational change in a cell can render it insusceptible to the controls which maintain normal structural and functional integrity of tissue.

(6) A clone-producing mutant is as liable as any

other somatic cell to have undergone other viable somatic mutations.

(7) Once a disproportionately large mutant clone has developed it will be subject in its turn to mutation and the same rule will hold that only mutants with an additional proliferative advantage will be in a position to dominate the population and change the character of the clone. This is the process of sequential mutation by which, according to hypothesis, full malignant character emerges in a series of steps. Progression in experimental cancer represents the same process.

This approach, which was first stated in detail in 1957 (Burnet, 1957b), probably contains nothing which had not been implicit for many years before that, but I believe that the 1957 presentation brought it more nearly in line with modern genetic ideas. What is more important is that I can see no discrepancy that has been introduced in the decade that has elapsed since it was written.

Immunological status of the cancer cell

It is axiomatic that transplantation of a spontaneous primary tumour to another host follows the same rules as transplantation of normal skin. The histocompatibility antigens are identical for all practical purposes and in fact the concept of histocompatibility antigens first arose from the study of tumour transplantation in pure line strains of mice. No biological statement can ever be made in absolutes. Probably no primary tumour will "take" in 100% of nominally syngeneic recipients even when a substantial inoculum of cells is used. With accurate cell dosage the proportion of takes will diminish as the dose is reduced. There is always a measurable possibility that the syngeneic line is still partly heterozygous and it is necessary to

consider, first, that a proportion of tumour cells are liable to damage while being prepared for transplantation, and second, that there is a factor which can be called invasiveness or virulence of the tumour which to some extent can override histocompatibility differences. The limit of that capacity is reached in the Ehrlich ascites tumour which can be transferred to mice of any breed.

Working in the opposite direction is the appearance of tumour antigens, i.e., of antigens in cells of the tumour clone which are not present in the host (autochthonous or syngeneic) and which under optimal circumstances can provoke a specific immune response. The evidence for this is patchy and it is too early to say that there are specific antigens in every tumour cell. The best evidence is in relation to methylcholanthrene-induced sarcomas in mice, where each tumour appears to have a specific antigenic character differentiating it from other tumours induced in the same host. Similar results have been obtained with other chemical carcinogens and other animal species and the methylcholanthrene results can be accepted as prototype of a group of findings which will justify extensive theoretical analysis.

For the present we can accept the experimental evidence that specific immunity can be induced, as meaning that a new antigen is present on the surface of cells of the malignant clone. By a new antigen we mean one which is sufficiently dissimilar from any antigenic determinants in the body to allow the existence of immunocytes which, while inactive against any normal cells can be stimulated by the new antigen. This accepts the axiom that tolerance, natural or acquired, corresponds to the *absence* of reactive immunocytes.

There are three ways by which the new antigen may arise:

(1) It may necessarily be associated with the appearance of the malignant character, in the sense that the new antigenic determinant is part of some new or modified cell component – an enzyme, perhaps, or some part of the genome

controls – which, by its action, makes the cell malignant. The specificity of different sarcomas produced by the same carcinogen in syngeneic mice speaks strongly against this. If a certain chemical change is directly associated with the development of malignancy, surely it should be common to all the tumours.

(2) There are present amongst the fibroblasts (or more strictly the cell population in the limb from which the ancestral malignant cell is drawn) a wide range of minor differences in chemical structure of surface components, any one of which is so dispersed amongst a relatively small number of isolated cells that it cannot effectively serve as an antigenic stimulus to the body. Only when the amount of one such antigen is sufficiently increased by the development of a clone containing a significant number of cells does the possibility of antigenicity arise. The main interest of this hypothesis lies in the implication that there is a somatic-genetic process allowing the generation of a diversity of antigens analogous in some way to what must be postulated for the production of antibody patterns. It would add much to the symmetry of the concept on which this book is based if the characteristic process by which antibody pattern diversification arises is demonstrable in respect to other protein components of the cell surface and, more particularly, in those cell surface components which, as histocompatibility determinants, have played a complementary role to antibody pattern in the evolution of adaptive immunity.

(3) The third possibility is that as soon as the cell becomes malignant there is some relaxation of

> control within the genome. Things can happen which are not allowable in a normal cell. There are hints that there may be abnormal de-repressions, i.e., activation of the cell to synthesize a protein properly produced by cells in some part of the body but not by the cell immediately responsible for the malignant clone. If any such explanation of the emergence of the new antigen is accepted, however, any observable consequence must be stabilized after it occurs. Phenotypic restriction must be operative, since the new antigenic quality persists on passage of the tumour.

Looking at the three alternatives, I find myself settling for the second, mainly because of the detailed analogies that can be drawn from what has been learnt of the nature of multiple myelomatosis and Waldenstrom's macroglobulinaemia. The only acceptable interpretation of the pathogenesis of myelomatosis is that a cell already committed to the production of a certain immunoglobulin takes on malignant quality which in this instance is an almost trivial change. Instead of multiplying for 5-10 cell generations and then becoming a mature non-proliferating plasma cell, the clone retains the proliferating immature quality indefinitely.

The hypothesis would take a similar form when applied to the individual specificity of sarcomas induced in mice by methylcholanthrene. The histocompatibility antigens form part of a lipoprotein complex on the cell surface, the specific molecular configurations which confer the antigenic determinant quality are unknown. It is simplest to think of them as short amino acid sequences of protein or as configurations arising in secondary or tertiary folding of protein chains. If they are polysaccharide in character or in some other way have their antigenic pattern determined by an indirect method, the argument merely becomes a little more complex. The pattern one

way or another is fully determined by information in the genome.

It would be unjustified to assume that the surface antigens of cells that we speak of as histocompatibility antigens (HCA) were as clearly defined as some of the tabulations of H2 HCA in mice would lead us to believe. Even in mice there are many other indications of histocompatibility differences outside of the H2 system. In all probability we are dealing with a dynamic surface pattern of lipoprotein and complex polysaccharide which is liable to be modified, in regard to its significant antigenic determinants by a variety of somatic mutations or other intragenomic changes. There is no doubt that, in mice, mutations or equivalent genetic changes at the germinal cell level occur with considerable frequency.

At the somatic level there is the requirement that such changes will never be sufficiently numerous to allow the establishment of tolerance against any but the genetically determined HCAs. It would therefore be outside of any normal probability for a tumour to arise with any large deviation from the major HCA of its carrier. The differences we are concerned with must be at a lower level of significance. Probably what we should look for is a process by which there is a high probability of producing a very minor change but only a low probability that occasionally a major change can occur. Point mutation involving a single nucleotide seems to be the most likely source but one could also imagine a process involving interaction and crossing over amongst a number of duplicated genes each differing only by one or two nucleotides from any other. In line with our general approach, it would seem probable that the eventual solution will take the form of the same basic mechanism which is responsible for generating the diversity of immune patterns but keyed to give a much lower frequency and range of minor modifications.

The deeper significance of this type of diversification will need much further discussion; here it is only appropriate to make the point that any development in detail of the surveillance concept must somehow account for the two complementary diversification mechanisms, both involving components on the

surface of body cells. It is economy of hypothesis to postulate that both may be manifestations of similar processes. The present suggestion, then, is that at the somatic level there is extensive diversification of HCA pattern but the great majority of the changes are of minor character. This means that tumours arising, presumably in each case from a single cell, in each of six or ten mice will have a very high probability of being detectably different at the immunological level but not sufficiently different to be rejected immediately by nominally syngeneic recipients. On the other hand, if a somatic cell produces a very distinctively different antigen and under the impact of malignant change proliferates as a clone, the cell mass will be rejected as foreign at an early stage by the autochthomous host and will never be perceived as a tumour by the experimenter. It is probably true that only malignant cells which do not differ too widely in HCA structure from the host will ever emerge as tumours.

Are there antigens specific for tumours as tumours?

It is now accepted that under the influence of murine or hamster oncogenic viruses, tumours are induced which after passage in virus-immune animals can be obtained free of virus.

There have been suggestions that by special methods, virus can be recovered from some ostensibly virus-free stocks of such tumour cells but the weight of evidence is in favour of the view that tumours induced by virus can be obtained as malignant cell lines free of virus. The important finding is that each virus with this general quality induces virus-free tumours with a new HCA – the so-called T-antigen – which is characteristic of the virus used to induce the tumour. Here we have a condition differing sharply from that of the MCA sarcomas. The T-antigens are no "stronger", careful quantitative experiments being needed to detect them, but they are common to all tumours produced by one virus type. The generally accepted interpretation is that a portion of the viral DNA has become incorporated into the cell genome where it can code for the T-antigen. As is the almost

invariable rule amongst experimentalists, a positive cause for the phenomenon – the intrusion of portion of the virus genome – is sought without regard to the alternative possibility always present in such biological situations, that the uniform type of abnormal cell which emerges is selected for survival from a widely heterogeneous group or population of susceptible cells whose genome had been damaged or modified by intrusion of the viral genome. Any variant cell with a special capacity (a) to proliferate freely, (b) to tolerate relatively large amounts of proliferating virus without damage to its own viability and proliferative capacity, will inevitably be selected by the manipulations of the experimenter for survival and further study. From the standpoint of a general biologist this seems to be a much more reasonable interpretation than the conventional one. What must be emphasized repeatedly is that experimental cancer virus research has developed almost wholly as a naive exploitation of the immense selective power of an experimenter seeking proliferative activity of the viruses and cells with which he is working. It is a new chapter of the studies initiated by Darwin in his *Variation of Plants and Animals under Domestication.* In nature there is no evidence whatsoever that an oncogenic virus of the type of polymona, SV40 or Adenovirus 12 makes any use of its power to induce tumours as a means of survival. Polyoma virus appears to be a relatively common respiratory virus of mice whose only demonstrable effect is to provoke antibody production. There is no suggestion that any of the adenoviruses survive by inducing malignancy in man. It is possible for a virus to survive in association with a proliferative lesion of the skin; plantar warts and molluscum contagiosum of man and the Shope papilloma of the Silvilagus (cottontail) rabbit are the classical examples. None produces malignant disease, although by experimental manipulations of the usual sort it has been possible to produce malignant tumours in *Oryctolagus* rabbits from cottontail papilloma virus. There is no conceivable way by which the production of internal malignant tumours could evolve as a standard method of virus survival. This does not, of course, rule