Biological Basis of Medicine
1969
236
ROE 1969

CHAPTER 13

Mechanisms of Carcinogenesis

FRANCIS J. C. ROE
Chester Beatty Research Institute,
London, England

I. Introduction and Definitions

The first need in any area of research is to define the problem. Lack of precise definition is hampering the elucidation of mechanisms of carcinogenesis both by epidemiological and by experimental means.

Most cancer research workers would agree that "carcinogenesis" refers to "the induction of cancer", but differences between their individual images of "cancer" are legion. Doubtless most share a similar picture of the usual course of the terminal stages of cancer in man – of a slowly killing, invasive and disseminating process, associated with the proliferation of abnormal body cells. But, of the earlier stages of the disease, either in the clinic or in the laboratory, views differ according to knowledge and particular experience. Lack of anything like a common image is currently making cancer research a much more confused subject than it need be.

Although numerous infective agents that cause a wide variety of disease states are recognized, it is still useful to be able to use the term "infectious disease". Undoubtedly an equally large number and even wider range of aetiological agents are involved in the genesis of the cancerous state. Nevertheless, even some of those who could be expected to know better still speak of "the cause" of cancer, as though cancer were a single disease entity.

Because cancer is in reality a group of diseases, and because multiple factors may contribute to its causation, it can only be defined in general terms which avoid any reference to aetiological mechanisms. Elsewhere (Roe, 1966*a*) we have suggested as a definition: "Cancer is a disease of

multicellular organisms which is characterized by the seemingly uncontrolled multiplication and spread within the organism of apparently abnormal forms of the organism's own cells". Unfortunately neither this nor any other general definition meets the needs of all cancer researchers of all disciplines.

In some senses the morbid anatomist is in the best position to lay down the criteria that have to be fulfilled for a diagnosis of cancer to be made. He has the opportunity to examine the whole body macroscopically and microscopically and to establish the complete pattern of the disease state at one point in time – the time of death. Despite these advantages, even he is sometimes in doubt with regard to the diagnosis. If death is incidental to the disease state that is suspected of being cancerous (e.g. a possibly cancerous lesion is discovered at necropsy on a patient who died from some other cause), his findings may well be equivocal and subject to differences of opinion between himself and his colleagues. There are no hard and fast lines which separate the appearance of inflammatory, hyperplastic and neoplastic states. The classification of cancers into microscopically distinguishable types is the subject of even greater and more frequent differences of opinion between pathologists. For most experienced pathologists the areas of uncertainty are not wide, but they exist. When errors are made they are reproduced by the epidemiologist who bases his survey on necropsy findings – so that a distorted picture of mortality from a particular type of cancer may emerge. In the laboratory the cancerous nature of a lesion may be checked by seeing if it will grow as a tumour on transplantation into other genetically similar (syngeneic) animals of the same species. Growth of the transplant should certainly not be accepted as an absolute criterion of malignancy, but it is a procedure that may be helpful in some circumstances. Clearly this aid to precision is not available to the human pathologist.

In clinical practice the pathological diagnosis of biopsy specimens is more open to error than necropsy in so far as the information and amount of tissue available to the pathologist is much less. In many cases his report is in reality a prognostication based on previous experience of patients with lesions of similar macroscopic and microscopic appearance. The use of the word "malignant" in the pathological report is to be interpreted as "treat radically". But the "previous experience" on the basis of which such reports are made is sometimes ill-defined. Often it is not personal, but based mainly on the work of other observers. If radical treatment is recommended and followed, there is no means of knowing whether it was justified or not. Hence there is no feed-back of information that tells a pathologist that he is overdiagnosing malignancy. Under the most favourable conditions errors of judgement by pathologists are probably infrequent, but the potentiality for them increases sharply with distance from the necropsy department of the teaching hospital, and the margin of error in relation to epidemiological studies not based on pathological diagnosis is very wide.

Two aspects of cancer diagnosis are especially relevant to the present discussion, namely the concepts of the pre-invasive carcinoma and of latent carcinoma. The criteria for diagnosis of such lesions are far from generally agreed. Franks (1954), from a study of serial sections, has reported the presence of small foci of latent carcinoma in a considerable proportion of human prostates, the proportion increasing with age. He and others have described latent carcinomas in the lung and a number of other tissues. On the other hand, Whitwell (1955) and Cunningham *et al.* (1958) decided that the small neoplastic-like lesions or "tumourlets", often seen in association with bronchiectasis or chronic lung abscess are *not* true neoplasms. If an unknown proportion of such small and symptomless lesions were to be counted as examples of cancer, then it would become more or less impossible to arrive at a meaningful estimate of the human cancer burden.

In relation to this burden, ideally one would like to know the numbers and times of appearance of each type of cancerous lesion in every individual within a defined population. The study of correlations between genetic factors, environmental factors, and the incidence and progress of particular types of lesion would constitute the first logical step in the elucidation of the mechanisms of carcinogenesis involved.

The experimentalist is much better placed in the search for meaningful associations between the incidence of cancerous lesions and the operation of genetic and environmental factors. He can formulate, and rigidly adhere to, diagnostic criteria for malignancy and can, to a large extent, study the effects of single genetic or environmental factors whilst controlling the rest. Unfortunately, individual experimentalists often fail to take full advantage of these opportunities and the literature on experimental carcinogenesis is unnecessarily confused because of this.

As will be concluded below, it may well be that the value of many laboratory experiments in the field of carcinogenesis lies not in the fact that they reveal information of any basic relevance to the mechanism of induction of any form of cancer in man, but that they point to methods whereby the aetiology of particular forms of human cancer may be investigated. The study, however detailed in other respects, of pathologically and epidemiologically ill-defined lesions in genetically uncharacterized populations of laboratory animals is not very likely to be of much value in this latter connection.

II. Multi-factorial Causation of Cancer

If exposure to a particular agent is regularly associated with the subsequent development of cancer under a wide variety of circumstances and in a wide variety of species, it seems reasonable to regard the agent as "carcinogenic". Experiments with such agents are easy to perform and results that are both acceptable and quantifiable are more or less assured even if only scant attention is paid to the control of background genetic or environmental factors. More careful studies usually show that the effects of such agents may be modified by other agents operating simultaneously or

sequentially. Depending on the direction of the modification, such agents have been regarded as exerting *co-carcinogenic* or *anti-carcinogenic* effects. In a recent review (Roe and Rowson, 1968) an attempt was made to list some of the ways in which modification may be brought about (see Table I).

Many examples are known in which the mechanism of modification is so obvious that one would hesitate to use the term co-carcinogenesis or anti-carcinogenesis to describe them. Thus in skin carcinogenesis experiments, lipophilic solvents which aid absorption, enhance carcinogenesis by polycyclic aromatic hydrocarbons in comparison with oily solvents which retard it (Riska, 1956). In germ-free mice the oral administration of cycasin gives rise to no neoplasms, whereas in conventionally maintained animals whose intestines contain bacteria which degradate cycasin to a potent carcinogen, intestinal tumours arise (Laqueur *et al.*, 1967). The elucidation of the reason why germ-free and conventional animals behave differently obviates the need to regard the bacterial flora as co-carcinogenic. What would have been the position if the mechanism was not apparent?

This question raises a more general problem. There are situations in which the distinction between carcinogens and factors which enhance the co-carcinogenicity of other agents is difficult. The induction of malignant lymphoma in mice may be taken as an example. A high proportion of mice of the AK strain develop the disease spontaneously. A peculiarity of the strain is the poor development of the adrenal cortex and low level of adrenocortical secretion. Administration of cortisone or corticosteroids reduces the incidence of the disease. Associated with the poor development of the adrenal cortex in AK strain mice there is a hypertrophy, or more correctly, a persistence, of the foetal state of the cortex of the thymus. A group of viruses (including Gross Passage A virus, Maloney virus, and Graffi virus), carried regularly by mice of several strains, may react with the primitive cells of the thymic cortex in such a way that lymphoma eventually develops. Any factor or agent which favours the persistence of a wide zone of foetal cortical cells in the thymus favours the development of malignant lymphoma. Adrenalectomy and the administration of oestrogens do this; in other words, these treatments make mice of various other strains similar to the AK strain in respect of thymic status. X-radiation causes first a partial destruction, and then a rebound and fairly persistent hyperplasia of the thymic cortex. It is unlikely that any of these procedures would predispose to lymphomagenesis in the absence of one of the lymphoma viruses. Genetic factors influence not only the extent of the thymic cortical overgrowth in response to stimuli, but also the susceptibility of mice to the carrying of the necessary viruses.

It is now known that viruses, such as the Gross Passage A virus, may act as helper viruses by providing the information necessary for the production of the protein coat for yet another virus, the mouse sarcoma virus (Harvey and Maloney strains), though there is no evidence that the mouse sarcoma virus is itself involved in lymphomagenesis. At present, therefore, the closest we can get to identifying the true basic cause of malignant

TABLE I

Some mechanisms by which viruses and other agents may interact in carcinogenesis

True multifactorial carcinogenesis	Factors operating at the cellular level to modify		Factors operating at the tissue level to modify	
	Cell transformation	Tumour development	Cell transformation	Tumour development
Both agents act simultaneously or sequentially on the genetic apparatus of cells	1. Facilitation of entry of inducing agent into cell or its transport to the target site within the cell 2. Interference with mechanism whereby cell resists transformation, e.g., inhibition of the production of interferon, or of detoxification enzyme systems 3. An alteration in the physiological state of the cell such that it is more susceptible to transformation 4. Interference with balance between replication of cells and of the viruses they carry in favor of the latter	1. Protection of the transformed cell against immunological defence systems 2. Reduction of susceptibility of transformed cell to other homeostatic forces	1. Facilitation of absorption of inducing agent into body or of its transport within the body to the target tissue, e.g., by suppression of circulating antibody levels or of cellular defence systems 2. Interference with excretion or detoxification of carcinogen 3. Enhancement of exposure of target tissue to inducing agent by prior establishment of inflammatory state 4. Increase in proportion of cells susceptible to inducing agent as a result of metaplasia or hormone activation	1. Inhibition of immunological defence system 2. Alterations in hormonal or nutritional status 3. Induction of hyperplasia (e.g., by an irritant) such that clones of cells of critical size (Berenblum, 1954a) develop from transformed cells

lymphoma in mice is that it is one of a group of viruses. In the past, however, many other agents, including some of those mentioned above, have been regarded as causative.

Morton and Mider (1938) reported that certain carcinogenic polycyclic hydrocarbons predispose to malignant lymphoma in mice. Later Kawamoto *et al.* (1958) reported a similar response in respect of urethane. The administration of quite small doses of these agents to mice when they are newly born is especially evocative of lymphomas (Pietra *et al.*, 1959). The lymphomas that arise in response to these chemical agents are indistinguishable from "virus-induced" lesions which suggests that the chemicals do no more than enhance the lymphomagenic effect of viruses carried naturally by the mice. Thus, although there is abundant evidence from experimental studies in other systems that the polycyclic hydrocarbon and urethane referred to above may act as true carcinogens, it seems that in relation to the induction of lymphoma in mice their usual or sole role is one of co-carcinogenesis.

There are other examples of known carcinogens acting as co-carcinogens under certain circumstances. The administration of immuno-suppressive agents, such as X-radiation, prednisolone, cortisone, 6-mercaptopurine or methotrexate, change the response of rabbits to Shope fibroma virus from a trivial local reaction to one of generalized fibromatosis (see Roe and Rowson, 1968, for review). Coal tar, or carcinogens such as 3-methylcholanthrene (MC) or 3,4-benzopyrene (BP), may bring about the same type of enhancement (Ahlström and Andrewes, 1938). The evidence suggests that MC and BP bring about the enhancement not by reason of their carcinogenicity, but because they too suppress immune responsiveness (Stjernsward, 1965; Ball *et al.*, 1966; Weston, 1967).

There are, then, many examples both of multifactorial carcinogenesis and of known carcinogens modifying carcinogenesis by other agents. It follows that there is a real risk of confusing carcinogens and co-carcinogens, and that, in general, it is wrong indiscriminately to use as adjectives terms such as "carcinogenic" or "co-carcinogenic" in relation to individual agents without reference to the particular biological systems and conditions in which the adjective applies.

III. Investigation of Mechanisms

Because of the diversity of ways in which numerous factors may interact to bring about cancer, it would be presumptive for any worker to assume that a particular mechanism that he has unravelled in one biological system is directly, without modification, applicable to any other. If he is lucky he may discover a phenomenon of general application, but the onus is on him to demonstrate its wider significance and he should never assume it. This applies both to extrapolation between various laboratory species and extrapolation from the laboratory to man. History tells us that it is not always easy to demonstrate carcinogenesis by exposing laboratory animals to agents known to be potent carcinogens for man. Thus coal tar (soot) was recognized

as carcinogenic for human skin 140 years before the first tumours were induced in animals by it. Investigators made many difficulties for themselves by failing to observe their animals for long enough, or by exposing them to doses of tar that were too small to elicit an effect. Even today there is one agent, arsenic, associated with the induction of cancer in man, which has not yet been shown to induce cancer in other species. It is possible that the apparently peculiar sensitivity of man to carcinogenesis in response to arsenic depends on the existence of another peculiarly human factor. If this is so the latter may be the true carcinogen, and arsenic merely a co-carcinogen, albeit an important one.

It is not always easy to arrange a laboratory model in which human exposure is satisfactorily mimicked. This is especially true in relation to the induction of lung cancer by tobacco smoke. Other animal species lack the higher faculties used by man in his voluntary inhalation of the smoke and it is difficult passively to introduce a comparable dose of smoke into the lungs of laboratory animals (see Roe and Walters, 1965, for review). Despite these difficulties, adenomas and adenocarcinomas of the lungs have been induced in mice by prolonged exposure to tobacco smoke (Essenberg, 1952; Mühlbock, 1955; Harris and Negroni, 1967). In man the types of cancer mostly associated with exposure to tobacco smoke are squamous carcinomas and undifferentiated (oat-cell) carcinomas. The difference in type of tumour induced may well be attributable to anatomical differences between mouse and man (Roe, 1966*b*).

The basic requirements of a laboratory model include comparable anatomy, the possibility of comparable exposure (dose, dose-schedule, route of administration), comparable transport to the target tissue and comparable observation time. In addition, if the agent administered is not the proximate carcinogen but only a precursor of it, there should be in the model relevant enzymic and metabolic pathways similar to those in man. These latter requirements frequently beg the question in the sense that neither the identity of the proximate carcinogen nor the need for metabolizing enzymes are known.

In practice, it is encouraging that, despite all the possible stumbling blocks, many potent carcinogens act similarly in a variety of species and under a variety of conditions. Differences in response that cannot be attributed to lack of comparability in anatomical structure, dose at the target site, or enzyme pathways should perhaps lead one to suspect that the agent concerned is not acting as a true carcinogen in any of the systems studied.

Smithers (1962) has attacked "cytologism", or the preoccupation with changes at the cellular level in relation to cancer. He points out that cancers often arise in pathologically changed tissues or organs, and that their appearance is frequently accompanied by evidence of hormonal derangement or immunological disturbance, etc. On the other hand, all cancers grow by the multiplication of cells, and there is abundant evidence that the cells of which tumours consist are themselves abnormal. If it is

accepted that multiple factors may contribute to the genesis of cancer, then Smither's argument presents no conflict. What in effect he is recommending is a closer study of modifying (co-carcinogenic or anti-carcinogenic) factors which influence the risk that cancer will develop. From a practical point of view the distinction between carcinogen and modifier may be unimportant. If, as we know from animal experiments, it may be the modifier rather than the true carcinogen that determines whether cancer develops, then it is logical to conclude that the modifier deserves special study.

Several examples of what may be called "whole tissue carcinogenesis" are known. If the ovaries are transplanted into the spleen, the oestrogen it produces is destroyed in the liver and never reaches the general circulation (Li, 1948). Under these circumstances the normal feedback mechanism whereby circulating oestrogen inhibits the secretion of gonadotrophic hormones by the pituitary is interrupted. The latter are produced in excess and the ovaries become hyperplastic and eventually neoplastic. An exogenous agent may induce cancer indirectly by first producing an effect on a whole tissue, thus the administration of a sufficient dose of cadmium causes complete destruction of seminiferous tubules in the testes of rats, possibly through interference with the blood supply (Gunn *et al.*, 1963). Later, probably because of interference with a feedback mechanism, Leydig-cell tumours arise in the atrophied testes (Roe *et al.*, 1964). If the dose of cadmium is insufficient to cause testicular atrophy, then no Leydig-cell tumours are induced. There is no reason in this case to regard cadmium as a direct cause of the tumours since they also appear as a consequence of testicular atrophy from causes which involve no exposure to the metal.

IV. The Relationships Theory, Method between and Fact

Since the end of the last century there has been a succession of general theories of carcinogenesis, some of them based on facts gleaned from studies in only a small part of the wide field, and some of them based on no verifiable facts at all. A number of the more patently absurd theories are discussed in Oberling's excellent book, *The Riddle of Cancer* (Oberling, 1948).

That man should want to discover general patterns in relation to natural phenomena is one of his strengths. That he should imagine that he has done so when his only information stems from very limited experience is all too often a besetting weakness. At one time the microbiologists and protozoologists were the most productive of general theories of carcinogenesis, then the geneticists, embryologists and virologists came to the forefront. In recent years biochemical theories have predominated, but many of these already look outdated because of the emergence of molecular biology as a basic discipline. In fact, recent integrated observations in the fields of molecular biology, virology and immunology make virtually all the earlier general theories of carcinogenesis seem either wrong or irrelevant; not because they have led to the discovery of a new, generally applicable,

mechanism, but because they have shown how complex and how numerous the possible aetiological mechanisms are.

Not only are multiple factors implicated in the aetiology of individual tumours, but the spectrum of factors, and the pattern of their interaction, is different for different tumours. It follows that *knowledge of aetiological mechanisms is intimately dependent on the methods that have been used to investigate them: fact is dependent on method.*

Theories that are too many steps ahead of facts and methods are usually too vague to be helpful. At the present time, therefore, the most stimulating theories of mechanisms of carcinogenesis are fairly closely related to observations made on specific tumour systems by the use of specific methods of investigation. The gap between this level of theory and the level of general theory of carcinogenesis is both wide and widening. In other words, it is becoming more difficult to conceive of a useful general theory that is likely to explain the increasing diversity of mechanisms which are being shown to operate in various test systems.

There are of course many repeating patterns in carcinogenesis. Certain chemical and viral agents freely cross inter-species barriers in relation to an ability to induce cancer. If this were not so, it would hardly be justifiable to hope that studies on laboratory animals may lead to knowledge of the aetiology of human cancer. Certain modifying stimuli such as "wound-healing" also operate in a variety of species. No doubt other bits of information will in time be pieced together to form patterns; but there is no reason to expect that the patterns so formed will necessarily ever fit together to make a single simple whole concept. The mechanism of carcinogenesis like nature itself is likely to prove an infinitely variable phenomenon.

V. Cancer as a Type of "Response"

Many writers have suggested the cancerous change should not be regarded so much as a positive response – an event which results from stimulation of a specific type – but rather as failure in response. The quality of the organic as opposed to the inorganic is that it has a capacity to reproduce itself. For most primitive form of life, reproduction is limited only by the exogenous environment – lack of nutriments, etc. In multicellular organisms different cells specialize in different functions, even though each cell has all the information necessary for performing all the functions of every cell in the body. The elegant proof of this comes from experiments in which the nuclei from differentiated cells derived from the blastula stage of the frog embryo were substituted for the nuclei of fertilized ova before the first cleavage had occurred. The ova with the substitute nuclei developed into normal frogs (Briggs and King, 1952).

It follows that, in the multicellular organism, the capacity of each cell to divide and to produce, either a replica of the whole organism or any part of it, or just more cells of the same differentiated variety as itself, must be inhibited, restrained or controlled.

At present little is known of the mechanisms of inhibition, restraint or control other than that they must be both multiple and of a variety of types. Some, no doubt, are built-in to cells in the course of embryogenesis and tissue differentiation. Others, possibly mediated by nervous impulses or local cell-contracts, act continuously throughout life, and yet others, such as hormones, act intermittently at one time permitting cellular proliferation, at other times inhibiting it. Every cell in the body, then, has the information necessary to enable it to divide, but it also has the structures necessary to enable it to respond to factors which normally restrain cell-division.

The cancer cell stands convicted of proliferating when it should not do so. Theoretically its failure to behave normally in this respect may stem from a failure in the generation of the restraining mechanism, or in its transport to the cell, a failure of the cellular apparatus responsible for receiving the restraining message, or a failure of the cell to act on the message even though it received it.

There is evidence that the process of carcinogenesis is sometimes associated with the escape of cells from the state of suppression of a large part of their genetic information which is normal in relation to the differentiated cell-status. It is now well established, for instance, that certain oat-cell carcinomas of the bronchus secrete hormones, e.g. ACTH and antidiuretic hormone, which it is normally the prerogative of the pituitary gland to produce (see Roe and Walters, 1965, for review).

However, it would be wrong to give the impression that most cancers could be explained simply in terms of failure of suppression of proliferation (i.e. failure of homeostatic mechanisms). It is possible that some of the cancers of early childhood represent developmental failures in the switching off of the capacity of particular cell types to divide, but the majority of cancers of adult life cannot be explained on this basis, first, because there is ample evidence that the cells are abnormal, and do not resemble cells seen at any time during the development of the organism; second, because individual cells within the same tumour may differ from each other. The significance of these observations, however, may be clearer if the phenomena of the latent interval and of tumour progression are first considered.

VI. The Latent Interval and Tumour Progression

A puzzling and complicating aspect of carcinogenesis is that a variable, and sometimes long, interval separates exposure to a causative agent and manifestation of its effect in the form of the development of a visible cancer. Part of this interval is taken up by the submacroscopic stage of tumour development, but in many cases calculations based on the observed rate of growth of a visible tumour indicate that, either its origin from a single cell occurred long after the time of exposure to the supposedly causative agent,

or that the rate of growth must have got quicker during the course of the early growth of the tumour.

After a tumour has reached the visible stage, its rate of growth may suddenly increase. Microscopic examination of the tumour at this stage shows tissue of two types. Part of the lesion consists of less rapidly dividing cells and part, often to one side, of a mass of more rapidly dividing cells. The chemical history and the microscopic appearances both suggest that the more rapidly growing tumour has arisen by a sudden change in one (or possibly more) of the cells of the more slowly growing tumour. Perhaps in rather the same way the more slowly growing tumour arose in one of a mass of normal cells. The process by which a tumour undergoes successive changes towards greater and greater malignancy is referred to as "tumour-progression" (Foulds, 1954, 1957). Elsewhere (Roe, 1966*a*) we have suggested that the latent interval and tumour progression should be considered as related phenomena.

Unequivocal examples of tumour-progression, though not rare, are certainly not common. On the other hand, cellular pleomorphism is commonly encountered. Thus cells in any one neoplasm may differ in size, shape and chromosome number and indeed in every measurable parameter. This suggests a certain instability in the process involved in cellular reproduction.

The frequency of disorders of mitosis in some malignant tumours provides evidence of such instability. A consequence of this instability is that at any one time a neoplastic lesion consists of a mixed population of cells that compete, one with the other, for the available nutrients. Under these circumstances a process of natural selection is likely to operate, such that the most aggressive cells, i.e. those that can grow and divide most vigorously, tend to survive at the expense of the less aggressive. If this is an accurate representation of the situation, the predictable outcome would then be for tumours to become more and more malignant with the passage of time. By the same token, if one looks retrospectively into the history of a tumour one would expect the average growth rate of the constituent cells to be slower and slower the earlier in time after the inception of the neoplastic focus. The phenomenon of tumour-progression, therefore, may partly explain the length of the latent interval.

This biological concept of a tumour as a changing mixture of cell types has other important implications. For instance, it makes it unreasonable to expect that biochemical measurements made on homogenates of large pieces of tumour tissue, containing, perhaps, a wide variety of cell types, will necessarily lead to really meaningful results. The mixed cell-populations theory can also help to explain the difficulty of treating cancers with chemotherapeutic agents, particularly the fact that drug-resistant cell types appear sometimes relatively soon after the start of therapy. Cell variants that are less susceptible to a particular drug survive its initial onslaught and, without the competition of the cells that succumbed to the drug, flourish more than they might otherwise have done.

So far, it has been suggested that during the latent interval the rate of cellular proliferation increases because less and less "restrainable" cell lines emerge within the tumour focus. However, it is also possible that one or more of the restraining mechanisms becomes less and less effective. Berenblum (1954) postulated, for example, that it was necessary for a "cancer" cell to have given rise to a cellular mass or colony of "critical size" before it was assured of giving rise to cancer. He suggested that certain agents which cause hyperplasia could, by enabling proliferation beyond the critical colony size to be achieved, act co-carcinogenically in the induction of cancers.

It is natural to think of immune mechanisms at this point since it is conceivable that this is a type of restraining mechanism which may suddenly fail if the antigenic stimulus rises above a certain critical level, or if the capacity for immune response is reduced.

VII. Immune Mechanisms in Carcinogenesis

It is no part of the object of the present discourse to review the present state of knowledge with regard to immune mechanisms. However, a brief survey of what is known and not known may help to dispel premature belief in certain naively simple theories.

By the use of modern techniques it has been shown that abnormal antigens are associated with most, possibly all, neoplasms. In the case of some cancers there is also evidence of loss of antigens. The use of the term "antigens" in this context stems from the methods used for detecting their presence, and offers the advantage that no one expects the precise chemical structures to be known. All antigens are in fact proteins or polypeptides, and the possible variety of abnormal proteins that may occur in cells is legion. The presence of abnormal proteins in cells is by no means unique for cancer. There is, however, comparatively little information concerning the presence of new proteins or lack of normal proteins in non-cancerous diseases.

The most readily interpretable information on new antigens comes from studies in viral oncogenesis. Specific proteins are produced in the course of virus replication within the cells of the host. The proteins are of two types, those that form the protein envelope of the virus itself, and those (probably enzymes) that are involved in the replicating process itself. Both are coded for in the nucleic acids of the virus.

Cells that undergo malignant transformation as a result of infection with an oncogenic virus show surface changes including the appearance of new proteins. New proteins that appear in cells transformed by a particular virus are specific in the sense that they are identical irrespective of the species, strain or tissue from which the transformed cell originated. The specificity of the new proteins enables the virus concerned to be identified. It is broadly assumed that "transformation" which refers to a morphological and behavioural change of cells grown *in vitro* corresponds to an

in vivo change from normal to cancerous. There is much evidence that there is some truth in this, but not enough information, particularly with regard to states intermediate between normal and full transformation, for one to be completely committed to this assumption at the present time.

Much less is known concerning the presence of new proteins in cells transformed by chemical or physical agents. It is not yet fully established that exposure of cells *in vitro* to chemical agents or to X-irradiation can bring about malignant transformation. The success claimed in various published papers by some (e.g. Berwald and Sachs, 1963, 1965; Borek and Sachs, 1966; Heidelberger and Iype, 1967; Sanders and Burford, 1967) is offset by an unknown large number of unpublished negative observations. It is possible that success has only been achieved where a potentially oncogenic virus, capable of being activated by the test chemical, was also in the flask with the cells at the time of exposure to X-rays or to the "carcinogenic" chemical agent. However, if this were so, one could expect that the transformed cells would possess common virus-determined new antigens. According to Sachs (1966, personal communication), however, this is not the case. Cells of the same type and in the same flask, transformed as a result of exposure to the same chemical agent, produce different arrays of new antigens. The variety of new antigens is probably large, but it is not yet certain whether it is finite or infinite, nor whether different chemicals ever give rise to the same new antigens as each other. More recent studies of Reiner and Southam (1967) suggest that different sarcomas induced by 3-methylcholanthrene may possess some common antigens though the margin of antigenic overlap indicated by their findings is not very wide. Precise knowledge concerning *in vitro* transformation by chemical agents is urgently awaited.

There is no evidence at the present time of the presence of particular new proteins especially correlated with cancer nor is there evidence of a correlation between the number or concentration of new proteins in transformed cells or in cancer cells and their malignancy: it is probable that most of the new proteins, especially those that appear as a result of the induction of cancer by X-rays or chemical agents are non-functional and quite incidental by-products of the cancerous change.

So far in this section the assumption has been made that proteins that appear during the course of carcinogenesis are not only "new" but also "abnormal". This assumption is usually unwarranted in the light of the information available. During the course of embryogenesis and differentiation of tissues it is likely that a wide variety of proteins are produced transiently. The information for their production thereafter normally remains unexpressed in the "adult" differentiated cell. It would not be surprising if, in future, some of the proteins that appear in association with carcinogenesis are shown to be identical with proteins produced transiently during early embryonic life.

This possibility has important implications, because if true, it then

follows that some of the so-called "cancer antigens" are not foreign to the cancer-bearing host, and no immunological reaction against them is to be expected.

Where the new proteins are foreign to the host, the immunological response to them is likely to vary. However, no sure method for predicting the extent of the response to particular antigens is at present available. These remarks apply both to soluble antigens that can escape from cells and to cell-surface, "transplantation", antigens.

There is plenty of evidence that experimental animals may mount an immunological attack against induced cancers (Klein *et al.*, 1960), but no evidence, in terms of spontaneous regression, that chemically-induced tumours ever succumb to this attack. However, perhaps success is really the rule at the submacroscopic level, and perhaps the appearance of a visible tumour is a relatively rare expression of failure. Alternatively, as suggested by Old and Boyse (1964) and others, perhaps the problem is that the growth of malignant cells outpaces the immune responses of the host: inocula as small as 40 malignant cells that are capable of rapid multiplication can produce a tumour in a isologous host (Old *et al.*, 1962).

Both in man and in experimental animals there are examples of regression of cancer, occasionally entirely spontaneously, but more usually following large-scale, though incomplete, destruction or removal of the tumour (Everson and Cole, 1966; Boyd, 1966). It is interesting that chorio-carcinoma, a rare malignant tumour arising because trophoblastic cells derived from a foetus invade the mother, comes high in the list of types of tumour that exhibit spontaneous regression. It is presumed that antigenic differences between the cancer and the maternal host eventually stimulate a successful immunological response (though hormonal factors may also be important in this case). Long-term survival of patients with Burkitt's lymphoma following treatment with cytotoxic drugs has also been attributed to immunological rejection of the small residue of tumour cells left after drug treatment. But these examples are the exception and not the rule.

Some potent chemical carcinogens, most cytotoxic drugs, corticosteroids, and a variety of other agents and procedures such as neonatal thymectomy suppress immunological responsiveness. This may influence the process of carcinogenesis by other agents in a number of different ways (see Roe and Rowson, 1968, for review). However, there is no indication that any of these agents, or procedures induce cancer. By the same token, there is no good evidence that the natural immunological response of an animal against its tumour can ordinarily be so boosted that the tumour is rejected (Old *et al.*, 1961). If, however, newer approaches to the treatment of cancer by the introduction into the tumour-bearing host of large numbers of lymphocytes (Alexander, 1965) that have been exposed to tumour-specific antigens prove successful, then it may be necessary to reconsider the role of immunological factors in carcinogenesis. At present it seems unlikely that they play more than a secondary, and often comparatively minor, role.

VIII. Possible Modes of Action of Potent Chemical Carcinogens

Cancer and carcinogenesis are four-dimensional: the finished article, the tumour, is the result of a series of changes taking place over many cellular generations and subject to many influences. Many of the measurable consequences of first exposure to a potent carcinogen are probably incidental and irrelevant to the carcinogenic process. Others may influence the rate at which tumours grow or appear, or the likelihood that they will appear and yet not be responsible for the primary change. We do not yet know for certain that the cancerous change necessarily involves an alteration (mutation) in the genetic information coded in the nucleic acids of cells. It is possible, as suggested by the work of Brookes and Lawley (1964 – review), that carcinogenic alkylating agents react principally with the N-7 position of the guanine moiety of both DNA and RNA and that, in the case of bifunctional alkylating agents, two such reactions could link the two strands of DNA in such a way that the genetic code is altered. But such a theory cannot easily explain carcinogenesis by mono-functional alkylating agents such as ethyl methane sulphonate (Walters *et al.*, 1967). Indeed it may be that such observations amount more to a definition of the reactivity of different parts of the guanine molecule rather than to a meaningful theory of carcinogenesis. By the same token, the elaborate calculations of the theoretical chemists with regard to the relation between chemical structure and carcinogenic activity (e.g. Pullman and Pullman, 1955) never seem capable of supporting a theory of carcinogenesis which applies to more than a very narrow range of chemical structures.

A priori it seems unlikely that the important reactions which lead to cancer induction will be learned from studies on highly reactive carcinogenic agents capable of combining with many cellular components. The perfect chemical carcinogen for study purposes would be one that was capable only of one reaction, namely that basic to the induction of cancer. Perhaps no such substance exists. But while we are waiting to find out if it does, we might stand a better chance of discovering the essential alterations in cells that lead to carcinogenesis by careful studies of the effects of single low doses. Under these circumstances some of the irrelevant effects will not be produced and can therefore be ruled out for the purposes of further consideration.

IX. Résumé and Conclusions

Cancer is a general term that refers to a large number of diseases. Many factors in many and various combinations contribute to the causation of different types of the disease. In some cases a single factor seems to be of such predominant importance that it is justifiable to regard its activity as being carcinogenic. However, it is not justifiable to assume that carcinogenicity in one biological system implies carcinogenic potential for others. Even when an agent that is known to be carcinogenic in other systems

appears to contribute to the induction of cancers in a new system, it cannot be assumed that its contribution relates to its carcinogenic potential.

It is difficult to distinguish between carcinogenic and co-carcinogenic activity. Only when information from a variety of test systems is available may it become justifiable to regard a particular agent as "carcinogenic". In any event, the use of such a term should normally relate only to the findings under the actual conditions of testing. In this connection there is a serious need, especially in relation to biochemical and molecular biological studies, constantly to define the "cancers" that are the objects of investigation. Lack of precision in this is both a constant handicap to the detection of aetiological mechanisms and a source of increasing confusion in experimental cancer research. Oversimplification is a continuing danger in relation to the elucidation of mechanisms of carcinogenesis. This is particularly obvious in the search for carcinogenic metabolites from apparently non-carcinogenic precursors. Examples of conclusions having been based on inadequate experimental data, and of the over-ready extrapolation from one biological test system to another are all too numerous. Theories of carcinogenesis in which an attempt is made to explain the causation of all forms of cancer in terms of a single mechanism are either too broad to be of much value, or simply ridiculous in the extent to which pertinent facts about cancer are overlooked. The latter include the width of the array of cancer types that may be derived from a single tissue, the fact that relatively similar cancers may arise after exposure to quite different aetiological agents, the extent of the variation between tumours in every measurable property, the latent interval that often separates cause and effect, and the phenomenon known as tumour progression.

Some of the variation between induced tumours in structure and behaviour is attributable to structural, physiological or pathological differences between target cells and some to differences in exposure to the relevant agent. Variation in response may also arise because of genetically or experimentally determined host factors such as hormonal or general immunological status. It is because of the multiplicity of these possibilities for variation that the theories of mechanisms of carcinogenesis must be tailored to each individual situation.

Detailed research on mechanism of action of potent carcinogens is still a fully justified pursuit, though the study of the effects of doses small enough not to cause general effects outside the target area is to be recommended. Investigators should be quite clear as to whether they are studying the immediate interaction between the carcinogenic agent and its biological target or the consequences, immediate or remote, of this interaction. The biochemical complexity of living matter is such that the longer the interval after exposure to an agent, the harder it will be to deduce the nature of the initial event from the changes found. In experiments that involve repeated exposure to an agent, it may well be impossible to discover the nature of the initial interaction amid the background of secondary effects in the cells concerned and of general effects on the tissue or organism as a whole.

The full examination of the mechanisms of induction of any single form of cancer includes investigations at the whole organism level, of tissues, of cells, and of subcellular constituents. Many disciplines are needed and the protagonist of one discipline ignores the rest at his peril. Elucidation is unlikely ever to be complete in so far as mystery is an intrinsic component of biology. However complete the knowledge of the mechanisms involved in the genesis of one particular form of cancer, the details may be largely irrelevant to the causation of any other form of cancer. But, with luck, the *methods* developed for tackling the problem will be applicable to other situations.

Acknowledgements

I am most grateful to Dr K. E. K. Rowson, Institute of Laryngology and Otology, London, for his expert advice, and to Mrs A. Englefield and Miss L. Barlow for their assistance in the preparation of the manuscript.

References

Ahlström, C. G. and Andrewes, C. H. (1938). *J. Path. Bact.* **47**, 65-86.
Alexander, P. (1965). In *The Biology of Cancer* (E. J. Ambrose and F. J. C. Roe, eds), pp. 91-123. Van Nostrand, London.
Ball, J. K., Sinclair, N. R. and McCarter, J. A. (1966). *Science, N.Y.* **152**, 650-651.
Berenblum, I. (1954). *Cancer Res.* **14**, 471-477.
Berwald, Y. and Sachs, L. (1963). *Nature, Lond.* **200**, 1182-1184.
Berwald, Y. and Sachs, L. (1965). *J. natn. Cancer Inst.* **35**, 641-662.
Borek, C. and Sachs, L. (1966). *Nature, Lond.* **210**, 276-278.
Boyd, W. (1966). *The Spontaneous Regression of Cancer.* Charles C Thomas, Springfield, Illinois.
Briggs, R. and King, T. J. (1952). *Proc. natn. Acad. Sci. U.S.A.* **38**, 455-463.
Brookes, P. and Lawley, P. D. (1964). *Br. med. Bull.* **20**, 91-95.
Cunningham, G. J., Nassau, E. and Walter, J. B. (1958). *Thorax* **13**, 64-68.
Essenberg, J. M. (1952). *Science, N.Y.* **116**, 561-562.
Everson, T. C. and Cole, W. H. (1966). *Spontaneous Regression of Cancer.* W. B. Saunders, Philadelphia.
Foulds, L. (1954). *Cancer Res.* **14**, 327-339.
Foulds, L. (1957). *Cancer Res.* **17**, 355-356.
Franks, L. M. (1954). *Ann. R. Coll. Surg.* **15**, 236-249.
Gunn, S. A., Gould, T. C. and Anderson, W. A. D. (1963). *Am. J. Path.* **42**, 685-702.
Harris, R. J. C. and Negroni, G. (1967). *Br. med. J.* **4**, 637-641.
Heidelberger, C. and Iype, P. T. (1967). *Science, N.Y.* **155**, 214-217.
Kawamoto, S., Ida, N., Kirschbaum, A. and Taylor, G. (1958). *Cancer Res.* **18**, 725-729.
Klein, G., Sjögren, H. O., Klein, E. and Hellström, K. E. (1960). *Cancer Res.* **20**, 1561-1572.
Laqueur, G. L., McDaniel, E. G. and Matsumoto, H. (1967). *J. natn. Cancer Inst.* **39**, 355-371.
Li, M. H. (1948). *Am. J. Obstet. Gynec.* **55**, 316-320.
Morton, J. J. and Mider, G. B. (1938). *Science, N.Y.* **87**, 327-328.
Mühlbock, O. (1955). *Tijdschr. Diergeneesk.* **99**, 2276-2278.
Oberling. C. (1948). *The Riddle of Cancer.* Oxford University Press, Oxford.
Old, L. J. and Boyse, E. A. (1964). *A. Rev. Med.* **15**, 167-186.

Old, L. J., Benacerraf, B., Clarke, D. A., Carswell, E. A. and Stockert, E. (1961). *Cancer Res.* **21**, 1281-1300.
Old, L. J., Boyse, E. A., Clarke, D. A. and Carswell, E. A. (1962). *Ann. N.Y. Acad. Sci.* **101**, 80-106.
Pietra, G., Spencer, K. and Shubik, P. (1959). *Nature, Lond.* **183**, 1689.
Pullman, A. and Pullman, B. (1955). *Adv. Cancer Res.* **3**, 117-169.
Reiner, J. and Southam, C. M. (1967). *Cancer Res.* **27**, 1243-1247.
Riska, E. B. (1956). *Acta path. microbiol. scand.* Suppl. **114**, 1-110.
Roe, F. J. C. (1966*a*). In *The Biology of Cancer* (E. J. Ambrose and F. J. C. Roe, eds), pp. 1-32. Van Nostrand, London.
Roe, F. J. C. (1966*b*). In *Lung Tumours in Animals* (L. Severi, ed.), pp. 101-126. University of Perugia, Perugia.
Roe, F. J. C. (1968). *Fd. Cosmet. Toxicol.* (In the press.)
Roe, F. J. C. and Rowson, K. E. K. (1968). *Int. Rev. exp. Path.*, **6**, 181-227.
Roe, F. J. C. and Walters, M. A. (1965). *Prog. exp. Tumor Res.* **6**, 126-227.
Roe, F. J. C., Dukes, C. E., Cameron, K. M., Pugh, R. C. B. and Mitchley, B. C. V. (1964). *Br. J. Cancer* **18**, 674-681.
Sanders, F. K. and Burford, B. O. (1967). *Nature, Lond.* **213**, 1171-1173.
Smithers, D. W. (1962). *Lancet i*, 493-499.
Stjernsward, J. (1965). *J. natn. Cancer Inst.* **35**, 885-892.
Walters, M. A., Roe, F. J. C., Mitchley, B. C. V. and Walsh, A. (1967). *Br. J. Cancer* **21**, 367-372.
Weston, B. J. (1967). *Nature, Lond.* **215**, 1497-1498.
Whitwell, F. (1955). *J. Path. Bact.* **70**, 529-541.