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Cancer inducing agents

Francis J. C. Roe

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Cancer inducing agents

It has become increasingly difficult to state whether a given agent will or will not induce cancer. It is possible that, given the appropriate conditions, all substances are carcinogenic

Some carcinogens in industry and every day life

Aniline dyes
Antioxidants such as benzidine and β-naphthylamine
Asbestos
Certain metals: arsenic, beryllium, cadmium, chromium, cobalt, nickel and selenium
Certain medicines, particularly some of those used in the treatment of cancer; also some hormones
Creosote
Epoxides, used widely as adhesives and in the manufacture of plastics
Exhaust gases from petrol and diesel engines
Smoked foods and charcoal steaks
Soot, coal tar and smoke
Tobacco smoke and raw tobacco used by 'chewers'
Ionizing radiation
Sunlight
Cancer producing viruses ?

WIDELY DIFFERING agents have been shown to be capable of causing cancer in man. In the past they have been arbitrarily divided into chemical, physical and viral. For many reasons this division is not very meaningful. Thus asbestos may cause cancer because of its physical rather than chemical nature

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CANCER can be induced in laboratory animals by deliberately exposing them to a wide variety of physical, chemical or viral agents some of which are known to cause cancer in man. It has become customary to regard the agents concerned as 'carcinogens'. Yet this may not be the best way of looking at the situation, because it suggests too strongly that the many different cancer inducing agents share a common feature which enables them to bring about the induction of cancer by a specific mechanism.

Instead it may be better to regard carcinogenesis as an intimate '*pas de deux*', with the living tissue and the cancer inducing agent as the principal dancers on a stage which is the environment provided by the multi-cellular organism in which cancer will eventually arise. Both the dancers can appear in other roles at other times and the stage can be used for other purposes. Other dancers can act as substitutes, but the *pas de deux* itself always requires two dancers and a stage on which to appear.

THE ANIMAL BODY consists of a complex community of cells all derived originally from one cell, the fertilized ovum. Within each cell, coded in the arrangement of bases in its nucleic acids (deoxyribonucleic acid, DNA, and ribonucleic acid, RNA), is a plan of the whole body but, under normal circumstances, only a fragment of the plan is expressed by each cell. It may be presumed that the expression of the majority of the plan is actively suppressed by a pattern of control mechanisms. These include long distance hormonal and neural systems; local systems involving tissue antigen-antibody reactions and cell to cell contacts; and intracellular gene-suppressor systems, built-in to cells during their differentiation from the undifferentiated state (see "The control of genes", SCIENCE JOURNAL, March 1966).

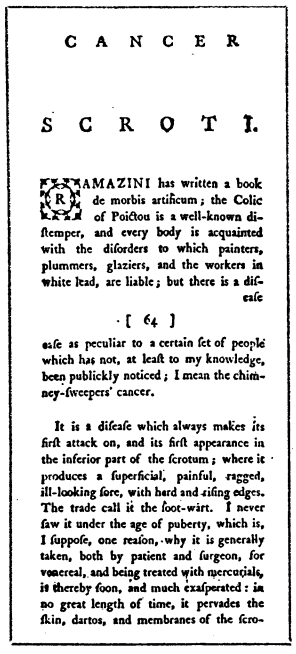
The normal steady state is maintained in the body by the continual operation and interplay of these control mechanisms, each acting as a negative feed-back system. Repair following injury is controlled by the same mechanisms, the combined function of which is to maintain, or in this case, re-establish the *status quo*. It is convenient, therefore, to apply the adjective 'homeostatic' to these control mechanisms.

Each body cell may be regarded as an individual living unit of which the genetic potential is constantly fettered by the chains of homeostatic controls.

Whatever else carcinogenesis may involve, it certainly involves an effective breaking out from these chains.

Observation indicates that, even under normal circumstances, the two daughter cells resulting from the division of a tissue cell are not completely identical. Their inequality may be trivial, non-heritable and correctable during subsequent cell divisions. But if inequitable cell division proceeds on a large scale, and if sequential abnormal divisions occur because the mechanism for getting rid of defective cells cannot operate quickly enough, then a mixed population of cells will arise. Under the pressure of the limited availability of nutrients, and the operation of homeostatic mechanisms, a process of natural selection will then operate: the most vigorous cells—those least susceptible to homeostatic suppression—and their progeny will become relatively more numerous than the less vigorous members of the population. Once a cell which carries a heritable defect has gained a measure of autonomy, natural selection operating among its progeny will tend to lead to the dominance of progressively more vigorous and autonomous cells. In keeping with this view is the fact that it is a characteristic of cancer that it is progressive—in the sense that cancerous tissue tends to become more malignant with time. Furthermore it is usual for a time interval, which may be very long, to separate exposure to carcinogenic stimulus and the appearance of cancer. It now seems likely that this interval is occupied by the process of natural selection and the slow emergence of cells sufficiently autonomous to be regarded as cancerous.

This is the background against which carcinogenesis should be viewed. In theory, at least, cancer may be induced either directly by an effect on the genetic material of cells or indirectly by interference with extracellular homeostatic control mechanisms. This distinction is far more meaningful than one based on the chemical, physical or viral nature of the agents concerned and it may in fact throw light on the mechanisms by which these agents operate.



CANCER

SCROTI.

RAMAZINI has written a book de morbis artificum; the Colic of Poictou is a well-known diſtemper, and every body is acquainted with the diſorders to which painters, plummers, glaziers, and the workers in white lead, are liable; but there is a diſeaſe

[64]

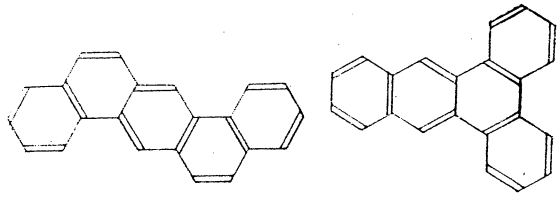
eaſe as peculiar to a certain ſet of people which has not, at leaſt to my knowledge, been publickly noticed; I mean the chimney-ſweepers' cancer.

It is a diſeaſe which always makes its firſt attack on, and its firſt appearance in the inferior part of the ſcrotum; where it produces a ſuperficial, painful, ragged, ill-looking ſore, with hard and riſing edges. The trade call it the ſoot-wirt. I never ſaw it under the age of puberty, which is, I ſuppoſe, one reaſon, why it is generally taken, both by patient and ſurgeon, for venereal, and being treated with mercurials, it thereby ſoon, and much exaſperated: in no great length of time, it pervades the ſkin, dartos, and membranes of the ſcro-

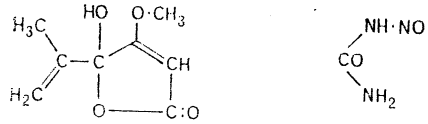
PERCIVALL POTT first described the association between exposure of chimney sweeps to soot and cancer of the scrotum in 1775. His book, first page of text illustrated, was the first in a long and continuing line of works on occupational and industrial medicine

IN 1775 PERCIVALL POTT recorded an association between the occupational exposure of chimney sweepers to soot and cancer of the scrotum: a majority of young men in their teens or twenties with this form of cancer had a history of having been employed as 'climbing boys'. One hundred and forty years later two Japanese workers reported the induction of skin cancer in rabbits by the repeated application of coal tar. In 1930, a team under the late Sir Ernest Kennaway detected and synthesized the first chemical carcinogens of the type present in coal tar. These were aromatic polycyclic hydrocarbons made up of four or five benzene rings. In time many hundreds of such substances were synthesized. Some proved active in the induction of cancer in animals, others—often closely related chemically to active compounds—proved quite inactive. Theoretical chemists developed hypotheses associating structure and activity. Calculation of the electron charge densities of each of the various active molecules showed that all possessed a region of high density, the 'K-region', in their double bond system. In these areas the probability of bond formation occurring with other molecules through charge transfer is greatest. However, not all 'K-region' positive compounds proved to be active as carcinogens: high electron density in a second area of the molecule, the 'L-region', rendered such compounds inactive. By the use of this theory, and subsequent modifications of it, it was possible to predict carcinogenic activity within the limited class of the polycyclic hydrocarbons.

In the meantime, however, a wide variety of quite different chemical agents were found to be carcinogenic. Their extreme diversity led to the abandonment of the hope that the theoretical chemists would quickly solve the cancer problem.

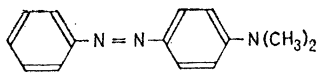


SIMILAR COMPOUNDS can have dissimilar effects. Thus 1, 2, 5, 6-dibenzanthracene, left, is a potent carcinogen whereas the related substance 1, 2, 3, 4-dibenzanthracene, right, is carcinogenically inactive

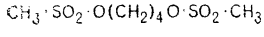


PENICILLIC ACID is the basis of a variety of penicillins. Causes cancer at injection site in rats

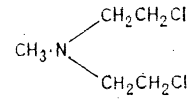
NITROSOUREA causes tumours at various sites including the brain in some laboratory animals



4-DIMETHYLAMINOAZOBENZENE (butter yellow) was formerly used to give colour to margarine. However, it has since been found to cause liver cancer in rats

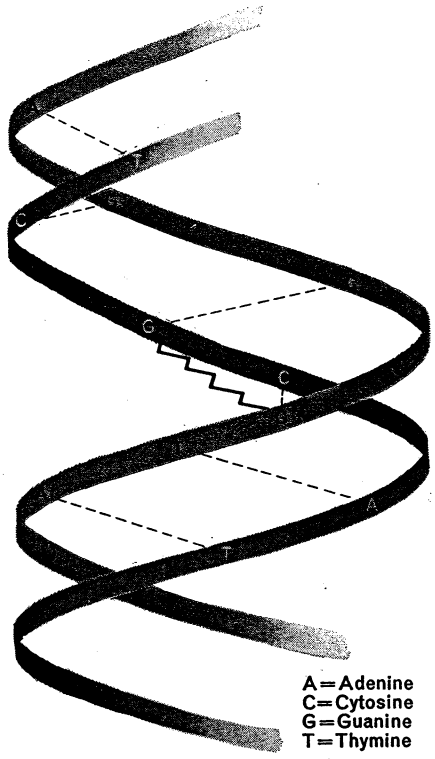


MYLERAN (BUSULFAN) is of value in the treatment of myeloid leukaemia —a blood cell cancer

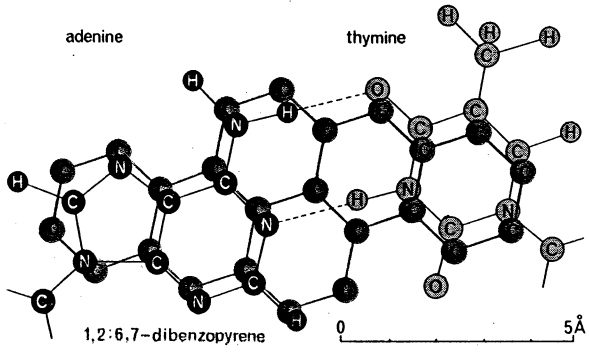


NITROGEN MUSTARD has been found of value in the treatment of several different types of cancer

ANOTHER LANDMARK in the history of the subject was the observation of Alexander Haddow in the late 1930s that there is an association between carcinogenic activity and ability to inhibit growth, including tumour growth. Not only this, but it seemed that carcinogenic compounds often had a selective toxic effect on the process of cell division, and were able to produce mutations in the cells. During the past 20 years this observation has led to the discovery of new carcinogens such as the aminostilbenes and to substances such as the biological alkylating agents which both induce cancer and inhibit cell multiplication. The essential property of alkylating agents is their ability to introduce alkyl



TWO STRANDS OF DNA may become linked together by a bifunctional alkylating agent such as myleran, the molecule of which becomes attached at each end to a nucleotide base (A, C, G or T). The effect of such a linkage may lead either to a reduction in the capacity of affected cells to reproduce or to the induction of cancer



SOME CARCINOGENS, such as 1, 2: 6, 7-dibenzopyrene, shown coloured, are similar in chemical structure to parts of the DNA molecule, for example adenine and thymine, black. This suggests that this type of compound may be able to cross link the DNA

radicals, such as $—CH_3$ or $—C_2H_5$, into molecular components of cells. The effect of such introduction leads to a dramatic reduction in the capacity of cells to reproduce and, in some cases, to the induction of cancer. The former effect is immediate and the latter delayed. Alkylating agents capable of introducing a single alkyl radical are termed monofunctional; those able to introduce two or more are termed bifunctional or polyfunctional respectively. When bifunctional or polyfunctional compounds react with two or more molecules the end result may be that the molecules become linked together by the alkylating agent.

It is now believed that both the carcinogenic and tumour inhibiting effects are due to reactions of this type with the genetic material of the cell, deoxyribonucleic acid (DNA). In recent years an ingenious theory has been developed that the most significant effect of the polyfunctional alkylating agents is to cross-link the two strands of DNA. If this were true, it would be possible to explain, in terms of a single chemical mechanism, all three types of biological activity of these agents: carcinogenicity, mutagenicity and growth inhibiting activity. As a result of similar thinking a new suggestion was made with regard to the possible mode of action of carcinogenic polycyclic hydrocarbons, and their distinction from inactive analogues. It was pointed out that some of the carcinogenically active compounds, such as 1, 2:6, 7-dibenzopyrene, were similar in chemical structure to part of the DNA molecule. This suggests that this type of compound might also cross-link the two strands of DNA.

These theories are still actively stimulating new work but, clearly, they do not apply to all forms of carcinogenesis. As more compounds have been examined, the association between carcinogenicity, growth inhibition and mutagenicity has become less clear cut. Monofunctional alkylating agents, capable of reacting with DNA but not of cross-linking its two strands, have been shown to be carcinogenic, though less so than related polyfunctional compounds.

There are grounds for believing that most potent carcinogens affect the DNA or RNA of cells more or less directly and specifically. The most potent cancer inducing agents of all, namely the tumour viruses, consist simply of DNA or RNA. Carcinogens may be weak either because their reaction with nucleic acids is non-specific, or because they affect nucleic acids indirectly rather than directly. A third possibility is that their primary effect is not on cells at all, but on their environment by interference with the homeostatic control systems.

Two facts concerning chemical carcinogenesis deserve special mention. First, several metals are known to be capable of inducing cancer, either in men exposed to them in industry, or in laboratory animals under experiment. A human cancer hazard is now recognized in relation to arsenic, beryllium, chromium and nickel. In the laboratory the latter three, together with cadmium, cobalt and iron, have been shown to induce cancer. Curiously, no one has yet succeeded in inducing cancer by exposing laboratory animals to arsenic. Secondly, in recent years a number of naturally occurring carcinogens have been recognized. These include cycasin, an alkaloid present in the cycads—types of palm the leaves, stems and roots of which provide starch, for example sago, and form a staple item of diet in some parts of the world; the senecio alkaloids present in some species of ragwort; safrole, a component of several essential oils including sassafras oil, which is extensively used in North America to flavour 'root beer'; and aflatoxin, a lactone formed by some species of the mould *Aspergillus flavus* when it grows in ground nuts and cereals stored under the hot, humid conditions of the tropics.

IT HAS LONG BEEN PUZZLING that hormones, produced naturally by the body, act as carcinogens under certain circumstances. For instance, cancers of many kinds may be induced in animals by giving them excessive amounts of oestrogen, one of the hormones normally produced by the ovary. Many attempts have been made to show structural resemblances between particular hormones and carcinogens of the polycyclic hydrocarbon type. Indeed, some of the latter have been shown to exhibit weak oestrogenic activity. Nevertheless, the links between carcinogenesis by hormones and by other agents are very tenuous. It is much more likely that the primary action of hormones is not on cells but on one of the homeostatic mechanisms which control their growth and self-expression. The continued presence of excessive amounts of a hormone may, by blocking a normal suppressing mechanism, permit the proliferation of a tissue. This may favour the emergence of autonomous cells in one of two ways. First, the con-

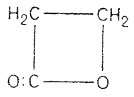
ditions existing in a proliferating tissue may be more favourable to the survival of cells which deviate from normal. Secondly, if because of previous exposure to a carcinogen the cancerous process has been started and there is already a mixed population of cells, the process of selection of more vigorous and autonomous cell lines will operate more quickly under conditions of active proliferation.

IT HAS BEEN SHOWN in the laboratory that cancer may be induced by exposing an animal to two agents, A and B, in that order, whilst exposure to A alone, B alone, or B followed by A does not lead to cancer. Despite several attempts to do so, no one has yet found an agent which has only A-type activity (tumour initiating activity) or only B-type activity (tumour promoting activity). According to the theory presented here, tumour initiators act primarily on cells and tumour promoters primarily on homeostatic control mechanisms. Doses of carcinogens too low to complete the process of carcinogenesis may, nevertheless, be sufficient to initiate it. It is not surprising that pure promoters have not been demonstrated since it is not possible to escape background exposure to initiating agents in the form of low doses of environmental chemical carcinogens and ionizing radiation. It is reasonable to suspect that homeostatic control mechanisms are weakened as a result of the ageing process. The tumour promoters may do no more than bring forward in time a process which would eventually occur spontaneously as a result of ageing. There is some experimental evidence that this is the case. The term 'co-carcinogen' has been applied to an agent which enhances the activity of a carcinogen. There are many possible mechanisms by which such enhancement may be brought about; for example, the co-carcinogen may aid the absorption of carcinogen or block its breakdown to non-carcinogenic metabolites. All tumour promoters are co-carcinogens, but not all co-carcinogens are tumour promoters.

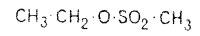
UNTIL THE PUBLICATIONS of F. C. Turner in 1941 and of B. S. and E. T. Oppenheimer and A. P. Stout from 1948 onwards the term physical carcinogenesis was applied, for the most part only, to the induction of cancer by agents such as ultraviolet light, ionizing radiation, physical trauma and chronic irritation of various kinds. It was fairly easy to interpret the effects of both ultraviolet and ionizing radiation in chemical terms, and the interpretation was supported by the introduction of the idea of 'radiomimetic agents' in connection with chemical agents whose effects, in general, resembled those of ionizing radiation.

The fact that physical trauma seems to be able to initiate or precipitate cancer has never been very palatable to those who seek a neat chemical explanation of all forms of carcinogenesis. To them the discovery by Peyton Rous that wounding the ears of rabbits enhanced carcinogenesis by coal tar, though it did not induce cancer on its own, was a windfall. Physical trauma could then be dismissed as a co-carcinogenic factor and need not be explained directly in chemical terms. On the other hand, the demonstration by first Turner and later the Oppenheimers that cancer may be induced by the implantation of chemically inert materials into the tissues made it necessary once more to consider physical carcinogenesis seriously in its own right. At the Chester Beatty Research Institute we have induced cancer in the bladders of mice by the implantation of simple glass beads. Some have argued that nothing is so chemically inert that chemical carcinogenesis is ruled out. Nevertheless, the induction of cancer by the implantation of objects of various shapes, and the simultaneous failure to induce cancer by implanting the powdered chemicals from which the objects are made, have compelled the acceptance of the view that the implanted objects induce cancer because of their physical and not their chemical characteristics. In our researches at the Institute we have found that a high proportion of a group of rats developed cancer at the site of implantation in the subcutaneous tissues of pieces of polyvinyl sponge (as used in plastic surgery) measuring $20 \times 20 \times 5$ mm, whereas only 1 out of 24 rats did so in response to implants measuring $33 \times 33 \times 2$ mm. The amount of sponge was the same in both cases but the shape was different. What possible relation can this type of cancer induction have to that due to the various chemical agents discussed above?

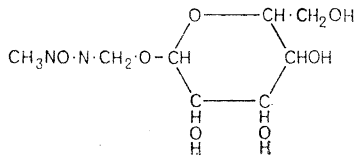
In the past the distinction between chemical and physical carcinogenesis has tended to be artificial. A better distinction is between agents which primarily affect cells and those which primarily affect homeostatic control mechanisms.



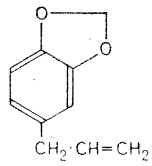
β-PROPIOLACTONE is an alkylating agent with virus killing properties. It induces cancer on injection into rats or when it is applied to the skin of mice



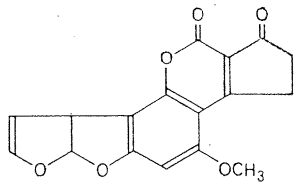
ALKYLATING AGENT (ethyl methanesulphonate) has been shown to induce formation of tumours of the lung when injected into infant mice



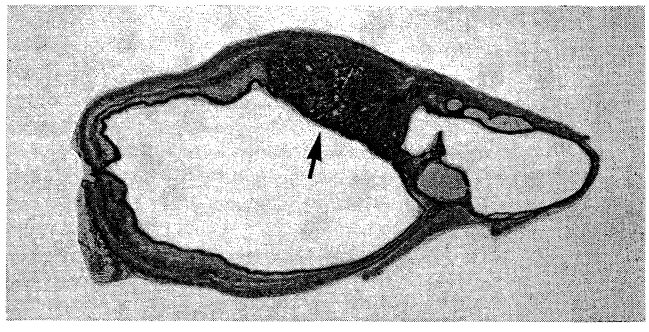
CYCASIN—an alkaloid found in various species of cycad palm which are an important source of starch in many tropical countries—can induce cancers in rats



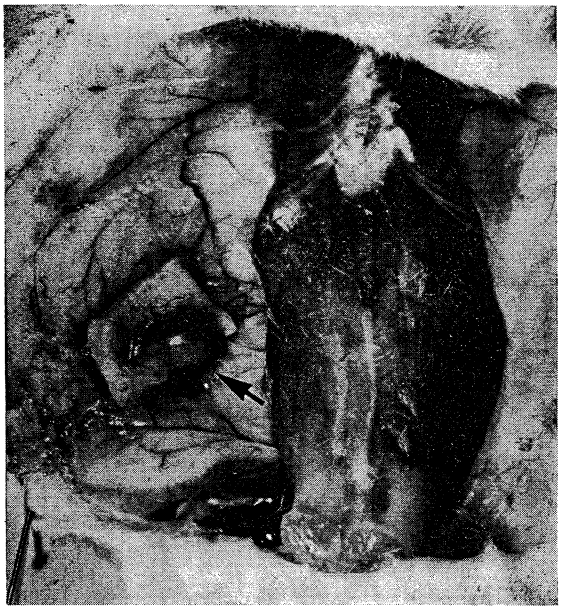
4-ALLYL—1, 2-METHYLENEDIOXYBENZENE (safrole) is a natural ingredient of sassafras tea and has been used to flavour 'root beer' in North America. It has been shown to cause liver tumours when fed to rats



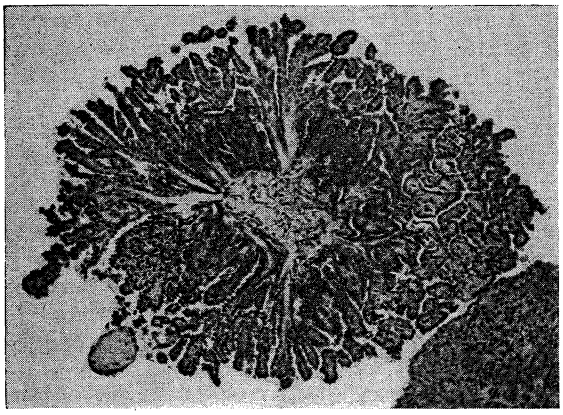
AFLATOXIN B is one of a group of toxins produced by strains of the mould *Aspergillus flavus* which grows in ground nuts and cereals stored under hot, humid conditions. Besides acting as a poison—it killed more than 100,000 turkeys and game birds in 1960—it can induce cancers of the liver and kidney in rodents



CANCER, arrowed, in the bladder of a mouse following the implantation of a glass bead 40 weeks earlier



IMPLANTED OBJECTS can induce cancer because of their physical characteristics. The cancer, arrowed, arose in the region of a piece of plastic sponge implanted in the subcutaneous tissue of the rat several months earlier. A similar piece of sponge of the same weight but of different shape was found to induce cancer far less frequently



ASBESTOS induces cancer possibly because of its physical rather than chemical properties. This nodule of cancerous tissue from the abdominal cavity of a rat arose several months after the last of a series of four subcutaneous injections of asbestos

FURTHER READING

CHEMICAL CARCINOGENESIS by David B. Clayson (*J. and A. Churchill, London, 1962*)

MECHANISMS OF CARCINOGENESIS: CHEMICAL, PHYSICAL AND VIRAL (in *British Medical Bulletin, 20, No. 2, 1964*)

CHEMICAL CARCINOGENESIS AND CANCERS by W. C. Hueper and W. D. Conway (*Charles C. Thomas, Springfield, Illinois, 1964*)

ACKNOWLEDGEMENT

E. Boyland (page 5, bottom)

It is now suggested that, as in the case of the induction of cancer by the administration or withdrawal of hormones, carcinogenesis by inert objects is brought about by interference with an extracellular homeostatic mechanism. It is possible, for instance, that the presence of the object interrupts tissue communication and negative feed-back control mechanisms over an area. From studies on cells grown in tissue culture it is apparent that an environment in which cells may divide and move freely leads sooner or later to the appearance of cancer-like cells.

It is possible that this is also the explanation of some examples of what has up to now been regarded as chemical carcinogenesis. Certain iron-carbohydrate complexes, such as iron-dextran, iron-dextrin and saccharated iron oxide, induce local cancer when injected subcutaneously or intramuscularly into animals. The carbohydrate moieties of the complexes do not themselves induce cancer, nor does iron in other forms do so. The complexing of iron with the carbohydrates leads to the formation of very large molecules. These are ingested by scavenging white blood cells which may remain for long periods at the site of injection, particularly in animals which are not generally deficient of iron. The question is do these large masses of iron complex loaded cells act in the same way as implanted inert objects and do they interfere with trans-tissue communication in the same way as such objects? Certainly the whole sequence of events which precedes the development of cancer is very similar in the two instances.

AT FIRST SIGHT it may seem that this is a merely academic problem but, in fact, it has important practical implications. In most countries, governments now require that constituents of cosmetics and pharmaceutical preparations, substances added to food, and substances such as pesticides or herbicides which may contaminate food should be tested for carcinogenicity in laboratory animals. A positive result in any carcinogenicity test renders a substance, if not completely unacceptable, then at least suspected of being dangerous for man. Should a potentially useful food additive or drug, intended for oral administration, be banned on the grounds that it induces cancer at the site of its subcutaneous or intramuscular injection in animals? Might not the latter be an implantation-induced effect rather than an example of true chemical carcinogenesis?

AS MORE AND MORE different types of agent—chemical, physical and viral—have been shown to be capable of inducing cancer, the avoidance of exposure to known carcinogens which was once easy became at first difficult and then impossible. Substances capable of inducing cancer in laboratory animals are present in vehicle exhausts, tobacco smoke and the general atmosphere—even where there is no marked pollution. They have also been isolated from certain cooked foods and even from so called 'health' foods. Dangerous materials are used in many branches of industry. They are present in freshly mined minerals, such as asbestos, in the materials used for household or garden maintenance, such as creosote, and in a variety of pharmaceutical preparations. No man, be he primitive or civilized, can completely avoid exposing himself to potentially carcinogenic factors. Why then bother to try? Why stop smoking? Why take precautions? The answer is that it is a matter of dose and probability: the larger the quantity of carcinogen taken into the body the greater the risk of cancer.

In the consideration of an environmental factor the question "Is this carcinogenic?" is the wrong one—since it only permits of two answers, "Yes" or "No". The better question is, "What is the risk that exposure to a measured dose of this particular agent by a specified route of exposure will lead to cancer during a stated interval of time after exposure?" The question framed in this way takes into account the vital part played by the exposed tissue in the carcinogenesis process. It also stresses the distinction between major and minor carcinogenic hazards. Such a distinction can be made tentatively on incomplete evidence and given as an expression of informed opinion, whereas the inflexibility of the 'all' or 'none' distinction between 'carcinogenic' and 'non-carcinogenic' stultifies clear thinking and paralyses legislative action. For those who insist on an 'all' or 'none' distinction perhaps the most reasonable one is that there are two types of substances, materials or agents, those which have been shown to induce cancer and those which have not *yet* been shown to do so. In other words there may be no agents in the 'none' category.