CIBA FOUNDATION SYMPOSIUM

ON

CARCINOGENESIS

Mechanisms of Action

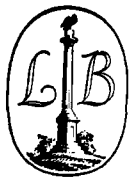
Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.*, M.A., M.B., B.Ch.

and

MAEVE O'CONNOR, B.A.

With 48 Illustrations



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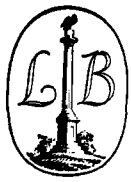

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This volume is respectfully dedicated by the Chairman and members of the Symposium to the memory of the late

SIR ERNEST KENNAWAY,
D.M., D.SC., F.R.C.P., F.R.S.
1881–1958

Printed in Great Britain

THE Ciba Foundation, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle. However, being established under British trust law, it enjoys complete independence in practice and policy.

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

PREFACE

AFTER the VIth International Cancer Congress in Brazil in 1954, the Director of the Ciba Foundation was approached with a suggestion that immediately before the VIIth Congress in London in 1958, a symposium on carcinogenesis might be held at the Foundation. The Director readily agreed to this proposal. The organization of the symposium was undertaken by the Deputy Director, who was greatly helped by the constant and invaluable advice of Professor A. Haddow, F.R.S., and Professor E. Boyland.

The conference was designed on established Ciba Foundation lines and Professor Haddow, who acted as Chairman on this occasion, directed its course with the lightest but surest of touches. The individual members of the group represented different disciplines and countries, but all were actively engaged in some aspect of cancer research. A sad loss to the meeting was the death of Sir Ernest Kennaway on January 1st, 1958, which robbed the symposium of one who would have been a most valuable contributor.

When the programme was drawn up ample time was allowed for informal discussion of the papers offered. Such thorough discussion, which is a feature of these symposia, is only made possible by limiting the number of those taking part. The editors therefore hope that the complete record of the proceedings which is presented here will afford the pleasure of vicarious participation to all those working on cancer research who could not be invited to attend this meeting.

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List of those participating in or attending the Symposium on "Carcinogenesis: Mechanisms of Action", 24th–26th June, 1958.

P. ALEXANDER	Chester Beatty Research Institute, London
I. BERENBLUM	Dept. of Experimental Biology, Weizmann Institute of Science, Rehovoth, Israel
F. BIELSCHOWSKY	Cancer Research Dept., University of Otago, Dunedin
GEORGIANA BONSER	Dept. of Experimental Pathology and Cancer Research, University of Leeds
E. BOYLAND	Dept. of Biochemistry, Chester Beatty Research Institute, London
A. BRUES	Argonne National Laboratory, Lemont, Illinois
SIR MACFARLANE BURNET	Walter and Eliza Hall Institute of Medical Research, Melbourne
H. DRUCKREY	Chirurgische Universitäts-Klinik, Freiburg im Breisgau
J. FURTH	Children's Cancer Research Foundation, Boston, Massachusetts
H. N. GREEN	Dept. of Experimental Pathology and Cancer Research, University of Leeds
C. HACKMANN	Inst. of Experimental Pathology, Farbenfabriken Bayer A. G., Wuppertal-Elberfeld
A. HADDOW	Chester Beatty Research Institute, London
C. HEIDELBERGER	McArdle Memorial Laboratory, University of Wisconsin, Madison, Wisconsin
I. HIEGER	Chester Beatty Research Institute, London
E. S. HORNING	Dept. of Experimental Pathology, Chester Beatty Research Institute, London
H. S. KAPLAN	Dept. of Radiology, University of Stanford, San Francisco, California
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P. C. KOLLER	Dept. of Cytogenetics, Chester Beatty Research Institute, London
R. LATARJET	Laboratoire Pasteur de l'Institut du Radium, Paris
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R. H. MOLE	Radiobiological Research Unit, Harwell
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M. H. SALAMAN	Dept. of Cancer Research, London Hospital Medical College, London
L. SEVERI	Division of Cancer Research, Istituto di Anatomia e Istologia Patologica, Perugia
R. TRUHAUT	Institut Gustave-Roussy, Villejuif, Seine
A. C. UPTON	Biology Division, Oak Ridge National Laboratory, Tennessee
A. L. WALPOLE	Pharmaceuticals Division, I.C.I. Ltd., Macclesfield, Cheshire
E. C. W. WEILER	Division of Biology, California Institute of Technology, Pasadena; and Max-Planck-Inst. für Virusforschung, Tübingen

CHAIRMAN'S OPENING REMARKS

A. HADDOW

THIS symposium was arranged in relation to the VIIth International Cancer Congress, to provide an opportunity, in these very agreeable surroundings, for more detailed discussion than might be possible during the Congress itself. When the symposium was originally planned we obviously had hoped and expected that Sir Ernest Kennaway would be with us. In fact he had accepted, but it was not to be. In this audience, there is no need for me to say what his loss has meant. No-one had made a vaster contribution to this subject. In tribute, some of us had the thought that we might inscribe these proceedings, when published, to him.

The second paper this morning is on the Oppenheimer effect and here again we have had a very sad loss in Dr. Oppenheimer's death a couple of weeks ago. I do not know how many here knew him personally. He was a New York physician, always tremendously interested and active in research, but mostly in his own subject of cardiology. As you know, some years ago—comparatively late in his life—he made the discovery to which the second paper refers. While he was studying the production of hypertension in the rat, investing the kidney with cellophan to induce it, and keeping these animals for long periods of time, he unexpectedly noted the development of sarcomata in relation to the cellophan sheets. The observation has been very widely confirmed, and gave Dr. Oppenheimer (and Mrs. Oppenheimer, his helper) great delight, coming as it did towards the end of his career. That delight has certainly been shared by a great many people who through it became his friends.

In the '20s and '30s I think it is true to say that our subject was in the stage of the discovery and identification of carcinogenic agents. In the late '40s and in the '50s there took place

a marked shift of emphasis from the agents themselves towards the question of their mechanism of action. As far as we can see, there is no fundamental reason why we should not ultimately be able to decipher the process in chemical terms. As a corollary, we would then know the precise biochemical nature of the differences between normal and malignant cells, with all its implications for the control of cell division. Things are very different from the early '20s, in that we now have a tremendous range and variety of carcinogenic agents. These are so varied that their initial routes of action must inevitably be different—hence the title of this symposium, the *mechanisms* of action. Nevertheless the question is still quite open, whether malignancy may depend upon some key biochemical lesion or loss—about that we will be hearing something from Dr. Weiler later in the proceedings.

I think most of you will have seen the *British Medical Bulletin* for May of this year, devoted to the causation of cancer. This succeeds a similar issue in 1947, and there is a good deal to be learned in comparing the present issue with that of 11 years ago. We can never be satisfied or complacent, but there is certain evidence of a move towards greater precision in our knowledge now as compared with then, and there are many parts of the subject which are entirely new—for example, the carcinogenic action of many alkylating agents and their mechanism. It is of interest that even since this recent issue was completed, several developments have taken place which are altogether new. One I particularly have in mind is a sudden great increase in interest in the carcinogenicity of metals, and in the rôle of metals in carcinogenesis, about which I should like to say something further, later in our proceedings.

[The Chairman then made reference to the fact that during the course of the meeting Sir Macfarlane Burnet would go to Buckingham Palace, where the award of the Order of Merit would be personally bestowed upon him by Her Majesty the Queen.]

THEORIES OF CARCINOGENESIS

I. HIEGER

Chester Beatty Research Institute, Royal Cancer Hospital, London

THERE are at least two ways of interpreting carcinogenesis: first, that there is a limit to the functional integrity of the cells of long-life in the sense of an interval which is a substantial fraction of the lifespan of the body as a whole, after which the organizing capacity diminishes; or alternatively that some factor generated internally or introduced from the outside acts as a triggering device for the carcinogenic process. So far, our knowledge of carcinogenesis refers exclusively to the second case, and I shall confine myself therefore to carcinogens of three kinds, chemical, viral and environmental.

But before leaving the possibility of carcinogenesis without a carcinogen, one might ask whether senescence is conceivable without a gerontogen, or differentiation without a differentiator? The organizers are not much heard of nowadays—did someone find that a much simpler agent could be as effective as the sterol fraction of the dorsal lip of the blastopore?

Since carcinogens of completely different character exist, it follows that they are certainly not the ultimate stage in the chain of events between their application and neoplasia, and the gaps in our understanding, or, might I say, the missing links, are as elusive as the steps in any other drug action.

The problem of carcinogenesis has been faced—it is really too early to call it attacked—by an arsenal of scientific concepts, such as quantum mechanics, electronic characteristics of chemical molecules, and mutation of a biochemical, subcellular or cellular kind according to which level of organization is being considered.

Physicists and chemists having come to the aid of cancer research have naturally tried to find correlations between the

potencies of chemical carcinogens and their physicochemical properties such as the electronic configuration of the molecule, excess charge on the K region and the reactivity to osmium tetroxide. A table showing values for the two properties side by side does suggest an approximate agreement between biological and physicochemical order, and of course "order" is the operative word here.

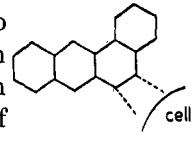
In my opinion, the awkward inconsistencies which occur, the very small margins of physical characteristics which sometimes separate powerful and weak carcinogens, and the failure of the exponents to predict on physical grounds what compounds should or should not be carcinogenic, let alone the prediction of the structure of a "super carcinogen", make it difficult to believe that electronic theory has yet very much to offer as an explanation of the mechanism of carcinogenesis.

To begin with, the electronic characteristics are described by a number calculated to the second or third place of decimals, that is, defined to one part in 100 or 1,000. But the order in which the potencies can be arranged is quite a different matter. An experimental pathologist would consider himself lucky if he could reproduce his results to within 25 or 50 per cent, which is not good enough for the order of arrangement. Except for a few carcinogens there is no completely satisfactory way of comparing their potencies, and most workers would consider becoming involved in such studies as unrewarding. At the risk of telling my audience what they already know too well, might I point out that in attempting to arrive at an estimate of the relative potencies of carcinogens it is quite mistaken to employ doses larger than optimal ones or to place much reliance on crude average latent periods—the sensitive part of the curve relating dose and response should be used; skin does not react the same as the other tissues; species and strains and groups of animals have different sensitivities, even siblings of the same group of the same pure strain of mouse respond with different degrees of susceptibility. Tumour induction assays are often reproducible only by a factor of 2 or 5; that is to say that if a preparation of carcino-

gen produces 10 per cent of tumours, a repetition of the test can often yield only 5 per cent or 2 per cent.

Physical chemists tend to ignore these difficulties. Coulson[1] in his article, "Electronic Configuration and Carcinogenesis", says "... quantum-mechanical principles ... must inevitably lead to a better understanding of ... cocarcinogenesis, anti-carcinogenesis, drug action, chemical mutation, and the mechanism of estrogenic and other hormone activity". He attaches importance to the total charge (or electron density) at the K region of hydrocarbons on Pullman's theory. In a table of 42 compounds arranged in order of total charge the carcinogenic activity does not increase parallel to the charge but shows fluctuations; there are no less than six maxima of activity sinking in between to minima or even to zero activity. Nevertheless the general idea seems to be that the K region of the molecule has some specially reactive properties. Coulson deduces that ... "There is thus no kind of conflict with the tentative view that carcinogenic action begins by a bond addition to some enzyme". The logical steps for this deduction I regret I have not been able to discover. He finally decides that total charge on the K region is insufficient for the correlation with carcinogenic activity and that the calculation should include bond order, but even this correction does not overcome the difficulties for he concludes that ... "The fact that there are some serious failures in this correlation suggests that there may be two or more ways in which the carcinogenicity is shown, or that there are two or more stages in the complete phenomenon, and our K-region analysis deals with only one of these stages—the remaining stages may be governed by an entirely different index, corresponding to an entirely different mechanism".

The chemists have an innocent way of regarding carcinogenesis as a kind of chemical process. They use a structural formula which is intended to indicate what part of the carcinogen acts on the cell. To my mind such a simplification helps no more than to say that the molecules of



penicillin or morphine become attached to the cell, but where do we go from there? Why do carcinogens act on some tissues and not on others? Since benzpyrene is highly carcinogenic for mouse tissue and only very slightly so for the tissues of the monkey and rabbit, their cells must be lacking in something fundamental, and what is wrong with guinea pig cells which are so strongly resistant to anything but the most powerful carcinogens?

But the most serious difficulty for any purely chemical theory of carcinogenesis is the very wide range of compounds which can induce tumours, including a variety of plastic films (cellophan, nylon, dacron, polythene, perspex, polyvinyl-chloride, polystyrene and silk). Oppenheimer[2, 3], still clinging desperately to chemical theory, suggested that the polymer film is degraded to the accompaniment of free radical formation and that it is this supply of free radicals which is the immediate carcinogen. However, in his 1958[4] paper he describes an admirably contrived control experiment where nylon film was put in one side of the animal and nylon powder in the other: the film gave 58 per cent of sarcomas, the powder gave none. It looks as though the mechanism is via physical conditions and not via direct chemical processes, especially as he finds films of quite different materials to be active, such as silver, tantalum and stainless steel. Oppenheimer now proposes that the film acts as a confining system which creates a restricted metabolic environment. Such an idea would fit in not too badly with Earle's[5] important discovery of transformation *in vitro* in the absence of carcinogen and also with a still nebulous explanation of cholesterol carcinogenesis which could operate by bringing about abnormal conditions in the capsule surrounding the injected material.

The guinea pig difficulty could suggest that the process of carcinogenesis does not depend primarily on the lines shown in the simple sketch on the previous page, but is determined by factors of the type involved in species differences, which amount to immunological differences. A theory of carcinogenesis based on immunological ideas was

put forward by Green[6] in his paper of December, 1954. I confess that I found it difficult to understand; as far as I can follow the argument it is something like this: first the carcinogen acts on the labile lipoproteins of the cells about to become cancerous in such a way as to convert them to a kind of simplified antigen; secondly, this antigen is not sufficiently energetic immunologically to bring about the formation of neutralizing antibodies, otherwise cancer cells would be destroyed as fast as they were created; while the new antigens are masked in some way, they confer sufficient specificity on the altered cells to make them behave abnormally, that is neoplastically. If I understood Green's theory at all, it seems to be a variation of the mutation hypothesis, with this difference: it puts immunological changes first and neoplastic transformation as a consequence, while the usual versions of the mutation hypothesis assume that some genic change occurs first. Green[7] maintains a critical attitude to his theory for he states . . . "Moreover, it is always possible to object that antigenic simplification, if found, is an expression of the immaturity of the cancer cell and not primarily of the malignant state. The most convincing evidence would be to discover new facts predicted by the theory." I would put it slightly differently and say—the test of the theory would be to confirm the deductions made from it. I believe that immunity theory is bound to have an important place in cancer ideas in the future—for example, Earle has reported that some immunological changes slowly occur during his transformation experiments *in vitro*.

When we pass from the protein molecule to the gene—that is to the next higher level of organization—the hypothesis to be considered is somatic mutation. Since it is postulated that the chromosomal structure of all the cells of the body is derived by template replication from the zygote, they should have chromosomal identity; how then to explain the differences between a brain cell and, say, a toenail basement-membrane cell—by the terminology "differentiation"? Might not malignant transformation belong then to the same genus

as differential change? This question could be answered on one condition—that we knew something about differentiation. Huxley [8], whose intellectual scope we all admire, has regrettably gone in for the creation of some recondite terminology in "The Biology of Cancer"; he speaks of pluripotent de-differentiation, hypoplastic de-differentiation, and anabiotic de-differentiation. But I seem to remember looking through the histological descriptions of tumours and finding the terms —"well-differentiated mammary carcinoma" and "epithelioma showing keratin pearls". If that is not differentiation, what is? To equate malignancy with de-differentiation or a-maturation is too crude a simplification.

Let us return to somatic mutation. To anyone who has looked through a microscope at a section of tissue that has undergone carcinogenic change, surely it must be obvious that a whole area of tissue has been transformed. If gene transformation adequately describes the process it must be occurring in hundreds or thousands of cells practically simultaneously. Such a situation is very far from the conventional picture of random gene mutation, and how can mutation theory cope with the fact that in late age groups one-third or even a half of the population develops clinical cancer, and if the non-clinical cases be included we would be dangerously near the 100 per cent level.

Moreover, if each mutation is assumed to be independent of the preceding ones, Crile [9] points out that for the carcinogenic action of uracil we should require a mutational step for the conversion of normal thyroid to hyperplastic thyroid, another for the change to benign tumour, another for metastasizing tumour, and one for autonomous cancer independent of a supply of uracil. Failla [10] calculates that if the mutations are independent of the preceding ones the probability of mutation is decreased in proportion to the number of genes required. If we assume 25,000 genes per cell, then if cancer mutation depends upon two genes the probability is decreased by a factor of the order of 10^4 to 10^5. Clearly, a seven-mutation system would decrease the probability to practically zero.

Yet Doll[11], supporting Nordling's hypothesis, postulated on mathematical grounds that a seven-stage mutational change would meet the case, by examining the age-specific mortality rates for 17 types of cancer. This seven-stage idea depends on the 6th-power relation between log of death rate and log of age: but unfortunately the same kind of relation holds for cerebral haemorrhage, coronary thrombosis and gastric ulcer.

Clearly, some alternative approach to genic mutation is needed, and something on the lines of differentiation is our nearest analogy.

After Haddow's[12] classical paper in 1944, the somatic mutation theory became dominated by the idea of extranuclear genes. But is the advantage a real one when we transfer the difficulties from a malignant transformation in nuclear genes to mutation in the cytogenes? Since we are now embarked, or should I say adrift, on hypothesis, only one further step is required to suggest that the mutated indigenous cytogene is parallel to the action of a cytoplasmic virus introduced from the outside. In its simplest form the argument runs like this: (1) chromatin contains replicating-polymer, i.e. deoxyribonucleic acid (DNA), and therefore cytogenes should consist of DNA; (2) viruses contain a high proportion of DNA; (3) bacterial transforming principles consist of DNA; (4) Dmochowski[13, 14] and Bittner[15] carefully try not to be quoted as saying that virus arises *de novo* from preformed normal subcellular elements, but notwithstanding their caution that is exactly what their statements do suggest. The late Dr. R. N. Salaman[16] with the candour of self-confidence said that it was an "almost inescapable conclusion" that the virus of paracrinkle in the potato is formed *de novo*.

Generalizations on carcinogenesis have been formulated from a still higher level in the work of Berenblum, Rous, Mottram and Twort. The widest known theory is that of Berenblum, whose thesis is that carcinogenesis is a two-stage affair, initiation and promotion. Berenblum, in agreement with Rous, is now quite content with two stages; until 1947 he used a three-stage scheme.

But is this two-stage arrangement unavoidable? Salaman[17] and colleagues have found that croton oil, the promoter *par excellence*, is itself a weak carcinogen. Berenblum[18] admits that croton oil has proved a broken reed and finds a standby in urethane, which has not yet been shown to be a carcinogen for mouse skin but is an initiator when fed or when injected subcutaneously or intraperitoneally. Now, acetylaminofluorene and 9 : 10-dimethylbenzanthracene can act in the same way. The dialectic position is becoming a little unsure, for we now have to postulate that urethane can act as a complete carcinogen for one tissue, the lung, but is only an initiator for the skin. Would it not be just as adequate to say that skin is a highly specialized tissue as to say that urethane (first croton oil) is a special kind of semicarcinogen? This line of work needs to be extended to other tissues—its great interest is self-evident.

I would venture to conclude that while we have at present a number of theories of carcinogenesis, there is as yet no theory of carcinogenesis; that these hypotheses have scarcely proved more than re-statements of the facts of experiment or observation; and that the very tentative nature of these ideas is a measure of the difficulties of our formidable and wonderful problem.

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[Discussion of this paper was postponed until after the paper presented by Dr. P. Alexander.—EDS.)

OBSERVATIONS ON THE OPPENHEIMER METHOD OF INDUCING TUMOURS BY SUBCUTANEOUS IMPLANTATION OF PLASTIC FILMS

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THE concept that carcinogenic chemical substances act directly on those cells which subsequently become malignant is usually taken for granted but lacks direct experimental evidence. Except perhaps in the case of cancer viruses there is no proof that malignant transformation can be brought about *in vivo* by a substance entering a cell and producing an irreversible change (somatic mutation) which persists in the daughter cells. On the other hand, a number of well-defined stimuli are now recognized where there is no direct pharmacological interaction between the carcinogen and the cells that become malignant; discussion of one of these indirect processes of inducing cancers forms the subject of this paper.

Ten years ago, B. S. and Enid Oppenheimer and Stout (1948) published their chance observation that highly malignant fibrosarcomata had been found in rats, the kidneys of which had been wrapped with cellophan to produce hypertension. In their subsequent paper in 1952 they provided sufficient data to justify the claim to have discovered an entirely new group of carcinogenic agents for rats and mice. Whatever the chemical composition, a thin film of plastic when introduced subcutaneously proved carcinogenic and so far no plastic material has been found which did not produce sarcomata in approximately 25 to 45 per cent of the animals. Oppenheimer and co-workers (1953*a*) eliminated decisively the possibility that adventitious impurities were responsible by

establishing that the carcinogenicity was quite independent of the degree of purity of the polymer (e.g. the most pure polyethylene and polystyrene were somewhat more active than the commercial samples), and also by showing that all the most probable impurities, such as the monomeric starting materials and the catalysts (e.g. benzoyl peroxide), used in the preparation of some of the polymers were not carcinogenic when painted or embedded in pellets. The conclusion can therefore not be resisted that the induction of tumours by these films must be due to the macromolecular components which are completely insoluble in physiological fluids.

Elimination of a chemical mechanism

Although the Oppenheimers themselves have refrained from speculations, a number of *ad hoc* hypotheses for a chemical mechanism have been advanced by other workers who have confirmed and extended their observations. On finding films of nylon carcinogenic Druckrey, Schmähl and Danneberg (1952*a*) attributed this action to the ability of the polymer to form hydrogen bonds with body proteins. With polyethylene, which cannot form hydrogen bonds, the formation of epoxides during aerial oxidation was invoked (Druckrey, Schmähl and Danneberg, 1952*b*), since some low molecular weight epoxides are carcinogenic (cf. Haddow, 1953). However, there are many polymers to which neither of these hypotheses can apply and a different mechanism might have to be postulated for nearly every type of plastic. Even the most enthusiastic would find it difficult to put forward a chemical reaction in which polytetrafluoroethylene ("Teflon") could be involved under physiological conditions since its inertness is such that it has the lowest coefficient of friction* of any structural material and is used for this reason to line skis: yet it is carcinogenic (Oppenheimer *et al.*, 1953*a*). Another decisive experiment is that films of silk, prepared from solutions of the

* Friction between two surfaces requires that there is some chemical interaction between them.

fibres, are as carcinogenic as films made from synthetic plastics (Oppenheimer *et al.*, 1955); it would be difficult to find a reaction of silk which is not shared by some physiological proteins.

A suggestion (Fitzhugh, 1953) which has been considered by the Oppenheimers (Oppenheimer *et al.*, 1953*b*) is that free radicals or free radical-forming substances might be involved since many of the polymers are prepared by free radical mechanisms.* Recent physicochemical measurements on occluded polymer radicals (Ingram, Symons and Tapley, 1958) show, however, that there is no correlation between free radical content and carcinogenicity; for example, polyacrylonitrile contains a concentration of free radicals a hundred times greater than that in most other vinyl polymers while its carcinogenic activity is less than that of most other films. But the real difficulty in any such hypothesis is the migration of the free radical polymer out of the film and its survival in a reactive form during the transport to the cell on which it acts. In molecular terms this distance is huge since the film floats inside a sheath of connective tissue without making contact with any cells.

Oppenheimer and co-workers (1955) believe that the very slow breakdown of some of the films *in situ*, which they were able to detect with radioactively-tagged polymer—the attack is so slight that no visible erosion of the film occurs—provides some support for the hypothesis that a diffusible free radical may be the causative agent. But again the extreme range in chemical reactivity and constitution of the plastic films, all of which are carcinogenic, would appear to exclude this possibility since the metabolites would have no properties in common and would also be formed at quite different rates (e.g. contrast the breakdown of silk with that of a silicone plastic!).

A very strong argument against any hypothesis which

* There are many polymers in which no radicals are introduced during their synthesis (e.g. the condensation of an acid and an amine to give nylon) and for these oxidation at secondary and tertiary carbons to give free radical peroxides has been postulated (Fitzhugh, 1953).

requires the chemical interaction between a compound of the film and its neighbouring cells is the importance of the physical shape of the foreign substance. The same plastic materials when embedded in the form of powder or fibres fail to produce any tumours at all and when introduced as plain film, perforated film, woven or knitted cloth the tumour incidence invariably falls in this order (see Table I). When foils of six

Table I

(after Oppenheimer *et al.*, 1953*a*, 1955)

Material	% *Incidence of tumour* *Plain film*	*Perforated film*	*Textile*
Polyester (terylene)	20	5	0
Polyamide (nylon)	27	7	0
Polyethylene	20	14	2·5
Polytetrafluoroethylene	24	19	—

different metals were used, malignant tumours were obtained with five, the exception being tin (Oppenheimer *et al.*, 1956). The failure of this metal was attributed to its friability and in every case it was found to be "broken up and crumbled into a fragmentary mess". When thicker tin foil was used tumours were produced (private communication from Mrs. E. T. Oppenheimer). This is another instance of the importance of the physical shape.

The importance of the size of the film

In view of the possibility that local anoxia may be playing a part we decided to examine the carcinogenicity of cellophan film as a function of size. Visking sausage tubing 0·4 mm. thick was used after soxhlet extraction for 48 hours with both water and ethanol. The squares were steam-sterilized prior to their subcutaneous implantation. Squares of 2 cm. × 2 cm., 1 cm. × 1 cm., and ½ cm. × ½ cm. were introduced subcutaneously into the abdominal wall of six-week-old albino rats. The numbers of tumours which developed in the three groups of treated rats and the average latent period of

tumour induction are seen in Table II. This experiment was terminated after 20·5 months.

Palpable subcutaneous lesions began to develop in the first group of rats approximately 12 months after the insertion of the 2 × 2 cm. films, whereas in the second group bearing the 1 × 1 cm. films no palpable lesions arose until 17 to 18 months.

Table II

Size of film	*Total numbers of rats used*	*No. of animals surviving one year*	*No. of animals with tumours*	*Average latent period of tumour induction in weeks*
2 cm. × 2 cm.	18	18	10	78
1 cm. × 1 cm.	24	18	6	89
½ cm. × ½ cm.	24	22	1	106

The only tumour which arose in the third group with the ½ × ½ cm. plastics did not develop until 20·5 months after insertion. From these experiments it appears that a correlation exists between the actual size of the surface area of the implanted plastic film and tumour induction. The fact that both the latent period and the incidence of tumour induction is dependent upon the size of the film is of special interest. The 2 × 2 cm. films in several of the rats failed to give rise to tumours. They were excised and examined histologically when it was found that in every single instance these films had accidentally become folded during their insertion into the subcutaneous tissues of their hosts, thus considerably reducing their surface area. The true incidence of tumours arising from 2 cm. × 2 cm. films is therefore considerably greater than the value indicated in Table II and may approach 100 per cent.

Histological examination

Oppenheimer and co-workers (1955) noticed that the film, when introduced subcutaneously, was very rapidly (two to three weeks) enclosed by a sheath of connective tissues in which it floated without being attached to it in any way. No