Section of Comparative Medicine

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Advances in the Study of Chemical Carcinogenesis

PRESIDENT'S ADDRESS

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IT will be easy for you to understand how it is that I, more perhaps than most, should wish to acknowledge a special debt to comparative pathology and comparative medicine, inasmuch as the field of cancer investigation, more than almost any other, has been dependent—indeed utterly dependent—upon the comparative method. Although we have by far the greater part still to learn, our accumulated knowledge is already vast, and we recall with gratitude—to mention only a very few examples—the early work upon tumour transplantation, such names as that of Leo Loeb, the unravelling of cancer genetics in the mouse, Peyton Rous' discovery of the avian tumour virus which bears his name and still provides us with a great opportunity as well as an enigma now forty years old, Bashford's great work on the natural history of cancer in the animal kingdom, and the much more recent demonstration of the Bittner virus as a factor in the causation of mammary cancer in mice—all discoveries of the first order in the comparative field. Little do they know of cancer who only cancer know: the subject is in fact almost coterminous with cell biology itself and equally dependent for its advance upon advances in the basic sciences as a whole. Here indeed lies one of its greatest attractions—not only do we draw upon the basic sciences in applying them to our special problem, but we may also hope, partly by labour but more often by good fortune, to repay the debt. This is strikingly so too in the case of comparative pathology, which has at once catalysed and fostered the growth of our knowledge of cancer and at the same time been itself abundantly enriched.

Chemical carcinogenesis is the subject in which one is most engrossed; it is a research in which the chase becomes ever more enthralling as the months and years go by. In this field comparative pathology again provides us with our first lesson—namely, that carcinogenic potency is no absolute property of a given chemical substance but is dependent for its expression upon a great range of factors amongst which specific, genetic, and organ and tissue differences rank as the most important. Many years of the most patient research into the metabolic history and fate of individual carcinogenic substances, when these are introduced into the tissues of different animal species, are only now beginning to yield a glimpse of the reasons why such substances may readily provoke the appearance of tumours in one species or in one set of circumstances, and not at all in another.

When we speak of recent advances in the field of chemical carcinogenesis we forget that the bulk of work in the subject has been carried out only in the past three decades, in the whole of which period the pace of advance has been considerable. Thus it is only a matter of thirty-five years since the first experimental chemical production of cancer by the Japanese; only twenty-one years since the first production of cancer by a pure chemical individual in the shape of 1:2:5:6-dibenzanthracene, by my predecessor Sir Ernest Kennaway; and only fifteen years since we first developed the view that the carcinogenic hydrocarbons may operate by specific damage to the growth mechanism of the normal cell, which then reacts by the adaptive development of a new mechanism—the nature of which still eludes us—and the emergence of what is for all practical purposes a new cell race. In the meantime. we have collected a vast quantity of information relating to hundreds of chemical carcinogens—the carcinogenic hydrocarbons, concerning which so much was contributed by Kennaway, Cook, Hieger, Hewett and others; the carcinogenic azo dyestuffs, to the study of which we again owe so much to the Japanese; and a large number of aromatic amines, including β-naphthylamine, 2-acetamidofluorene, and most recently a whole series of aminostilbenes.

In all these cases we have acquired a great deal of knowledge as to the relationship between chemical structure and biological action—both within a series and sometimes linking one series and another. But in no case—a striking fact—do we know the place in the cell at which they act—whether the cell surface, the cytoplasm, the nuclear membrane, the nucleus itself—or the nature of the receptors with which they combine. Only very recently have we obtained our first hints as to (1) the more precise nature of the mechanism of action, and (2) the site in the cell at which it takes place. These hints—and of course they are for the moment no more—have arisen entirely in the past three years and very largely from the discovery, in this short interval, of the carcinogenic potency of the nitrogen mustards.

Most here are no doubt familiar with the history of the nitrogen mustards as potential chemical warfare agents, and with their limited therapeutic application especially in Hodgkin's disease. It appeared not unreasonable to expect to improve the therapeutic efficiency of these substances by chemical modification, and in the past three years some 250 variants have been synthesized, by Kon, Ross and others at the Royal Cancer Hospital, and have been tested biologically to this end. Although the chemical possibilities are certainly far from exhausted, therapeutic usefulness has proved difficult to increase. On the other hand, the wider biological investigation of these substances has led to considerable advances of a more fundamental kind, which are, in fact, beyond anything we had in mind, or could, perhaps, have expected. The first approach was to decide in which aromatic amines, if any, *bis*chloroethyl or similar groups could be inserted and still confer the cytotoxic activity characteristic of the aliphatic nitrogen mustards. From extensive clinical trials carried out with a few of these substances (and particularly with the *bis*chloroethyl derivative of β-naphthylamine), it appears that the therapeutic effects are very largely confined to those tumour types already known to be responsive to the aliphatic mustards. From cytological evidence it appears too that the action of these substances, as in the aliphatic series, is very largely direct, as shown by the production of chromosome breaks, of bridges at anaphase, and of defects in chromosome spiralization; the last effect is specially important in view of what I shall have to say of the mechanism of action. The chromosome fragments appear to be ejected into the cytoplasm, where they agglomerate as "micronuclei," this process being repeated in successive divisions in each of which the cell accumulates further nuclear damage until it is no longer viable: the essential damage—of which the aberrations during mitosis are the sequel—is, however, believed to occur in the so-called resting stage between divisions.

All the biological phenomena included in their clinical and cytological effects are such as to justify the description of these compounds as radiomimetic, and the same analogy is apparent in the damage to cell division which they can produce in hæmopoiesis and spermatogenesis, as also in a remarkable and apparently permanent greying or bleaching or hair which appears over the site of intracutaneous or subcutaneous injection in coloured mice; the last effect is quite indistinguishable from that induced by X-radiation or the subcutaneous injection of a radioelement such as plutonium. These observations have led to the development of the concept of radioequivalence as between ionizing radiations and such chemical agents, not only as regards their effects *in vivo* but also *in vitro*, where the reactions brought about by both types of agent with deoxyribonucleic acid show a quantitative correspondence greater than would appear to be due to chance alone. These facts, as also the observation of Elson that the growth-inhibitory effects of these aromatic mustards, like those of the carcinogenic hydrocarbons and aminostilbenes, appeared to be increased by a sufficiently low concentration of protein in the diet, made it desirable to determine whether the aromatic nitrogen mustards might, equally with ionizing radiations, be capable of producing tumours, as a further expression of radiomimetic action. Tests of selected compounds were therefore carried out in the rat, mouse, and hamster, and with abundantly positive result, tumours having been produced in all these species at the site of application. A feature of the tumours induced by subcutaneous injection has been the frequent coincident appearance of sarcoma and carcinoma, and it is also possible to induce intestinal carcinoma, by administration orally. As a class, all these tumours have proved of the greatest interest cytologically, on account of the high proportion with nuclear abnormalities (of the same general types as those produced by the mustards acutely). Indeed it would seem in these cases as though the tumour cells bear the imprint of the causal carcinogen, and it is of additional interest that in certain tumours the visible chromosomal abnormalities may be perpetuated through many transplanted generations, although they tend to die out, no doubt through some selective process, after a shorter or longer time.

These facts and findings have certain implications, and allow certain inferences. In the first place, while cytological abnormalities have frequently been observed in individual tumours induced by other carcinogens, such as the cyclic hydrocarbons, their interpretation has been difficult, and their significance doubtful, on account of their inconstancy and the fact that they might, in other cases, be entirely absent. In the present instance we have been compelled to study these nuclear changes more closely, even if only on account of their relative frequency, and admitting that they are unlikely to be causally connected with tumour induction and propagation, and no doubt only associated. One may conjecture whether the frequency of such abnormalities in the mustard-induced tumours may be a reflection of the high chemical reactivity of these substances, in contrast say with the carcinogenic hydrocarbons. Again, in the nitrogen mustard series there might appear to be a greater prospect than with the hydrocarbons, on account of this high reactivity and their relatively simple molecular structure, of deciphering the mode of action. In a long series we were impressed by the apparent dependence of biological activity on a certain degree of chemical reactivity, and on the presence in the molecule of a minimum of two reactive side-chains: this bifunctional or polyfunctional requirement had previously been commented upon by others, for the sulphur mustards as well as their nitrogen analogues. In an endeavour to interpret this situation, Goldacre, Loveless and Ross had suggested, from general considerations of the adsorption of drugs to proteins, from the aspect of chemical cytology, and from kinetic studies of the reactions of "two-armed" compounds, that such bi- or polyfunctional agents might operate through a process of chemical cross-linking between the constituent linear macro-molecules of the chromosome structure itself. We now know that this

explanation is unduly exclusive, and we cannot for instance dismiss a two-armed combination along the length of such molecular fibres, rather than between them, or other processes involving regular polymerization (such as has been suggested by Rose), or indeed a more random molecular arborization. The main importance of the hypothesis lies in its suggestion of direct chemical combination of the carcinogenic molecule with genetic material, and in this sense it has proved highly fertile in development. In this connexion a great deal of help has come from the field of textile cross-linking, as for instance from Speakman's suggestion of the di-epoxides, many of which do in fact duplicate the biological action of the nitrogen mustards and are the first of a whole series of chemical types all yielding the same carbonium ion. The cross-linking potentiality is not, however, sufficient, and reaction must occur first of all under mild conditions, and, secondly (according to Ross), with acid groups preferentially, and not for example with sulphydryl or amino groups alone. As indicated, however, there are undoubtedly other possibilities, such as reaction at two sites on a single fibre to give rings of varying stability, polymerization, arborization, and even swamping of the biological sites or receptors by one-armed compounds.

Until recently, our picture of the carcinogenic process envisaged damage of the normal growth mechanism as the primary effect. At present (at least so far as the mustards are concerned), we now picture damage to the chromosome by direct combination with genetic material, followed maybe by the generation of a new and self-duplicating chemical and genetic rearrangement. A primary effect on the nucleus would not of course be surprising, although we are so far quite ignorant as to whether such combination, through cross-linking or other means, is between say polypeptide chains or via the nucleoprotein. All this has greatly influenced our recent thinking upon the subject of carcinogenesis, has to some extent clarified it, and is already leading to fresh development. The process I have hypothetically described is not dissimilar from that of globulin and immune body production from a fibre, surface or template secreting by replication, or liberating a specific protein, and in this connexion it is not without interest that the cell types most sensitive to the action of the nitrogen mustards (the lymphocyte, the plasma-cell, and reticulo-endothelial cells more generally) include those which may be, according to one view, responsible for immune body production. More recently, certain of the compounds under discussion have been shown (by Ford and by Revell) to have a varying degree of preferential action at certain specific chromosome regions, especially the so-called heterochromatic regions known to have characteristic chemical and genetic properties, the latter associated not so much with Mendelian inheritance as with the quantitative inheritance of growth-rate and differentiation features and their mutation. It is perhaps a sign of the times, and some indication of progress, that while the mouse was selected some thirty years ago as the most suitable test object for carcinogenicity studies, on account of its availability, susceptibility, short life-span and ease of maintenance, we are now searching for both animal and plant material with cytological features of the greatest advantage. Much help is also being gathered from studies of the relationship between carcinogenesis and mutation. Here again there is great need for a more detailed knowledge of chromosome structure, such as can only become available in the decades ahead. Meantime, however, as has been the case with other cytogenetic problems—and cancer is one such—we hope to acquire further information from the concomitant changes induced in the giant chromosome of the *Drosophila* salivary gland. It is clear that an immense amount still remains to be accomplished, but from what I have said there would appear to be—and for the first time—some prospect (if only a prospect) of correlating the chemical properties of a given carcinogen with the chemical properties of specific chromosome regions, with the resultant cytological effects, and with the genetic consequences to the cell which thus ensue.