CHEMICAL CARCINOGENESIS

A SERIES of six lectures entitled "Chemical Carcinogenesis" was given at the Northern Polytechnic, London, between October 7 and November 14, 1965. Their purpose was to publicize the carcinogenic hazards of chemicals, particularly to those engaged in the production and marketing of chemical, rubber and pharmaceutical products. The course was inspired by an article by Prof. A. Haddow (*New Scientist*, 25, 348; 1965) entitled "Control of Cancer-inducing Chemicals", and Prof. Haddow very kindly helped to plan the course in its initial stages. The need for these lectures was shown by the high level of attendance at the meetings, and by letters from people expressing regret at not being able to attend and asking for abstracts. The lecturers, who kindly supplied abstracts for inclusion in this report, were: Prof. A. Haddow, director of the Chester Beatty Research Institute, London; Prof. E. Boyland and Dr. F. J. C. Roe (Chester Beatty Research Institute); Dr. P. N. Magee (Toxicology Research Unit Medical Research Council Laboratories, Carshalton, Surrey); and Miss Joan Davies (The Royal Marsden Hospital, London).

The meetings began with a lecture by Dr. P. N. Magee on carcinogenesis by nitroso compounds.

A large number of *N*-nitroso compounds are known to be capable of inducing cancer in experimental animals[1–5]. These compounds are among the most potent of all known carcinogenic substances[6,7]. The simplest member of the dialkylnitrosamine series, dimethylnitrosamine, is a powerful liver poison, causing necrosis of the liver in all the common species of laboratory animal[8]. When continuously fed to rats, mice and hamsters it induces malignant tumours of the liver in high incidence[1]. When given to young adult rats for shorter periods at higher levels, and even in survivors of single large doses, kidney tumours are induced but no liver tumours[2]. Injection into newborn rats is followed after about a year by kidney and liver tumours[9]. Dimethylnitrosamine is rapidly metabolized[10] and a large part of the carbon of the injected dose is excreted in the expired carbon dioxide[11,12]. The liver appears to be the main organ concerned with metabolism which is enzymatically mediated[13,14]. The kidney and probably some other organs in the rat are also capable of metabolism of dimethylnitrosamine but much less actively than the liver. One metabolic pathway appears to involve oxidative removal of a methyl group with the formation of the very unstable monomethylnitrosamine which decomposes spontaneously to yield an active alkylating intermediate, possibly the corresponding diazoalkane[15,16]. There are certainly other metabolic pathways of dimethylnitrosamine but these have been less thoroughly investigated.

A hypothesis to explain the toxic, mutagenic and carcinogenic action of the nitroso carcinogens suggests that the active agent concerned is the alkylating intermediate. Evidence in support of this hypothesis comes from examinations of carcinogenic activity in relation to chemical structure with a large number of nitrosamines[4], although certain discrepant features remain unexplained. Direct evidence of alkylation of tissue constituents has been obtained with the demonstration that proteins and nucleic acids become alkylated in animals injected with dimethyl and other nitrosamines[15,16]. Alkylation, mainly on the 7-position of guanine, occurs in the DNA and RNA of liver and kidney in the treated animals[16]. The methyl group in both the methylated DNA and RNA is unstable and rapidly disappears from the nucleic acids *in vivo* at a rate which cannot be explained by normal metabolic turnover[17]. This alkylation reaction is the same, chemically, as that which is suggested to underlie the mutagenic action of the biological alkylating agents. Whether it underlies the carcinogenic action of the nitroso carcinogens remains to be established.

Prof. E. Boyland devoted two lectures to discussing the biochemistry of a wide range of chemical compounds.

As most cancer is caused by chemical substances in the environment, study of the biochemistry of chemical carcinogens is of interest because it should lead to the prevention of the disease. Some carcinogenic compounds act without previous metabolic change and are considered to be direct carcinogens. Alkylating agents, which include mustard gas, nitrogen mustards, methylsulphonyl esters, lactones and epoxides, are probably direct carcinogens of this type. They react with the purine, guanine residues of nucleic acid. The nucleic acid is thus changed so that mutations and cancer result.

On the other hand, the aromatic amines, 2-naphthylamine, benzidine, and 4-aminobiphenyl, which induce cancer of the bladder, are carcinogenic after metabolic activation and are therefore indirect carcinogens. The active metabolites are derivatives of arylhydroxyamines and *ortho* aminophenols which can induce cancer of the bladder directly.

Most chemical carcinogens have been first recognized through accidental industrial exposure. An early example of this was the recognition of scrotal cancer in chimney sweeps as a consequence of exposure to the soot of chimneys (Percival Potts, 1775). The observation that soot and coal tar were carcinogenic led to the isolation of the active polycyclic hydrocarbon, 3,4-benzpyrene. Following this, a number of related polycyclic hydrocarbons were synthesized and tested by Cook and Kennaway, and the relationship of chemical structure and biological activity investigated. It is not known whether these hydrocarbons require metabolic change before exerting their biological action.

Urethane, which was used as an anaesthetic, as a solvent for drugs and in the treatment of leukaemia and myeloma, is also carcinogenic. Urethane is converted by mammals to *N*-hydroxyurethane, which has many biological activities that urethane does not possess. *N*-hydroxyurethane and some related hydroxylamine derivatives are carcinogenic and also inactivate the Shope fibroma virus and other viruses *in vitro*. The mechanism of action of *N*-hydroxyurethane and its analogues is probably due to reaction with the pyrimidine base, cytosine, of nucleic acids.

Many of the compounds used in the chemotherapy of cancer (such as nitrogen mustards, urethane and oestrogens) are carcinogenic. Knowledge of the mode of action of these substances is therefore of value both in prevention and treatment of cancer.

Prof. A. Haddow spoke on the heterogeneity of carcinogens and questions of the mechanism of action. An outstanding feature in carcinogenesis was the great number and variety of causative agents, whether chemical, physical or viral. This made it certain that such agents operate by biochemical routes which, initially at least, must be correspondingly different. However, it could by no means be excluded that they could lead to a key cellular event or alteration, similar, or perhaps even identical, in the different cases. Hence there was a prospect of the emergence, from a picture of great complexity, of a more synthetic comprehension of the carcinogenic process, with all that this could mean towards elucidating the essential biochemical differences between normal and cancer cells, and perhaps in turn, to rational

methods of control and even prevention. Prof. Haddow illustrated this theme by accounts of the carcinogenic hydrocarbons, aminostilbenes, aromatic amines, the biological alkylating agents, metals and metal–carbohydrate complexes, and of such miscellaneous examples as asbestos, the cycads, and the recently discovered aflatoxins. It was remarkable that, even now, so little was known with any precision as to the mode of action of the carcinogenic hydrocarbons. Their molecular planarity had suggested a possible interaction with the nucleotide plates, but this view might require to be modified or abandoned in view of the more recently recognized non-planarity of the potent 9 : 10-dimethyl-1 : 2-benzanthracene. However, other collateral evidence (for example, solubilization of the polycyclic hydrocarbons by purines and nucleic acids) still suggested some type of interaction with DNA, and a special case being widely investigated was that of the possible intercalation of hydrocarbon molecules between adjacent base-pair plates.

Of all the classes of chemical carcinogen, it was perhaps in the field of the alkylating agents (such as nitrogen and sulphur mustards, *bis*-epoxides, polyethyleneimines, dimesyl compounds, lactones and alkylnitrosamines) that most progress had been made in the past 15 years—largely as a result of the relative simplicity and higher chemical reactivity of these agents. Most remarkable was the demonstration by Brookes and Lawley of alkylation in guanylic acid at position 7, and of *bis*-guanyl and similar products almost certainly derived from inter-chain cross-linking of the DNA helix. That many of the DNA-viruses might act by modification of the genome similar to the cytogenetical changes induced by chemical agents was one of the considerations prompting hopes of an eventual synthetic understanding.

Miss Joan Davies spoke about occupational bladder tumours. More than 2,000 men die with bladder tumours every year in England and Wales, and some of these tumours are of occupational origin. If a bladder tumour is diagnosed at an early stage, treatment may be simple and effective, but often a tumour does not cause any symptoms until it is comparatively advanced, and treatment is then more difficult. This is why early diagnosis by means of cytological screening (regular microscopic examination of urine samples) is important for groups of workers who run an enhanced risk of developing the disease—groups such as certain chemical workers for whom the Association of British Chemical Manufacturers set up a screening service in 1956.

In 1954 a high risk of bladder tumours among men working in firms manufacturing alpha-naphthylamine, beta-naphthylamine and benzidine had been demonstrated[18]. Between 1921 and 1952, 243 out of 2,500 workers had developed the disease and half these had died as a result (at an average age of about 55). Another 250 would probably develop tumours in due course, for some 18 years usually elapsed between starting work with the chemicals and the onset of the disease. Beta-naphthylamine was the most dangerous chemical. The longer a man worked with these substances, the more likely he was to be affected, but even brief and indirect contact could cause tumours.

Until 1949 the rubber industry made extensive use of antioxidants containing alpha- and beta-naphthylamine, and in 1954 Case and Hosker[19] directed attention to an excess risk of death from bladder tumours among skilled rubber workers. However, no full survey has ever been made to determine the size of the risk or the range of workers affected. In 1956–57 the Rubber Manufacturing Employers' Association set up a screening service, but by no means all member firms made use of this service and many exposed rubber workers were not screened until very recently.

The same anti-oxidants were used until 1949 in insulating rubber in the electric cable industry, but here the risk apparently went unheeded until recent publicity following the inquest on a former cable worker[20]. The severity of the risk can be gauged by the finding[24] that in the rubber mill of one cable factory thirty out of 139 men had worked for 10 or more years in contact with such an anti-oxidant, and six of the thirty have so far developed bladder tumours (four of these dying of the disease). Apparently no cable workers were screened until after the inquest mentioned. As well as definitively identifying the carcinogens responsible, a full survey of the rubber and cable industries might identify the workers running the greatest risk of developing the disease, and so enable screening priorities to be established.

In 1953, bladder tumour was made a prescribed industrial disease under the Industrial Injuries Acts, but through a general lack of awareness many rubber and cable workers with the disease (or their widows) have never been advised to claim the extra financial benefits to which they are entitled.

The final lecture was given by Dr. F. J. C. Roe, who reviewed the evidence that some metals, minerals and pharmaceutical preparations are capable, under certain conditions, of inducing cancer in laboratory animals or man. Among the metals, clear evidence of tumour induction has been obtained in the cases of arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel and zinc. Arsenic is undoubtedly carcinogenic for man but it has not been shown to be so for other species. Some of the other metals, such as lead and zinc, have not yet been shown to be carcinogenic in man. Asbestos has recently been found to be the principal cause of a rare type of human tumour, the mesothelioma. Also, it is now clear that the association between lung cancer and asbestosis is both definite and strong.

A wide variety of pharmaceutical preparations have been shown to be carcinogenic in laboratory animals. A few have been shown to induce cancer in man. Where the use of such preparations is life-saving, it may be necessary to accept a risk that cancer will be induced in a proportion of patients. But where non-carcinogenic alternatives exist, or where the benefits to be expected from the use of a drug are small, the continued prescribing of carcinogenic agents cannot be justified. A full report of the preparations considered is due to appear shortly elsewhere[22].

Of special importance in relation to the carcinogenic hazard from drugs is the difficulty of extrapolation from animal experiments to man. Whereas potent carcinogens are frequently active in almost all species, weaker agents may not be so. Thus, a weak effect observed in one animal species may be unmatched by a similar response in another. It is important, therefore, to have information on tests in more than one species. An experiment on animals is more likely to be relevant to the human situation if the dosage used is realistic and the route and mode of administration the same, or comparable. One of the most contentious problems is knowing how to interpret the induction of cancers in animals by the parenteral administration of a substance normally given orally to man. In general, the need for greatest concern relates to drugs which, if they are taken at all, tend to be taken in large doses over long periods, and to drugs freely available without medical prescription. The real risk of cancer induction in man by such substances should be ascertained by epidemiological methods, though this may be impossible where a drug can be bought without prescription. Epidemiological surveys are expensive and often slow to yield results. If a new drug were introduced to-day, it may be more than 20 years before its carcinogenic action in man could be detected by epidemiological means, because of the long latent periods generally required for the induction of human cancer. In the meantime, the best use must be made of experimental findings to guide prescribing practice.

The importance of the subject was underlined by Dr. Roe, who suggested that as much as 75 per cent of human

cancer is attributable to exposure to carcinogenic agents in the environment and therefore potentially preventable. Potent carcinogens tend to reveal their activity sooner or later, even if no careful investigation is made. The real difficulty relates to weaker agents, none of which by themselves has a maked effect on cancer incidence, but which, together, may very materially increase the cancer burden.

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[1] Magee, P. N., and Barnes, J. M., *Brit. J. Cancer*, **10**, 114 (1956).
[2] Magee, P. N., and Barnes, J. M., *J. Path. Bact.*, **84**, 19 (1962).
[3] Schoental, R., *Nature*, **188**, 420 (1960).
[4] Druckrey, H., Preussmann, R., Schmähl, D., and Müller, M., *Naturwissenschaften*, **48**, 134 (1961).
[5] Magee, P. N., and Schoental, R., *Brit. Med. Bull.*, **20**, 102 (1964).
[6] Druckrey, H., Schmähl, D., and Schildbach, A., *Naturwissenschaften*, **48**, 56 (1961).
[7] Druckrey, H., and Schmähl, D., *Naturwissenschaften*, **49**, 217 (1962).
[8] Barnes, J. M., and Magee, P. N., *Brit. J. Indust. Med.*, **11**, 167 (1954).
[9] Terracini, B., and Magee, P. N., *Nature*, **202**, 502 (1964).
[10] Magee, P. N., *Biochem. J.*, **64**, 676 (1956).
[11] Dutton, A. H., and Heath, D. F., *Nature*, **178**, 644 (1956).
[12] Heath, D. F., *Biochem. J.*, **85**, 72 (1962).
[13] Magee, P. N., and Vandekar, M., *Biochem. J.*, **70**, 600 (1958).
[14] Brouwers, J. A. J., and Emmelot, P., *Exp. Cell. Res.*, **19**, 467 (1960).
[15] Magee, P. N., and Hultin, T., *Biochem. J.*, **83**, 106 (1962).
[16] Magee, P. N., and Farber, E., *Biochem. J.*, **83**, 114 (1962).
[17] Craddock, V. M., and Magee, P. N., *Biochem. J.*, **89**, 32 (1963).
[18] Case, R. A. M., Hosker, Marjorie E., McDonald, Drever B., and Pearson, Joan T., *Brit. J. Indust. Med.*, **11**, 75 (1954).
[19] Case, R. A. M., and Hosker, Marjorie E., *Brit. J. Prev. Soc. Med.*, **8**, 39 (1954).
[20] *Lancet*, i, 306, 328 (1965).
[21] Davies, Joan M., *Lancet*, ii, 143 (1965).
[22] Roe, F. J. C., *Clin. Pharmacol. Therap.* (in the press).

RECENT ADVANCES IN THE MICRO-IRRADIATION OF CELLS

RESEARCH workers who are interested in the effects of microbeams of radiation of a variety of biological materials met recently to discuss problems which are currently important in the development of this subject. The meeting was arranged by Dr. M. Bessis and was held in the Laboratoire de Pathologie Cellulaire, le Kremlin-Bicêtre, Paris.

Dr. C. L. Smith (Cambridge) was chairman for the first session, which was concerned with recent advances in the design and construction of microbeams and opened with some comments by H. Glubrecht (Hannover) on the development of an ultra-violet microbeam coupled with an ultra-violet microspectrophotometer of the Caspersson type. He emphasized that the dose of ultra-violet radiation which was used for microspectrophotometric measurements on living cells should be not more than one-thousandth of that which had been used in the microbeam to irradiate the cells. In answer to L. Trapp (Zeiss, Oberkochen) he said that ultra-violet absorption measurements had been made over areas varying from 0·7μ to 12μ and dose-rates had been obtained by taking into consideration the reflexion and transmission coefficients of all the relevant surfaces. G. Nomarski (Paris) indicated the importance of increasing the ultra-violet reflecting power of aluminized mirrors above the normal figure of 85 per cent, particularly in arrangements where there could be as many as ten to sixteen reflexions.

Nomarski then gave details of developments in laser techniques. It is now possible to produce a beam which is constant in intensity over an area approximately equal to that of a single cell, and dose reproducibility has been improved to about 10 per cent by surrounding the laser with a 0·5-mm thick water jacket. The available wavelengths may soon be extended by harmonic generation from two fundamental wave-lengths at 6940 Å and 10600 Å with the aid of a new crystal and, in the near future, repeatable pulses varying in length from 50 μsec to 6 msec at 0·1-sec intervals may be available. Glubrecht suggested that when this became possible dose-rate effects would be very interesting, and drew an analogy with pulse radiolysis.

There was then a discussion on the importance of localization of the radiation delivered by a microbeam system, and Nomarski indicated that, for ultra-violet light, precision of the order of 0·15μ was possible for a thin target. P. P. Dendy (Cambridge) and A. Forer (Copenhagen) both supported the importance of precise physical location but drew attention to biological difficulties which include the unavoidable irradiation of membranes and other cell parts in addition to the target, and the ease with which biochemical changes can be transferred from the site of the primary lesion.

M. Ernst (Hannover) described a recently constructed X-ray microbeam which uses 5-keV X-rays to irradiate an area approximately 4·5μ in diameter. The whole apparatus is contained in a constant-temperature box which can be sterilized. In this way, uncovered biological specimens can be used, and this, in turn, allows the use of lower-voltage X-rays which give a better localization of energy. The apparatus was compared with the earlier models of Seidel and Buchholtz[1] and Seed[2].

A discussion on the production of small holes followed and Nomarski indicated that 1μ-diameter holes in 10μ-thick tungsten could be produced by laser radiation. H. Mel (Paris) told the meeting about a relatively new procedure in which small areas of a surface can be weakened by the deposition of certain elements followed by neutron irradiation. Suitable chemical treatment leads to the production of very small regularly shaped holes at the weakened sites.

Smith gave a brief outline of a new electron microbeam which is being built in Cambridge. It is hoped ultimately to obtain a 3μ spot of 50-keV electrons, and incident illumination coupled with phase contrast will be used to view the biological material and control the site of irradiation.

The session ended with a description by Forer of a modified reflecting objective which permits the observation of a cell in polarized light under phase contrast conditions during ultra-violet microbeam irradiation. Nomarski pointed out the advantage of circularly polarized light for this type of work, particularly at high numerical apertures.

In the second session, the results of some recent experiments were presented. Prof. H. Glubrecht (Hannover) took the chair and suggested that the speakers should try to show how their experiments had extended ancillary techniques and quantitative methods for assaying the biological effect, both of which are strictly limited in microbeam work. Bessis described some work in which mitochondria in *KB* cells had been stained with various vital stains. At staining concentrations which were twenty times lower than the limit of visibility, mitochondria became highly sensitive to laser microbeam irradiations. An electron microscope study of the irradiated cell showed that mitochondria which had been hit were extensively damaged. Certain mitochondria which were not in the irradiated region showed structural changes 2–3 h after irradiation but they had recovered by 24 h.

A discussion followed on valid criteria for deciding if a cell is alive, and Bessis stated that, in their work, the ability to take up nucleic acid precursors and the activity of enzymes had been used as criteria. This point was taken up by the next speaker when Dendy discussed some experiments which had been performed at Cambridge. He referred briefly to earlier micro-irradiation