Meeting Report

MODERN ASPECTS OF CHEMICAL CARCINOGENESIS
A report of the FEBS Advanced Course No. 20 held at Middlesex Hospital Medical School, London, W1P 5PR, U.K. on 19–21 April 1972

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1. Introduction

P.N. Magee (London, U.K.). It is now established that many types of cancer in man are caused by environmental factors and that these factors include carcinogenic chemicals. Since the pioneering work of Pott, Yamagiwa and Ichikawa, and Kennaway, a large number of pure chemicals has been shown to cause cancer in experimental animals, often at very low dose levels. Certain chemical carcinogens are known which are active after only one dose, the tumours becoming clinically apparent months later. An obvious feature of any list of chemical carcinogens is their widely differing chemical structures and this has led to the realization that many chemicals are not carcinogenic themselves but only become so after metabolic conversion in the body to more biologically active forms. In the terminology of J.A. and E.C. Miller the parent compound is a precarcinogen, the metabolites with greater carcinogenic potency are proximate carcinogens and the final active molecular species is the ultimate carcinogen. There are, of course, some chemicals which do not require metabolic activation for carcinogenic action. It is widely assumed that the reactive ultimate carcinogen, which is usually an electrophile, reacts with nucleophilic centres in the cell and thus induces the change to malignancy. It is further assumed that the crucial cellular interaction for carcinogenesis is between the ultimate carcinogen and a nucleic acid or a protein or both. There is no general agreement which of these macromolecules is the crucial cellular target. Reaction with DNA could produce mutant somatic cells, some of which might have the required properties for malignant growth and which could escape the postulated immunological surveillance mechanisms which are concerned with the elimination of cancer cells from the body. If DNA is the primary target for carcinogenesis, mechanisms by which the initial damage could be repaired might be of great importance in determining whether transformation occurred or not. Transfer RNA has also been suggested as the primary target in carcinogenesis and aberrant tRNA methylation implicated in its modification. Proteins were the first targets of chemical carcinogens to be reported and certain cytoplasmic proteins of the 'h' and similar types are under continued study in several laboratories.

There is great current interest in viral carcinogenesis and possible inter-relationships with chemical carcinogenesis are being explored. There is evidence that carcinogenic chemicals, like X-rays, can activate oncogenic leukaemogenic viruses but it is not established whether such a process occurs in the induction of all types of tumour. It has been postulated that the cells of most or all vertebrate species contain genomes of RNA tumour viruses that are vertically transmitted from parent to offspring. This hypothesis suggests that chemical carcinogens may act directly on oncogenic information contained in the viral genome, the so-called oncogene.

Aspects of these ideas on the mechanism of chemical carcinogenesis were discussed at the Course and are briefly reported below.

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2. Metabolism of chemical carcinogens and interaction with cellular macromolecules

2.1. *First Session.* (Chairman: *P.N. Magee*, London, U.K.)

The first part of this session was concerned with carcinogenesis by nitroso compounds and was opened by *R. Preussmann* (Heidelberg, W. Germany) who discussed structure–activity relationships of these and related carcinogens including hydrazo-, azo- and azoxy-alkanes and 1-aryl-3-mono- and dialkyl triazenes, emphasizing their remarkable organ specificity in experimental animals. Malignant tumours have been induced in liver, lungs, kidney, urinary bladder, oesophagus, stomach, intestine, nasal cavity, brain, spinal cord and peripheral nerves of rats, the target organ depending on the compound used. In all the groups of compounds small changes in the chemical structure often lead to striking differences in the organs in which tumours are induced. Thus the organ specificity is largely governed by the chemical structure of the carcinogen and to some extent also by the animal species, the route of administration and the doses used. Many of these carcinogens are active after single doses. Nitrosamines, hydrazo-, azo- and azoxy-alkanes, as well as 1-aryl-3,3-dialkyltriazenes require metabolic activation to form the proximate and ultimate carcinogenic intermediates while nitrosamides, such as *N*-nitroso-*N*-methylurea and 1-aryl-3-mono-alkyltriazenes are activated by hydrolytic processes without involvement of enzymes. Alkylation *in vivo* of nucleic acids and proteins occurs after administration of these carcinogens and the probable ultimate carcinogen is an alkylating agent. Some of these compounds, especially those with ethyl groups, have powerful transplacental carcinogenic effects, leading to a high incidence (up to 100%) of tumours in the offspring of treated mother rats. Such tumours usually appear at an early age and are predominantly located in the nervous system.

P.F. Swann (London, U.K.) discussed the usefulness of *N*-nitroso compounds in studies designed to elucidate the mechanism of the induction of cancer, underlining their chemical simplicity and high carcinogenic activity. He described experiments on the alkylation of nucleic acids *in vivo* by the nitroso compounds which supported the view derived from structure–activity relationships (see above) that these carcinogens owe their carcinogenic activity to the production of alkylating intermediates during their breakdown in the animal. The critical cellular target for this alkylation is not known and the problems of identifying it were discussed. Some criteria for an experimental approach to these problems were proposed based on observed differences in carcinogenic behaviour of different alkylating agents. The induction of kidney tumours in the rat by single doses of several alkylating carcinogens was suggested as a model for study since dimethylnitrosamine and *N*-nitrosomethylurea were both active under these conditions but methyl methanesulphonate was not. Since the latter compound gave rise to a similar degree of methylation of kidney nucleic acids *in vivo* the difference in carcinogenic activity provided a potential means for distinguishing between biochemical effects which were essential or inessential for the induction of the tumours. In contrast to the behaviour of these methylating compounds, the corresponding ethyl derivatives, diethylnitrosamine, *N*-ethylnitrosourea and ethyl methanesulphonate all induced kidney tumours in the rat.

C. Heidelberger (Madison, Wisc., USA) then changed the topic to polycyclic hydrocarbons and carcinogenesis *in vitro* using cell cultures of fibroblastic lines derived from adult C3H mouse prostate. After describing criteria for transformation *in vitro* he showed that this phenomenon was unrelated to toxicity. Single individual cells could be transformed with high efficiency and different colonies of transformed cells did not show common surface antigens. In this system there was strong evidence that the polycyclic hydrocarbons required metabolic activation to produce transformation. Experiments had shown that K-region epoxides were formed which were more active than the parent polycyclic hydrocarbons in producing malignant transformation and were probably proximate carcinogenic derivatives of the hydrocarbons. The epoxides reacted with DNA, RNA and protein of the cells to a greater extent than the parent hydrocarbons or other derivatives including phenols and *cis* and *trans* dihydrodiols. Emphasis was placed on binding of the epoxides to 'h' proteins which was thought to play some role in chemical oncogenesis.

2.2. *Second Session.* (Chairman: *E. Hecker*, Heidelberg, W. Germany)

P. Brookes (London, U.K.) reviewed earlier work on the binding of carcinogenic polycyclic hydrocarbons to macromolecules of mouse skin emphasising the necessity of metabolic activation and the significance of DNA binding. Experiments with rodent cells in tissue culture had advantages for quantitation of data on extents of binding. Both carcinogenic and non-carcinogenic compounds were metabolised but covalent binding to nucleic acids was only significant with the carcinogens. Human embryo lung cells were able to metabolise polycyclic hydrocarbons to products which became bound to DNA.

E. Kriek (The Netherlands) outlined previous work on the metabolism of the carcinogenic aromatic amine, 2-acetylaminofluorene, discussing the role of the endoplasmic reticulum of the liver and the formation of derivatives which react with cellular macromolecules. The first metabolic reaction is *N*-hydroxylation of the parent compound followed by formation of the *N*-sulphate ester. This ester is a highly reactive electrophile that attacks nucleophilic groups in the cell, the main site of action in the nucleic acids being the C-8 position of guanosine. The major part of the carcinogen bound to tRNA and rRNA in rat liver is acetylaminofluorene but about 70% of that bound to DNA is deacetylated. Another derivative of acetylaminofluorene has been isolated from hydrolysates of DNA containing bound carcinogen but it has not yet been characterized. This minor reaction product was still detected in rat liver DNA two months after injection of *N*-hydroxy-acetylaminofluorene but the carcinogen bound on the C-8 position of guanosine was removed more rapidly with a biological half-life of approximately 7 days.

B. Ketterer (London, U.K.) returned to the binding of chemical carcinogens to cellular proteins with reference to the azo dye, *p*-dimethylaminoazobenzene. After injection into animals this compound is bound to a soluble protein in the liver cell cytoplasm which is identical with the protein known as ligandin which also binds steroids, bilirubin and various exogenous anions. Most of these compounds are not bound covalently but the binding with the azo dye is covalent. Other compounds which bind to ligandin include haematin and stilboestrol. Ligandin is a major protein component of liver cell sap and can be induced with agents such as phenobarbitone and DDT. It also occurs in kidney intestinal mucosa, urinary bladder, lung and skin. The function of this protein is not yet established. It may not play a direct role in carcinogenesis but may have a transport function or play a part in cellular control mechanisms.

2.3. *Third Session.* (Chairman: *D.B. Ludlum*)

P.D. Lawley (London, U.K.) developed the discussion of the relation of alkylation to carcinogenesis further, suggesting that the initiation stage of carcinogenesis may be a somatic mutation and that relationships between alkylation mutagenesis and reactivity of alkylating agents may therefore apply to carcinogenesis. Several sites in nucleic acids are now known to be reactive towards alkylating agents, including N-1, N-3 and N-7 of adenine; N-3, N-7 and O-6 of guanine and N-3 of cytosine. There are differences in the relative reactivities of these sites for different alkylating agents which can be correlated with their classification, following Ingold, into S_N1 and S_N2 types. However, the simple prediction that S_N1 agents should react to a greater extent at the sites of lower nucleophilicity than should the S_N2 agents is not confirmed experimentally. Recent work by Loveless and by Ehrenberg and their colleagues has suggested that S_N1 character is positively correlated with mutagenic activity. It seems possible, therefore, that a further correlation between the spectrum of reaction products in DNA and induced mutation will be discernible. The suggestion that alkylation on the O-6 position of guanine may be important in mutagenesis is attractive since it gives rise to a mispairing base and also fits the correlation between S_N1 reaction and mutation. Ludlum has reported that 3-methylcytosine in polynucleotide templates can cause miscoding *in vitro* and has proposed this as a promutagenic group. The differing capacities of nitroso compounds and methyl methanesulphonate to induce kidney tumours in the rat reported by Swann and Magee (see above) also supports a relationship between carcinogenesis and S_N1 reactivity. It seems likely, however, that a gradation of both the chemical and biological properties of these agents will be found rather than a clear-cut distinction between two types.

V. Wunderlich (Berlin-Buch, G.D.R.) discussed the possible role of mitochondrial DNA in carcinogenesis. After reviewing some earlier findings suggesting in-

volvement of mitochondria in carcinogenesis such as respiratory impairment, loss of swelling–contraction functions, etc., he concluded that these were probably secondary events and suggested that changes in mitochondrial DNA might be more relevant. So far, however, physicochemical studies of DNA from tumours have not revealed any specific changes. Studies were reported on the reaction of liver mitochondrial DNA with *N*-nitrosomethylurea or dimethylnitrosamine after these carcinogens were administered to rats and hamsters. Mitochondrial DNA was more highly methylated than nuclear DNA, mitochondrial RNA and mitochondrial protein. These results were considered to support the hypothesis that mutations at the mitochondrial level may be particularly involved in carcinogenesis, as first proposed by Graffi. Since several nitroso compounds are known to mutate mitochondria in micro-organisms and plants, such organisms might provide useful models for study of interactions between chemical carcinogens and mitochondria. Mitochondrial DNA has advantages over nuclear DNA for the study of the effects of chemical carcinogens on functional activities of DNA such as DNA synthesis, repair synthesis and transcription. Carcinogen induced changes in tertiary structure are readily measureable as changes in superhelix density.

B.R. Rabin (London, U.K.) introduced a new topic by describing work exploring the mechanism of the inhibition of protein synthesis in rat liver by the powerful carcinogen aflatoxin. In the experimental system used the binding of ribosomes to the endoplasmic reticulum was monitored by measuring the apparent activity of a membrane bound disulphide interchange enzyme, normally masked by bound ribosomes. Oestradiol was shown to be necessary for the binding of ribosomes to the endoplasmic reticulum of male rats and testosterone for binding to female endoplasmic reticulum. Aflatoxin, which appeared to act without metabolic activation, displaced ribosomes from the reticulum in an irreversible manner. Prior treatment with oestradiol protected the male endoplasmic reticulum and testosterone protected the female reticulum from degranulation by aflatoxin. Similar results were obtained with other carcinogens suggesting that interaction with steroid binding sites in the endoplasmic reticulum may be a common feature of such compounds. The possibility that these reactions might be used as a test for chemical carcinogens was discussed.

E. Hecker (Heidelberg, W. Germany) gave a concise survey of present ideas on initiation and promotion mechanisms in chemical carcinogenesis describing in detail the procedures used in his laboratory. A single initiating dose of 7,12-dimethylbenz[a]anthracene (DMBA), 10 μmole, is administered to mice by stomach tube followed by twice weekly topical applications of an active tumour promoting agent such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), 0.02 μmole, to the skin of the back. This treatment yields 4 to 5 skin tumours per mouse within three months. In order to distinguish between specific carcinogenesis and non-specific biochemical changes, other groups of mice are given chemicals of the same type which lack initiating activity such as dibenz[a,c]-anthracene or promoting activity such as 4α-phorbol-12,13-didecanoate (4α-PDD). Experiments on the fate of radioactively labelled DMBA and TPA were described including investigation of their binding to nucleic acids and proteins of mouse skin. Since the active promoter was bound to skin proteins to the same extent as the inactive compound, it was concluded that covalent protein binding had nothing to do with promotion. Incorporation of labelled choline into lecithin of mouse skin was markedly stimulated by the active promoter but the inactive compound had no such effect.

3. The possible role of nucleic acid methylases in carcinogenesis

(Chairman: *E. Borek*, Denver, Colo., USA). The Chairman reviewed the current status of knowledge about the enzymes that modify tRNA with particular emphasis on the tRNA methyl transferases. He pointed out that these enzymes present in crude tissue extracts from tumours were aberrantly hyperactive when compared to enzymes from normal tissues and that several workers had reported an increased content of methylated bases to be present in tRNA of tumour tissues. Dr. Borek described the regulatory functions attributed to tRNA and stressed that even minor changes in tRNA structure could affect such regulatory functions. He, therefore, suggested that alteration of tRNA could modify animal cells in a manner at present hidden in the multiplicity of functions of tRNA but leading to tumour growth.

Dr. Borek stressed that the induction of a virus integrated within the genome of the cell might be the mechanism of chemical carcinogenesis and thought that the high frequency of 'carcinogenesis' of cells in tissue culture was more consistent with this idea than mechanisms involving mutation. He proposed that the only permanently and constantly altered macromolecule in tumours is tRNA and noted that tRNA's were altered in bacteria after lysogenic induction. Therefore, chemical carcinogenesis might be attributed to activation of a virus leading to an altered tRNA possibly by the action of virus-specified tRNA methylases.

Valda M. Craddock (Carshalton, U.K.) supported Dr. Borek's suggestion by showing that during feeding of dimethylnitrosamine there was increased methylation of liver tRNA *in vivo* and enhanced activity of tRNA methylases present in liver homogenates assayed *in vitro*. The increased methylation of tRNA was not due merely to increased nucleic acid synthesis as the amounts of the N^2-substituted guanines produced were increased to a greater extent than the other methylated bases. In contrast, the methylation of DNA corresponded to that of controls when the rate of DNA synthesis was taken into account. Increased methylation of tRNA was not seen in regenerating liver or in liver from animals treated with nitrosamines which became cirrhotic rather than malignant. Dr. Craddock thought it unlikely that there was any direct connection between alkylation of tRNA caused by reaction with alkylating carcinogens and the altered activity of tRNA methylases (both Dr. Craddock and Dr. Pegg emphasized the difference in the methylated bases produced by the different pathways). Dr. Craddock postulated that reaction of the carcinogen with the chromatin, possibly by producing a mutation-like change, was responsible for the carcinogenesis and that the altered tRNA methylase activity is one of the ways in which the alteration in the genome expresses itself.

A.E. Pegg (London, U.K.) described studies on the specificity of tRNA methyltransferases from mouse colon and colonic tumours induced by administration of dimethylnitrosamine. Purified tRNA's from yeast and bacteria were utilised as substrates for methylation and the nucleotide sequences methylated identified by standard methods for sequencing RNA. Although the tumour cell extracts catalysed methylation of these tRNA's to a greater extent and at a faster rate than extracts from normal cells, there were no differences in the sites methylated within the tRNA's tested as substrates. Dr. Pegg concluded that an increased extent of methylation of tRNA *in vitro* by crude tissue extracts from tumours did not necessarily represent a change in specificity of the tRNA methyltransferases and therefore that further analysis of tRNA sequences was required before the conclusion could be drawn that tRNA methylases from tumour could methylate tRNA molecules at sites not methylated by enzymes from control tissues.

Dr. Pegg also described some studies on the ethylation of nucleic acids following the administration to rats of a single dose of ethionine. Analysis of the ethylated bases in tRNA and direct experiments *in vitro* with liver tRNA methyltransferases were consistent with the view that ethylation occurred by the action of these enzymes utilising *S*-adenosyl-L-ethionine as an alkyl donor. However, some evidence that a minor proportion of the ethylation of tRNA occurred by another pathway was obtained and a small amount of ethylation of DNA producing 7-ethylguanine was also noted. Dr. Borek thought that this small amount of ethylation of DNA was insignificant in carcinogenesis but Dr. Pegg and Dr. Swann pointed out that ethionine is not a potent carcinogen and must be fed at high doses for a considerable time before producing tumours and that since the degree of ethylation of DNA under conditions required to produce hepatomas was unknown the question of the significance of ethylation of DNA during carcinogenesis by ethionine was difficult to evaluate.

4. Nucleic acid repair and its possible role in chemical carcinogenesis

(Chairman: *M.M. Elkind*, Brookhaven, Conn., USA).

B.S. Strauss (Chicago, Ill., USA) opened this session, pointing out the good correlation between the ability of compounds to induce cancer and their ability to induce mutation. Since mutagens must necessarily change nuclear DNA to produce their effect, it is often assumed that carcinogens must also interact with DNA and that the mechanisms of carcinogenesis and mutagenesis are similar. The discovery of cellular systems which can repair damage produced by mutagens suggests that there may be some connection

between the ability to repair damage produced by a carcinogen and its carcinogenic activity. Repair of damage induced in bacteria by ultraviolet light (U.V.) was described in some detail, emphasising the importance of analysis of UV-resistant mutants leading to the recognition of several different repair processes. The process of excision repair involves a series of steps including incision, excision, repair synthesis and joining. A second repair mechanism occurs after DNA synthesis (post replication repair) but its detailed mechanism is not understood. Other repair systems participating in recombination have been described. The most important evidence that repair, or more precisely lack of repair, is involved in carcinogenesis has been the observation that Xeroderma pigmentosum patients, who lack the incision enzyme, are very likely to develop tumours in parts of the body exposed to light. If there is a connection between carcinogenesis and repair it is most probably a negative one. Carcinogenesis might arise from the failure of the repair mechanisms to recognize and to remove a potentially carcinogenic lesion in DNA.

B.W. Fox (Manchester, U.K.) briefly reviewed published work on the damage induced in mammalian cells by X-rays, U.V. light, alkylating agents and some chemical mutagens and carcinogens in an attempt to discover any characteristics of the timing of the repair processes following damage by these agents that could be related to their carcinogenic activity. Repair synthesis of DNA in P388F lymphoma cells was compared after exposure to methyl methanesulphonate (MMS) and to *N*-methyl-*N*-nitrosourea (NMUrea). The time required for rejoining of single strand breaks was longer after MMS treatment than after X-ray or UV damage and repair after NMUrea treatment was even more protracted. It was suggested that this protracted repair synthesis following treatment with carcinogenic chemicals, with consequent attempted replication over damaged strands, might be a significant factor contributing to their carcinogenic effects. An efficient repair system might thus decrease the risk of carcinogenesis.

J.J. Roberts (London, U.K.) continued the discussion of DNA repair in carcinogenesis, mentioning repair deficient cells, as in Xeroderma; the possibility that co-carcinogens might act as inhibitors of repair; the possible consequences of faulty repair and the possibility that the repair process might release an integrated viral genome. Manifestations of excision repair of alkylated DNA can be demonstrated in mammalian cells in culture following treatment with alkylating agents. The amount of non-semiconservative synthesis is related to the extent of initial alkylation of cellular DNA and this "repair synthesis", for which both purine and pyrimidine precursors can be used, continues for many hours after the initial alkylation. No difference was found in excision repair of DNA in Yoshida sarcoma cells which were sensitive or insensitive to cytotoxicity by alkylating agents, suggesting that a deficiency of this type of repair cannot be the basis of the difference in sensitivity. Similar results were obtained with HeLa cells which were sensitive or insensitive to *N*-methyl-*N*-nitrosourea. Evidence was presented that another form of repair, possibly related to post-replication repair, was responsible for these differences in sensitivity of cells to alkylating agents. This repair could be inhibited by caffeine.

D. Bootsma (Rotterdam, The Netherlands) reviewed the defective DNA repair in Xeroderma pigmentosum cells in greater detail. Reduced repair DNA synthesis has been reported after treatment of xeroderma cells with 4-nitroquinoline 1-oxide, 4-hydroxyaminoquinoline 1-oxide and *N*-acetoxy-2-acetylaminofluorene. In contrast, repair DNA synthesis following X-irradiation, methyl methanesulphonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine occurred at the normal level in xeroderma cells. The clinically distinct forms of Xeroderma pigmentosum (the classic form with skin lesions and the De Sanctis Cacchione form with neurological abnormalities in addition) might indicate the presence of different mutations all resulting in U.V. sensitivity of the skin. The genetic heterogenicity of Xeroderma pigmentosum was studied by fusion of cells from different patients and measuring repair DNA synthesis in binucleate cells. Repair synthesis was found in cells derived from fusion between the De Sanctis Cacchione cell strains and the strains from classical forms of xeroderma but it was absent from the parental cells and from fusions between cells from different classic forms or different De Sanctis Cacchione patients indicating that two different genes are involved. It is possible that incomplete repair might result in the presence of DNA lesions

at the time of DNA replication previous to cell division which might initiate mutagenesis and carcinogenesis.

5. Possible inter-relations between chemical and viral carcinogenesis

(Chairman: *A.C. Allison*, London, U.K.). *L. Sachs* (Rehovot, Israel) discussed his experiments on the chromosomal control of malignancy and the use of carbohydrate-binding lectins such as concanavalin A to elucidate the structure and activity of the cell surface membrane. His chromosome analysis of normal cells, cells transformed by viral or chemical carcinogens, and transformed cells that had been induced to revert to a normal phenotype, has led to the conclusion that viral and chemical carcinogens induce malignancy by inducing chromosome rearrangements. Evidence was presented to show that these rearrangements produce a change in balance between chromosomally located factors that determine expression and suppression of malignancy. His experiments with lectins have shown that changes in the structure and activity of the surface membrane that are required for a gain of agglutinability by concanavalin A are associated with cell malignancy *in vivo*. These changes are not shown by the lectins from soybean and wheatgerm, and the same changes are found in cells transformed by chemical or viral carcinogens.

R. Weiss (Los Angeles, Calif., USA) discussed the induction of RNA tumour viruses in normal cells by chemical carcinogens and mutagens. Avain tumour viruses were induced in normal embryonic chicken cells which were treated in culture with ionizing radiations or chemical carcinogens and mutagens. The induced viruses possess a buoyant density, DNA polymerase, polypeptides, and 70 S RNA typical of RNA tumour viruses. They act as "helper viruses" in complementing a strain of Rous sarcoma virus defective for replication and they undergo phenotypic mixing and genetic recombination with other avian tumour viruses. The induced viruses belong to subgroup E as judged by host range, envelope antigens and interference patterns. These findings show that the induced viruses belong to the group known as avian RNA tumour viruses. However, they do not transform fibroblasts and their oncogenic potential *in vivo* remains to be demonstrated.

Nucleic acid hybridization studies suggest that the viral genome exists in the normal cell in DNA form. Endogenous viral gene expression is under control of an autosomal locus with a dominant allele, gs^+, for partial expression. When the gs^+ allele is present, chick cells synthesize group-specific antigens of avian tumour viruses, complement certain defective mutants of Rous sarcoma virus and frequently become resistant to exogenous infection with viruses of the same subgroup specificity.

In chicken cells lacking the gs^+ allele, viral functions are activated in more than 50% cells by chemical carcinogens or mutagens, but less than 10^{-4} cells are induced to release infectious virus particles. 4-Nitroquinoline-1-oxide and 5-iododeoxyuridine appear to be the most effective inducing agents, though positive results were obtained with a variety of other agents. Recent studies on established lines of mouse cells show that they similarly possess endogenous viral genomes which can be activated by chemical mutagens. It is reasonable to speculate that all higher vertebrates possess endogenous viral information as suggested by Huebner and Todaro in their oncogene hypothesis. The relationship between chemical induction of RNA "tumour" viruses and chemical carcinogenesis is far from clear, but it would be unwise to ignore the presence of endogenous viral information in discussing mechanisms of carcinogenesis.

6. Conclusion

The meeting concluded with a wide-ranging discussion, covering all the topics discussed, under the chairmanship of *A.C. Allison* (London, U.K.).

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