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Chemical carcinogens and their mechanism of action

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CHEMICAL CARCINOGENS AND THEIR MECHANISM OF ACTION

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

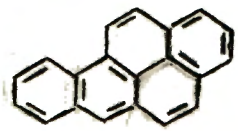
INTRODUCTION

It has been known for many years that certain chemicals are carcinogenic. These chemicals may be separated into groups of similar structure. Although common mechanisms of action have been postulated for the carcinogenic compounds within a particular group, the structural variation between groups is great enough that a common mechanism applicable to all of the groups would seen unlikely. Accordingly, a number of different theories have been suggested by the investigators in this field. However, striking similarities in both the known and postulated actions of these chemicals can be found.

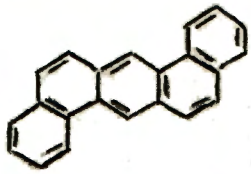
The purpose of this paper is to present the better known groups of chemical carcinogens, to relate some of the experimental work done with certain of these chemicals concerning their fate in the body or their effect on biological processes, and to discuss the various theories of malignant transformation of the cell

CARCINOGENIC HYDROCARBONS

In this group of chemical carcinogens much experimental work has been done with benzpyrene and dibenzanthracene.



3,4-benzpyrene



1,2,5,6-dibenzanthracene

Miller (1951) found that crude epidermal protein preparations from the skin of mice treated with 3,4-benzpyrene contained fluorescent substances which appeared to be derived from the carcinogen. These protein-bound derivatives were not found in the dermal portion of the skin or in untreated areas of skin. Calcutt and Payne (1953), in their experiment on the intracellular distribution of 3,4-benzpyrene in mouse liver, separated cellular components by centrifugation techniques. They found that this carcinogenic hydrocarbon appeared in considerable quantity in the nuclear fraction up to twenty-one weeks while the mitochondria contained the carcinogen only up to five days. No hydrocarbon was detected in the supernatant fraction. Extraction with 70% alcohol subsequently removed the benzpyrene from the mitochondria with one washing, but the nuclei required three or four separate washings for complete removal.

Weist and Heidelberger (1953) demonstrated that, following topical application of 1,2,5,6-dibenzanthracene-9,10-C^{14} to mouse skin, an irreversible chemical binding takes place between the carcinogen and nucleoproteins, but that this binding does not occur with nucleic acids. Weist and Heidelberger (1953) also demonstrated that, following injection of 1,2,5,6-dibenzanthracene into the submaxillary glands of mice, the amount of carcinogen bound to the nuclear fraction is small compared to the cytoplasmic and mitochondrial fractions. Haddow (1958) believes that such evi-

dence as the above of the interaction of carcinogens with cellular proteins points to a mechanism whereby key proteins, such as nucleoproteins or enzymes, can be deleted from the cell.

Fiala and Fiala (1959) also conclude that there is inactivation of certain cellular proteins following their binding with carcinogenic hydrocarbons. In their experiment with mouse epidermis treated with 3,4-benzpyrene, they reported, in contrast to Weist and Heidelberger, that the carcinogen-bound proteins are distinct from nucleoproteins. They also found no protein-bound carcinogen in the mitochondria and, therefore, felt that 3,4-benzpyrene does not interfere with the enzymes of cellular respiration. However, the carcinogen was found to inhibit formation of the enzymes tryptophan peroxidase and glucose-6-phosphatase. For a number of years the view has been widely held that carcinogenic hydrocarbons interact with the sulfhydryl groups of enzymes (Rhondoni, 1955). Mills and Wood (1953) believe that such an action is responsible for the urease inhibiting property of benzpyrene.

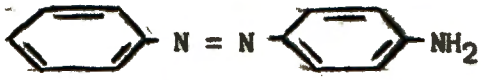
The interresting effect of growth inhibition on a young rat fed 1,2,5,6-dibenzanthracene was demonstrated by Elson (1958). During the time that the animal received this carcinogen, its growth was completely inhibited. Nitrogen balance studies showed that, although the total intake of food nitrogen was reduced by 20%, the excreted nitrogen, mainly in the form of urea, remained as great as before treatment with the carcinogen. Elson postu-

lated that the effect of the carcinogen was to prevent the utilization of amino acids for protein synthesis and growth, while their utilization for energy metabolism was unaffected. Thus, in producing an adult type of metabolism, he considered the carcinogen to have an "ageing" effect on the animal.

In the latter case it is tempting to contemplate the possibility that a loss of enzyme function via binding with the carcinogen was the factor preventing protein synthesis.

AZO CARCINOGENS

There is a great deal of evidence that cellular proteins are the site of attack in carcinogenesis by the aminoazo dyes as well as by the carcinogenic hydrocarbons (Miller and Miller, 1954).



p-aminoazo benzene

3'-methyl-4-dimethylaminoazobenzene

It has been shown that when C^{14}- labeled carcinogenic azo dyes are administered to rats, a high isotope content is observed in the proteins isolated from the microsomal fraction of liver cells (Hultin, 1957). Gelboin, Miller, and Miller (1959) demonstrated <u>in vitro</u> that rat liver slices, homogenates, and either isolated

mitochondria or microsomes formed a protein bound dye when incubated with either 3'-methyl-4-monomethylaminoazobenzene or 3'-methyl-4-aminoazo benzene. They also demonstrated that 3-6 times as much bound dye was formed if the animals were previously injected intraperitonially with 3,4-benzpyrene or 3-methylcholanthrene. Whitcutt, Sutton, and Nunn (1960) fractionated rat liver proteins by chromatography and electrophoresis after feeding the carcinogen 3'-methyl-4-dimethylaminoazobenzene. The fraction of "slow moving proteins" was then resolved into eight components. Only one of these components contained covalently bound azo dye and the properties of another component were found to have changed. In view of the above data it is easy to see why the suggestion has been made that interference with cellular preteins is a basic step in carcinogenesis by the azo dyes (Miller and Miller, 1952; Haddow, 1958).

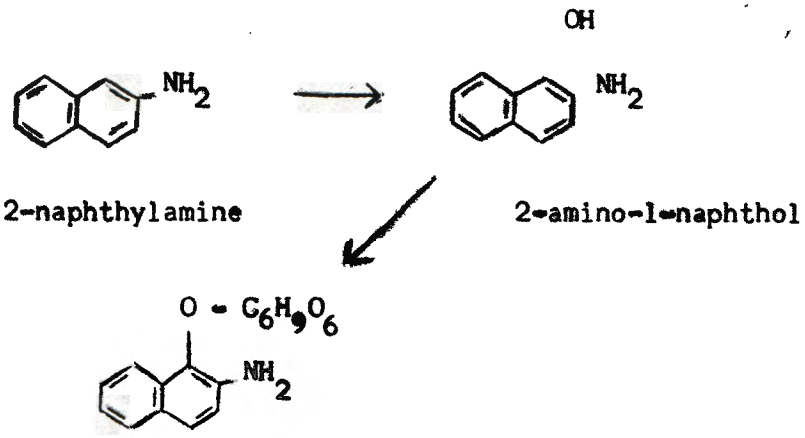
Fiala and Fiala (1959) give evidence that 3'methyl-4-dimethylamineazobenzene, like 3,4-benzpyrene, inhibits tryptophan peroxidase and glucase-6-phosphatase.

Orr (1958) has observed that cancer frequently developes after a long period of time following a single application of an aminoazo dye to epidermis, while the epithelial cells seem histologically and histochemically normal in the meantime. Therefore, Orr suggests that the primary effect of the carcinogen is to produce a "field" change affecting all the elements in the treated area, including the supporting and nutrient stroma, and that the

neoplastic change in the epithelial cells is the result of the altered metabolic conditions to which they have become exposed.

AROMATIC AMINES

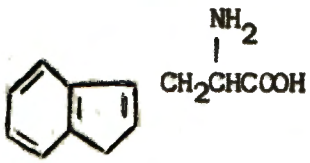
The aromatic amines are a group of compounds with diverse chemical structure. They have been found to produce tumors in many areas of the body, the site apparantly depending on the structure of the compound. There is much evidence implicating some of their metabolities as the agents for their carcinogenic activity. Many investigators believe that their conversion to o-aminophenols is the basis for carcinogenesis (Badger, 1956). On this basis Boyland (1958) believes that the derivative 2-amino-1-naphthol is the cause of bladder cancer by the aromatic amines. He suggests the following mechanism to explain the fact that 2-naphthyl-amine induces cancer of the bladder but not of other organs.



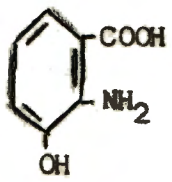
2-amino-1-naphthyl glucosiduronic acid

2-naphthylamine is converted to 2-amino-1-naphthol in the liver, and the latter is so rapidly conjugated with sulfuric or glucuronic acid that its concentration in the free form is minimal. The 2-amino-1-naphthyl glucosiduronic acid is then excreted in the urine and not reabsorbed, so that its concentration in the urine is higher than in other body fluids. In the urine, soluble, excreted β-glucuronidase at a pH of 5-6, which is nearer to the optimum pH (4.5) of the enzyme than are the neutral body fluids, liberates some 2-amino-1-naphthol which penetrates the bladder mucosa.

Boyland (1958) also points out that among the O-aminophenols known to induce bladder cancer are three compounds which are metabolites of tryptophan and have been found in human urine. 3-Hydroxyanthranilic acid has been especially implicated and has been found in higher than normal concentration in the urine of patients with bladder cancer.



Tryptophan



3-Hydroxyanthranilic acid

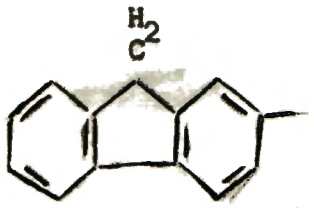
He, therefore, suggests that abnormal tryptophan metabolism may be responsible for bladder tumors in persons who have had no industrial exposure to aromatic amines.

Elson (1958) believes that preferential excretion of the aromatic amines as glucuronides is an important factor in carcinogenesis, the active carcinogen being then liberated by the glucuronidase present in the body cells. He reports that a sharp change in preferential conjugation of some amines from ethereal sulfate to glucuronide occurs on passing from the monocyclic aromatic amines to the polycylic amines. Although none of the amines excreted preferentially as sulfates have been found to be carcinogenic, all of the amines conjugated as glucuronides have produced cancers in rats (Walpole, Williams, and Roberts, 1952; Spitz, Maguigan, and Dobrines, 1950).

Boyland (1958), on the other hand, reports that carcinogenic aminophenols conjugate with both sulfuric and glucuronic acids, and he presents evidence that sulfatase and especially β-glucuronidase are found in higher concentrations in some patients with bladder carcinoma.

Burke and Miller (1960), in their experiment with rats fed the hepatic carcinogen 2-acetylaminofluorene, demonstrated that isolated perfused livers of these rats have a decreased ability to synthesize urea and to produce carbon dioxide from added histidine-2-C^{14}. They also demonstrated that protein synthesis in these livers, as measured by histidine-2-C^{14} incorporation into liver and plasma proteins, was greater than normal. From these results they postulated that there is a loss of enzyme activity involved in the conversion of amino acids to urea and carbon dioxide and a

corresponding increased availability of amino acids for protein synthesis. They suggested that possible enzymes lost are liver catalase, choline oxidase, glutaminase, and arginase. Elson and Hoch - Ligete (1954) confirmed that an intermediate oxidation product of the aromatic amines, probably of a quinonoid structure, is responsible for their enzyme inhibiting properties. They showed that this oxidation of the tissues is brought about by the cyto-



2-Acetylaminofluorene

Quinone

chrome oxidase component of the succinoxidase system.

The fluorene derivatives of the aromatic amines are considered to be more strongly carcinogenic than the diphenyl derivatives.

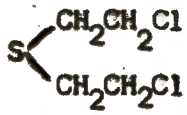
4-Acetylaminodiphenyl

Roe (1955) believes that the greater carcinogenic power of the fluorene derivatives is associated with the - CH_2 - bridge which helps to maintain a coplanar arrangement of the benzene nuclei. Spectrographic evidence (Badger, 1956) confirms that planarity is

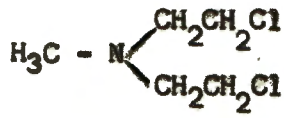
greater in 2-acetylaminofluorene than in 4-acetylaminodiphenyl.

ALKYLATING AGENTS

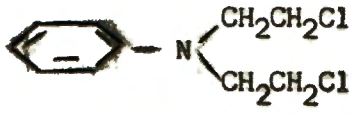
The mechanism of action with this group of carcinogens is perhaps more clearly understood than with any other group. They are frequently referred to as radiomimetic chemicals because they produce the same effects as ionizing radiation. The carcinogenic agents of this group in clude the mustards, ethylineimines, epoxides, and methane sulfonates (Haddow, 1958; Badger, 1956). Alexander (1960) published an excellant article on the action of the alkylating agents, emphasizing their similarity to high energy radiation in that they produce chromosome damage and inhibit cellular mitosis. He states that the chief chemical groups in living material with which the alkylating agents react are the sulfhydryl group (SH), the amine group (NH_2), and the acid group (COOH), the acid group being the most important. The following formulas demonstrate the structure of several representative alkylating agents and show how they may combine with cellular proteins.



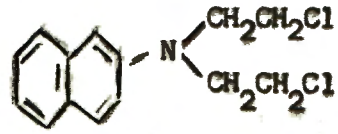
Mustard gas



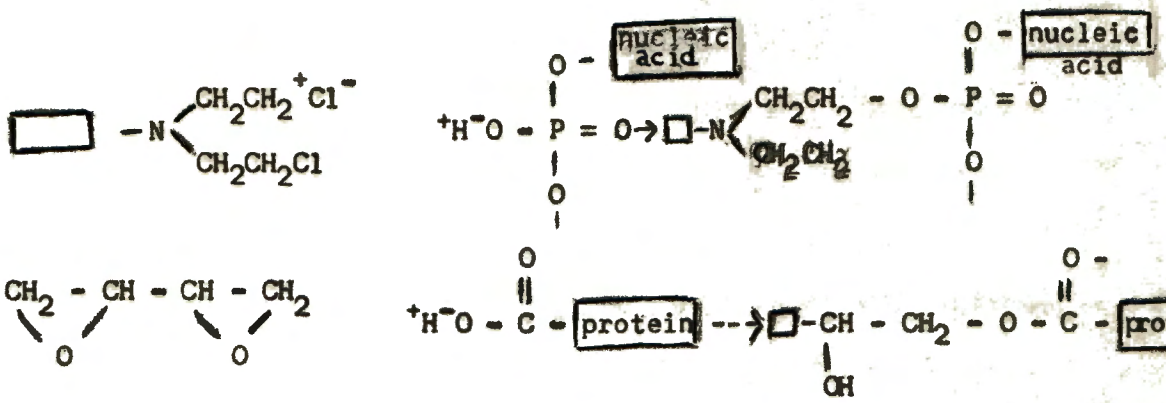
Nitrogen mustard



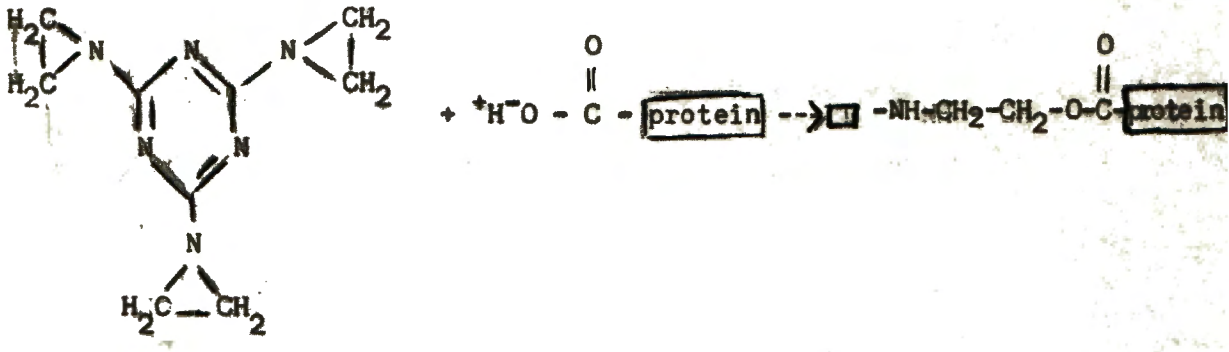
NN-di-2-chloroethylaniline



2-naphthyl-di-2'-chloroethylamine



1,2,3,4-diepoxybutane



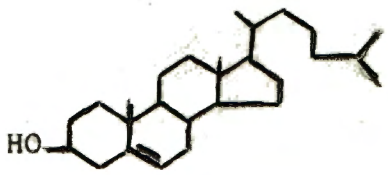
2,4,6;triethyleneimino-1,3,5-triazine

It has been found that two alkylating centers per molecule of carcinogen are more effective than one center in inhibiting cellular mitosis and producing chromosome damage, although, molecules with one alkylating center may cause damage to chromosomes. Alexander postulates that in the case of the molecules with two alkylating centers, two cellular molecules (such as two strands of DNA) could be cross-linked resulting in a genetic abnormality leading to inhibition of mitosis. Cross-linking of DNA by alkylating chemicals has been demonstrated in salmon sperm. In the case of molecules with one alkylating center, he suggests that alkylation of one of

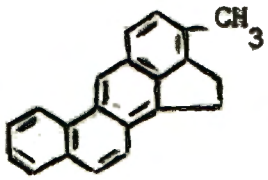
the reactive groups on a DNA molecule complicates the process of chromosome duplication and increases the likelihood of a copying error during cell division.

CHOLESTEROL AND RELATED COMPOUNDS

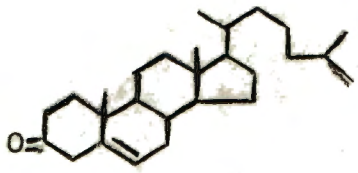
Although cholesterol is included in the tumor producing chemicals, relatively little is known about its mechanism of action. Hiegar (1958) was able to produce seventy sarcomata in 1,434 mice by subcutaneous injection of oily solutions of cholesterol. Badger (1956) suggested that many spontaneous cancers might be the result of a chemical carcinogen formed *in vivo* by an abnormal mechanism of cholesterol metabolism. In this connection, methylcholanthrene and Δ^5 - cholestene-3-one have been formed from cholesterol and have been found to be carcinogenic in animals.



Cholesterol



Methylcholanthrene



Δ^5-cholestene-3-one

Fieser and Fieser (1944) call attention to the fact that methylcholanthrene has been produced by an unusual pyrrolytic degradation of a derivative of cholesterol and that several steroids have been similarly transformed into actively carcinogenic hydrocarbons. They, therefore, submit the possibility that a comparable process occurs in the body.

PLASTICS AND OTHER POLYMERS

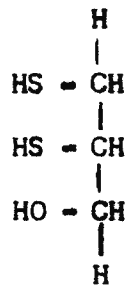
Although these substances have been found to induce tumors, they are another group about which little of the mechanism is understood. In this group, sarcomas have been produced by subcutaneous implantation of bakelite discs and films of cellophan, polyethylene, dacron, teflon, silk, and polytetrafluoroethylene (Haddow, 1958). Haddow points out that the inert nature of these materials makes a chemical theory of action seem unlikely. However, it has been shown that polyethylene may be cross-linked by oxygen, and it has been postulated that residual valencies on the polymer surface may bind with cellular proteins. On the other hand, polytetrafluoroethylene is considered to be too chemically inert to enter into any reaction.

Oppenheimer, Willhite, Danishefsky, and Stout (1961) demonstrated that polyethylene powders, in contrast to films, are noncarcinogenic when imbedded subcutaneously. As a result of this experiment, they suggest that no chemical action is involved in polyethylene carcinogenesis since a higher incidence of tumor pre-

duction would be expected from the powder because of the increased surface available for chemical reaction.

INORGANIC CARCINOGENS

Badger (1956) includes arsenic, beryllium, chromium, cobalt, nickle, and zinc in this group. He suggests that, with the exception of beryllium, all of these elements would be expected to combine with sulfur groups in cellular proteins. In this respect the inorganic ions may resemble the carcinogenic hydrocarbons in their mode of action. The reactivity of these ions toward sulfur groups is reflected in their action toward British antilewisite.



2,3-dimercaprol-1-propanol
(BAL)

DISCUSSION

An understanding of chemical carcinogenesis requires an understanding of the nuclear control of the cell. An excellent discussion of this subject has been published by Gay (1960) and by Pfeifer (1960). The DNA of the chromosomes is the primary hereditary material, the sub-

stance of which genes are made. DNA consists of two interconnected molecular strands, each strand serving as a template on which a new strand is reproduced, providing the building blocks are present. Thus, DNA provides the blueprint for the construction of living cells. It has been suggested that DNA presides over the synthesis of RNA in accord with the genetic plan borne by the chromosomes and that RNA plays the key role in the elaboration of proteins in the cytoplasm. RNA molecules cause amino acids in the cytoplasm to become attatched to the RNA molecule at particular points and thereby become aligned to form a particular protein. Via this mechanism the metabolic chores of any cell are guided by DNA control. Electron microscopy of giant salivary - gland cells of the fruit fly larva has shown a sequence of events that carries chromosomal material through the nuclear membrane into the cytoplasm. In some cases this process occurs at the heterochromatic region of the chromosome. Biochemical tests have shown this material from the nucleus to contain both DNA and RNA. This suggests that in such a cell a new biochemical process may be initiated under the domination of a special region on one of its chromosomes.

Barr and Moore (1957) have demonstrated that the heterochromatic regions of sex chromosomes are large compared with those of autosomes and that the heterochromatic region is well developed in females and insignificant in males. They believe that the visibility of the heterochromatic region in the intermitotic nuclei of females is accounted for by the fact that the two X-chromosomes are

in apposition. They have further demonstrated that, in a significant number of malignant cells from tumors in female hosts, the heterochromatin was identified with a lower frequency than was encountered in nonmalignant tissues. Therefore, they feel that the effect of chemical carcinogens on chromosomal synapsis or exchange of genetic material is worthy of further investigation as a mechanism in carcinogenesis.

Although the exact mechanism of chemical carcinogenesis is not yet known, Haddow (1958) thinks there is little doubt of the importance of the combination of these carcinogens with genetic material. He suggests that the primary step may be inhibition of certain fundamental processes of genetical or enzyme synthesis, followed by the generation of a new self duplicating DNA template. Hrader (1959) studied the effect of various carcinogens on the incorporation of labelled amino acids into proteins and found that some doses of carcinogens stimulate this process while other inhibit it. Rhondoni (1955) believes that in neoplastic tissue there is an inhibition of the mechanism controlling protein synthesis so that cellular proteins are built up at an increased rate. He also believes that a new template is originated which directs the synthetic process toward the formation of a degraded living matter. He likens carcinogenesis to protein denaturation, both processes resulting in despecialization of a protein system.

Haddow (1957) and 1958) postulates that carcinogenesis may be a case of biological mutation by loss, especially in the case

of the alkylating agents which interfere with gene reproduction. He believes that the resulting deficiency is enzymic in nature. The loss of enzyme systems, which normally control the synthesis of substances essential to cell division, may result in unregulated accumulation of these substances and so convert the normal cell into one of unimpeded growth.

Angelleti, Moore, and Suntzeff (1960) have demonstrated a high degree of similarity in the protein and enzyme composition of various neoplastic tumors. When they chromatographed soluble proteins of different types of tumors, they obtained similar patterns for the proteins and for the enzyme activities. They thought it especially significant that not only were the enzyme patterns similar; but also the multiple peaked pattern of each particular enzyme was strikingly similar for all of the tumors. They suggest that the close resemblance of the enzymatic patterns reflects the tendency of all tumors to approach a common metabolic type.

Many believe in an electronic basis of carcinogenesis, Pullman and Pullman (1955) being two of the main investigators in this field. Chalvet, Daudel, and Moses (1958), in discussing the interaction of aromatic hydrocarbons with cellular proteins, state that it seems probable that a necessary condition for a substance to be carcinogenic is that a substantial amount be fixed by one of its bonds to the protein. Mason (1958) suggests that induction of electron mobility in the protein part of carcinogen - protein complexes may lead to a partial breakdown in the hydrogen bond system, thus af-

fecting the protective action of the protein part of a cellular nucleoprotein. He also postulates that subsequent photon induced reactions may be responsible for changes in the nucleic acid configuration which will enable it to transmit an altered code.

SUMMARY

The more commonly investigated groups of chemical carcinogens have been presented along with evidence which suggests their mechanism in initiating malignant transformation of the cell.

It was shown that the carcinogenic hydrocarbons form an irreversible chemical bond with cellular proteins and that the nucleoproteins are probably the fraction affected. These hydrocarbons were also found to inhibit enzymes, and two explanations for this action were suggested. The carcinogen may prevent formation of an enzyme or it may inactivate an enzyme by combining with it. Both explanations are certainly plausable. It can be seen that the carcinogen may primarily inactivate an enzyme, thereby altering cellular metabolism, or it may first combine with a nucleoprotein, thereby interferring with nuclear control and protein formation in the cell. Decreased protein synthesis may also explain the growth inhibitory effect of the carcinogenic hydrocarbons.

The azo carcinogens were found to be similar to the carcinogenic hydrocarbons in combining with cellular proteins and inhibiting enzymes, and a similar mechanism of action may be applied to both groups.

In the case of the aromatic amines, the site of tumor formation in the body apparantly depends on variations in chemical structure. The importance of their conversion to O-aminophenols and their preferential excretion as glucuronides was discussed. There is much evidence that the O-aminophenols are active carcinogens. On the other hand, there is a lack of convincing evidence that the carcinogenic aromatic amines are excreted preferentially as glucuronides. It is of interest to note that increased enzyme activity (in the form of β-glucuronidase) was considered to be a possible factor in bladder carcinogenesis while loss of enzyme activity was a factor in hepatic carcinogenesis. In the latter case, enzyme inhibition was thought to result in an increased availability of amino acids for protein synthesis in contrast to the decreased protein synthesis thought to be produced by the carcinogenic hydrocarbons. In addition, there is some evidence that the more planar the aromatic amine molecule, the more strongly carcingenic it is.

It has been fairly well established that the alkylating agents combine with DNA and thus produce genetic abnormalities of the cell. Here again there may be interferrence with the nuclear control mechanism of the cell.

Cholesterol and related compounds have been converted to carcinogenic hydrocarbons and there is a possibility that this may occur in the body as a result of an abnormal type of metabolism.

The plastics and other polymers are known to produce tumors, but they are, in general, such inert substances that a chemical theory of action seems unlikely.

Little investigation has been done on the metalic compounds of the inorganic group of carcinogens. In view of their reaction with the sulfhydryl groups of BAL it is likely that they may react similarly with the sulfhydryl groups of enzymes and therby cause enzyme inhibition.

CONCLUSION

Chemical carcinogens or their carcinogenic metabolites combine with cellular proteins, probably the nucleoproteins, and inhibit enzyme activity. Enzyme inhibition may well be secondary to combination of the carcinogen with DNA. By combining with DNA, the carcinogen interferes with the master control of metabolic processes in a cell and its descendents, thereby initiating malignant transformation.

Similar protein and enzyme patterns are found to be present in different types of tumors suggesting a tendency for neoplastic tissues to approach a common metabolic type.

Spontaneous cancers in persons not exposed to the chemical carcinogens may be the result of an abnormal metabolism which converts normal body chemicals to carcinogenic substances.

ACKNOWLEDGEMENT

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