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Mutagenic and Carcinogenic Properties of Polycyclic Aromatic Hydrocarbons

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The rapid development of the chemical industry, combustion of fossil fuels, and smoking of tobacco have resulted in contact of the general population with benzo(a)pyrene and other carcinogenic aromatic hydrocarbons. Persons especially at risk occupationally are those engaged in thermal processing of oil shale, coal, and heavy residual petroleum.

It has been shown that polycyclic aromatic hydrocarbons require metabolic activation before they can act as mutagens or carcinogens. This metabolic activation results from interaction with microsomal enzymes present in many body cells, yielding reactive epoxides which react with DNA and produce mutations in the count frame shift or participate in covalent bonding. While opinions differ regarding the relative role of these processes in mutagenesis, considerable evidence exists which links mutagenesis and carcinogenesis.

Metabolites of the polycyclic aromatic hydrocarbons which are carcinogenic are usually mutagenic, which supports the hypothesis that damage to chromosomes plays an important role in carcinogenesis. These facts open the possibility to monitoring the spread of carcinogenic substances in the biosphere by relatively simple tests whose endpoint is mutagenesis.

The rapid development of the modern chemical industry has resulted in the introduction of a large number of polycyclic aromatic hydrocarbons into the environment. These substances have been detected in air, soil, reservoir water, marine sediments, and in some types of food products.

Production processes related to heat treatment and incomplete fuel combustion of combustible shale in particular serve as the primary sources of environmental pollution by polycyclic aromatic hydrocarbons.

Eleven carcinogenic polycyclic aromatic hydrocarbons were detected in the air of industrial cities (*1*, *2*). These substances are released into the environment in the exhaust gases of internal combustion engines, in various types of smoke, in the smoke from tobacco, and in fly ash. The most widespread of these is benz(a)pyrene. Fly ash from coke contains 0.2 μg benz(a)pyrene per gram of coke ash, from 7.5 to 9.2 μg benz(a)pyrene per gram of coal ash, 1.4 to 1.5 μg benz(a)pyrene per gram of brown coal ash, and 11.5 to 20.1 μg of benz(a)pyrene per gram of wood ash (*3*). Benz(a)pyrene is also found in various tars which are dumped into water reservoirs with wastewater from chemical or petroleum processing and wastes from the coal tar chemical and shale industries (*4*). The following amounts of benz(a)pyrene were detected in the wastewater of the following industrial enterprises: coal-tar chemical enterprises, 0.092-2.65 mg/l. (*5*); petroleum processing enterprises, 0.58-0.181 μg/l. (*6*); production of acetylene, 0.061 mg/l. (*7*). It should be stated that the wastewater treatment facilities did not remove benz(a)pyrene.

Since benz(a)pyrene is frequently found in drinking water, the World Health Organization has established that its concentration should not exceed 0.2 μg/l. (*8*).

A large number of people in industry come into contact with products containing polycyclic aromatic hydrocarbons in the following forms: workers at enterprises which thermally process combustible shales, hard coal, petroleum and also at enterprises utilizing products from the thermal processing of fuels (hard coal, pitch, tars, bitumins and oils, shale tar and oils, certain types of petroleum and products obtained from processing pitch, residual heavy oil, bitumins and oil of petroleum origin), along with products from the wood chemical industry and the processing of peat. As a consequence, it has been established that benz(a)pyrene is contained at a

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level of 0.001–1%, in various types of coal tar 1.5–2% in coal pitch, and 0.2% in shale tars, particularly of the Kamara type (*9*). Other hydrocarbons are also found in the majority of the above industrial products, but at lower concentrations.

Polycyclic aromatic hydrocarbons represent a danger due to their potential carcinogenic and mutagenic capabilities. It has been shown that approximately 90% of the cancer occurring in man is of chemical origin (*10*). Data exist which indicate that 60–80% of various forms of cancer in man are the result of the effect of the environment (*11, 12*). It is probable that polycyclic aromatic hydrocarbons make their contributions in this respect.

There have been a significant number of studies to investigate the mechanism responsible for the mutagenic effect of polycyclic aromatic hydrocarbons in *in vitro* test systems and their capacity for malignant transformation of mammalian cells in *in vitro–in vivo* tests and to determine the carcinogenic potential of these compounds for animals *in vivo*.

It was shown that polycyclic aromatic hydrocarbons are among those compounds which by themselves are neither active mutagenic agents nor active carcinogens, but only after transformation through the mammalian metabolism are they converted into chemically reactive derivatives which damage genetic material and evoke malignant changes in cells. It is shown that the potentially carcinogenic polycyclic aromatic hydrocarbons manifest their mutagenic properties after activation by microsomal liver enzymes in mammals (*13–18*). Later it turned out that the activating capacity is not possessed solely by liver enzymes but also by the enzymes of other organism tissue, such as lung (*19, 20*), along with the epithelial bronchial cells in man (*21*).

Many studies have concerned the problem of how polycyclic aromatic hydrocarbons are metabolized in the mammalian organism. It is shown that polycyclic aromatic hydrocarbons are metabolized into epoxides (*13, 22-24*). Epoxides couple with nucleic acids to a greater extent than do the corresponding hydrocarbons. It is on this basis that their significant activity in inducing malignant transformation can be explained (*23*). It is supposed (*25*) that the metabolic transformation of polycyclic aromatic hydrocarbons is necessary for the covalent bond formation of their metabolites with DNA, RNA, and albumins.

Cavalieri and Calvin (*26*) proposed the effect of enzymes served as the mechanism for activating polycyclic aromatic hydrocarbons. Results from a study of the reactivity at various points of the ring system of the three strongest hydrocarbon carcinogens, i.e., benz(a)pyrene, 7,12-dimethylbenzoanthracene, and 3-methylcholanthrene, showed the most reactive positions to be those at which the proton is replaced easily. It is most likely that the bonds with nucleic acids are formed at these positions.

Studies concerned with the degree of hydrocarbon bonding with macromolecules relate to the establishment of a relationship between the number of bonds and the level of cellular metabolism, the carcinogenic activity of the chemical compound, the state of DNA, and other aspects. A positive correlation has been established between the cellular metabolic activity and the bonding dimension of the polycyclic aromatic hydrocarbons with DNA (*27*). The highest number of bonds noted for DNA was observed for DNA which was obtained from cells with high metabolism (*28*).

Quantitative studies were also undertaken to investigate the bonding of polycyclic aromatic hydrocarbons which differ with respect to their degree of carcinogenicity with DNA, RNA, and albumin. A positive correlation was established between the carcinogenic activity of chemical substances and the number of bonds with macromolecules (*29–31*). This correlation was considered as a bond between the hydrocarbon molecules and the macromolecule at a dosage of 1 *mM* of hydrocarbon/mouse. It turned out that the bonding level with polycyclic aromatic hydrocarbons is characteristically greater for DNA than for protein or RNA. For a given dose, it took 0.1–1.0 molecules of hydrocarbon/molecule of DNA while it took 0.01–0.02 molecules in the case of protein. In studying the interaction of dimethylbenzanthracene (DMBA) at various concentrations with normal cages of rodents (*32*) it was also established that the highest activity in bonding hydrocarbon is inherent to DNA. At doses of 0.01–0.1 mg/ml, the number of bonds with RNA was two times less and with proteins four times less than with DNA. At the highest possible saturated concentrations of DMBA, the bonds were equivalent to one molecule of hydrocarbon/molecule of DNA, while in the case of protein, the ratio was one molecule of hydrocarbon/1250 molecules of protein.

Besides the capacity to form covalent bonds, another mechanism was determined for the interaction with DNA. Epoxides can be physically situated in the DNA molecule (*13, 22, 33–35*). This appears to be the reason for the insertions in the replication process and in the repair synthesis of DNA. Obviously shift stabilization of the repeating sequence of bases is observed. The effectiveness of the building agent as one which has shifted the mutagen count framework declines, if this agent is capable of

reacting covalently with DNA. Obviously this ability to be incorporated in the molecule can be fixed by this covalent reaction.

Studies have also been undertaken concerned with the malignant transformation of mammalian cells *in vitro* under the effect of hydrocarbons. In treating the cell cultures of mouse prostate gland with various polycyclic aromatic hydrocarbons, such malignant transformation were observed as the formation of multilayer cultures with chaotic cell orientation, the manifestation of cells with multipolar mitoses, increase in the capacity of cells to stick to glass, and their increased sensitivity to heat. On transplantation of such cells in animals, the latter as a rule develop tumors (*36–40*).

In studying the quantitative aspects of malignant transformations after treating the cell cultures of mice with carcinogenic and noncarcinogenic hydrocarbons, a clear correlation was observed between the carcinogenic activity of the polycyclic aromatic hydrocarbons and the transformation frequency. Powerful carcinogens conditioned the maximum frequency of transformations, while noncarcinogenic polycyclic aromatic hydrocarbons did not produce transformed cell colonies (*38, 41*).

As a consequence, DNA, RNA, and proteins serve as the primary targets for carcinogenic hydrocarbons. Reactive epoxides are formed in the mammalian organism. These reactive epoxides are obviously those terminal forms on which malignant transformation of cells depend. The epoxides can react with fundamental DNA and RNA and also build themselves in between the bases of DNA, evoking mutations in the count frame shift.

It was shown that DNA in the most sensitive receptor for polycyclic aromatic hydrocarbons, RNA and proteins being less sensitive. This corresponds to the mutation theory of carcinogenesis. In dealing with the problem as to which of the two processes, i.e., the formation of the covalent bonds or the location (intercalation), is of greater significance in carcinogenesis, the first process is considered by a number of authors to be the most important factor in the biological phenomena of carcinogenesis and mutagenesis (*27, 42*), while other authors (*14, 16*) show that carcinogenic polycyclic aromatic hydrocarbons manifest themselves in test strains which have been designated for determining mutagens, shifting the count frame and that it is primarily the intercalation of hydrocarbons between the DNA bases which is the reason for carcinogenesis, while the covalent bonds intensify the carcinogenic potential of the chemical compound.

It has been hypothesized that the manifestation of mutations in somatic cells may be the reason for carcinogenesis (*43–45*). It was also shown that the terminal metabolites of many carcinogens (including those of polycyclic aromatic hydrocarbons) are mutagens (*46*). The parallel effect of the mutagenic and carcinogenic action of hydrocarbons was noted by Avertsev (*47*) and Fridlyanskaya (*48*), who showed the carcinogenic polycyclic aromatic hydrocarbons evoke chromosomal aberrations in a rat cell culture, while their noncarcinogenic analogs do not have an effect from a practical point of view on chromosome mutation frequency. On the basis of these studies, the authors conclude that mutations participate in carcinogenesis.

In studying the quantitative relationship between the carcinogenic and mutagenic properties of polycyclic aromatic hydrocarbons, a parallel effect was found between the ability of 12-benzoanthracene derivatives to damage the chromosomes of bone marrow cells and the ability to cause sarcoma when rats were injected with these substances (*49*). The results of the work clearly show the quantitative correlation and make it possible to suppose that damage to chromosomes plays an important role in carcinogenesis. In addition, Teranishi et al. (*17*) used the Iball index (*50*) to evaluate the carcinogenicity of polycyclic aromatic hydrocarbons in polluted air. Their mutagenic potential was studied on *Salmonella* by using the procedure of Ames et al. (*13*). Three of the most powerful carcinogens yielded a substantial number of histidine positive revertants. The weak carcinogens did not have any demonstrable effect on the strains studied. As a consequence, the parallel effect between the carcinogenic and mutagenic action of chemical compounds can serve as an indirect proof of the mutagenic process of malignancy (*51, 52*).

The results of testing chemical carcinogens for mutagenicity in various test systems show that many, up to 80% (*53*) and even up to 90% (*54*), and perhaps all of the chemical carcinogens are mutagens (*55*). In this respect, the study of the mutagenic properties of chemical carcinogens and in particular of polycyclic aromatic hydrocarbons opens up a great possibility to monitor the spread of substances in the biosphere which are dangerous from the carcinogenic point of view.

From the point of view of practice, the use of already studied genetic methods which define environmental mutagens will make it possible in a short time by use of mass screening methods to determine environmental pollutants which are harmful from a carcinogenic point of view and which would otherwise require a great deal of time and labor to determine by ordinary methods.

In its entirety, problems associated with the prevention of environmental pollution by carcinogenic substances should be solved by a complex of

means. Under these conditions, genetic methods could play a decisive role in solving the individual aspects of this problem.

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