On the Binding of Benz[a]pyrene to DNA “in vivo”

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The Possibility that “in vivo” a binding takes place between DNA and benz[a]pyrene was examined. After oral administration of [3H]-benz[a]pyrene, radioactive DNA was isolated from the skin, spleen and liver of NCLmice, and especially from the liver. This suggest that covalent binding between DNA and benz[a]pyrene takes place.

Many studies described in the literature suggest a relationship between the chemical structure of several hydrocarbons and their carcinogetic power. It has been known since 1930 1 that it is possible to induce tumors by local treatment with 1,2,5,6-dibenzanthracene. The possibility that light may influence carcinogenesis due to benz[a]pyrene has also been studied 2.

Many authors have reported that the carcinogetic activity depends upon a binding between the hydrocarbon, or one of its metabolites, and the cellular constituents. Some time ago it was suggested that binding to proteins was the beginning of the carcinogenesis; it has, moreover, also been suggested that an important part of the tumoral process may be attributed to a binding between hydrocarbons and nucleic acids. There are different opinions on the possibility that such a binding occurs, both “in vitro” and “in vivo”. With regard to experiments “in vitro”, HETDELBBERGER 3 up till 1964 excludes that the responsibility for the beginning of the carcinogetic processes could be attributed to the binding between hydrocarbons and DNA. The results of BROOKES and LOWLEY 4 are noteworthy; when mouse skin was treated with a set of tritiated hydrocarbons, radioactive nucleic acids were isolated. BROOKES 5 studied in 1966 the problem quantitatively, and GOSMAN and HEIDELBERGER considered it again in 1967; finding that the radioactivity supplied by tritiated hydrocarbons of high specific activity was bound to DNA, RNA, and to proteins isolated from mouse skin after topical treatment. In this case benz[a]pyrene was not considered. BROOKES and HEIDELBERGER 7 found in 1969 that reaction between hydrocarbons and nucleic acids was obtained using 7,12-dimethyl-benz[a]anthracene as a hydrocarbon, and rodent embryo cells in culture as a source of DNA.

PRODI et al. 8 examined the radioactivity of DNA, RNA, nuclear and cytoplasmic proteins of various organs of rat after i.p. injection of tritiated 7,12-dimethylbenz[a]anthracene and benz[a]pyrene. All the cellular constituents examined showed the presence of radioactivity, indicating binding, although in limited measure.

With regard to the experiments “in vitro”, we recollect that the studies of BOYLAND and GREEN 9.

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200 F. CARLASSARE, C. ANTONELLO, F. BACCICHETTI, AND P. MALFER

16 J. MARMUR and P. DOTT, J. molecular Biol. 6, 109 [1962].
20 J. EIGER and P. DOTT, J. molecular Biol. 12, 549 [1965].
21 J. A. HARPS, A. I. KRASNA, and B. H. ZIMM, Biopolymers 6, 595 [1968].
Liquori et al. and Ts'o e Lu confirmed the formation of a binding between carcinogenic hydrocarbons and DNA, whereas those of Heidelberger found no evidence of any binding.

Later, Rice demonstrated the formation of photoadducts after irradiation of benz[a]pyrene in the presence of the purine and pyrimidine bases present in DNA.

In 1968, following investigation of various opinions as to the reactions which take place “in vitro” between purine and pyrimidine bases present in nucleic acids and some photosensitizing substances, we have also confirmed the formation of photoadducts after U.V. irradiation of these bases in the presence of benz[a]pyrene.

The analysis of the substance isolated after irradiation of the mixture of benz[a]pyrene and thymine in solution has demonstrated that it was the product of a C,C-cyclo-addition between a molecule of base and one of hydrocarbon.

Kodama and Nagata, by irradiating an alcoholic solution of benz[a]pyrene and other hydrocarbons in the presence of DNA as the cetyltrimethylammonium salt, in addition to a photooxidation of the guanine moieties, found a covalent linkage between hydrocarbons and DNA.

In 1970 we have isolated a photoadduct benz[a]pyrene-thymine from DNA hydrolized after irradiation at 365 nm in the presence of benz[a]pyrene.

The aim of the present work has been to contribute to research whether “in vivo” formation of a covalent binding between the hydrocarbon or its metabolite and the nucleic acid is possible. We have considered the DNA extracted from mouse skin, spleen and liver, after oral administration of tritiated benz[a]pyrene.

Materials and Methods

Benz[a]pyrene. Benz[a]pyrene, m.p. 176°—178°, \( \lambda_{\text{max}} 296 \text{ m} \mu (\epsilon > 5000), \lambda_{\text{max}} 320 \text{ m} \mu (\epsilon > 2800), \) was purchased from Fluka AG Buchs, Switzerland. Benz[a]pyrene\(^3\text{H} \) was supplied by the Radiochemical Centre, Amersham, England.

Radioactive measurements. These were performed with a liquid scintillation system, Beckman 150 SL, using for each counting 10 ml of the following solution: 4 g of 2,5-diphenyloxazole; 0.075 g of 2,2'-paraphenyl-bis(5-phenyloxazole); 120 g of naphthalene; dioxane up to 1000 ml of solution.

Male and female NCL mice were used, weighing about 20 g each, 0.4 mg and 0.1 mC benz[a]pyrene in 0.4 ml olive oil was administered per os to each mouse. Five experiments were performed, treating groups of 10 mice at a time, with the back previously shaved. After 15 hours the mice were killed and the skin of the back, liver and spleen of each one were removed. The biological material was rapidly weighed, frozen and then stored at \(-20^\circ\) until processed. The extraction of DNA was performed essentially as described by Szyszkowska and Szyszkowski. The organic material was homogenized with saline solution containing 0.02 M citrate sodium and 0.15 M sodium chloride.

The skin was homogenized in 4 times its weight of saline solution, the spleen in 100 times and the liver in 10 times its weight. The homogenates, treated with 2% of sodium dodecyl sulphate, was strongly shaken for 2 min and made up to a 1 M concentration with sodium chloride.

The suspensions, after storage at 0° for a night, were extracted with equal volumes of chloroform: butanol (4/1, \( v/v \)), repeating the extractions until no trace of sediment remained between the organic and aqueous layer.

From the aqueous clear solutions the DNA was precipitated with 2 volumes of absolute ethanol, washed again several times with ethanol 95%, then with ethanol 70% and then with dry acetone.

The samples of DNA obtained were separately dissolved in smaller amounts of 2 \( \cdot \) \( 10^{-3} \) M NaCl. The solutions were made up to pH 8 with Tris buffer, trypsin added and, after standing 12 hours at 37°, dialyzed against water for 48 hours. After dialysis, the DNA, precipitated with ethanol, washed and dried as described, was dissolved again in water and determined quantitatively by a spectrophotometric method. The average values of DNA obtained were, for each group
of 10 mice, about 1.5 mg from the skin, 30 mg from the liver and 40 mg from the spleen.

Fractions of aqueous solution, corresponding to 100, 200, 400, 800 µg of DNA for the skin and to 100, 200, 400, 800, 1000 µg for the spleen and the liver, were taken and the solution was made up to 1 ml with water and then to 11 ml with scintillator solution.

The counting was performed for 10 min per sample.

To confirm that the radioactivity could not be ascribed to a product due to the presence of the light, we also carried out some extractions of DNA from tissues in the dark, obtaining values agreeing with those reported in Fig. 1.

**Conclusion**

Fig. 1 shows that in skin, spleen and liver a certain amount of radioactivity is bound to DNA, after treatment per os of the NCL mice with labelled benz[a]pyrene. On the basis of radioactivity of DNA isolated, it was possible to calculate, with respect to nucleotides present in DNA, that this product was obtained with a molecular ratio of 1:875.000 from the liver; of 1:1.910.000 from the skin, and 1:3.150.000 from spleen. This means that, considering the average molecular weight of DNA as 6.000.000, one molecule of benz[a]pyrene was bound to every 46.8 molecules of DNA in liver, every 101 molecules of DNA in skin, and every 168 of DNA in spleen. Such radioactivity was never present in high values, but in the case of liver it appears significant, a fact suggesting that at least in liver a covalent binding of benz[a]pyrene to DNA takes place.

3. C. HEIDELBERGER, J. cellular Comparat. Physiol. 64, sup. 1, 129 [1964].
6. L. M. GOSHMAN and C. HEIDELBERGER, Cancer Res. 27, 1678 [1967].