CHANGES IN 7,12-DIMETHYLBENZ(a)ANTHRACENE CAUSED BY VITAMIN A

K. L. Kkhandudzha, T. D. Esakova,
B. K. Semin, Yu. M. Petrusevich,
and B. N. Tarusov

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The metabolism of 7,12-dimethylbenz(a)anthracene (DMBA) was studied in vitro on incubation with liver microsomes and liver and mammary gland homogenates of rats kept on a diet enriched with vitamin A. Vitamin A inhibited the formation of lipophilic metabolites and increased the output of water-soluble metabolites. The concentration of lipophilic metabolites in the microsomes and liver and mammary gland homogenates was two, two, and five times less, respectively. The amount of unmetabolized DMBA in the liver microsomes of the control and experimental animals was the same.

KEY WORDS: vitamin A; 7,12-dimethylbenz(a)anthracene and its derivatives; carcinogens; liver and mammary glands of rats.

By the metabolism of polycyclic hydrocarbons both in vivo and in vitro water-soluble and lipid-soluble products are formed. It was shown previously [14] that K-region epoxides which are intermediate products in this process, are toxic and are more active in cell transformation in vitro than the original hydrocarbons, dihydrodiols, and K-region phenols. Studies of lipid-soluble metabolites revealed highly toxic and carcinogenic products [1, 7, 9].

The addition of vitamin A to the diet of experimental animals has a prophylactic and, in some cases, a therapeutic action also on malignant growth. It has been suggested that the anticarcinogenic action of vitamin A may be either hormone-like in character [3] or may take place through changes in metabolism of carcinogenic polycyclic hydrocarbons [13].

The object of this investigation was to test this second hypothesis.

EXPERIMENTAL METHOD

Noninbred albino rats weighing about 125 g were used. One group of animals was kept on a normal diet while the animals of the other group were given vitamin A palmitate in a dose of 2.5 mg daily with the diet for 10 days.

Liver microsomes were isolated and suspended in sucrose–phosphate buffer [11]. Protein was determined by the biuret method [6].

The ^{3}H-labeled 7,12-dimethylbenz(a)anthracene (DMBA-^{3}H) for incubation was obtained from the Radiochemical Centre, Amersham, England, and its specific radioactivity was 6.4 Ci/mmole. Microsomes and liver and mammary gland homogenates were incubated in the presence of 2 μM NADPH and DMBA-^{3}H (7.8 nM for microsomes and 1.95 nM for liver or mammary gland homogenate) for 20 min at 37°C. To assess the nonenzymic oxidation of the carcinogen, boiled microsomes or homogenates were used. DMBA and its metabolites were extracted from the incubation mixture with ethyl ether, and aqueous impurities were removed with potassium sulfate. The extracts were fractionated by two-dimensional thin-layer chromatography on Silufol (Czechoslovakia) in petroleum ether (first direction) and in a system of benzene and ethanol (19:1) (second

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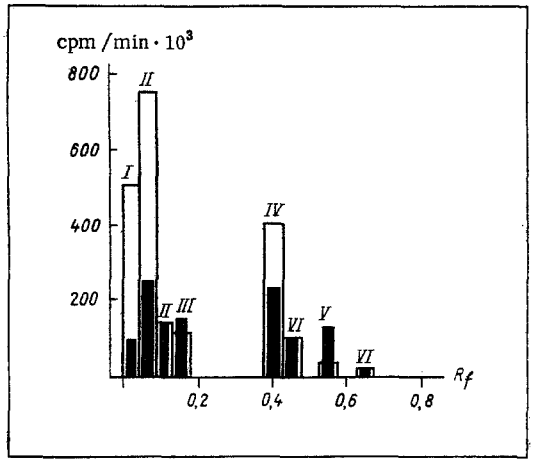

Fig. 1

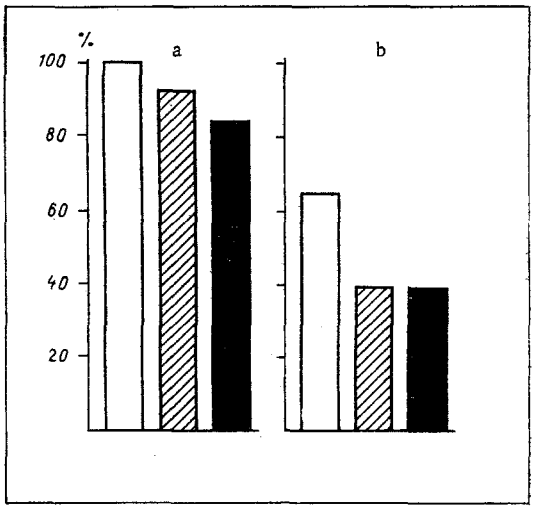

Fig. 2

Fig. 1. Quantitative composition of metabolites after incubation of DMBA-^{3}H with liver microsomes of control and experimental animals. Unshaded columns represent control animals, shaded columns animals on vitamin A-enriched diet. I) Polar metabolites; II) unidentified metabolites; III) 7,12-di-OH-MBA; IV) 7-OH,12-MBA; V) 12-OH,7-MBA; VI) unidentified metabolites. Abscissa, relative chromatographic mobility (R_f); ordinate, radioactivity (in cpm $\times 10^3$).

Fig. 2. Quantity of total radioactivity (a) and radioactivity of unmetabolized DMBA (b) extracted with ethyl ether after incubation of liver microsomes (4 mg protein) at 37°C with DMBA-^{3}H. Unshaded columns represent boiled microsomes; obliquely shaded columns liver microsomes of control animals; black columns liver microsomes of animals receiving vitamin A.

direction). To detect DMBA and its metabolites the chromatograms were tested in UV light. The radioactivity of the excised areas of the chromatograms was measured with a liquid scintillation spectrometer.

EXPERIMENTAL RESULTS

The composition of the metabolites formed by incubation of DMBA-^{3}H with the microsomes of the control and experimental animals is shown in Fig. 1. The corresponding standard compounds were used to identify 7,12-dihydroxymethylbenz(a)anthracene (7,12-di-OH-MBA), 7-hydroxymethyl, 12-methylbenz(a)anthracene (7-OH, 12-MBA), and 12-hydroxymethyl, 7-methylbenz(a)anthracene (12-OH, 7-MBA). The relative quantity of polar and unidentified metabolites and of 7-OH, 12-MBA in the experimental series was 7.3 and 1.5 times less, whereas the relative quantity of the metabolites 7,12-di-OH-MBA and 12-OH, 7-MBA was 1.5 and 3.5 times greater, respectively, than in the control. The quantity of an unidentified metabolite with high chromatic mobility was virtually the same in both cases. The total content of lipid-soluble metabolites in the control was almost twice that in the experimental series.

Results showing the relative quantity of total radioactivity extractable with ethyl ether from boiled microsomes and native microsomes of the control and experimental animals after incubation with DMBA-^{3}H are given in Fig. 2a. The amount of DMBA-^{3}H metabolized into water-soluble compounds was 8% greater in the microsomes isolated from the liver of rats kept on a diet rich in vitamin A than in the control. Data showing the relative amounts of unmetabolized DMBA-^{3}H are given in Fig. 2b. In boiled microsomes, 33% of the DMBA was oxidized in the course of 20 min at 37°C, whereas in the experimental and control samples up to 60% of the DMBA was oxidized.

The distribution of metabolites formed in liver and mammary gland homogenates of the control and experimental animals is shown in Fig. 3. The principal identified metabolites of DMBA in the experiments with liver homogenate were 7-OH, 12-MBA; 12-OH, 7-MBA; and 7,12-di-OH-MBA; whereas in the experiments with mammary gland homogenate only 7-OH, 12-MBA was identified. The quantity of the polar compounds 7-OH, 12-MBA and 12-OH, 7-MBA in the experimental series was four, three, and two times less, respectively, than in the control; the amount of 7,12-di-OH-MBA was the same, and there was twice as much of the unidentified metabolite.

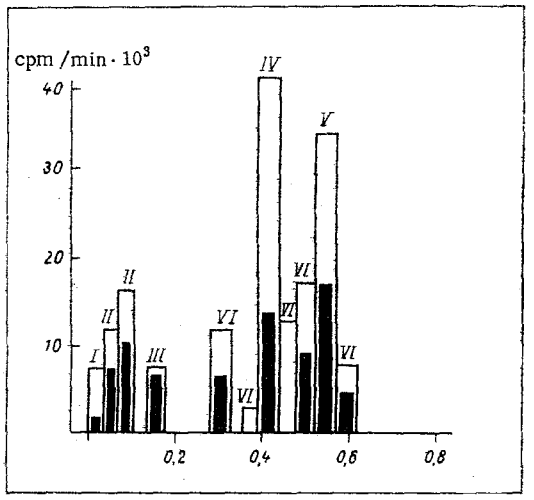

Fig. 3. Quantitative composition of metabolites after incubation of DMBA-^{3}H with liver homogenate from control and experimental animals. Legend as in Fig. 1.

A diet rich in vitamin A thus modified DMBA metabolism in the direction of reducing the lipid-soluble metabolic products of the carcinogen. The quantity of the single metabolite 7-OH, 12-MBA in the mammary gland homogenate of the control animals was five times greater than in the experimental group.

Radioactive label, not extracted from the reaction mixture, could belong either to highly polar metabolites or complexes of the polycyclic hydrocarbon with protein or nucleic acids [2, 14]. Water-soluble products of polycyclic hydrocarbons are formed by conjugation with glucuronic and sulfuric acids [9]. This conjugation requires preliminary metabolism of the carcinogen [4]. The first stage of metabolism of the hydrocarbon is the formation of K-region epoxides [10], which then are either transformed by a nonenzymic route into phenols or form dihydrodiols and conjugated derivatives with the participation of enzymes [5]. All these products, including conjugates, are extracted by organic solvents and they constituted the greater part of the organic extract in the present experiments also.

The quantitative and qualitative composition of the metabolites formed in the liver homogenate and investigated in these experiments correspond to data in the literature [15, 16]. The level of 7-OH, 12-MBA, which is an active metabolite of DMBA and causes necrosis of the adrenals, was much lower in the present experiments (hypervitaminosis A diet); the quantity of 12-OH, 7-MBA in the liver microsomes was higher, although in the liver homogenate it was 50% lower than in the control. This may have been due to changes in the rate of its metabolism. It follows from Fig. 2a that the quantity of radioactive label extracted from experimental samples with ethyl ether was less than in the control, whereas the quantity of unmetabolized DMBA was the same. This means that more of the carcinogen was metabolized into products which either react with intracellular macromolecules or into highly polar products, which therefore remain in the water. Experiments in vitro have shown [8] that retinol and some of its derivatives inhibit the formation of metabolites bound with microsomal components.

It can be postulated on the basis of these results that vitamin A stimulates the formation of enzymes metabolizing the carcinogen into water-soluble products to a greater degree than into lipid-soluble products, or metabolizing lipophilic metabolites into water-soluble products. Inhibition of metabolism of polycyclic hydrocarbons into lipophilic products is evidently not the only action of vitamin A as an anticarcinogenic agent. This effect of vitamin A may be linked with its hormone-like action on differentiation of tissue cells [3].

Besides investigations in which vitamin A acted as an anticarcinogenic factor, in other investigations tumor development has been found to be stimulated by vitamin A [12]. In those experiments vitamin A was applied to the skin either before or after application of DMBA. It is possible that the mechanisms of action of vitamin A when applied to the surface or administered by mouth are different and that the manner of its participation and its role in chemical carcinogenesis depend on its portal of entry into the body.

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EFFECT OF ESTROGENS ON THE BLOOD SERUM LEVEL OF CARCINO-EMBRYONIC ANTIGEN IN RATS WITH NEOPLASMS OF THE INTESTINE AND NONSPECIFIC INJURIES OF ITS MUCOSA

V. B. Okulov, V. N. Anisimov, and K. M. Pozharisskii

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Carcino-embryonic antigen (CEA) was detected in the blood serum of 70% of rats with tumors of the large intestine induced by 1,2-dimethylhydrazine and with posttraumatic regeneration of the mucosa of the large intestine. After injection of estrogen (diethylstilbestrol propionate, 0.57 μg daily for 6 days) the frequency of appearance of CEA in such animals increased, as also did the CEA level in the blood serum. In rats with injury to the mucosa of the large intestine, injection of estrogen prevented the natural decrease in the CEA concentration as the intensity of the regenerative process diminished.

KEY WORDS: carcino-embryonic antigen; large intestine; estrogens.

An antigen which, in some of its physicochemical features, is analogous to the carcino-embryonic antigen (CEA) of similar tumors in man has been found in the blood serum of rats with induced tumors of the large intestine and in the tumor tissue itself [1]. This antigen is also found with high frequency (about 70%) in rats with chronic nonspecific lesions of the intestinal mucosa [5].

During the study of the role of the endocrine system in experimental carcinogenesis in the intestine [4], the writers found CEA with high frequency and in a high titer in animals receiving injections of estrogens. The investigation described below was carried out to study this phenomenon.

Laboratories of Experimental Tumors and Endocrinology, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

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