COMPARATIVE PENETRATION OF POLYCYCLIC HYDROCARBONS THROUGH THE RAT PLACENTA INTO THE FETUS

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In experiments on rats the permeability of the placenta was studied to the following carcinogen polycyclic hydrocarbons: 7, 12-dimethylbenz(a) anthracene (DMBA), benz(a)pyrene (BP), and 3-methylcholanthrene (MC). The compounds were administered to the rats by gastric tube in the form of a suspension in sunflower oil in a dose of 200 mg/kg body weight on the 21st day of pregnancy and their content was determined after various times in the liver of the pregnant animals, the placenta, and the fetuses, using a fluoresecence-spectral method. Accumulation of DMBA in the fetal tissues reached a maximum 2-3 h after its administration, when its level was 1.53-1.6 μg/g. FP passed through the placenta to the fetus in considerable concentrations (2.77 μg/g), but MC did so only in traces. The same ratio between the concentrations of the compounds also was found in the liver of the pregnant rats, an organ with a rich blood supply. It can accordingly be concluded that MC is absorbed from the gastrointestinal tract into the blood stream of rats to a much lesser degree, and BP to a greater degree than other polycyclic hydrocarbons, with the result that corresponding quantities of the carcinogens reach the fetus.

The possibility of development of tumors in the progeny of mothers exposed during pregnancy to the action of chemical carcinogenic agents has not been proved experimentally [3-6]. The sensitivity of the fetus to carcinogenic agents is particularly great in the late periods of development [1, 6], i.e., at a time when the various exchange functions between the fetus and mother are performed by the placenta. This phenomenon has been called transplacental carcinogenesis.

One of the factors determining the sensitivity of the fetus in experiments to study transplacental carcinogenesis is the penetration of carcinogens or their active metabolites into the fetus in sufficient quantities to produce a visible effect. In this connection the state of the placental barrier with respect to substances harmful to the fetus, including those with carcinogenic properties, is interesting.

In a previous investigation the high permeability of the placenta in rats to 7,12-dimethylbenz(a)anthracene (DMBA) when the compound was injected intravenously at the end of pregnancy was proved [2].

This paper describes the results of a study of the penetration of carcinogenic polycyclic hydrocarbons – DMBA, 3-methylcholanthrene (MC), and benz(a)pyrene (BP) – through the placenta to the fetus after administration of the substances by gastric tube to female rats in the late stages of pregnancy. A fluorescence-spectral method was used to determine the concentrations of DMBA, MC, and BP in the fetal tissues, the placenta, and the liver of pregnant rats.

EXPERIMENTAL METHOD

Sexually mature noninbred female albino rats obtained from the Rappolovo nursery, Academy of Medical Sciences of the USSR, were used. From the 21st day after fertilization the rats received a single dose of DMBA, MC, or BP, made up as a suspension in sunflower oil, in a dose of 200 mg/kg by gastric tube. In

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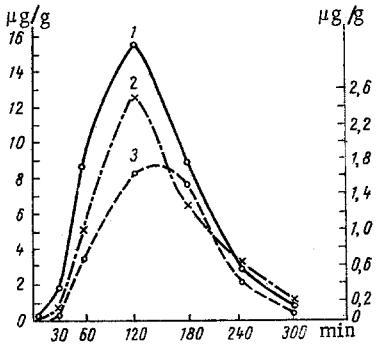

Fig. 1

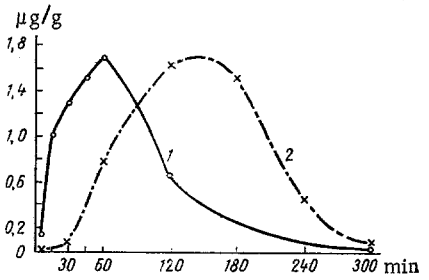

Fig. 2

Fig. 1. Concentration of DMBA in liver (1) of pregnant rats, placentas (2), and fetuses (3) at various times after administration of the compound in a dose of 200 mg/kg by gastric tube on the 21st day of pregnancy. Abscissa, time after injection of DMBA (in min); ordinate: left – DMBA concentration in liver (in μg/g), right – in fetuses and placentas (in μg/g).

Fig. 2. Concentration of DMBA in fetal tissues after intravenous injection of the compound into rats in a dose of 25 mg/kg (1) and after its administration by gastric tube in a dose of 200 mg/kg (2) on the 21st day of pregnancy. Abscissa, time after administration of DMBA (in min); ordinate, DMBA concentration in fetal tissues (in μg/g).

the experiments with DMBA the rats were killed at various times after administration of the compound: 5 and 30 min and 1, 2, 3, 4, and 5 h. In the experiments with MC and BP the rats were killed 3 h after receiving the compound. At each of the above times at least four animals were killed. The uterus with the fetuses was extracted and washed to remove blood in 0.9% NaCl solution. The uterus was opened, the fetuses exposed and freed from membranes, and then washed consecutively with 96% ethanol, acetone, and ether in order to prevent polycyclic hydrocarbons from falling on the surface of the fetuses from outside during the manipulations of dissection and removal. The concentration of polycyclic hydrocarbons was determined separately in the fetuses and their placentas taken from the same rat, and also in its liver. The method of extracting the tissues, of thin-layer chromatography, and of recording quasilinear fluorescence spectra of the fractions containing DMBA was described earlier [2]. By comparing the records obtained with the quasilinear spectrum of pure DMBA, MC, and BP it could be deduced whether any of these compounds were present in the sample; if so they could then be determined quantitatively by the use of an internal standard. 1,2-Benzyrene (analytical line 3878 Å) was used as the internal standard for DMBA and MC, and 1,12-benzperylene (analytical line 4063 Å) for BP. The analytical lines of the spectra of the compounds were: DMBA 3983 Å, MC 3926 Å, and BP 4030.5 and 4085 Å. The sensitivity of these methods was about 0.01 μg of the compound.

EXPERIMENTAL RESULTS

The results of the quantitative determination of DMBA in the liver of the pregnant rats and in the placentas and also the dynamics of penetration of the unchanged substance into the fetal tissues are illustrated in Fig. 1. Clearly during the first 30 min after administration of DMBA to the pregnant rats the compound could be detected in the fetal tissues but only in traces. An increase in the concentration of the carcinogen in the fetal tissues was evident after 1-4 h; it reached a maximum after 2-3 h when its level was considerable, namely 1.53-1.6 μg/g. Only traces of DMBA could be found in the fetuses 5 h after its administration to the mother.

Changes in the DMBA concentration in the tissues of the liver and placenta in relation to the time elapsing after administration of the compound to the pregnant rats were similar to those observed in the fetuses. However, the ratio between the DMBA concentrations determined after 2 h in the liver, placentas, and fetuses was 10 : 1.5 : 1.

The penetration of other polycyclic hydrocarbons (BP and MC) was compared only 3 h after administration of the compounds, for preliminary experiments showed that the dynamics of their uptake by the fetus

TABLE 1. Concentration (in μg/g) of DMBA, BP, and MC in Tissues of 21-Day Fetuses, Their Placentas, and the Liver of Pregnant Rats 3 h after Administration of the Compounds in a Dose of 200 mg/kg by Gastric Tube

Carcinogen administered	Fetuses		Placentas		Liver	
	M and limits of variations	No. of analyses	M and limits of variations	No. of anlyses	M and limits of variations	No. of analyses
DMBA	1,53 (0,63—3,50)	4	1,28 (0,86—1,60)	4	8,80(5,50—11,80)	4
BP	2,77 (1,67—5,00)	5	3,94 (2,24—6,15)	5	23,50 (11,10—50,20)	4
MC	0,0013 (traces—0,002)	6	0,009 (traces—0,03)	6	0,10 (0,03—0,22)	6

was similar in principle. The quantitative results of this series of experiments are given in Table 1. After administration of the same dose (200 mg/kg) to pregnant rats BP passed through the placenta to the fetus in considerable amounts (2.77 μg/g), greater than those for DMBA. On the other hand, MC passed to the fetus only in trace amounts. The results with MC, it should be noted, were not explained due to the wrong choice of time of recording after administration of the compound. In a special series of experiments in which the accumulation of MC in the fetal tissues was determined after 1, 2, and 5 h, only traces of the compound likewise were detected (0.014-0.035 μg/g).

The differences found between the concentrations of the polycyclic hydrocarbons in the fetal tissues do not reflect their ability to cross the placental barrier. The concentration of MC in the liver of the pregnant rats was extremely low, but that of BP was much higher then the concentration of DMBA (Table 1). Nevertheless the concentration of a compound in the blood can be estimated from its concentration in an organ like the liver, with a rich blood supply. On these grounds it can be concluded that MC is absorbed to a much lesser degree from the gastrointestinal tract into the blood stream in rats than the other polycyclic hydrocarbons, as a result of which only very small quantities of it reach the fetus.

Comparison of the dynamics of the passage of DMBA into the fetal tissues after its intravenous and intragastric administration to pregnant animals (Fig. 2) showed the following results: first, the maximum of accumulation of DMBA in the fetal tissues occurred 1 h after intravenous injection but 2-3 h after intragastric administration; second, the maximum of its accumulation after intravenous injection was limited to a short period of time (about 15 min in the interval between 45 and 60 min), whereas after intragastric administration the maximum was much more prolonged (about 2 h in the interval between 2 and 4 h); third, the concentration of the compound in the fetal tissues was roughly of the same magnitude (1.5-2 μg/g) when administered by both ways, even though the dose of DMBA injected intravenously was 25 mg/kg, compared with 200 mg/kg by the intragastric route. These differences can evidently be explained by the absorption of DMBA in much smaller quantities from the gastrointestinal tract into the blood stream of the pregnant female and the consequent delay in reaching its maximal concentration.

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