CHANGES IN CYTOCHROME P-450 CONTENT AND MICROSOMAL AND CYTOSOL GLUTATHIONE TRANSFERASE ACTIVITY IN GUINEA PIG LIVER FOLLOWING CUTANEOUS APPLICATION OF PETROLEUM-DERIVED MINERAL OIL CONTAINING POLYCYCLIC AROMATIC HYDROCARBONS

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Prolonged contact of the skin of laboratory animals with mineral oils, isolated from petroleum under mild conditions (distillates) and containing a considerable quantity of polycyclic aromatic hydrocarbons (PAH), induces an increase in the frequency of formation of malignant skin and other tumors [2, 5]. Initiation of these malignant processes is associated with the induction of cytochrome P-450 (P-450) and of dependent monooxygenases in these organs, which participate in the oxidation of PAH with the intermediate formation of epoxide derivatives, which possess high genotoxic potential [1, 9]. Two pathways of enzymic detoxication of PAH epoxides exist in liver cells. The first is connected with epoxyhydrolase activity and the formation of diols of PAH [7], whereas the second is connected with glutathione transferase (GT) activity and with the formation of glutathione conjugates of PAH. Diols of PAH thus formed, under certain conditions, may once again become substrates for oxidation by microsomal monooxygenases, with the formation of even more genotoxic diol-epoxides of PAH [10], which are the final carcinogenic form for many PAH. Glutathione conjugates are true detoxication products of PAH epoxides. The aim of this investigation was to determine changes in the P-450 level and GT activity in microsomes (GTM) and cytosol (GTC) of guinea pig liver under the influence of prolonged application of various doses of Mark D-11 mineral oil distillate (MOD), obtained from Baku petroleum and containing up to 10% of PAH, and also to study dependence of values of the P-450/GT_m and P-450/GT_c ratios, each established relative to control values of P-450, GT_m, and GT_c, on the dose of MOD.

EXPERIMENTAL METHOD

Experiments were carried out on speckled male guinea pigs weighing 300-350 g. The animals were kept on a balanced protein diet. Food and water were provided ad libitum. Altogether there were five groups of guinea pigs, to a depiled area of whose skin measuring 20 cm^2 0.4 ml of one of the following substances was applied daily for 20 days: 0.9% NaCl (control), 100% MOD, 50% solution of MOD in furfurol, 3% solution of MOD in ethanol, and 0.5% solution of MOD in ethanol. Each of the above substances was applied to the skin by the method of open epicutaneous application. A single exposure lasted 4 h. The substances applied to the skin were then removed with ethanol. During exposure to the test substances the guinea pigs were kept in individual cages. On the 21st day of the experiment, 1 day after the final exposure of the guinea pigs' skin to MOD, the animals were decapitated. The microsomal and cytosol fractions of the liver were obtained by differential centrifugation of the crude homogenate [3] on a Beckman L8-70 ultracentrifuge (USA) in the SW×55Ti rotor. The microsomal residue thus obtained was washed free from adsorbed proteins by precipitation under the same conditions. All procedures involved in isolation of subcellular fractions were carried out at 4°C. Concentrations of cytochrome P-450 and cytochrome b_5 (b_5) in the microsomes

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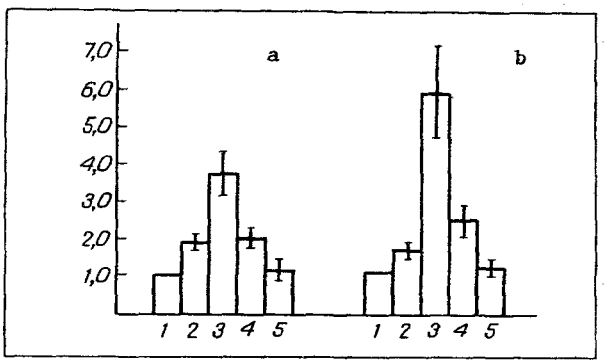

Fig. 1. Ratios P-450/GT_c and P-450/GT_m in guinea pig liver after 20 applications of different doses of MOD to skin. Ordinate, ratios of levels of cytochrome P-450 and GT_c or GT_m activity: a) P-450/GT_c, b) P-450/GT_m. Abscissa, groups of animals: 1) control, 2-5) receiving MOD as 100, 50, 3, and 0.5% solutions.

TABLE 1. Concentrations of Cytochromes P-450 and b_5 and GT_m and GT_c Activity in Guinea Pig Liver after Long-Term Application of Various Doses of Petroleum-Derived MOD ($M \pm m$)

Group of animals	Preparation	Dose of MOD, mg	Cytochrome		GT_m	GT_c
			P-450	b_5	nmoles/min/mg protein	
			nmoles/mg protein			
1- (n=5)	0,9 % NaCl	0	0,719±0,028	0,507±0,027	443,2±23,08	3123±94,6
2- (n=5)	100 % MOD	460	1,274±0,084***	0,495±0,026	492,0±22,22	3194±136
3- (n=7)	50 % MOD	210,1	1,603±0,145***	0,506±0,016	179,7±38,99***	1900±183**
4- (n=8)	3 % MOD	13,8	1,172±0,038***	0,508±0,020	305,6±53,75*	2500±154**
5- (n=7)	0,5 % MOD	2,3	0,992±0,068**	0,503±0,010	519,1±90,21	3074±124

Legend. Number of animals in group given in parentheses. Significance of differences between control and experimental groups: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

were determined by the method in [8]. Activity of GT_m and GT_c was determined by the method in [4], with 1-chloro-2,4-nitrobenzene as the substrate, at 36°C. Microsomal and cytosol protein was determined by the method in [6]. All spectral measurements were made on a "Specord" spectrophotometer (East Germany) with constant-temperature cuvette. The results were subjected to statistical analysis by the Fisher–Student parametric test. Differences were considered to be statistically significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

The results showed that application of various doses of MOD to the skin for 20 days causes a marked increase of 38-123% in the P-450 concentration. The level of induction was highest on application of 50% MOD solution and lowest on application of a 0.5% solution of MOD. In three groups receiving MOD solutions, dependence of the level of P-450 induction on the dose of MOD was linear, and differences between the groups were significant. The mean degree of induction of P-450 under the influence of 100% MOD may be attributable to the very low permeability of the skin barrier for P-450 inducers from 100% MOD, which gave rise to a marked inflammatory reaction. The b_5 concentration was not significantly changed by various doses of MOD.

Prolonged application of 50% and 3% solutions of MOD to the skin reduced GT_m activity by 59 and 31% and GT_c activity by 39 and 20%, respectively. The degree of inhibition of the two GT isozymes depended on the dose of MOD, and it was 1.5-2 times higher in the group receiving a 50% solution of MOD than after application of the 3% solution of MOD. On application of the 0.5% MOD solution to the skin no changes were observed in GT_m and GT_c activity.

Since it has been suggested that significant correlation exists between the rates of formation and removal of genotoxic metabolites of PAH, present in the composition of MOD, values of the P-450/GT_m and P-450/GT_c ratios were determined, with the stipulation that the parallel control value be taken as 1 (Fig. 1). The greatest values of these ratios were observed in the group receiving a 50% solution of MOD, where they amounted to 5.9 and 3.7, respectively. In the group receiving the 3% MOD solution these ratios were significantly lower, namely 2.45 and 2.05, respectively. In the group of animals receiving the 0.5% solution of MOD, the ratio P-450/GT_m did not exceed 1.54 and the P-450/GT_c ratio did not differ from the control. Thus long-term application of various doses of MOD to the skin gave rise to significant changes in the activation and inactivation of intermediate metabolites of PAH. With an increase in the dose of MOD, activation processes predominated increasingly over detoxication processes. It can be tentatively suggested that the threshold of the toxic action of MOD lies within the region from 2.3 to 13.8 mg per animal.

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