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Effect of the Benzene Fraction of Petroleum on Protein Content in Rat Liver and Kidney

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Barr-Nea and Wolman (1977) and Van Duuren et al. (1979) in their studies on petroleum toxicity and carcinogenesis rubbed petroleum into the skin of mice and found that the animals developed malignant tumors of the lymphatic system and papilloma. The authors suggested that the effect observed was due to the presence in petroleum of aromatic hydrocarbons as well as hydrocarbons of different degree of oxidation.
Although the benzene fraction of mineral oil contains only trace amounts of these types of hydrocarbons, it was found to cause hypertrophy of hypophysis and adrenal cortex in animals subjected to chronic intoxication (Przybyłowski et al. 1978).
In the present work we demonstrated that the R-33 benzene fraction of petroleum caused in the rat hypertrophy of liver and kidney, changes in total protein content in these organs and changes in the protein synthetizing system, both when it was applied intraperitoneally and in the form of chronic inhalation of vapors.

MATERIALS AND METHODS

White, male Wistar rats weighing 160 - 200 g were used for the experiments. Animals were fed with LSM standard mixture (Bacutil,Warsaw) and had water ad libitum. The LSM standard mixture contained: bran, ground grain, defatted milk powder, fodder yeast, mineral salts and vitamins.
Animals were intoxicated with the R-33 benzene fraction of petroleum from Masovian Petrochemical Works in Płock.
The characteristics of the fraction given by the Analytical Laboratory of Masovian Petrochemical Works is as follows: boiling range: 98 - 163°C, chemical composition: nonaromatic compounds (paraffin and cycloparaffins) - 92.59%, benzene - 0.31%, toluene - 1.96%, ethylbenzene - 0.93%, para-xylene - 0.41%, meta-xylene - 1.27%, orthoxylene - 0.75%, kumene - 0.24%, C_9-aroma-

tic compounds - 1.54%.
Animals were given an intraperitoneal injection of the R-33 benzene in a single dose of 3.09 ml/kg body weight (LD_{25}) . Control animals were injected with the same volume of 0.9% NaCl.
Animals were exposed to vapors of benzene at a concentration of 33.75 g/m^3 in an inhalation chamber (Rusiecki et al.1977) for 6 hrs daily, 6 times a week for a period ranging from 3 to 90 days.
Rats were decapitated without anaesthesia and liver and kidney were isolated.
Tissues were homogenized in 2 volumes of water in a Potter-type glass homogenizer. Homogenate was used for determination of total protein content.
Tissue (liver or kidney) was cut into small pieces and homogenized in 4 volumes of 50 mM Tris HCl buffer pH 7.6 containing 25 mM KCl, 10 mM $MgCl_2$ and 0.25 M sucrose.Homogenate was centrifuged for 15 min at 15 000 x g. Mitochondrial pellet was discarded and the supernatant centrifuged for 60 min at 105 000 x g; the microsomal pellet obtained was homogenized in 50 mM Tris HCl buffer pH 7.6 containing 80 mM KCl, 6 mM $MgCl_2$, 4 mM DDT and 0.25 M sucrose and used for [^{14}C]leucine incorporation as a cell-free system. The supermatant after 105 000 x g centrifugation served as the cytoplasmic fraction.
The incubation mixture in a total volume of 0.25 ml contained: 60 mM Tris HCl pH 7.6, 60 mM KCl, 8 mM $MgCl_2$, 4 mM mercaptoethanol, 0.2 mM GTP, 5 mM ATP, 10 mM phosphenolpyruvate, 40 µg of pyruvate kinase, mixture of non-radioactive aminoacids (10^{-5} M each) , 50 mM [^{14}C]leucine, microsomal fraction equivalent to 200 mg of protein and cytoplasmic fraction equivalent to 1 mg of protein. After 20 min of incubation at 37°C 3 ml of 7% TCA was added and the mixture left for several hours in an ice-bath. Then it was filtered through Whatman GF/C filter paper. The collected precipitate was washed three times with 5 ml portions of 7% TCA, dried, and radioactivity was counted.
Filter paper discs with the applied precipitate were placed in liquid scintillator composed of 0.5% PPO, 0.01% POPOP in toluene. Radioactivity was counted in Tri-Carb liquid scintillation counter (Packard) with 80% efficiency. The amount of incorporated [^{14}C]leucine was expressed as [^{14}C]dpm.
Total protein was estimated by the method of Lowry et al. (1951) with bovine serum albumin as standard.

RESULTS AND DISCUSSION

In rats subjected to a single intraperitoneal dose of the R-33 benzene fraction of petroleum a hypertrophy of

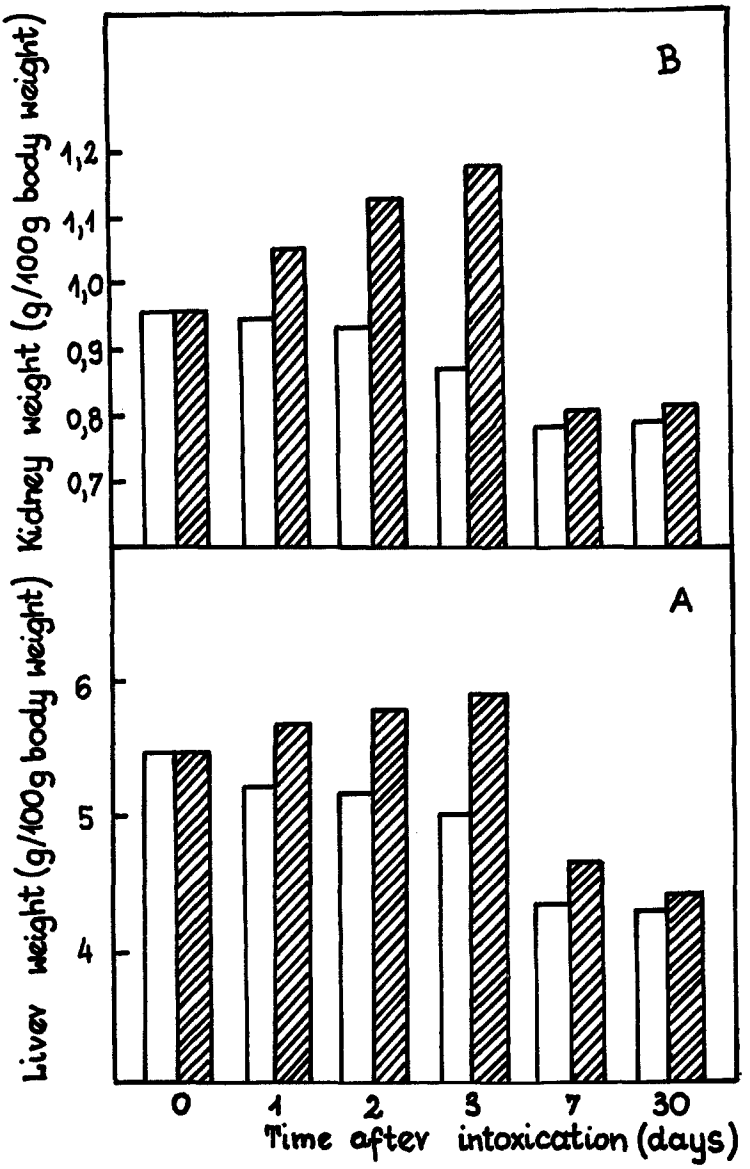


Fig.1. Hypertrophy of liver (A) and kidney (B) in rats intoxicated with a single dose of R-33 benzene fraction of petroleum LD_{25} applied via intraperitoneal injection. Outlined columns - control animals, dashed columns - intoxicated animals.

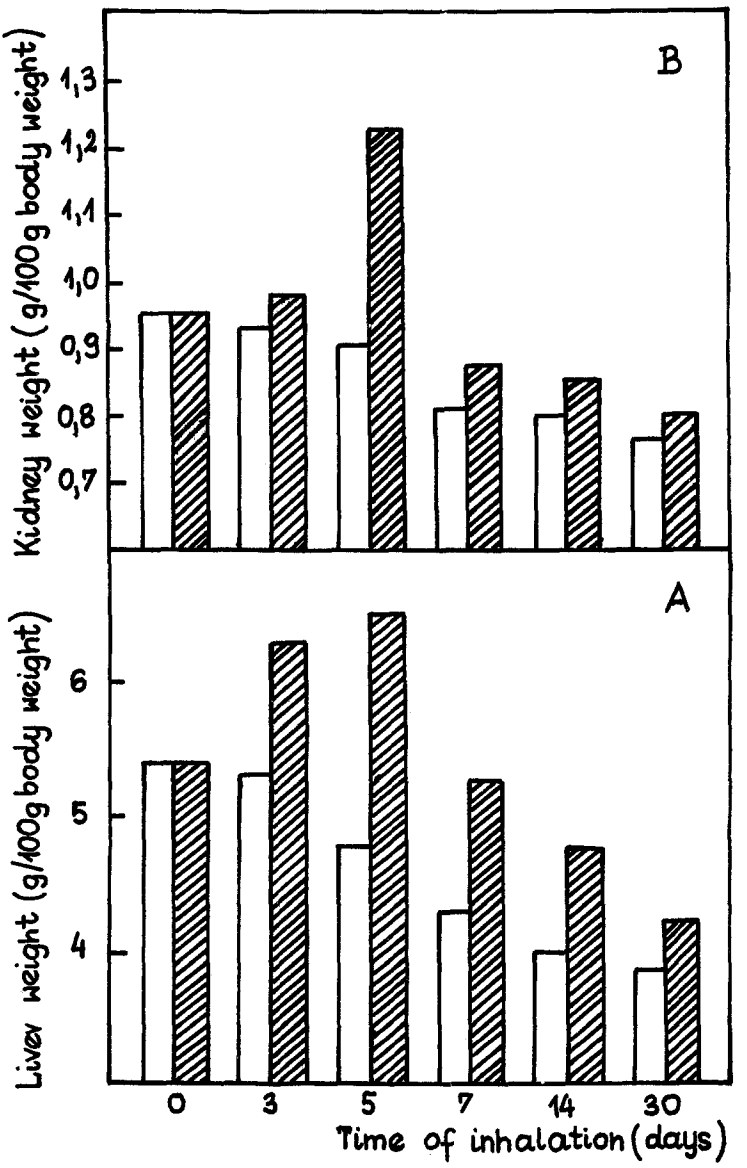


Fig. 2. Hypertrophy of liver (A) and kidney (B) in rats exposed to inhalation of vapors of the R-33 benzene fraction of petroleum for 6 hrs daily, 6 days a week for the time indicated. Outlined columns - control animals, dashed columns - intoxicated animals.

liver and kidney was observed on the first day after intoxication (Fig.1A and 1B).
The hypertrophy reached a maximum on the third day, and declined after a week. Liver and kidney hypertrophy was also observed in animals subjected to inhalation of vapors (Fig. 2A and 2B).
In this type of intoxication, hypertrophy appeared after three days and reached a maximum after five days. Small differences in liver and kidney weight, were as compared with controls, observed even after three months of inhalation.

Table 1. Effect of single intraperitoneal injection with a LD_{25} dose of the R-33 benzene fraction of petroleum on total protein content in rat liver and kidney.

Time after intoxication (days)	Number of animals	Protein content			
		liver		kidney	
		mg/g of tissue	% control	mg/g of tissue	% control
0	15	156.1±4.8	100	97.6±4.3	100
1	7	161.4±5.6	102	108.1±3.1	110
2	7	161.6±3.8	104	104.6±3.7	108
3	7	x180.5±5.2	116	x160.1±4.8	165
5	8	157.4±8.3	101	x135.9±3.4	139
30	8	158.1±7.8	101	98.2±8.1	101

Experimental conditions as described in Methods.
Results are mean values ± mean standard deviation.
x $p \leq 0.05$ (Student's "t" test)

The increased weight of organs could result from retention of water or from increased content of total protein. In the case of intoxication by intraperitoneal injection the increase in organ weight was paralleled by the increase in protein content (Table 1).
On the third day after intoxication, protein content was increased by 16% and 65% in liver and kidney, respectively. In liver the protein level was normalized by the end of the fifth day but in kidney it remained still increased after a month. The above results suggest that the increased organ mass observed after intraperitoneal intoxication could be a result of increased cellular protein level. However, in the case of intoxication by inhalation of vapors there were no changes of protein level were observed either in liver or in kidney (Table 2).

Table 2. Effect of intoxication by inhalation of vapors of the R-33 benzene fraction of petroleum on total protein content of rat liver and kidney.

Time of intoxication (days)	Number of animals	Protein content			
		liver		kidney	
		mg/g of tissue	% control	mg/g of tissue	% control
0	52	162.3±1.2	100	102.7±0.9	100
3	16	163.9±1.5	101	x113.2±1.1	110
5	16	164.7±1.3	101	x115.3±1.1	112
14	14	169.3±1.4	104	105.9±1.2	103
30	8	170.9±1.7	105	105.3±1.2	102
90	8	172.4±2.1	106	104.9±1.4	102

Experimental conditions as described in Methods. Intoxication was performed in an inhalation chamber for 6 hrs daily, 6 days a week for the time indicated. Results are mean values ± mean standard deviation.
x $p \leq 0.05$ (Student's "t" test)

Table 3. Effect of intoxication by intraperitoneal injection and by inhalation of vapors on the protein level of microsomal fraction from rat liver and kidney.

Type of intoxication	Protein mg/g of tissue		
	control animals	intoxication animals	% control
Intraperitoneal (third day after injection)			
liver	33.72±1.98 (10)	x51.04±3.02 (9)	151
kidney	15.90±0,93 (10)	x19.77±0,98 (9)	124
Inhalation (fifth day after inhalation)			
liver	34.62±2.01 (12)	x48.06±2.89 (12)	139
kidney	16.08±0.95 (12)	x21.41±0.94 (12)	133

Experimental conditions as described in Methods. Number of animals is given in brackets. Results are mean values ± mean standard deviation.
x $p \leq 0.05$ (Student's "t" test)

In both types of intoxication the protein content of microsomal fraction was considerably increased for both in liver and kidney (Table 3).
A similar increase in protein content was reported by Sladek and Meannering (1969) and Nelson and Kearney (1977) in their studies on toxicity of lipid-soluble substances. The benzene fraction studied by us could therefore induce the biosynthesis of enzymatic systems active in biotransformation of xenobiotics.

Table 4. Effect of intoxication by intraperitoneal injection with the R-33 benzene fraction of petroleum on incorporation of [^{14}C]leucine into protein in cell-free systems from rat liver and kidney.

Microsomes	Supernatant	Specific activity dpm/mg protein	% control
Liver			
control	control	4995 ± 240	100
intoxicated	intoxicated	x8353 ± 320	167
intoxicated	control	x4095 ± 210	82
control	intoxicated	x8018 ± 310	161
Kidney			
control	control	5046 ± 260	100
intoxicated	intoxicated	x10834 ± 380	215
intoxicated	control	x14227 ± 420	282
control	intoxicated	x4220 ± 230	84

Microsomes and supernatant were isolated from control and intoxicated (3 days after intoxication) animals as indicated. Experimental conditions as described in Methods. Results of 6 experiments (12 animals in each group) are expressed as mean values ± mean standard deviation.
x $p \leq 0.05$ (Student's "t" test)

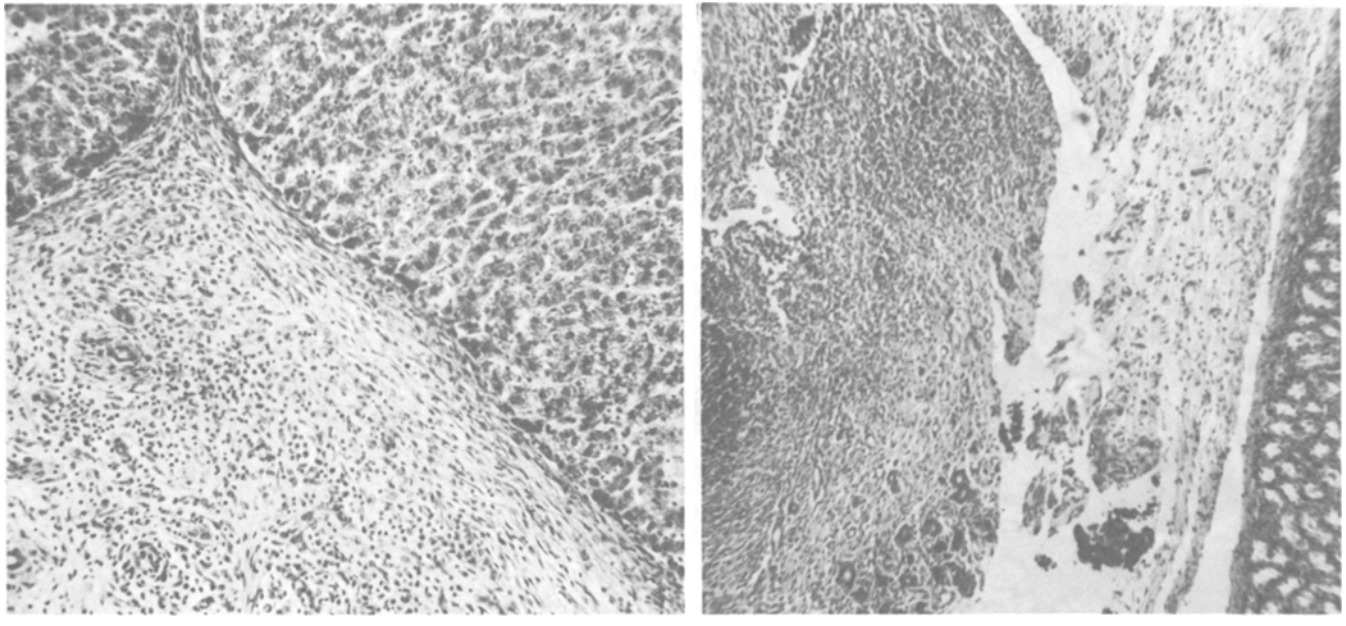
On intoxication by intraperitoneal injection the rate of [^{14}C]leucine incorporation into proteins differed for cell-free systems isolated from liver and kidney (Table 4).
In liver for which a 50% increase was observed the rate of incorporation was dependent on cytoplasmic elements

Table 5. Effect of intoxication by inhalation of vapors of the R-33 benzene fraction of petroleum on incorporation of [^{14}C]leucine into proteins in cell-free systems from rat liver and kidney.

Microsomes	Supernatant	Specific activity dpm/mg protein	% control
Liver			
control	control	5731 ± 280	100
intoxicated	intoxicated	x6291 ± 290	110
intoxicated	control	x4730 ± 240	83
control	intoxicated	x6060 ± 300	106
Kidney			
control	control	4275 ± 220	100
intoxicated	intoxicated	x5079 ± 270	119
intoxicated	control	x4947 ± 260	116
control	intoxicated	x3115 ± 280	73

Microsomes and supernatant were isolated from control and intoxicated (5 days) animals as indicated. Experimental conditions as described in Methods. Results of 6 experiments (12 animals in each group) are expressed as mean values ± mean standard deviation.
x $p \leq 0.05$ (Student's "t" test)

of the synthetizing system whereas in kidney, microsomes were responsible for a three-fold increase of incorporation. In the two types of intoxication studied the systems incorporating [^{14}C]leucine into proteins isolated from both organs showed the same pattern of changes except that for the intoxication by vapor inhalation the changes were less pronounced (Table 5).
On the basis of the above observations it can be suggested that hypertrophy of liver and kidney caused by the intoxication with the R-33 benzene fraction of petroleum is related with results from increased level of total protein. The latter could reflect the regeneration processes occurring in tissues damaged by intoxication. Such damage was demonstrated by histological examination of organs of intraperitoneally intoxicated rats (Fig.3).
Similar damage was also reported for birds fed with petroleum contaminated food (Szaro et al. 1978).
The observed stimulation of protein synthesis seems to result from increased activity of cytoplasmic factors in liver and of polysomes in kidney. The recessive character of changes and increased content of microsomal protein point to the possibility of induction



A B

Fig.3. Proliferation of fibroblasts in rats a week after a single, intraperitoneal injection of the R-33 benzene fraction of petroleum. A - within the liver sac, B - within the kidney sac. Magnification 100x, stained with hematoxyline and eosine.

in rat tissues of metabolic systems responsible for biotransformation of exogenous substances.

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