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Tyrosine Aminotransferase Activity in the Benzene Intoxicated Rat

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Petroleum and its lipophilic derivatives when accumulated in animal tissues (Lawler et al. 1978), cause changes in nervous and haematopoietic system (Browning 1965, Axelson et al. 1976) as well as structural and functional damage of liver and kidney (Przybyłowski 1974). The toxic effect of hydrocarbon solvents on hepatic metabolism manifests itself by changes in the enzymatic pattern of blood serum. Changes in the activity of phosphatases (Kala et al. 1978) as well as leucine aminopeptidase, glutamine aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase (Przybyłowski et al. 1978) were observed in rats intoxicated with different fractions of benzene.
Therefore it seemed reasonable to investigate the effect of benzene fraction of petroleum on cellular metabolism. The results of the present work concern the activity of tyrosine aminotransferase (EC 2.6.1.5.), the enzyme involved in catabolism of aromatic amino acid which is known to be under both hormonal (Gelehrter 1976) and stress dependent (Geller et al. 1969) control. Changes in tyrosine aminotransferase activity effect the level of tyrosine oxidation as well as the metabolic conversion of this amino acid into tyramine, tyroxin, adrenaline and noradrenaline.

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, chemi-

cal composition: nonaromatic compounds (paraffins, cycloparaffins) - 92.59%, benzene - 0.31%, toluene - 1.96%, ethylbenzene - 0.93%, para-xylene - 0.41%, meta-xylene - 1.27%, ortho-xylene - 0.75%, kumene - 0.24%, C_9-aromatic compounds - 1.54%.
Animals were given an intraperitoneal injection of 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, six times a week for a period ranging from 3 to 90 days.
Rats were decapitated without anaesthesia and liver and kidney were isolated.
Bilateral adrenalectomy was performed by the method of Brady and Bunger (1979). After the operation animals were kept for 5 days on standard diet and had the 0.9% solution of NaCl ad libitum. Control group consisted of sham-operated animals.
Hydrocortisone was given intraperitoneally in a single dose of 3 mg per 100 g body weight always at 6 hrs before sacrificing the animal.
Tissues were homogenized with 9 volumes of 0.25 M sucrose in a Potter glass homogenizer for 3 min at 4°C. Homogenate was centrifuged for 10 min at 600 x g. This was followed by 15 min centrifugation at 14 000 x g. The obtained supernatant was used for estimation of protein and the activity of tyrosine aminotransferase.
Tyrosine aminotransferase activity was determined by the method of Diamondstone (1966).
Total protein was estimated by the method of Lowry et al. (1951) with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Intoxication of rat with a single LD_{25} dose of the benzene fraction of petroleum injected intraperitoneally affected the activity of tyrosine aminotransferase in both liver and kidney (Table 1).
Activity of the enzyme in liver increased by 76% on the third day after intoxication and remained increased by 40% after a week. It returned to the control level after a month. In kidney a 50% increase of activity was observed on the third day after intoxication. The activity decreased after a week but then rose again and after a month reached the level by 15% higher than control.
The observed changes were correlated with changes in total protein content. In liver the increase in enzyme activity was paralleled by the increase in total protein (Fig.1A). In kidney such a correlation was observed up to the third day after intoxication but

Table 1. Activity of tyrosine aminotransferase in liver and kidney of rats intoxicated by intraperitoneal injection of a single LD_{25} dose of the benzene fraction of petroleum.

Time after intoxication (days)	Number of animals	Liver		Kidney	
		Activity nmole/g of tissue	% control	Activity nmole/g of tissue	% control
0	40	2163±46	100	347±14	100
1	18	x2544±71	118	x396±19	113
2	16	x2814±86	130	x473±23	136
3	18	x3816±97	176	x538±22	155
6	14	x3024±104	140	x303±20	87
30	16	2226±78	103	x401±17	115

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

not after 7 and 30 days (Fig. 1B).
In contrast to the effect of inoxication by intraperitoneal injection the inhalation of vapors resulted in a decrease of tyrosine aminotransferase activity (Table 2).

Table 2. Activity of tyrosine aminotransferase in liver and kidney of rats intoxicated by inhalation of vapors of the benzene fraction of petroleum.

Time of intoxication (days)	Number of animals	Liver		Kidney	
		Activity nmole/g of tissue	% control	Activity nmole/g of tissue	% control
0	40	2280±56	100	352±10	100
3	10	x2010±74	88	x290±12	82
6	12	x1962±68	86	x308±14	88
14	16	2360±79	104	x462±16	131
28	10	x2688±83	118	x405±19	115
90	10	2316±65	101	355±16	101

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

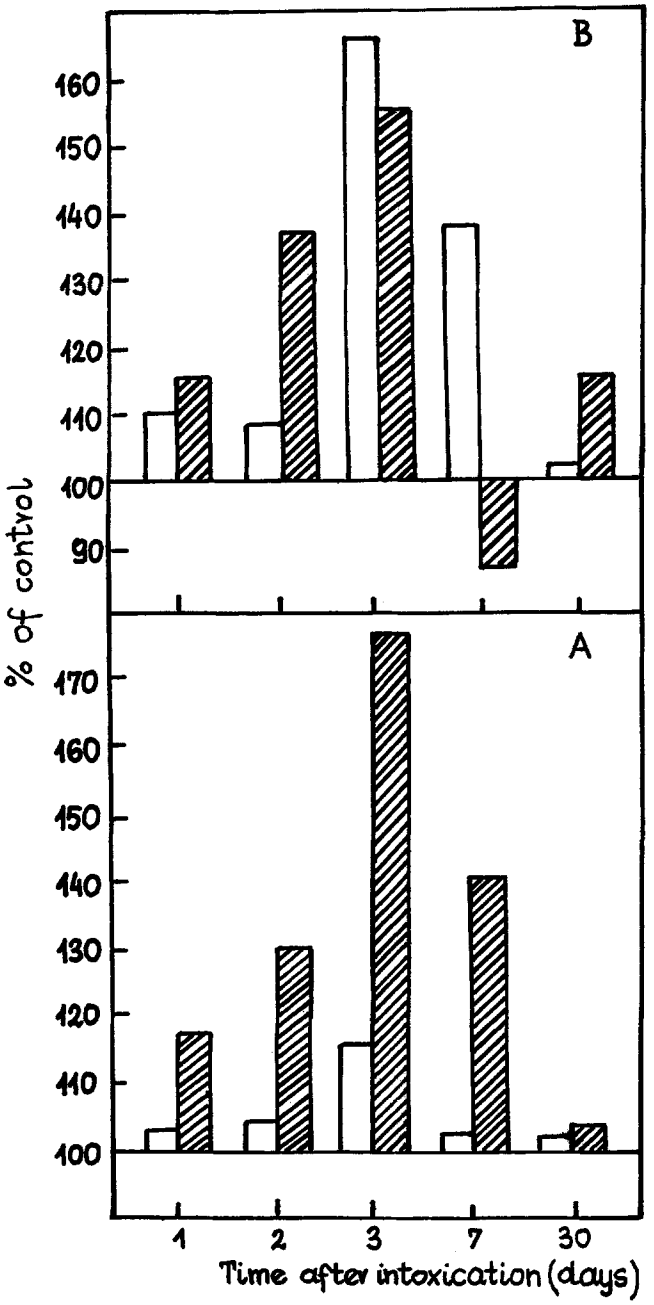

Fig.1. Changes in tyrosine aminotransferase activity and total protein content in liver (A) and kidney (B) of rat after intoxication with single intraperitoneal injection of the benzene fraction of petroleum. Outlined columns - total protein content. Dashed columns - activity of tyrosine aminotransferase.

During the first week of exposition to benzene vapors the decrease of the enzyme activity reached 15% in liver and 20% in kidney. Changes were not stable and recessed within several days when the inhalations were stopped (Table 3) .

Table 3. Reversibility of changes in tyrosine aminotransferase activity in liver and kidney of rats inhalated one week followed by days without treatment.

Time after intoxication (days)	Number of animals	Liver		Kidney	
		Activity nmole/g of tissue	% control	Activity nmole/g of tissue	%control
Control	40	2280±56	100	352±10	100
1	16	x1960±42	86	x309±11	88
2	15	x1824±46	80	x285± 9	81
6	13	2143±52	94	352±11	100
30	11	2462±57	108	380±13	108

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

Prolongation of the intoxication period up to one month resulted in a 20% increase in the enzyme activity in liver and 15% in kidney (Table 2) . The increase, probably of a compensatory nature, occurred in liver concomitantly with the increase in total protein content (Fig.2A) . This type of correlation was not observed in kidney (Fig.2B) .
In rats intoxicated for a period of three months the activity of tyrosine aminotransferase was the same as in control animals.
The character of changes occurring in the initial period of intoxication suggested that they could result not only from the direct toxic action of benzene but also from the stress due to by the application of an exogenous substance. Intoxication of sea-gulls by orally administred petroleum resulted in the appearance of stress symptoms in the form of adrenal hypertrophy and increased synthesis of steroids (Miller et al. 1978) . Geller et al. (1969) reported that tyrosine aminotransferase activity in liver increased in stress conditions due to steroid-hormones dependent induction of the apoenzyme. In animals subjected to adrenalectomy and hence with no stress-stimulated overproduction of

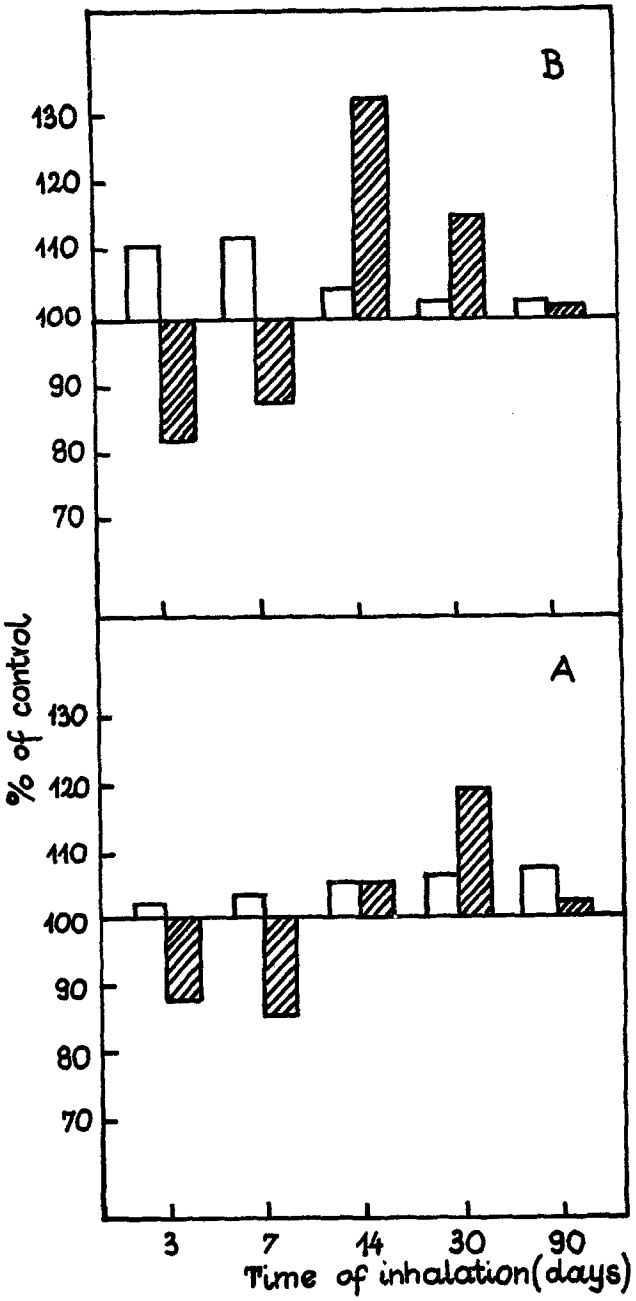

Fig.2. Changes in tyrosine aminotransferase activity and total protein content in liver (A) and kidney (B) of rat intoxicated by inhalation of vapors of the benzene fraction of petroleum. Outlined columns - total protein content. Dashed columns - activity of tyrosine aminotransferase.

steroids intoxication with the benzene fraction resulted not in an increase but on the contrary in lowering of the activity of liver aminotransferase (Table 4). The decrease in activity was prevented by administration of hydrocortisone. The increase in activity correlated with the increase in total protein content observed after intraperitoneal intoxication in a conditions of a severe stress, could result from steroid-dependent enzyme induction. On intoxication by inhalation of vapors, the animals were subjected by much weaker stress, hence the increase in steroid level was probably insufficient for induction of aminotransferase.

Table 4. Activity of liver tyrosine aminotransferase in non-operated rats and rats after bilateral adrenalectomy, intoxicated with the benzene fraction of petroleum.

Substance administered	Animals	Activity % control
benzene fraction (intraperitoneally)	non-operated	176
	after adrenalectomy	75
benzene fraction (intraperitoneally + hydrocortisone)	after adrenalectomy	176
benzene fraction (inhalation)	non-operated	88
	after adrenalectomy	32
benzene fraction (inhalation + hydrocortisone)	after adrenalectomy	93

Benzene fraction was administred to animals on the fifth day after bilateral adrenalectomy. Activity of liver tyrosine aminotransferase was determined on the third day after intraperitoneal intoxication or after three days of inhalation.
Hydrocortisone was administered in a single dose, intraperitoneally, always at 6 hrs before sacrificing.
Experimental conditions as described in Methods.
Results are mean from 6 experiments.

Therefore the observed decrease of activity could be ascribed directly to the effect of the benzene fraction. The above results could suggest the involvement of glucocorticoids in the metabolic response of liver to intoxication under the conditions studied.
According to Jerina and Daly (1974) the intensity of detoxication in the organism of hydrocarbon solvents as well as other environmental agents depends on micro-

somal hydroxylating monooxygenases linked cytochrome P_{450}. The observed reversible character of changes in tyrosine aminotransferase activity suggests the existence of a mechanism of metabolic adaptation to the hydrocarbons administred. Thus in our experiments can not be excluded the stimulation of detoxication systems, the more so that their induction in sea-gulls upon oral intoxication with petroleum was demonstrated by Miller et al.(1978).

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