Biochemical Changes Induced by Ethyl Acetate in Blood and Liver of Rat

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Industrial and environmental hazards due to widespread applications and abuse of organic solvents have widely been recognized (CAPURRO 1970; FODOR and WINNEKE 1971). Ethyl acetate used in the manufacture of plastics, pharmaceuticals, fruity essences, perfumes and silk has been shown to produce central nervous system depression and deep narcosis in experimental animals. Repeated exposures of rabbits to ethyl acetate results in secondary anemia with leucocytosis, hypermia, cloudy swelling and fatty degeneration. Fatal accidental ethyl acetate poisoning has also been reported (VON OETTINGEN 1960). However, at present little is known about the biochemical lesions produced by this common solvent. We have, therefore, studied the effect of intraperitoneal administration of ethyl acetate on the pyruvic acid content of blood and liver and also on the activity of certain liver enzymes.

EXPERIMENTAL

Twenty male albino rats of I.T.R.C. colony (average weight 200 g) kept on ad libitum diet were divided into two groups. Animals of one group were injected with ethyl acetate (intraperitoneally, 1.0 ml/kg) daily for eight days and the animals of second group served as controls.

Animals fasted for 20 hours were stunned by blow on head and blood was collected in 10% trichloroacetic acid solution by cutting juglar veins. The liver was removed free of adhering materials and a portion was homogenised in 10% trichloroacetic acid solution. Pyruvic acid and lactic acid were estimated in protein free filtrate of blood and liver by the method of Friedemann and Haugen (1943) and Barker and Summerson (1941) respectively. Liver glycogen was assayed as described by Montgomery (1957). From the remaining portion of the tissue 10% (W/V) liver homogenates were immediately prepared in cold 0.25 M sucrose solution using Arthur-Thomas tissue grinder. Activity of acid phosphatase and 5'-nucleotidase (WOOTON 1964), glucose-6-phosphatase (SWANSON 1955), lactic dehydrogenase (KORNBERG 1955) and adenosine triphosphatase

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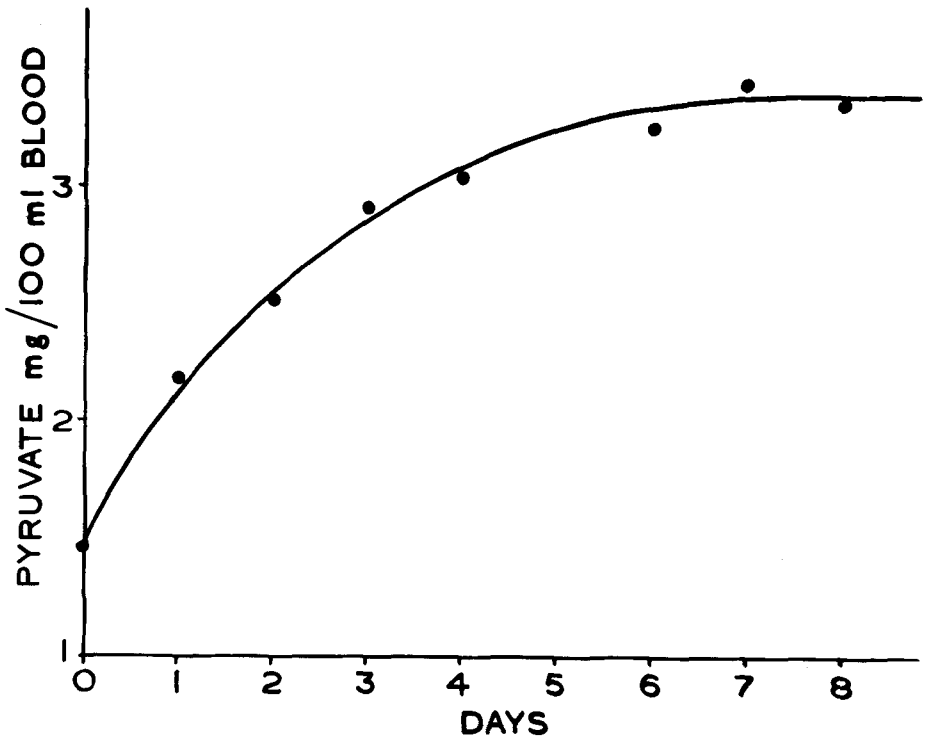
(SETH and TANGRI 1966) was measured after making suitable dilutions. Succinic dehydrogenase activity was estimated by a modification (DeROBERTIS et.al. 1962) of the method of Slater and Bonner (1952) and the proteins by the method of Lowry et al. (1951) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Effect of repeated administration of ethyl acetate on the pyruvic acid content of rat blood is shown in Fig. 1. The pyruvic acid content continued to increase steadily from 1st day to the seventh day and then probably levelled off, indicating that pyruvate metabolism is affected by this solvent. The level of blood pyruvic acid reflects roughly its concentrations in tissues from which it is derived. An

FIGURE I

Effect of Ethyl Acetate on Pyruvate Content of Rat blood at different time intervals



increase in its concentration may be due to either an increased synthesis or a decreased utilization or both. To understand this we studied the effect of ethyl acetate on the lactic acid content of blood and liver and on the glycogen content and activity of certain enzymes of liver. A significant decrease in the glycogen and increase in the pyruvic and lactic acid contents were observed in liver (Table I). The two-fold increase in the pyruvic acid and lactic acid levels was of the same order as observed in blood at this time interval.

TABLE I

Effect of ethyl acetate on pyruvic acid, lactic acid and glycogen levels

Estimation	Control	Experimental
Blood Pyruvic acid (mg/100 ml Blood)	1.46 ± 0.25 (6)	3.45 ± 0.11 (6)
Blood Lactic Acid mg/100 ml Blood)	9.20 ± 0.54 (7)	17.38 ± 0.44 (7)
Liver Pyruvic Acid (/ugm/gm Fresh tissue)	5.36 ± 0.43 (12)	10.70 ± 1.12 (12)
Liver Lactic Acid (/ugm/gm Fresh tissue)	121.00 ± 4.69 (7)	283.40 ± 9.59 (7)
Liver Glycogen (/ugm/gm Fresh tissue)	3.98 ± 0.21 (6)	2.21 ± 0.24 (6)

Figures in parentheses indicate number of animals used for estimations. All values are mean ± S.E.

Effect of ethyl acetate on the activity of certain rat liver enzymes is shown in Table II. Activity of glucose-6-phosphatase was significantly decreased indicating that more glucose-6-phosphate is available for other metabolic routes which are (i) its conversion to glucose-1-phosphate and glycogen (ii) its oxidation through the glycolysis and Kreb's cycle and (iii) its oxidation through hexose monophosphate shunt pathway.

TABLE II

Effect of ethyl acetate on the activity of certain liver enzymes

Enzyme	Control	Experimental
Glucose-6-Phosphatase[a]	64.53 ± 2.27 (9)	35.68 ± 4.71 (9)
ATPase[a]	67.52 ± 0.79 (7)	64.08 ± 0.62 (7)
5'-nucleotidase[a]	21.42 ± 0.19 (7)	20.79 ± 0.18 (7)
Acid Phosphatase[b]	19.94 ± 0.61 (6)	20.90 ± 1.20 (6)
Succinic Dehydrogenase[c]	33.70 ± 0.36 (6)	33.40 ± 0.66 (6)
Lactic Dehydrogenase[d]	692.92 ±19.27 (6)	1011.20 ± 53.24 (6)

Enzymatic activity expressed a nmoles of Pi liberated, b nmoles of phenol liberated, c nmoles of ferricynide reduced and d nmoles of NADH oxidised/minute/mg of protein. Figures in parentheses indicate number of animals used for estimation. All values are mean ± S.E.

Since a decrease in the liver glycogen content was observed (Table I) the conversion of glucose-6-phosphate to glycogen could be ruled out. A normal activity of succinic dehydrogenase in ethyl acetate treated animals suggests that the terminal oxidation of pyruvic acid also remains undisturbed. Thus the observed increase in pyruvic acid contents in liver and blood could be due to an increased rate of glycolysis. The increased activity of lactic acid contents supports this assumption. Effect of ethyl acetate on hexose monophosphate shunt remains to be investigated.

The activity of adenosine triphosphatase, 5'-nucleotidase and acid phosphatase were not significantly altered. This suggests that in general hydrolysis of phosphate esters is not affected by ethyl acetate.

Our results suggest that daily intraperitoneal administration up to eight days leads to biochemical

-alterations which might be responsible for its toxic effects.

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