

THE EFFECT OF LEAD ON PROTEIN AND LACTATE
DEHYDROGENASE ACTIVITY IN HEPATIC SLICES
CULTURED *IN VITRO*

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Slices of foetal and adult liver were cultured *in vitro* with various concentrations of lead ions. A decrease in protein content and an increase in lactate dehydrogenase activity in the culturing medium were found. The changes were dose-dependent, and the foetal liver tissue was found to be more susceptible to the effect of lead than the tissue of adult rats.

Toxic action of lead has been subject of numerous investigations. It is known that lead affects various organs of human and animal body. Hepatic changes in chronic lead poisoning have been reported in clinical studies (1). Pathological changes have also been observed with histochemical methods in the liver of animals exposed to lead compounds (1).

The aim of the present work was to investigate the toxicity of lead ions applied in various concentrations to hepatic slices cultures *in vitro*. The method of hepatic slice culturing was elaborated for estimation of drug hepatotoxicity and has been found useful in toxicological research (2—5).

MATERIAL AND METHODS

Hepatic slices for culturing were taken from foetal and adult rat liver. Rats of Wistar strain from the Central Experimental Animal House of the Silesian School of Medicine were used. Foetal tissue samples were taken from pregnant rats on 18—20th day of pregnancy. Liver samples were taken at autopsy from male, three-month-old, healthy rats.

Hepatic slices were cultured as described previously (5). Tissues were removed as fast as possible under sterile conditions. Samples were washed with phosphate buffered saline solution and cut with scissors

into small pieces. The crumbled tissue was weighted and 200 mg sample was transferred into a small conical flask containing 5 ml of Eagle's medium. The medium was supplemented with crystalline penicilline (100 U/ml) and streptomycine (100 µg/ml). The medium and saline solutions were purchased from the State Plant of Sera and Vaccines in Lublin. Cultures were aired with a mixture of oxygen and carbon dioxide (95 : 5 v/v), and were stored at 37 °C in a thermostat. The medium was changed every 24 hours and on the second day of culturing, lead acetate (Polish Chemical Reagents, Gliwice, Poland) was added to final concentrations of 0.20, 0.40, 0.60, and 0.80 mg of lead/L. After the second and the third change of the medium the same concentration of lead acetate were added. The control culture was supplemented with sodium acetate in the same concentration as the experimental cultures. The tissue culture without any additions to the medium served as control.

Protein content in the medium was measured after 24, 48, 72 hours of culturing with or without lead acetate using the method of *Lowry and co-workers* (6). Bovine serum albumin served as standard. In the same media, the activity of lactate dehydrogenase was determined with the spectrophotometric method of *Wróblewski and DeDue* (7) as described by *Kokot* (8) at the temperature of 37 °C.

All mean values were obtained from five cultures. Results were analysed by means of Student's »t« test.

RESULTS

Table 1

Protein content (µg/g wet tissue) in the medium in which slices of adult and foetal rat's liver were cultured. Results are expressed as means ± SEM of five experiments.

Concentration of lead ions (mg/L)	Duration of culturing					
	24 hrs		48 hrs		72 hrs	
	Adult	Foetal	Adult	Foetal	Adult	Foetal
0.00*	50 ± 4	30 ± 2	57 ± 3	30 ± 6	62 ± 4	32 ± 3
0.20	44 ± 2	28 ± 3	48 ± 2***	29 ± 3	56 ± 4	30 ± 2
0.40	40 ± 3**	24 ± 2**	44 ± 3***	26 ± 2	48 ± 4***	27 ± 2
0.60	38 ± 4**	23 ± 4	40 ± 2***	24 ± 1	45 ± 5***	24 ± 3**
0.80	38 ± 2***	20 ± 2***	39 ± 2***	21 ± 3	41 ± 4***	22 ± 1***

* Control culture without sodium acetate

** p < 0.005

*** p < 0.001

Table 2
Lactate dehydrogenase activity (IU/ml of medium) measured in the medium in which adult and foetal rat's liver slices were cultured. The activities are expressed as means \pm SEM of five experiments.

Concentra- tions of lead ions (mg/L)	Duration of culturing					
	24 hrs		48 hrs		72 hrs	
	Adult	Foetal	Adult	Foetal	Adult	Foetal
0.00*	12.4 \pm 2.5	20.5 \pm 3.4	13.0 \pm 1.7	21.5 \pm 2.5	12.8 \pm 1.65	19.5 \pm 2.4
0.20	13.0 \pm 1.2	21.1 \pm 0.9	15.2 \pm 1.2	23.2 \pm 1.2	19.0 \pm 1.72***	25.2 \pm 1.8**
0.40	13.5 \pm \pm 3	23.1 \pm 1.6	17.0 \pm 2.2	26.2 \pm 2.4	19.9 \pm 1.14***	29.1 \pm 2.1***
0.60	15.2 \pm 1.8	34.0 \pm 2***	16.9 \pm 1.2***	37.1 \pm 1.1***	19.0 \pm \pm . \pm 8***	39.8 \pm 1.1***
0.80	16.8 \pm 1.7	39.5 \pm 1.5***	17.7 \pm 2.1***	40.2 \pm 1.5***	24.2 \pm 1.18***	41.0 \pm 1.1***

* Control culture without sodium acetate

** p < 0.005

*** p < 0.001

Table 1 shows the details of protein release by hepatic slices taken from foetal and adult rats. Lead ions produced a decrease in protein release. The effect was found to be dose dependent.

Estimation of lactate dehydrogenase activity indicates that lead ions produce an increased release of the enzyme from the hepatic tissue. This phenomenon was found in cultures of adult and foetal tissue. It appears that foetal tissue is more susceptible to lead ions than adult tissue and that the release of lactate dehydrogenase is dose-dependent.

There was no differences in protein release and lactate dehydrogenase activity between cultures with various concentrations of sodium acetate and without sodium acetate (results are not shown).

DISCUSSION

The question of lead hepatotoxicity was raised in studies on hepatic lesions in chronic alcoholics. It was found that wine contains measurable amounts of lead (9, 10) and in alcoholics, due to excessive wine consumption, signs of lead intoxication were observed (11, 12).

Lead may damage the liver directly and may interact with the hepatic metabolism of other exogenous substances (13—16). Thus, lead is known to decrease cytochrome P₄₅₀ content in the liver (17). It also prolongs the hexobarbital-induced sleeping time by impairing drug-metabolising enzyme system (18, 19). On the other hand, lead compounds are known to protect against alterations induced by carbon tetrachloride or dimethylnitrosamine (20, 21) by inhibiting the metabolism of these toxins. Lead hepatotoxicity observed in animal studies is thought to be a complex of direct and indirect disturbances of metabolic pathways in the body.

The present study indicates that lead ions affect directly the hepatic tissue. Lead inhibits the release of proteins, presumably their synthesis, and produces cellular damage as shown by the increase of lactate dehydrogenase activity in the medium. This hepatotoxic action of lead seems to be dose-dependent. The mechanism of toxicity is unknown. It is suggested that lead inhibits the enzymes in the hepatocyte, and in this way produces the changes observed in the experiment.

To summarize, lead ions act on the liver tissue and the method of hepatic slice culturing has been found useful for investigations of heavy metal hepatotoxicity *in vitro*.

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Sažetak

UČINAK OLOVA NA SADRŽAJ PROTEINA I AKTIVNOST LAKTAT DEHIDROGENAZE U KULTURI JETRENOG TKIVA

Rezovi jetre fetusa i odraslog štakora održavani su *in vitro* u medijima kojima su dodavane različite koncentracije iona olova. Utvrđeno je značajno smanjenje količine proteina u mediju i povećanje aktivnosti laktat dehidrogenaze. Promjene su bile ovisne o koncentraciji olova u mediju. Jetra fetusa bila je osjetljivija na učinke olova negoli jetra odraslog štakora.

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