Changes in Activity of Malate Dehydrogenase and Glucosephosphate Isomerase in Serum of Rats Exposed Chronically to Inorganic Mercury and its Aryl and Alkyl Compounds**

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Whereas problems of absorption, retention and biotransformation in the living system of various mercury compounds are relatively well understood under conditions of chronic exposure /NORSETH and CLARKSON, 1970; NORSETH and CLARKSON, 1971; CLARKSON, 1972/, the question of sensitive biochemical indicators of intoxication with these compounds, when injury is clinically latent, forms still a subject of considerable interest.

Changes of activity of some enzymes in soft tissues of laboratory animals, as a consequence of exposure to mercury vapour, were found by means of histochemical methods /KOŚMIDER et al., 1963; JONEK and KOŚMIDER, 1964; JONEK and GRZYBEK, 1964; JONEK et al., 1964; JONEK, 1964/. In patients poisoned with mercuryc chloride an enhanced activity of D-glutamylotransferase /GGTP/ and leucine aminotransferase /LAP/ was found by JACYSZYN /1973/. Our previous studies of rats exposed over prolonged periods to various doses of methylmercurycyanguanidine have shown, that activities of malate dehydrogenase /MDH/ and glucosephosphate isomerase /PHI/ may form a sensitive enzymatic indicator of intoxication of these animals with methylmercury compounds /CHMIELNICKA et al., 1975; CHMIELNICKA and BALCERSKA, 1975/.

The aim of this study was an extension of this observation with regard to other mercury compounds /mercuric chloride, phenylmercuric chloride, ethylmercuric chloride/, administered chronically and also investigation whether activity of glutathione reductase /GSSG-R/ in erytrocytes, an enzyme with SH group in the active centre /ICEN, 1967/ is affected by such treatment. It was also interesting to see whether there is a relation between the amount and chemical form of mercury, accumulated in the liver in course of chronic exposure, and activity of the enzymes listed above /MDH, PHI, GSSG-R/.

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MATERIALS AND METHODS

Mercury compounds: Following compounds were used: /MetHg /-a fluid seed dressing preparation 08-containing 0,8 % Hg in form of methylmercurycyanguanidine /Chemical Works, AZOTY, Jaworzno/; /$HgCl_2$/ - mercuric chloride, /EtHg/ ethyl- and /PhHg/ - phenylmercuric chlorides /BDH, Laboratory Reagents/.

Animals. In the experiments 60 albino rats, females of Wistar strain of 200 g body weight were used. They were divided into 5 groups, 12 animals each. The rats were given orally eachsecond day solutions /0,5 ml/ of above listed Hg compounds in amount corresponding to 5 % of DL_{50}, in proportion to body weight. This corresponded to: 2,3 mg/kg of MetHg, 1,2 mg/kg of EtHg, 1,3 mg/kg of PhHg and 1,4 mg/kg of $HgCl_2$. The animals were given a standard LSM diet and tap water ad libitum.

Blood was sampled from the tail vein; over the first month every week later every second week. After 14 weeks of exposure the rats were sacrificed in ether narcosis - from each animal blood was withdrawn by heart puncture and liver was obtained by dissection.

Determination of enzymes activities: In serum, obtained by centrifugation of the whole blood /10 min. at 5000 r.p.m./ activity of following enzymes was determined: malate dehydrogenase - MDH /E.C. 1.1.1.37/ using L-malic-acid and NAD and colorimetric measurement acc. to SEVELA and TOVAREK /1960/ at A_{550}; the results were expressed in units of the original method: μmoles of liberated pyruvate $hour^{-1}ml^{-1}$ at 37°C. Activity of glucosephosphate isomerase - PHI /E.C. 5.3.1.9./ was determined acc. to BODANSKI /after SZCZEKLIK, 1973/ using glucoso-6-phosphate at A_{490}; the results were expressed in units corresponding to μmoles of liberated fructose $hour^{-1}ml^{-1}$.

Activity of glutathione reductase - GSSG-R /E.C. 1.6.4.2./ was determined in haemolysate of erythrocytes by means of the method modified from MASSEY and WILLIAMS /1965/. The haemolysate had been prepared acc. to BEUTLER /1969/. Activities are reported as the change in absorbance at 340 nm per minute per ml whole blood /$\triangle A\ min^{-1}ml^{-1}$ whole blood/, corrected for the blank.

Determination of mercury. Inorganic as well as total mercury in liver-tissue was determined by means of cold atomic absorption /MAGOS, 1971/ partly modified by the present authors /BALCERSKA et al., in press/. For the measurements a Hendrey Relays Mercury Vapour Concentration Meter Type E 3472 has been used. The liver homogenate was prepared in a following way: the tissue was homogenised in a small volume of 0,9 % NaCl solution for 5 min. at 100 r.p.m. After homogenization physiological saline was added at such amount as to obtain a 10 % suspension. For determinations 0,2 - 1,0 ml of the latter has been taken.

Significance of differences between corresponding results was tested by means of t-student test accepting per cent level of probability as the borderline.

RESULTS AND DISCUSSION

Influence of the studied compounds of mercury upon MDH activity in the blood serum in course of experiment is presented in fig. 1.

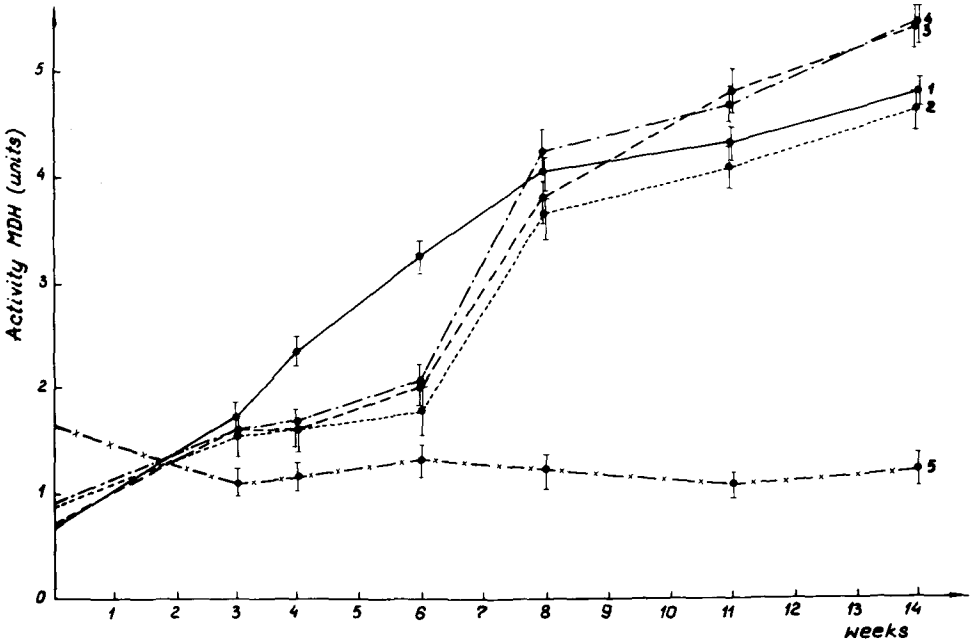

Figure 1. MDH activity in blood serum of rats in course of prolonged intoxication with mercury compounds. 1 - MetHg; 2 - $HgCl_2$; 3 - EtHg; 4 - PhHg; 5 - controls.

Already after 3 weeks an enhancement of activity was seen when MetHg had been administered; when other Hg compounds are considered an evident increase of the activity was observed after eight weeks. Fourteen weeks since initiation of the exposure /Table 1/ to MetHg, $HgCl_2$, EtHg and PhHg the levels of activity were significantly higher than for controls /1,2 units and reached values of 4,7; 4,6; 5,4 and 5,4 units, respectively

Dynamics of changes of PHI activity in the serum is presented in fig. 2.

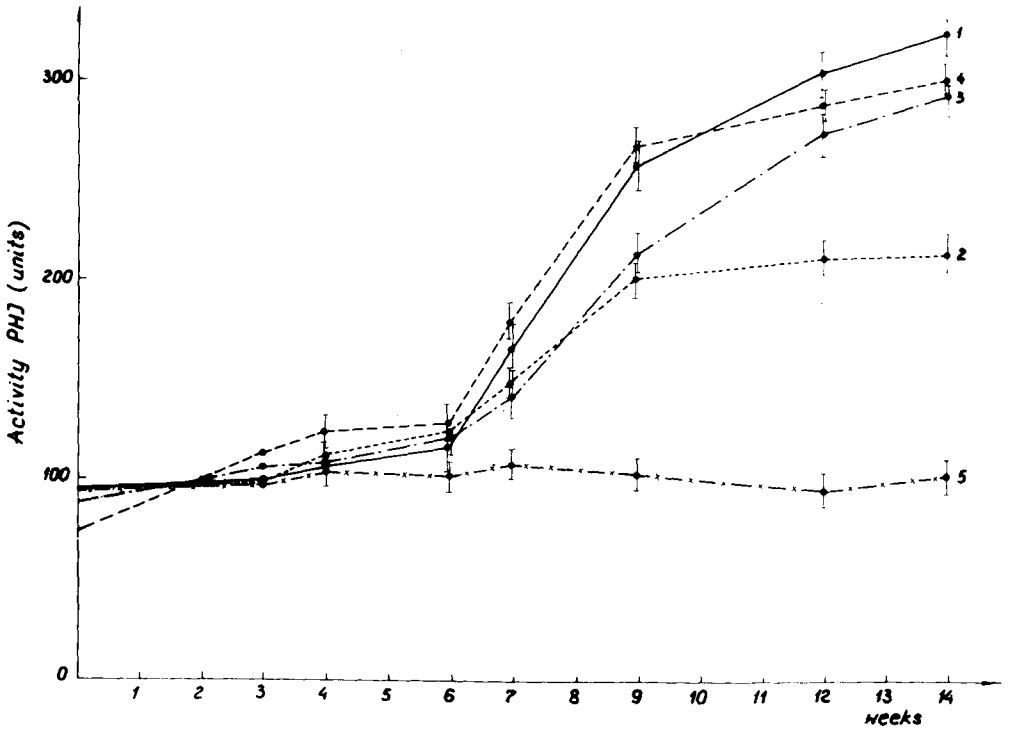

Figure 2. PHI activity in blood serum of rats in course of prolonged intoxication with mercury compounds. 1 - MetHg; 2 - $HgCl_2$; 3 - EtHg; 4 - PhHg; 5 - controls.

The rise of activity started around 7 weeks after initiation of the exposure and reached significantly higher values - relative to controls - after 14 weeks /Table 1/, The increase by a factor of 3 was observed in rats exposed to MetHg, EtHg and PhHg whereas after $HgCl_2$ treatment the activity was roughly doubled. In the same table 1 data are presented for GSSG-R from which it follows that there are no significant changes of the activity, attributable to administered mercury compounds.

For these studies enzymes such as MDH and PHI were selected because they penetrate to the blood after injury of cellular membrane of liver and kidney cells /BERGMAYER, 1970/. According to BARABAN /1961/ an enhanced activity of PHI in

Table 1
Enzyme activity after 14 weeks of exposure to various mercury compounds.

Compound administered	MDH /units/		PHI /units/		GSSG-R /ΔA/min/ml	
	95 per cent confidence limit	significance of the difference relative to controls	95 per cent confidence limit	significance of the difference relative to controls	95 per cent confidence limit	significance of the difference relative to controls
Controls	1,2±0,13		106,02± 8,55		6,71±0,16	
MetHg	4,7±0,16	+	344,7±±12,48	+	0,73±0,03	-
PhHg	5,4±0,19	+	305,8 ± 8,79	+	0,75±0,09	-
EtHg	5,4±0,22	+	294,17±11,32	+	0,65±0,14	-
$HgCl_2$	4,6±0,2	+	215,3 ± 9,79	+	0,91±0,25	-

+ difference significant at 5 per cent level

- difference insignificant at 5 per cent level

Each result represents mean of 6 determinations.

blood may form an early and sensitive sign of the nephrotic syndrome. Glutathione reductase in turn represents a group of enzymes possessing SH-group in the active center, which however are intimately associated with interior of erythrocytes. No changes of activity of GSSG-R in erythrocytes of rats exposed chronically to mercury may result from the fact that the latter, when enters the body, is bound predominantly by proteins of cellular membranes, or by others like haemoglobin, albumines and globulines which show great affinity to this element. According to a theory formulated by ROTHSTEIN /1973/ mercury compounds display no specificity of binding with particular proteins, the affinity resulting only from availability of sulphhydryl groups. Thus mercury may react with almost all proteins, as well the highly active ones as with those playing only a structural role.

Inhibition of enzymes by Hg compounds, seen in in vitro experiments is not always observed after respective in vivo treatment. This refers, among others, also to glutathione reductase /MYKKANEN and GANTHER, 1974/ and to some mitochondrial enzymes /MAGNAVAL *et al.*, 1975/.

From our experiments it seems to follow that after chronic exposure to mercury compounds no inhibition of so called indicator enzymes should be expected, but rather an increase of activity in the serum of those which due to their low molecular weight will easily penetrate from cytoplasm into the body fluids after damage inflicted by the exposure to cellular structures and membrane /liver, kidneys/. The experiments reported here demonstrated that MDH and PHI form a good example and lend support to the postulate of CLARKSON /1972/, that mercury compounds start their deleterious action by attacking and damaging cellular membranes.

Degree of the damage may depend on the concentration and chemical form of mercury, deposited in soft tissues in course of the exposure..

Table 2

Total mercury content of mercury in the liver / µg/g/ and organ weight in rats exposed to various Hg-compounds for 14 weeks.

No of animal	MetHg	EtHg	PhHg	$HgCl_2$
	µgHg/g liver			
1	29,1	7,8	1,9	1,2
2	27,7	8,3	2,0	1,3
3	37,6	8,5	2,1	1,1
4	36,0	8,4	2,1	1,2
5	34,2	9,6	2,1	1,4
6	37,0	8,2	2,1	1,3
95 % confidence limit	33,6±4,29	8,5±0,7	2,05±0,08	1,25±0,1
mean liver weight /g/	4,7±0,4	6,4±0,95	6,58±0,87	7,28±0,91

In table 2 data are assembled on total Hg content in the liver of rats, exposed to various compounds of this element for 14 weeks. At the same time mean weight of livers is reported. The lowest weight was observed in rats exposed to MetHg; similar but slightly higher values were found in those treated with EtHg and PhHg, and the highest weight of the organ was seen after prolonged administration of $HgCl_2$. The highest concentration of total Hg in liver was seen in animals given MetHg.

Fig. 3 depicts contribution of organic and inorganic Hg to the total in these livers, expressed in μg/g tissue, and in proportion /%/ of the total mercury.

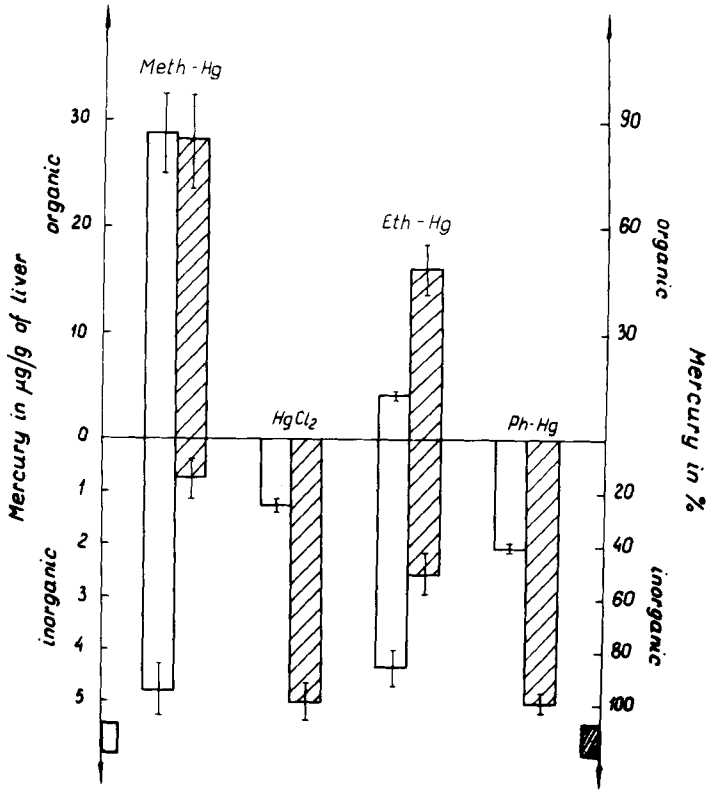

Figure 3. Level of organic and inorganic mercury in liver of rats exposed chronically to various Hg compounds /3 times weekly for 14 weeks/ at the dose corresponding to 5 % of DL_{50}.

Organic mercury was found only in livers of animals exposed to alkyl-mercurials and respective concentrations amounted to 28,8 and 4,1 μgHg/g fresh liver after MetHg and EtHg dosage. Inorganic mercury was found at levels of 4,7; 4,3; 2,0

and 1,2 µgHg/g for MetHg, EtHg, PhHg and $HgCl_2$, respectively. It seems remarkable that concentration of inorganic Hg in the livers after exposure to various compounds was of the same order, falling within the range of 1,2 - 4,7 µgHg/g.

Presumably this is inorganic mercury, formed also after biodegradation of organic compounds, which damages the cellular membranes of hepatocytes and kidney cells, and leads to outflow of cytoplasmatic indicator enzymes /MDH, PHI/ into the blood. KLEIN et al. /1973/ drew attention to the fact that nephrotoxic action in methyl-Hg results from influence of inorganic Hg, originating in vivo from the former.

Further similar studies in other animal species seem warranted with interspecies comparison of the effects as objective.

SUMMARY

In course of prolonged exposure /14 weeks/ to various mercury compounds /MetHg - a fluid seed-dressing preparation O,8, Phenyl and Ethyl chlorides and $HgCl_2$ in doses corresponding to 5 % of DL_{50}/3 times weekly/, enhanced levels of activity of malate dehydrogenase /MDH/ and glucosephosphate isomerase /PHI/ in blood serum were observed. After 7 weeks of exposure about fourfold increase of MDH and 2-3-fold enhancement of PHI activities were found relative to controls. After 14 weeks of exposure in livers of rats, given MetHg and EtHg, organic mercury was found at concentrations of 28,8 and 4 µg/g tissue, respectively. Inorganic mercury in liver was found in animals given all compounds and concentrations were in the range of 1,2 - 4,7 µg/g tissue.

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