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Effects of Methylmercury on the Lipid Components in Rats

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Methyl mercury is identified as an extremely toxic substance for man and his environment. Though the toxic effects of mercury have been widely studied from biochemical, physiological, and pathological points of view, there are very few reports which give clear elucidation of the mechanism for the occurrence of neurointoxication. On the other hand, YOSHINO *et al.* (1966) have reported that the incorporation of [U-^{14}C] leucine into the nervous system of mercury-intoxicated rats was markedly inhibited as a reduction of 43% of control. Some workers demonstrated that the attack by mercurials against the biosynthesis system of proteins was significantly associated with the occurrence of neurointoxication (CAVANAGH and CHEN 1971, BRUBAKER *et al.* 1973). Though the reduction of enzymatic activities by mercurials has been widely reported (YOSHINO *et al.* 1966, PATERSON and USHER 1971, CHANGE *et al.* 1973), few investigations have demonstrated the toxicity by mercurials through the functions of the biomembranes such as permeability and both ion- and metabolite-transport. Recently, several workers have attempted to elucidate the toxic effects of mercurials through the functions of the biomembranes (KASUYA 1972, YAGASAKI *et al.* 1975, HARA and NAKAO 1976).

In the present paper, we report the effects of methyl mercury chloride on the variation of both the content and fatty acid composition of lipids in the liver, kidney and brain of rats.

MATERIALS AND METHODS

Female rats of Wistar strain weighing 156gr to 178gr were randomly divided into four groups of five animals each. Three groups, A, B, and C were orally dosed with 1mg, 5mg, and 10mg of mercury per kilogram body weight as methyl mercury chloride in 0.9% NaCl solution. One group recieved 0.9% NaCl solution only for control. The rats were starved for 48 hours after the dose of mercurial, and then sacrificed via cardiac puncture under light ether narcosis. The liver, kideney, and brain were excised and stored at -20°C until use.

Total lipids were extracted from each organ with chloroform-methanol by the method of BLIGH and DYER (1959). One-tenths of total lipids was subjected to fatty acid analysis. To the remaining lipids, to separate phospholipids from neutral lipids as

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a precipitate, about 5ml of acetone with several drops of 0.1% $MgCl_2$ in methanol was added. Parts of the two lipid fractions were also subjected to fatty acid analysis. The each lipid fraction was determined gravimetrically.

Total, neutral, and phospho- lipids were saponified with 10% KOH-ethanol at 80°C for 2 hours, and then non-saponifiable substances removed with ethyl ether. Fatty acids were released by the acidification with 2N HCl and extracted from the aqueous solution with petroleum ether. Each fatty acid fraction was methylated with 3% HCl-methanol at 70°C for 2 hours for gaschromatographic analysis. Fatty acid composition was analysed with 10% DEGS (GC-grade, Nihon Chromato Kogyo L.T.D.) on 60-80 mesh Gaschrom Q at 170°C, by a Yanagimoto gaschromatograph (Model 1800G) equipped with a hydrogen flame ionization detector. The percentage composition of each fatty acid was calculated by peak area from gaschromatogram.

RESULTS

Table 1 shows the contents of neutral, phospho- and total lipids and the percentage composition of oleic acid in each lipid in the liver. It appears that the contents of neutral, phospho- and total lipids are slightly higher in groups A and B than in control group. However, there were no statistically significant differences between the groups with respect to lipid content. On the other hand, the percentage composition of oleic acid varied clearly with the dosage of mercurial. That is, the percentage composition of the acid in neutral lipid or total lipid was significantly lower in groups B and C than in control group. However, the percentage composition of the acid in phospholipid was not different between the groups.

Table 2 shows the content of each lipid fraction and the percentage composition of oleic acid in the kidney. The contents of neutral, phospho- and total lipids hardly changed by the dose of mercurial, while the percentage composition of oleic acid varied a little. That is, the composition of the acid in phospholipid in group A was significantly higher than in control group. On the other hand, the composition of the acid in total lipid of group B was significantly lower than that of control.

The lipid content and the percentage composition of oleic acid from each lipid in the brain are presented in Table 3. There were no significant differences between the groups with respect to neutral and total lipid contents while phospholipid content was significantly lower in group C than in control group. The percentage composition of oleic acid also varied significantly with the dose of mercury. That is, the percentage composition of the acid in phospholipid was significantly lower in groups B and C than in control group, whereas that in neutral lipid was significantly higher in group B than in control group. Consequently, group C was significantly lower than control group with respect to the percentage composition of oleic acid in total lipid.

Table 4 shows the percentage compositions of several kinds of fatty acids as well as oleic acid in neutral lipid of the liver.

TABLE 1

Liver Lipid Content of the Rats Dosed Methyl Mercury Chloride.

Groups	Lipid content per fresh matters (%)			% Composition of oleic acid in fatty acids		
	Neutral lipids	Phospholipids	Total lipids	Neutral lipids	Phospholipids	Total lipids
Control	0.97 ± 0.13	3.11 ± 0.20	4.08 ± 0.30	24.44 ± 3.14	6.80 ± 1.36	13.30 ± 1.57
A	1.08 ± 0.14	3.34 ± 0.19	4.42 ± 0.31	23.88 ± 0.74	7.04 ± 0.50	12.58 ± 0.60
B	1.10 ± 0.12	3.34 ± 0.15	4.44 ± 0.21	18.88 ± 3.05*	6.40 ± 1.34	9.98 ± 2.06*
C	0.93 ± 0.24	3.05 ± 0.18	3.98 ± 0.36	18.24 ± 3.35*	6.30 ± 0.77	9.52 ± 1.16*

The values are means ± S.D. from five animals.

* Significantly different from control group at $p < 0.05$.

TABLE 2

Kidney Lipid Content of the Rats Dosed Methyl Mercury Chloride.

Groups	Lipid content per fresh matters (%)			% Composition of oleic acid in fatty acids		
	Neutral lipids	Phospholipids	Total lipids	Neutral lipids	Phospholipids	Total lipids
Control	1.10 ± 0.06	2.72 ± 0.27	3.82 ± 0.28	17.64 ± 1.00	13.56 ± 0.58	15.22 ± 0.47
A	1.14 ± 0.08	2.84 ± 0.13	3.98 ± 0.08	17.72 ± 0.78	15.48 ± 1.12*	15.68 ± 0.57
B	1.19 ± 0.03	2.81 ± 0.09	3.94 ± 0.09	17.24 ± 1.06	13.42 ± 0.36	14.40 ± 0.62*
C	1.18 ± 0.17	2.76 ± 0.14	3.94 ± 0.27	17.48 ± 1.16	13.40 ± 1.11	14.66 ± 0.54

The values are means ± S.D. from five animals.

* Significantly different from control group at $p < 0.05$.

TABLE 3

Brain Lipid Content of the Rats Dosed Methyl Mercury Chloride.

Groups	Lipid content per fresh matters (%)			% Composition of oleic acid in fatty acids		
	Neutral lipids	Phospholipids	Total lipids	Neutral lipids	Phospholipids	Total lipids
Control	1.29 ± 0.24	6.49 ± 0.23	7.78 ± 0.41	25.26 ± 1.60	28.88 ± 0.72	28.84 ± 1.65
A	1.39 ± 0.15	6.59 ± 0.24	7.97 ± 0.30	25.08 ± 1.25	28.80 ± 2.97	27.12 ± 1.84
B	1.25 ± 0.13	6.11 ± 0.60	7.36 ± 0.68	29.26 ± 0.94*	24.94 ± 1.84*	28.56 ± 1.03
C	1.36 ± 0.12	5.66 ± 0.60*	7.02 ± 0.66	26.22 ± 1.19	24.84 ± 0.74*	25.54 ± 1.97*

The values are means ± S.D. from five animals.

* Significantly different from control group at $p < 0.05$.

TABLE 4

Fatty Acid Composition from Neutral Lipids of the Liver of the Rats Dosed Methyl Mercury Chloride.

Fatty acids	Groups and dosage levels of mercury (mg/body kg)			
	Control	A (1mg)	B (5mg)	C (10mg)
16:0	33.06 ± 2.20	34.30 ± 1.55	34.44 ± 1.68	34.52 ± 2.54
16:1 ω7	4.38 ± 1.08	4.08 ± 0.45	2.76 ± 0.87*	1.78 ± 0.36*
18:0	4.12 ± 1.71	5.50 ± 0.46	7.26 ± 2.03*	8.04 ± 3.00*
18:1 ω9	24.44 ± 3.14	23.88 ± 0.74	18.88 ± 3.05*	18.24 ± 3.58*
18:2 ω6	23.76 ± 1.91	22.64 ± 1.24	25.24 ± 3.14	24.66 ± 2.61
20:4 ω6	4.72 ± 1.68	5.18 ± 0.48	6.88 ± 2.00	7.08 ± 2.56
22:1	2.58 ± 0.84	2.48 ± 0.39	2.18 ± 0.66	2.64 ± 0.36
22:5 ω3	0.40 ± 0.12	0.30 ± 0.07	0.32 ± 0.04	0.32 ± 0.04
22:6 ω3	2.62 ± 1.29	1.82 ± 0.44	2.10 ± 1.04	2.74 ± 0.93
**	62.82 ± 1.99	60.40 ± 1.47	58.30 ± 2.02*	57.44 ± 3.32*

The values are means ± S.D. from five animals.
* Significantly different from control group at $p < 0.05$.
** A sum of unsaturated fatty acids.

TABLE 5

Fatty Acid Composition from Phospholipids of the Brain of the Rats Dosed Methyl Mercury Chloride.

Fatty acids	Groups and dosage levels of mercury (mg/body kg)			
	Control	A (1mg)	B (5mg)	C (10mg)
16:0	26.42 ± 4.11	28.74 ± 2.98	33.88 ± 1.65*	33.46 ± 0.96*
18:0	28.84 ± 1.59	29.26 ± 2.26	32.06 ± 1.55*	30.84 ± 0.57*
18:1 ω9	28.88 ± 0.72	28.80 ± 2.97	24.94 ± 1.84*	24.84 ± 0.74*
20:4 ω6	3.66 ± 0.56	3.82 ± 0.70	1.96 ± 0.61*	2.28 ± 0.37*
22:1	3.22 ± 0.48	3.43 ± 0.48	2.68 ± 0.57	2.44 ± 0.27
24:0	2.15 ± 0.54	2.96 ± 2.13	1.38 ± 0.74	1.28 ± 0.24
22:6 ω3	4.00 ± 0.87	3.99 ± 1.14	3.14 ± 1.31	3.86 ± 0.86
**	39.76 ± 1.06	39.04 ± 3.62	32.88 ± 2.87*	34.42 ± 1.63*

The values are means ± S.D. from five animals.
* Significantly different from control group at $p < 0.05$.
** A sum of unsaturated fatty acids.

16:0 palmitic acid, 16:1 ω7 palmitoleic acid, 18:0 stearic acid, 18:1 ω9 oleic acid, 18:2 ω6 linoleic acid, 20:4 ω6 arachidonic acid, 22:1 docosamonoenic acid, 24:0 tetracosanoic acid, 22:5 ω3 docosapentaenoic acid, 22:6 ω3 docosahexaenoic acid.

As is evident from the table, the percentage compositions of both stearic acid and palmitic acid increased with increasing dosage levels of mercury while those of both palmitoleic acid and oleic acid decreased with that factor. Statistical analysis revealed that the percentage compositions of both palmitoleic acid and oleic acid were significantly lower in groups B and C than in control group. On the other fatty acids, no consistent variation of percentage composition with dosage levels of mercury was recognized. Consequently, the percentage composition of the saturated fatty acids rised, while that of the unsaturated ones lowered by the dose of mercury.

Table 5 shows the fatty acid composition in the phospholipids of the brain tissue. It is also seen from the table that the percentage compositions of both palmitic acid and stearic acid as saturated fatty acid, were significantly higher in groups B and C than in control group while those of oleic acid, arachidonic acid and docosamoenic acid as unsaturated acids, were significantly lower in groups B and C than in control group.

DISCUSSION

Results obtained indicate that the lipid content in the tissues and the fatty acid composition in each lipid fraction varied with the dosage levels of methyl mercury. About the lipid content, single oral administration of methyl mercury caused a shift in the tissue lipid distribution, increasing or decreasing the content in each organ examined if not always significantly. At this point, we suppose that the slight increase of lipid fraction caused by methyl mercury may be attributable to the catalytic action of methyl mercury as a trace amount metal for the lipid biosynthesis rather than the toxic action of methyl mercury itself. Furthermore, the variations of total lipid content in each organ depended mainly on the variations of phospholipid content rather than neutral lipid one. KASUYA (1972) has reported that both sphingomyelin and phosphadidyl-L-serine from the brain of bovine tended to protect the outgrowth of Schwann cells and nerve fibers in the presence of organic mercurials. Considering the above facts, it is natural that the dosage of mercurial causes the decrease of the content of total lipids, especially of phospholipid in the organs of rats.

With the dosage of mercurial, the decrease of each lipid fraction was less in the kidney than in the liver and the brain. NORSETH and CLARKSON (1970) have reported that the retion of inorganic mercury to organic mercury after injection of methyl mercury chloride was larger in the kidney that in the liver and the brain. Therefore, the less decrease of each lipid fraction in the kidney may be attributable to the less accumulation of the dosed methyl mercury which was partly converted into inorganic mercury.

The percentage composition of oleic acid in each lipid fraction decreased with the dosage levels of methyl mercury except for that of oleic acid in the phospholipid from the kidney in group A. If the decrease of the percentage composition of oleic acid, or

unsaturated acids occurred as the toxic action of methyl mercury, we are forced to conclude that the increase of that of oleic acid in the phospholipid of the kidney reflected the control of bioprotection in the biomembranes of the kidney. It seems probable that the percentage composition of oleic acid in the phospholipid of the kidney increased with the protection against the presence of mercury, and simultanously decreased with the toxicity of mercury. Therefore,it is agreeable that groups B and C differed from group A but not from control group on the percentage composition of oleic acid in the phospholipid of the kidney.

In the liver, a significant decrease in the percentage composition of oleic acid was found in the neutral lipid and in contrast with the situation in the brain. The contrast variation was resulted in the different biochemical action of mercury to lipids among the different organs of rats. In fact, mercurials acted primarily on the decrease of the percentage composition of oleic acid in the liver, but in the brain they acted secondarily on the decrease of that of oleic acid after increase of that of the acid in the neutral lipid. This is because the composition in the neutral lipid increased with the increase of oleic acid which remained in the neutral lipid after inhibition of the incorporation into the phospholipid.

From the facts mentioned above, we propose the percentage composition of unsaturated fatty acids as well as oleic acid certainly decreased with the dosage of mercurials. Parts of the unsaturated acids might be reduced and/or peroxidided by a biochemical action of mercurials in the rat organs.

As a trace effect by the dose of mercurial was observed in the content of each lipid fraction, we assume that the starvation for 48 hours affects the lipid content stronger than the single oral dose of mercurials. Nevertheless, the significant decrease of the phospholipid content in the brain by the single oral dose of mercurials was distinguished from the comparable reduction in protein content by repeating administration of mercury, reported by KLEIN *et al.* (1972). We think that lipid content is more sensitive than protein one for evaluating the toxic effects of mercury. Furthermore, our experiment showed that the percentage composition of oleic acid and/or unsaturated acids varied more sensitively than the lipid content; the dose of 5mg of mercury level clearly affected the former but even the dose of 10mg of it hardly affected the latter. In addition to this, it is well known that enzymatic activities are sensitively changed by mercurials. We think, however, that the variations in the percentage composition of oleic acid contribute to the elucidation of the mercurial toxicity, because either the nature of the biomembranes affects more or less enzymatic activities or that of the biomembranes are widely controled by the composition of fatty acids, especially by the degree of unsaturation, of the phospholipid in the biomembranes.

REFERENCES

BLIGH, E.G. and W.J. DYER: Can. J. Biochem. Physiol. 37, 911 (1959).

BRUBAKER, P.E., R. KLEIN, S.P. HERMAN, G.W. LUCIER, L.T. ALEXANDER and M.D. LONG: Exp. Mol. Phathol. 18, 263 (1973).

CAVANAGH, J.B. and F.C.K. CHEN: Acta Neuropath. (Berl.) 19, 216 (1971).

CHANGE, L.W., R.A. WARE, and P.A. DESNOYERS: Fd Cosmet. Toxicol. 11, 283 (1973).

HARA, S. and T. NAKAO: Ann. Rep. Tokyo Metr. Res. Lab. P.H., 27-2, 161 (1976).

KASUYA, M.: Toxicol. Appl. Pharmacol. 23, 136 (1972)

KLEIN, R., S.P. HERMAN, P.E. BRUBAKER, G.W. LUCIER, and M.R. KRIGMAN: Arch. Path. 93, 408 (1972).

NORSETH, T., and T.W. CLARKSON: Arch. Environ. Health, 21, 717 (1970).

PATERSON, R.A., and D.R. USHER: Life Sci., 10, 121 (1971).

YAGASAKI, S., Y. HARA, nad T. NAKAO: Ann. Rep. Tokyo Metr. Res. Lab. P.H., 26-2, 148 (1975).

YOSHINO, Y., T. MOZAI, and K. NAKAO: J. Neurochemistry, 13, 1223 (1966).