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INTRACELLULAR DISTRIBUTION OF INORGANIC AND ORGANIC MERCURY IN RAT LIVER AFTER EXPOSURE TO METHYLMERCURY SALTS

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Abstract—The intracellular distribution of inorganic and organic mercury in rat liver after exposure to methylmercury salts has been reported. The results have been discussed comparing intracellular distribution of mercury after exposure to various mercury compounds. After exposure to methylmercury salts the lysosomes/peroxisomes contain the highest concentration of mercury followed by microsomes and mitochondria. Differences in distribution between various mercury compounds seem to be related to the stability of the carbon–mercury bond, lysosomes accumulating inorganic mercury by preference either injected as such, or released *in vivo* from the intact organomercurial. Toxicological and pharmacokinetic implications of these conclusions have been discussed.

DISTRIBUTION within the cell may explain differences in toxicity and pharmacokinetics of various mercury compounds. Studies of the intracellular distribution in the liver of mercury after exposure to methoxyethylmercury acetate and to inorganic mercury have been reported earlier.[1,2] In this paper is reported the intracellular distribution of mercury after exposure to methylmercury salts.

A new method for analysing inorganic mercury in the presence of methylmercury salts in biological samples was recently published.[3] The results indicate that the distribution of mercury in the cell after exposure to various organomercurials may be related to different stability *in vivo* of the carbon–mercury bond in these compounds. Some preliminary results analysing separately inorganic and organic mercury in cellular fractions after exposure to methylmercury chloride have therefore been included.

MATERIALS AND METHODS

Methylmercury dicyandiamide (MMDD) was a gift from A/B Casco, Stockholm, Sweden, all other chemicals were obtained through commercial sources.

For the distribution study of total mercury, 20 female rats kept on standard diet (Institute of Occupational Health, Oslo, Norway) without milk were given 200 μg MMDD/l. in the drinking water for 52–60 weeks. Rat weight at the end of the experiment was 200–300 g. No obvious toxic signs from the rats were observed. Three rats died during the exposure period, 10 rats were used for the distribution analysis.

All livers were homogenized and fractionated as described previously.[1] The marker enzyme determinations have been discussed earlier as have the statistical treatment and presentation of the results.[1,2] All methods were identical to those used in the previous

papers except the mercury determination after neutron irradiation by Institutt for Atomenergi, Kjeller, Norway, by their own method, and phosphate determination by the method of Martin and Doty.[4]

Inorganic mercury in cellular fractions after exposure to methylmercury chloride was determined in eight rat livers in four groups of two livers each. Female rats, 200 g, were intravenously injected with 1 mg Hg/kg (six rats) or 2 mg Hg/kg (two rats) as methylmercury chloride (MMC). The rats were killed after 4 days. The mercury concentration in the livers of these rats was higher than for the rats receiving methylmercury dicyandiamide in the drinking water, but smaller amount could not be used because of limitation by the specific activity of the available isotope.

Inorganic mercury in the presence of methylmercury salts was analysed by the method of Norseth and Clarkson,[3] and the isotopically labeled methylmercury chloride was prepared as described earlier.[3] Estimation of mercury content in cellular particles could not be done in this part of the experiment because of the assay design, but distribution among cellular fractions of mercury and of acid phosphatase activity was recorded.

RESULTS

The mercury content in the livers after exposure to MMDD was 0·82 μg Hg/g wet weight (SD = 0·16). No major differences in marker enzyme distribution was found compared to previous papers in this series (Fig. 1). The relative specific activity of acid phosphatase from the intravenously injected animals is also recorded in the figure. Mercury distribution may thus be compared for the different series of animals in the present and previous papers.

TABLE 1. RELATIVE SPECIFIC MERCURY CONTENT IN FRACTIONS

Mercury administration	No. of rats	Fractions N	M	L	P	S
Drinking water	10	1·03 ± 0·22	0·74 ± 0·09	1·31 ± 0·20	1·02 ± 0·16	1·27 ± 0·07
Injection	4*	1·20 ± 0·05	0·78 ± 0·09	0·63 ± 0·20	0·74 ± 0·02	1·19 ± 0·10

The values are computed as the ratio between relative distribution of mercury and relative distribution of protein in fractions and given as mean ± standard error.

* Four groups of livers from two rats each.

The concentration of mercury in the light mitochondrial fraction in livers from rats having been exposed to MMDD in the drinking water is higher than for the injected rats (rel.sp. content 1·31 vs. 0·63). The microsomal fraction also contained a slightly higher concentration of mercury, whereas no differences could be found in mitochondrial and nuclear fractions or in the supernatant (Table 1).

The results were statistically treated as described earlier.[1,2] For comparison to previously published results on other mercury compounds, protein distribution in pure particle populations was estimated by the same principles in order to calculate relative specific mercury content in cellular particles. Protein distribution values have not been recorded in previous papers. Table 2 shows the distribution coefficients applied in these

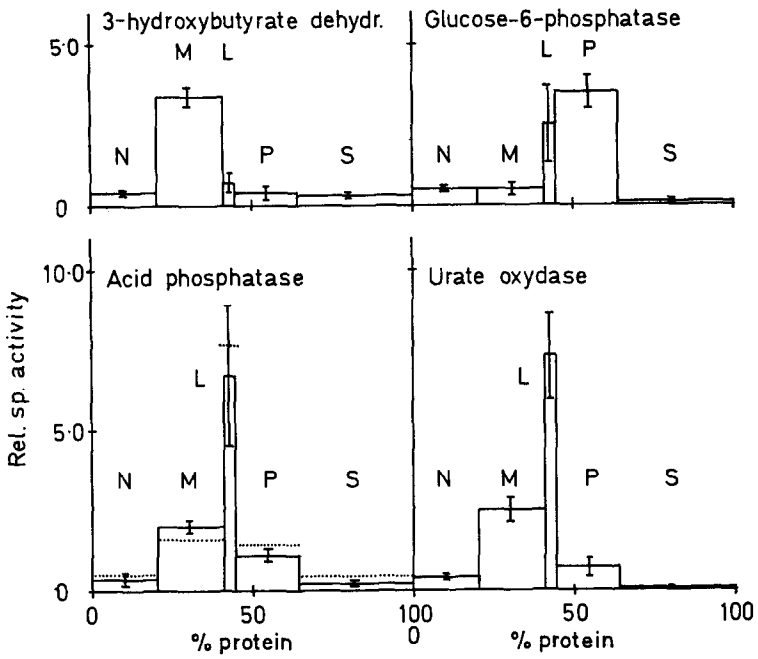

FIG. 1. Activity of marker enzymes in cellular fractions. The results are given as relative specific enzyme activity.

$$\text{Rel. sp. activity} = \frac{\%\ \text{enzyme activity}}{\%\ \text{protein}}$$

of total in the liver in a given fraction.

3-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate: NAD oxidoreductase 1.1.1.30) was used for mitochondria, correspondingly acid phosphatase (orthophosphoric monoester phosphohydrolase 3.1.3.2) for lysosomes, urate oxydase (urate: oxidoreductase 1.7.3.3) for peroxisomes and glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase 3.1.3.9) for microsomes.

The results are shown as mean ± S.E. (n = 10) for nuclear (N), mitochondrial (M), light mitochondrial (L) and microsomal (P) fractions and for the supernatant (S) from rats exposed to MMDD in the drinking water. The dotted line for acid phosphatase indicates methylmercury chloride injected animals (n = 4).

calculations and Tables 3 and 4 show the distribution of mercury and protein among cellular fractions and particles. These tables serve to underline the difference between fractions and particle populations which must be kept in mind to understand the principles of this method. Even if the lysosome/peroxisome population contains the highest concentration of mercury after exposure to MMDD, a considerably higher concentration of mercury in these organelles is found after exposure to MMC mercuric chloride (Table 5). A high mercury concentration in lysosomes/peroxisomes is also found after methoxyethylmercury acetate exposure, relative specific mercury content was 4·87, compared to 6·76 for inorganic mercury, but clearly higher than 1·89 for methylmercury. The mercury concentration in mitochondria did not vary dramatically when the three mercury compounds were compared, relative specific content was found to 0·77 for inorganic mercury, 0·58 for methoxyethylmercury and 0·52 for methylmercury. The mercury concentration in microsomes showed some variation, but with a different pattern compared to lysosomes/peroxisomes. Relative specific

TABLE 2. COEFFICIENTS COMPUTED FOR MERCURY AND PROTEIN DISTRIBUTION

Enzyme	Coefficient ± Upper limit of SD*	
	Mercury	Protein
3-hydroxybutyrate dehydrogenase (α_1)	0·1178 ± 0·0405	0·2285 ± 0·0140
Acid phosphatase and urate oxydase (α_2)	0·0423 ± 0·0142	0·0148 ± 0·0069
Glucose-6-phosphatase (α_3)	0·2556 ± 0·0495	0·2518 ± 0·0105

* The statistical method does not allow accurate determination of the standard deviation.

TABLE 3. MERCURY CONTENT IN CELLULAR PARTICLES AND FRACTIONS

Particle population	Fraction					Total
	N	M	L	P	S	
Mitochondria	1·0	8·3	0·3	0·9	1·4	11·9
Lysosomes and peroxisomes	0·6	4·1	0·9	1·0	0·4	7·0
Microsomes	2·8	2·8	2·4	17·8	0·7	25·9

Values are given as percentage of total mercury per gram liver.

TABLE 4. PROTEIN CONTENT IN CELLULAR PARTICLES AND FRACTIONS

Particle population	Fraction					Total
	N	M	L	P	S	
Mitochondria	1·9	16·0	0·6	1·7	2·7	22·9
Lysosomes and peroxisomes	0·3	1·7	0·9	0·6	0·2	3·7
Microsomes	2·7	2·8	2·1	16·8	0·7	25·1

Values are given as percentage of total protein per gram liver.

TABLE 5. DISTRIBUTION OF MERCURY EXPRESSED AS RELATIVE MERCURY CONTENT IN PARTICLES

	Methyl mercury	Methoxyethyl* mercury	Inorganic* mercury
Mitochondria	0·52	0·58	0·77
Lysosomes/peroxisomes	1·89	4·87	6·76
Microsomes	1·03	0·75	0·50

The values are computed as described from the relative distribution of mercury and protein among particles.

* Recalculated from previous papers.[1,2]

content for inorganic mercury was 0·50, for methoxyethylmercury 0·75 and for methylmercury 1·03. These values are estimated from mercury distribution in the present and previous papers and protein distribution from the present paper.[1,2]

The relative amount of inorganic mercury in the light mitochondrial fraction after injection of MMC was higher than in other fractions in all four assays reported (Table 6). The average value of 36·6 per cent was about twice as high as for the mitochondrial fraction, 19·9 per cent, which contained the second highest relative amount. The

TABLE 6. RELATIVE AMOUNT OF INORGANIC MERCURY IN CELLULAR FRACTIONS

Test no.	Total Hg (μg/g)	% of total in each fraction as inorganic				
		N	M	L	P	S
14/4	1·15	6·4	18·2	34·9	14·3	11·8
27/4	2·18*	6·6	14·9	48·5	8·8	6·6
25/5	0·95	6·9	15·2	20·3	10·5	9·2
16/6	0·88	10·7	27·3	42·8	18·1	20·0

All rats were killed 4 days after intravenous injection of 1 mg Hg/kg as methylmercury chloride. Each test no. consisted of livers from two rats.
* 2 mg Hg/kg.

nuclear fraction contained the lowest relative amount of inorganic mercury, 7·7 per cent, slightly lower than the supernatant and the microsomal fractions which contained 11·9 per cent and 12·9 per cent, respectively. The separate experiments are shown in the table because of the variation in total mercury content.

Based on the figures in Table 6 the amount of inorganic mercury in each fraction can be computed. Figure 2 shows that the distribution of inorganic mercury released in the liver 4 days after intravenous injection of MMC is similar to the distribution of mercury 4 days after injection of mercuric chloride. The highest concentration of mercury is found in the light mitochondrial fraction, and also the other fractions show the same distribution pattern.

DISCUSSION

The results demonstrate the importance of an analytical approach to the fractionation procedure as discussed in previous papers.[1,2] The method has been further improved by estimation of protein content in organelles. The protein values are in accordance with results obtained by other methods.[5–9] With ordinary preparative techniques, the high concentration of mercury in lysosomes after exposure to inorganic mercury, and in microsomes after exposure to methylmercury, with methoxyethylmercury in an intermediate position would not have been as easily demonstrated.

The results demonstrate that inorganic mercury, defined as mercury not bound covalently to a carbon atom, is accumulated by the lysosomal apparatus of the liver cell. The carbon–mercury bond in methoxyethylmercury compounds has been reported to be unstable in the rat.[10] Methylmercury salts also release some inorganic mercury in the rat liver, but probably at a slower rate than methoxyethylmercury salts.[11] The different concentrations of mercury in the lysosomes may therefore reflect the stability

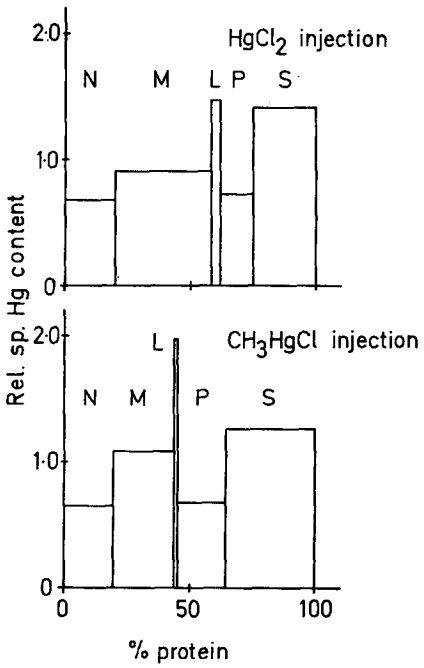

FIG. 2. Relative specific content of inorganic mercury in cellular fractions after injection of mercuric chloride and methylmercury chloride.

$$\text{Rel. sp. Hg content} = \frac{\% \text{ mercury}}{\% \text{ protein}}$$

of total in the liver in a given fraction. Fractions are indicated as in Fig. 1.

of the organomercurial. No mercury in covalent binding to carbon could be found in the rat liver after intravenous injection of mercuric chloride.* The intact organomercurials, both methoxyethylmercury and methylmercury compounds, on the other hand, are most efficiently concentrated in the microsomes.

The length of the exposure period for the three compounds was not the same. Mercuric chloride was intravenously injected, methoxyethylmercury added in the drinking water for 8–10 weeks and methylmercury dicyandiamide was added to the drinking water for about one year. These different exposure patterns were immediate needs and could not be controlled by the author. Some preliminary results on the separate distributions of inorganic mercury and the intact organomercurial were therefore included to support the above conclusions.

When methylmercury chloride is injected, a lower concentration of mercury is found in the light mitochondrial fraction after 4 days than for the drinking water exposed animals. Even if no calculation of mercury in the pure lysosome/peroxisome fraction can be done, this is evidence for the differences in exposure to be of no major consequence for the relationship between stability of the carbon–mercury bond and accumulation of mercury in lysosomes. The anion has been shown earlier to have no consequences for the distribution of methylmercury salts.[13] More mercury in the

* Author's own unpublished observations.

drinking water exposed animals may in fact be inorganic. An increasing relative amount of inorganic mercury with time after a single injection of methylmercury chloride was demonstrated in the liver in a previous paper.[11]

An accumulation of inorganic mercury in lysosomes with a distribution pattern of organomercurials related to their stability is further supported by a relative amount of inorganic mercury in the light mitochondrial fraction higher than in any other fraction after injection of MMC. The distribution pattern of this inorganic mercury is further the same as the distribution of mercury 4 days after injection of mercuric chloride.

These differences in intracellular distributions may have toxicological importance. Methylmercury cysteine excreted in the bile is efficiently reabsorbed from the small intestine.[12] Inorganic mercury excreted as a protein complex, possibly of lysosomal origin, is transported out with feces.[12] Mercury after exposure to methoxyethyl compounds is more rapidly cleared from the body than after exposure to methylmercury salts, and fecal excretion is important.[13] A small amount of mercury is also excreted in feces after exposure to inorganic mercury. Accumulation of mercury by the kidney may be related to lysosomal uptake. Lysosomal accumulation of mercury has been demonstrated in the kidney by histochemical methods.[14] Inorganic mercury is known to be efficiently trapped by the kidney, so is mercury after exposure to the unstable phenylmercury compounds.[15–17] Methylmercury exposure also leads to a retention of mercury by the kidney, probably to some extent related to release of inorganic mercury.[11] Exposure to methoxyethylmercury salts leads to a slow redistribution of mercury with time, indicating the same mechanism.[13] This accumulation may serve as a detoxification mechanism both in liver, kidney and brain, and may be the rate factor determining the toxicity of mercurials to the kidney. Inorganic mercury is known to be toxic at about 30 μg Hg/g kidney, while the same amount, or more inorganic mercury can be accumulated in the kidney from phenylmercury salts or by repeated dosing of inorganic mercury.[17,18]

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