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THE INTRACELLULAR DISTRIBUTION OF MERCURY IN RAT LIVER AFTER A SINGLE INJECTION OF MERCURIC CHLORIDE

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Abstract—The intracellular distribution of mercury in rat liver has been investigated at different times following a single injection of mercuric chloride. The centrifugal technique is based on marker enzyme distribution for characterizing the fractions, followed by a purification step for lysosomes and peroxisomes. The mercury content in cellular fractions has been examined and estimates are given of the mercury content in mitochondria, lysosomes, peroxisomes, and in microsomes. An accumulation of mercury is found in lysosomes with increasing time. The symptoms of mercury intoxication are compared with possible results of lysosomal injury. The possibility of studying physiological functions of lysosomes applying similar techniques is pointed out. Further the potential usefulness of the procedure is outlined for gaining additional information on the biochemical lesions in different forms of mercury intoxications.

THE INTRACELLULAR distribution of mercury may determine the clinical picture in mercury intoxication. An analytical centrifugal technique with marker enzymes for cell fractionation was recently adopted for a study on the distribution of mercury in rat liver after methoxyethylmercury exposure.[1] Comparison of intracellular distribution of different mercury compounds may supply additional information about biochemical lesions caused by these compounds.

In this report is presented the intracellular distribution of mercury in rat liver at different times after mercuric chloride injection. The previously described analytical centrifugal technique is extended with a preparative part for estimating mercury distribution between lysosomes and peroxisomes.

MATERIALS AND METHODS

Labelled mercury (^{203}Hg) with a sp. act. of 1·2–1·8 mC/mg was purchased as $^{203}HgCl_2$ from 'Institutt for Atomenergi', Kjeller, Norway. This solution was diluted with saline before injection, but always supplied more than 2000 cpm in the samples to be counted. All other chemicals were obtained through commercial sources in the purest form available.

Female rats weighing about 300 g and male rats weighing about 400 g were used. The weight difference was due to availability and did not affect the results. The rats were given a single injection of 0·5 mg mercury per kg in 100 μl saline in the exposed

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great saphenous vein when under light ether anesthesia. The animals were killed by decapitating, subsequently bled, and the livers were removed and homogenized as described by de Duve *et al.*[2] with slight modifications.[1] This method results in a nuclear fraction and a cytoplasmic extract.

For the analytical part of the study livers from two rats were pooled for each assay. The cytoplasmic extract was fractionated according to Appelmans *et al.* to give a mitochondrial fraction, a light mitochondrial fraction, a microsomal fraction, and a supernatant.[3]

One liver was used for each assay in the lysosome and peroxisome purification procedure. The rats were injected with Triton WR-1339 (1 g/kg) 4 days before being killed.[4] The injections were done as for mercury in the other saphenous vein. A combined mitochondrial and light mitochondrial fraction (M + L) obtained as the pellet after centrifugation by 25×10^4 *g*-min* and washed twice, was prepared for fractionation by gradient centrifugation. Care was taken not to contaminate the (M + L) fractions with microsomal components, but some contamination was still found. 250 μl (M + L) fraction was deposed on the top of an about 5 ml continuous sucrose gradient with density ranging from 1·16–1·26. The gradient was centrifuged for 180 min at 39×10^3 rpm applying Spinco model L and head SW-39.[4–6]

A solution of sucrose (2 g/ml) was pumped (0·5 ml/min) into the centrifuge tubes from the bottom to remove the gradient. The tubes were fitted to a tight-fitting cap, and the gradient was forced through a thin hose from the top. Before fractions were collected, the gradient passed through a Uvicord type 4701A equipment for transmittance recording. Fractions were collected at intervals of 1 min. Each gradient supplied 11 fractions of 0·5 ml, the last of which contained gradient material and a variable amount of heavy sucrose solution.

3-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate: NAD oxidoreductase 1.1.1.30) was used as marker enzyme for mitochondria,[7] correspondingly acid phosphatase (orthophosphoric monoester phosphohydrolase 3.1.3.2) was used for lysosomes,[2] urate oxydase (urate:oxidoreductase 1.7.3.3) for peroxisomes[6] and glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase 3.1.3.9) for microsomes.[8] The enzymic activities were determined as previously described with slight modifications for acid phosphatase when fractions from the continuous gradients were tested.[2, 7, 9, 10] Because of the limited amount of tissue suspension available, 20 hr incubation with thiomersalate and without Triton X-100 was applied. Proteins were estimated by the method of Lowry *et al.*[11]

The isotope concentration was measured applying an ordinary well type gamma scintillation counter or a Packard Tricarb model 3003 when large series from the gradients were counted. For the liquid scintillation counting the vials contained 100 μl tissue suspension, 1 ml Hyamine[12] and 10 ml Brays solution.[13] The tissue and Hyamine were incubated at 37° when necessary for getting transparent solutions without undissolved proteins. No quenching corrections were necessary in this counting system as all samples were quenched to the same degree. A counting standard, also with the same degree of quenching, was used for calculating total amount of mercury. The rats contained no mercury before the injections. Proportionality was assured by counting standard series with known amount of mercury in both counting systems.

* *g*-Min is average centrifugal force in the tube at constant rpm times centrifugal time in minutes.

RESULTS

Because of the differences in the experimental design for the analytical part and the preparative part in this study, the results for the two parts have to be considered separately. To apply the previously described statistical method for evaluating the general distribution pattern of mercury in the liver, determination of marker enzyme distribution is necessary in each fraction used for mercury distribution determination.[1] These results are found in Tables 1–8 and Fig. 1. In the purification step for lysosomes and peroxisomes the marker enzyme distribution is used for characterizing fractions without further computations. Consequently, some typical values for the enzyme distribution supply the necessary information (Tables 9–11 and Figs. 2–4).

Table 1 shows the mercury content in blood computed by comparing the samples with a known standard sample. Correspondingly, the mercury content in the liver and

TABLE 1. MERCURY CONTENT IN BLOOD

Time interval	No. of rats	μg Mercury/ml (mean ± S.D.)
1 hr	10	0·65 ± 0·11
1 day	10	0·39 ± 0·14
4 days	10	0·18 ± 0·09

TABLE 2. MERCURY CONTENT IN TOTAL LIVER AND IN LIVER CELL FRACTIONS

Time interval	No. of expts.	μg Mercury Mean ± S.D.	% recovery Mean ± S.D.	μg Mercury in fractions* N	M	L	P	S
1 hr	5	4·50 ± 1·50	84·1 ± 3·1	0·95	0·72	0·13	0·41	2·28
1 day	5	1·39 ± 0·52	88·2 ± 3·6	0·10	0·30	0·05	0·17	0·77
4 days	5	0·74 ± 0·67	82·8 ± 1·7	0·09	0·22	0·05	0·08	0·29

Values are given per g wet wt. liver. Recovery is computed from the mercury content of the nuclear, mitochondrial, light mitochondrial, and microsomal fractions, and from the supernatant.
* Corrected for recovery.

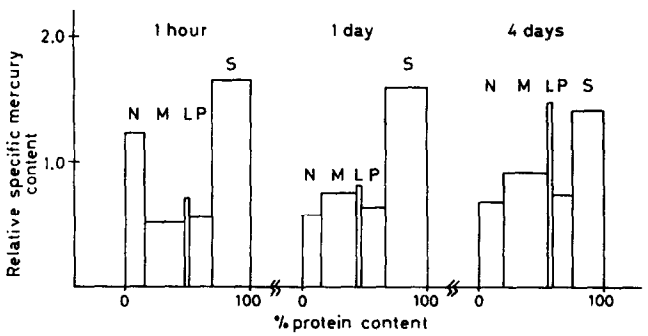

FIG. 1. The distribution of mercury in nuclear (N), mitochondrial (M), light mitochondrial (L), and microsomal (P) fractions, and in the supernatant (S) from rat liver cells at different time intervals after a single injection of mercuric chloride.

Rel. sp. mercury cont. = percentage mercury per percentage protein content.

in the liver cell fractions was estimated (Table 2). The low recoveries of mercury are difficult to explain. Loss by evaporation in connection with bacterial degradation is possible,[14] though all homogenates were kept refrigerated during storing and handling. However, as no specific loss of mercury was found examining the procedure, the recoveries were considered satisfactory for further calculations and conclusions. Fig. 1 shows the mercury distribution in fractions expressed as relative specific content*.[2] Table 3 shows the total activity[2] of the marker enzymes and the protein content of the liver homogenate. These values provide a test for the enzyme assay procedures. That there are two figures for 3-hydroxybutyrate dehydrogenase is due to the two series of rats. Both values agree with the findings in earlier reports.[1, 7] Table 4 shows the relative

TABLE 3. ENZYMATIC ACTIVITY AND PROTEIN CONTENT IN RAT LIVER

	No. of expts.	Total activity Mean ± S.D.	Per cent recovery
3-Hydroxybutyrate	10*	11·18 ± 2·99	104·7 ± 11·5
dehydrogenase	5†	4·57 ± 1·02	95·8 ± 19·1
Acid phosphatase	15	8·10 ± 1·14	86·2 ± 4·0
Urate oxidase	15	2·11 ± 0·75	75·0 ± 8·3
Glucose-6-phosphatase	15	20·64 ± 4·53	90·4 ± 10·1
Protein	15	209·50 ± 21·50	83·4 ± 5·2

Total activity is the sum of activity in the nuclear fraction and the cytoplasmic extract expressed as μmole of substrate decomposed per min under the conditions of the assay. Recovery is computed as in Table 2.
* Twenty male rats.
† Ten female rats.

TABLE 4. RELATIVE SPECIFIC ACTIVITY OF MARKER ENZYMES IN FRACTIONS

Enzyme	Time interval	Nuclear	Mitochondrial	Light mitochondrial	Microsomal	Supernatant
3-Hydroxybutyrate	1 hr	0·488	2·763	0·056	0·189	0·047
dehydrogenase	24 hr	0·327	3·180	0·022	0·183	0·064
	4 days	0·372	2·665	0·056	0·119	0·058
Acid phosphatase	1 hr	0·491	1·362	4·590	1·244	0·349
	24 hr	0·358	1·622	3·263	1·186	0·365
	4 days	0·344	1·238	4·435	1·378	0·424
Urate oxidase	1 hr	0·374	1·535	4·303	1·275	0·204
	24 hr	0·217	2·211	3·311	0·810	0·147
	4 days	0·214	1·957	3·335	0·657	0·195
Glucose-6-	1 hr	0·766	0·471	3·281	3·296	0·105
phosphatase	24 hr	0·567	0·564	2·845	3·169	0·107
	4 days	0·555	0·388	4·022	3·731	0·157

Values are computed from per cent distribution corrected for recovery as described in the text. Each value represents the mean of 5 experiments.

sp. act. of the marker enzymes at each of 3 time intervals. These figures express the purity of the fractions with respect to the cellular component represented by the marker enzymes.[2] This statement rests on the principle of biochemical homogeneity of cellular components.[15] The low recovery for some enzymes (Table 3) is of no account

* Rel. sp. cont. (rel. sp. act) is percentage of total enzymic activity (mercury content) per percentage of total protein.

as no major differences, compared with earlier reports, are found in the cellular component distribution.[2, 7, 16] The results were corrected for recovery before being used in the further calculations. Knowledge of the distribution of cellular components at the different time intervals is important for conclusions based on comparison of mercury content. The component distribution must be known for each fractionation because conclusions concerning the mercury content in components rest on a comparison between the component content in fractions and the corresponding mercury content in each liver homogenate.

The analytical part of the fractionation technique is in accordance with the principles necessary for applying the following set of equations for computing the mercury content in cellular components:[1]

$$\overline{Hg}_M = a_1\overline{X}_{1M} + a_2(\overline{X}_{2M} + \overline{X}_{3M}) + a_3\overline{X}_{4M}$$

$$\overline{Hg}_L = a_1\overline{X}_{1L} + a_2(\overline{X}_{2L} + \overline{X}_{3L}) + a_3\overline{X}_{4L}$$

$$\overline{Hg}_P = a_1\overline{X}_{1P} + a_2(\overline{X}_{2P} + \overline{X}_{3P}) + a_3\overline{X}_{4P}$$

$\overline{Hg}$ represents the mean relative specific mercury content, M, L, and P indicate the mitochondrial, light mitochondrial, and microsomal fractions respectively. $\overline{X}_1$, $\overline{X}_2$, $\overline{X}_3$, and $\overline{X}_4$ represent the mean relative specific activities of 3-hydroxybutyrate dehydrogenase, acid phosphatase, urate oxydase and glucose-6-phosphatase respectively. a_1, a_2, and a_3 represent unknown coefficients. The results in Tables 2–4 and in Fig. 1 thus provide 3 sets of equations, each comprising 5 fractionation assays for calculating the mercury content in cellular components after 1 hr, 1 day and 4 days.

Table 5 shows the above coefficients and the upper limit of their standard deviation.[1] The percentage distribution of mercury among cell components appears from Table 6, and for comparison Table 7 shows the amount of mercury in cellular components at different time intervals.

A corresponding statistical analysis provides a test for the amount of mercury bound to cellular components other than those characterized by the 4 marker enzymes in the 3 fractions used in the statistical estimations.[1] Table 8 shows that the computed and the found mercury contents are in accordance where this might be expected. This is essential for proving the reality of the statistical model in the present investigation.

Table 9 shows the protein content and marker enzyme activity in the typical (M + L) fraction in the Triton injected rats. Most observations are reported for acid phosphatase as lysosomes are the only cellular particles with changed properties in the applied procedure following Triton WR-1339 treatment as described. The mercury

TABLE 5. COEFFICIENTS FOR THE STATISTICAL DISTRIBUTION ANALYSIS AT DIFFERENT TIME INTERVALS

Enzyme	Coefficients ± upper limit of S.D. 1 hr	1 day	4 days
3-hydroxybutyrate dehydrogenase (a_1)	0·132 ± 0·013	0·132 ± 0·013	0·177 ± 0·023
Acid phosphatase and urate oxydase (a_2)	0·027 ± 0·007	0·067 ± 0·009	0·125 ± 0·032
Glucose-6-phosphatase (a_3)	0·134 ± 0·013	0·151 ± 0·012	0·125 ± 0·025

The probability model does not allow accurate calculation of the S.D.'s.

TABLE 6. MERCURY DISTRIBUTION AMONG CELLULAR COMPONENTS IN CENTRIFUGAL FRACTIONS AND IN THE CELL AT DIFFERENT TIME INTERVALS

Cellular component	Time after injection	Per cent (of total) mercury content in fractions N	M	L	P	S	Total	Per cent in components*
Mitochondria	1 hr	1·10	11·50	0·02	0·42	0·19	13·23	41·1
	1 day	0·62	11·80	0·01	0·45	0·29	13·17	31·8
	4 days	1·27	15·77	0·05	0·30	0·29	17·68	32·1
Lysosomes and peroxisomes	1 hr	0·41	2·52	0·80	1·17	0·47	5·37	16·7
	1 day	0·55	7·23	1·94	2·47	1·19	13·38	32·3
	4 days	1·35	13·34	4·47	3·66	2·17	24·99	45·3
Microsomes	1 hr	1·83	2·07	1·51	7·74	0·45	13·60	42·2
	1 day	1·22	2·40	1·89	8·85	0·56	14·92	36·0
	4 days	1·33	1·61	2·31	6·69	0·55	12·49	22·6

* Mitochondria, lysosomes, peroxisomes and microsomes taken as total (100%). Each value represents the mean of 5 experiments.

TABLE 7. MERCURY CONTENT IN INTRACELLULAR COMPONENTS

	Mitochondria	Lysosomes Peroxisomes	Microsomes
1 hr	0·60	0·24	0·61
1 day	0·18	0·19	0·21
4 days	0·13	0·18	0·09

Values are computed from the estimated percentage distribution and total content of mercury and given as μg per g wet wt. liver.

TABLE 8. COMPUTED AND FOUND MERCURY CONTENT IN FRACTIONS

Fraction	Time	Computed	Found
Nuclear	1 hr	3·2	21·2
	24 hr	1·4	7·3
	4 days	4·8	12·7
Supernatant	1 hr	1·1	50·6
	24 hr	3·5	55·6
	4 days	2·6	39·7
Microsomal	1 hr	8·9	9·3
	24 hr	10·5	11·9
	4 days	14·6	10·5

Values are given in percentage of total mercury content. The computed values are determined as in Tables 5 and 6, the found values are taken from Fig. 1 and the corresponding protein distribution.

content at different time intervals in the cytoplasmic extract with corresponding (M + L) fraction is shown in Table 10. Fig. 2 shows a density diagram of the gradient with Evans Blue as a visualizing agent and the typical Uvicord transmittance pattern of the gradient after centrifugation with tissue suspension. The transmittance is probably related to several factors including protein and nucleic acid content, and the size, form and concentrations of cellular particles, but it represents a test for the reproducibility of preparation procedures for gradients and cytoplasmic extracts.

TABLE 9. ENZYMIC ACTIVITY AND PROTEIN CONTENT IN THE TYPICAL CYTOPLASMIC EXTRACT AND M + L FRACTION

	No. of expts.	Cytoplasmic extr. Enzyme units* (mg)	M + L fraction Percentage of total
Protein	6	171·6 ± 3·6	24·5 ± 2·5
3-Hydroxybutyrate dehydrogenase	1	6·4	77·3
Acid phosphatase	6	2·5 ± 1·1	18·9 ± 5·6
Urate oxydase	1	1·1	54·3
Glucose-6-phosphatase	2	22·0	9·4

* One enzyme unit = 1 μmole substrate decomposed per min per g wt. liver under the conditions of the assay.

TABLE 10. MERCURY CONTENT IN CYTOPLASMIC EXTRACT AND M + L FRACTION AT DIFFERENT TIME INTERVALS

	1 hr	1 day	4 days	7 days	9 days	14 days
Cytoplasmic extract μg Hg per g wet liver	9·0	3·3	1·8	1·7	0·4	0·4
M + L fraction Percentage of total	9·7	10·0	10·5	9·1	12·0	13·8

Two rats were injected for the time intervals 1 hr and 1 day, otherwise the figures represent 1 rat.

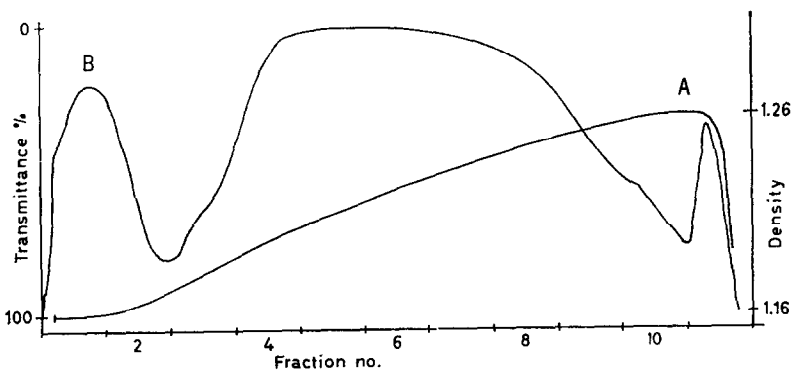

FIG. 2. A shows the gradient density visualized by adding Evans Blue to the 1·26 sucrose solution before blending with the 1·16 solution to make the gradient. B shows the density diagram of the typical gradient after having been centrifuged with tissue suspension as described in the text. No Evans Blue added.

The marker enzyme distribution in the gradient is shown in Fig. 3. The distribution of glucose-6-phosphatase was determined applying a modified (M + L) fraction. The usual fraction did not contain sufficient enzyme for the assays. The distribution was found to be almost equal throughout the gradient with a slight accumulation in fractions 4–6. These results are not shown in the figure, but they supply the necessary information about the microsomal contamination of the gradient. The recovery of enzymic activity from the gradient was low and variable for all enzymes. This was probably due to inactivation of enzymes during gradient handling, and to accumulation of errors related to small activity values and as much as 11 fractions. This low

recovery was found to be of no consequence as the activity patterns were almost identical for assays with different recovery. Typical values are shown in the table. The recovery of protein and mercury was satisfactory (Table 11). For the further calculations protein distribution analysis must comprise all assays used for mercury distribution analysis (Table 11) as this part of the calculations depends on the principles applied in the analytical part of the study. The complete assay procedure in this part of the STUDY is not in accordance with these principles because marker enzyme distribution analysis does not include all rats.[1]

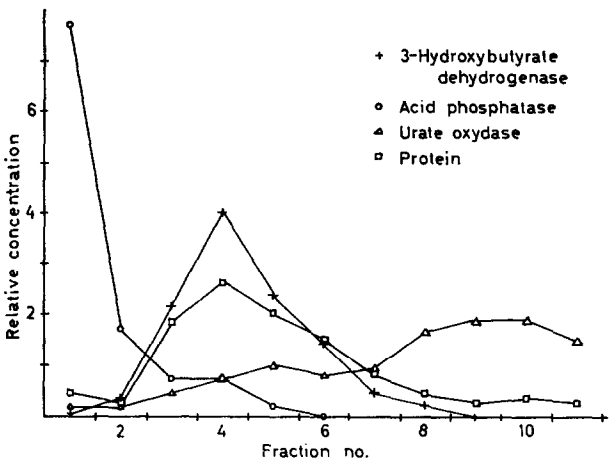

FIG. 3. Distribution of enzyme activity of marker enzymes in the gradient. Glucose-6-phosphatase activity is not shown as it was too small to be registered in fractions by the usual assay procedure. Relative concentration is the ratio of enzymic activity in a fraction to the computed activity per fraction provided equal activity in all fractions.

TABLE 11. RECOVERY OF PROTEINS AND MERCURY FROM THE GRADIENT

	No. of rats	No. of gradients	Recovery ± S.D.
Proteins	6	18	105·4 ± 8·9
Mercury	6	18	93·5 ± 1·2

Recovery is calculated as the sum of the 11 fractions from the gradient. The values represent 3 gradients from each of all rats used for the calculations in Fig. 3.

Fig. 4 shows the relative specific mercury content at different time intervals in fraction 1 and in the pooled fractions 7–11. Fraction 1 contains only traces of other marker enzymes than acid phosphatase. With the assumption that marker enzyme distribution represents distribution of the corresponding cellular component,[15] the relative specific mercury content in fraction 1 expresses the relation between mercury concentration in lysosomes and the corresponding (M + L) fraction. The relative

specific mercury content in the pooled fractions 7–11 thus represents the relative mercury content in peroxisomes. Fractions 2–6 contain mitochondria, most of the microsomal contamination and some lysosomes and peroxisomes. The relative specific mercury content in these fractions allows no conclusions concerning mercury distribution and is therefore not included in the figure.

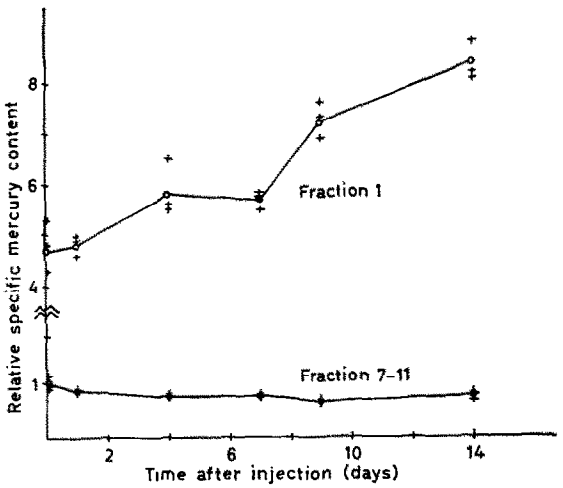

FIG. 4. The ratio of percentage amount of mercury to percentage amount of protein in fractions (rel. sp. mercury cont.) is shown for the pure lysosomal fraction (1), and for the pure peroxisome fractions (7–11) at different time intervals. Each of 3 gradients are indicated for each time interval. The curves are calculated from the mean values of 3 gradients from 1 rat.

DISCUSSION

The problems of centrifugal cell fractionation when investigating the distribution of enzymes within the cell have been thoroughly reviewed by de Duve.[15] The adoption of the same principles for distribution analysis of other compounds has been discussed in a previous paper.[1] Applying marker enzymes it is possible to draw conclusions concerning unknown distributions in relation to known cellular components. The value of such conclusions rests on the principle of biochemical homogeneity which has proved useful so far.[15] The marker enzymes also permit continuous testing of the fractionation procedure. Constant activity of marker enzymes in fractions indicates a constant content of cellular components. This is essential for obtaining comparable results in centrifugal cell fractionation investigations. Both the principle of biochemical homogeneity and that of standardization for comparison are of importance in the present study.

Almost pure fractions of lysosomes and peroxisomes can be obtained from rat liver by injecting the animals with Triton WR-1339 and fractionating the tissue by differential- and gradient centrifugation.[4, 6] This method is suitable for evaluating the mercury distribution among these cell components. The results of this preparative part of the distribution study of mercury cannot, however, without reservations be compared to the results obtained by analytical methods without Triton, but supply a valuable supplement.

When injected i.v., inorganic mercury accumulates transitorily in the liver.[17] This accumulation is present in all cell fractions as shown when comparing the mercury content at different time intervals in blood, liver and liver cell fractions. The relative amount of mercury in the cell fractions also changes with time. There are no major differences in the content of cellular components in corresponding fractions at the different time intervals. The differences in relative mercury content in fractions therefore represent a true difference in mercury distribution. Consequently, without further calculations it is found that the proportion of mercury in different cellular particle populations changes with time after a single injection of mercuric chloride. Constancy is assumed for the amount of protein in cellular components. Thus the distribution of mercury at different time intervals indicates a different mercury concentration in cellular organelles.

The high transitory accumulation of mercury in the nuclear fraction is not conclusive for an accumulation in the nucleus. This fraction is very heterogeneous and purified nuclear fractions are necessary for final conclusions. The transitory accumulation is also marked for the mitochondria and the microsomes, whereas the combined lysosome and peroxisome population retains the mercury. These conclusions depend on the statistical evaluation with use of marker enzyme distribution and illustrate the inadequacy of the preparative fractionation principles for analytical purposes. Besides the amount of mercury transitorily accumulated in the mitochondria, a certain more tightly bound amount is present. This is concluded from the increasing percentage of the total mercury in the cell found in mitochondria, the insignificant decrease after 1 day, and the almost constant percentage of mercury when computed from the content in components only.

The lysosome and peroxisome population has an almost constant mercury content throughout the 4-day period. Consequently, both the percentage taken of total mercury and taken as percentage of mercury in all components, increase. The results with Triton injected rats show this accumulation to be related to the lysosomes.

Triton injection increases mercury content in liver cells at all time intervals. The lysosomes may be responsible for this increase, but as no analytical study is done with Triton injection, the comparison is uncertain.

A lysosomal retention of mercury in the cell is obvious. The results are not without reservations conclusive for a corresponding accumulation without Triton treatment. The effect on lysosomes of the combined action of Triton and mercury are unknown. The S.D. of acid phosphatase activity in the (M + L) fractions indicates greater variation of lysosome content in the fractions than without Triton. This may be related to Triton uptake because of different injections or variation between animals to the effect of Triton, but may also result from mercury and Triton interaction as the acid phosphatase activity is computed from assays with different mercury content. Because of the experimental design analysis of variance or covariance estimation cannot be done. No indications were found for dependance between mercury and Triton judged by acid phosphatase distribution, but the results are too few for final conclusions.

Liver peroxisomes are unchanged after Triton WR-1339 treatment of the rats in this fractionation system.[6] No accumulation of mercury is found in this cell component. Although interactions between mercury and Triton on lysosomes may exist, it is unreasonable to assume a resulting complete deprival of a normal mercury

retention in peroxisomes. The combined results for lysosomes and peroxisomes in this part of the study therefore strongly suggest that lysosomes are responsible for the retention of mercury in the combined lysosome and peroxisome population demonstrated in the analytical part.

The lysosomal membrane may be temporarily damaged during the anesthesia because of ether or hypoxemia.[18, 19] Mercury may be taken up through the membrane and this in turn restored to normal with slight permeability for mercury. Some of the rats were anesthetized when killed to test this, but no differences in mercury distribution were found. Lysosomes may contain ligands binding mercury more tightly than other cell components, the ligands responsible for the retention of some mercury in the mitochondria excepted. Clarkson and Magos found two groups of ligands with different affinity to mercury in liver cell homogenates,[20] but both had greater binding capacity than the total amount of mercury in the cell in the present experiments. The retention in lysosomes may be a result of the normal scavenger function of lysosomes in the cell.[21]

Uptake and excretion of mercury by the cell may be related to the microsomal mercury content. The constant proportion of total mercury in the cell found in this cell component and the decreasing percentage amount if particle bound mercury is taken as total, indicate an equilibrium between cellular and microsomal content of mercury. The excretion of mercury may also be related to lysosome function.

Redistribution during homogenizing and handling of homogenates may obscure the results from this kind of distribution studies. Redistribution cannot be ruled out, but seems unlikely as different concentrations of mercury are found in cellular organelles at different time intervals. The total amount of mercury is different, but far from exceeding the capacity of the most active mercury binding ligand in liver homogenate described by Clarkson and Magos.[20]

The intracellular distribution of mercury at different time intervals may reflect the amount of mercury in different cell types in the liver. Accumulation of mercury may be expected in the Kuppfer cells or in other cells of the reticulo-endothelial system. Autoradiographic results show accumulation of mercury in the periportal connective tissue,[17, 22] but no specific accumulation was found in reticulo-endothelial cells.[22] The increased acid phosphatase activity found in rabbit liver during mercury intoxication is more prominent in the glandular cells than in reticulo-endothelial cells and connective tissue.[23]

These results may explain some of the aspects of mercury intoxication. Increased acid phosphatase activity is found histochemically in brain, liver, and kidney during mercury exposure in rabbit and rat.[23–25] The increased activity may be secondary to other cell damage by mercury, but the possibility exists for a primary lysosomal damage. Similar problems have been discussed in relation to carbontetrachloride exposure,[26, 27] but no final conclusions have been stated. An increased activity of acid phosphatase may depend solely on lysosomal injury as the substrate permeability of the lysosomal membrane is normally limited. Temporary or limited membrane damage may therefore cause increased enzymic activity with no consequences for other parts of the cell. With more severe membrane damage the lysosomal enzymes may escape, thus initiating cell injury or death.

The details in the multiple physiological functions of lysosomes are uncertain. Cell and tissue injury or impared cell function may be related to impaired lysosomal

function without morphological alterations.[21] The cell scavenger function of lysosomes may be impaired. The results will probably be most marked in cells with long turnover, as nerve cells. The lysosomal function in the thyroid gland related to the splitting of thyreoglobulin should be remembered as mercurialism shows some resemblance to hyperfunetion of this gland.[28–30] Deficient reabsorption of normally filtrated protein in the urine may explain the slight protinuria often found in mercury intoxication without extensive renal damage.[31] Mercury is found in the cells of the proximal tubuli corresponding to particles with acid phosphatase activity, and with centrifugal sedimentation corresponding to a light mitochondrial fraction.[32] Normal protein reabsorption also takes place in this region. This renal damage is reversible, clearly different from the nephrosis seen in relation to nephrotoxic syndromes with anuria resulting from extensive tubular or capsular injury in severe forms of mercury intoxication.

The interaction of mercury in energy metabolism may be explained by the retention of mercury in mitochondria. This will interfere with the active membrane transport of other substances than macromolecules and may be primary to lysosomal damage or not. Probably both lysosomal failure and other involved transport systems are responsible for impaired transport in the kidneys and in the gastrointestinal tract in mercury intoxication.[33, 34]

The similarity in organ- and subcellular distribution of mercury after methoxyethylmercury- and mercuric chloride exposure indicates a similar symptomatology in these forms of mercury intoxication.[1, 35, 36] Common symptoms are actually found, but may be associated with a removal of mercury from the methoxyethylmercury molecule, perhaps mainly done in the liver. The central nervous system and the kidney do not necessarily metabolize the molecule to the same degree, and this may explain some differences in the toxic symptoms. Evaluation, under the same conditions, of mercury in kidneys and the central nervous system may add valuable information. Determination of the amount of mercuric ion after exposure to different organic mercury compounds may supply further knowledge of interest. Investigation of the subcellular distribution of mercury after methyl- and phenylmercury exposure is useful in such comparisons because of the differences in organ distribution and symptoms between these and compared to mercuric chloride and methoxyethylmercury acetate.[36]

The present study indicates that further investigation of the lysosome function in different forms of mercury intoxication should give interesting results. The lysosome function may be impaired through a general intoxication of the cell, but also as a result of the normal cell scavenger function of lysosomes with active accumulation of the toxic substance. If lysosomes are found to be responsible for some symptoms in mercury intoxication, this may also supply further information on the normal function of lysosomes.

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