

Intracellular distribution of lead in the liver and kidney of the rat

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Castellino, N., and Aloj, S. (1969). *Brit. J. industr. Med.*, 26, 139-143. Intracellular distribution of lead in the liver and kidney of the rat. The distribution of lead in intracellular fractions of liver and kidney has been studied in rats for nine days after intravenous injection of 100 μg . of lead (^{210}Pb) per rat. The radioactivity and the protein nitrogen have been measured in the homogenates and in the nuclei, mitochondria, microsomes, and cell sap. Lead penetrates the cells quickly, being present in all fractions one hour after injection. During the first 72 hours the relative amounts of lead in the different fractions varied, which ruled out the possibility that all the differences could be explained by redistribution after homogenization. The distribution of the metal within the cell probably depends upon differences in the nature and the stability of bonding to intracellular structures. The binding of lead within the mitochondria seemed to be particularly stable, only 24.4% of the radioactivity being removed by washing with 0.25 M sucrose or 0.001 M ethylenediaminetetra-acetate or 0.001 M diethylenetriamino-penta-acetate in 0.25 M sucrose. This finding is discussed in relation to haem biosynthesis within the mitochondrion.

Previous work on the kinetics of distribution of lead in the rat indicated that the metal diffuses into the extravascular space and penetrates the cells, where it is probably fixed as an organic complex (Castellino and Aloj, 1964). Analysis of the data obtained in rats treated with a chelating agent, ethylene-diaminetetra-acetate, confirms the above hypothesis, since different rates of removal of ^{210}Pb from the tissues have been observed (Castellino and Aloj, 1965). These results are in agreement with the findings of Bolanowska, Piotrowski, and Trojanowska (1964), who described the complex kinetics of lead in rats injected with a single dose of lead labelled with ^{210}Pb in terms of three compartments with different exchange rates.

Miani and Viferbo (1958) studied the distribution of lead in tissues of the dog by histo-autoradiographic techniques. They reported that the metal is located in various intracellular structures.

Since one of the most important biological effects of lead is its interference with haem biosynthesis,

probably due to inhibition of cytoplasmic and mitochondrial enzymes (Goldberg, Ashenbrucker, Cartwright, and Wintrobe, 1956; Rubino, 1961; Rimington, 1951; Eriksen, 1955; Dresel and Falk, 1956; Gajdos and Gajdos-Török, 1958; Bernard, Gajdos, and Gajdos-Török, 1958; Grinstein, Bannerman, and Moore, 1959), it was of interest to examine the distribution of lead inside the cells of liver and kidney homogenates after injection of ^{210}Pb to rats.

Methods

Animals

Forty-six Wistar male rats weighing from 190 to 240 g. were used

Tracer compounds

^{210}Pb acetate, specific activity 50 mCi/mM, was obtained from the Radiochemical Centre, Amersham, Bucks. For injection this was diluted with 0.0001 N acetic acid in 0.9% NaCl in water. Radioactivity determinations were carried out employing a well-type crystal scintillation

counter (Nuclear, Chicago) by estimating the gamma emission of ^{210}Pb .

Treatment of rats

Injections were given into the femoral vein under ether anaesthesia. Each rat was given 0.2 ml. of lead acetate solution, pH 4.7, containing 100 μg . of lead. Radioactivity was 10^6 counts per minute (c.p.m.). The distribution of lead in the intracellular fractions of the liver and kidney was studied in six groups of five rats. The animals were killed, at intervals up to nine days after injection. An additional group of 16 rats, killed 24 hours after treatment, was used only to prepare liver and kidney mitochondria.

Analytical methods

The liver and kidneys were removed, minced thoroughly, and washed several times in cold 0.25 M sucrose, thus eliminating most of the contaminating blood. Since it had been previously shown (Castellino and Aloj, 1964) that the concentration of lead per gram of blood accounted for only 1.23 and 0.03% of the injected dose at one hour and nine days after injection respectively, perfusion of the organs was considered unnecessary. Separation of intracellular particles was accomplished by the method of Hogeboom, Schneider, and Pallade (1948). Homogenization was carried out on small amounts of tissue in a Potter-Elvehjem homogenizer with a Teflon pestle spinning at 700 r.p.m. Each portion was homogenized for 30 seconds. The homogenate was suspended in 0.25 M sucrose, 6 ml./g. fresh weight of tissue. Smears were observed under the phase contrast microscope to ensure that the cells had been broken. By centrifugation at 700 g for 10 minutes the nuclear fraction was isolated. Mitochondria were sedimented from this supernatant by centrifuging at 21,000 g for 10 minutes. Finally, the microsomal fraction was isolated at 105,000 g for 60 minutes. The remaining supernatant will be called the soluble fraction. The nuclear fraction and mitochondria were washed twice with 0.25 M sucrose. Each fraction was weighed and the volumes of washing liquid were measured.

The radioactivity and the protein nitrogen (Gornall, Bardawill, and David, 1949) were determined in the homogenates, in each intracellular fraction, and in the washing liquids. The amount of ^{210}Pb was expressed in counts per minute (Table 1) while the values shown in the figures are corrected for nitrogen content by the formula:

$$\frac{\text{c.p.m./mg. N in fraction}}{\text{c.p.m./mg. N in the homogenate}} \times 100$$

In order to study further the removal of the metal from the mitochondrial fraction, liver and kidney mitochondria from 16 rats injected 24 hours previously were prepared as above and divided into four equal parts, one of which was used as a control. The others were washed twice either with 0.25 M sucrose or with ethylenediamine tetra-acetate (E.D.T.A.) or diethylenetriaminopenta-acetate (D.P.T.A.) both 0.001 M in 0.25 M sucrose. The radioactivity was determined in the mitochondria and in the washing liquids. In Table 2 the values are reported as percentages of radioactivity present in the unwashed control fraction.

Results

Intracellular distribution of ^{210}Pb in liver and kidney homogenates

Table 1 shows the radioactivity of the homogenates and of each fraction at different times. Each result is the mean of five rats. Recoveries ranged from 100.2 to 121.0% in the liver, and from 106.5 to 117.5% in the kidneys. In Figs 1 and 2, radioactivities are shown calculated from the formula in the Methods section, i.e., radioactivities relative to the radioactivity of the homogenate on a scale such that 100 indicates equal radioactivity/mg. N.

At all times after injection all fractions contained lead. In the liver (Fig. 1) during the first 24 to 72 hours we observed a decrease of the relative radioactivity in the nuclear fraction and an increase in the mitochondria, indicating different rates of lead removal from these intracellular structures. In contrast, no

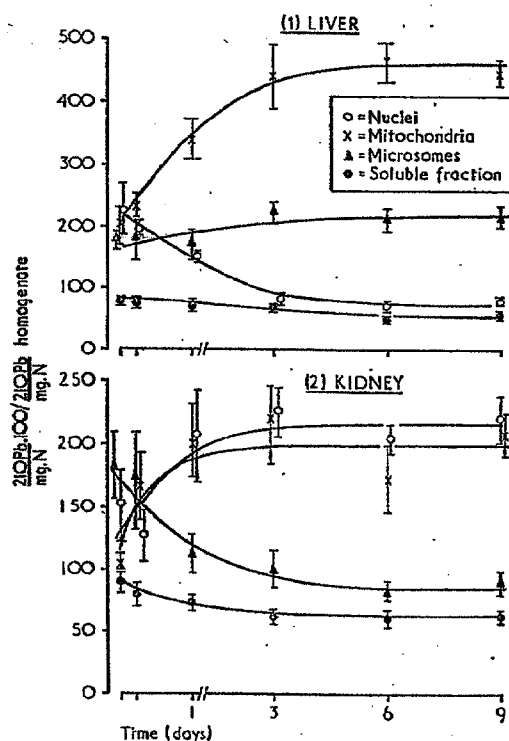


FIG. 1. Distribution of ^{210}Pb in liver fractions relative to ^{210}Pb in the whole homogenate corrected for nitrogen content. S.E.M.s are shown.

FIG. 2. Distribution of ^{210}Pb in kidney fractions; as for Figure 1.

TABLE 1

AMOUNTS OF ^{210}Pb IN LIVER AND IN KIDNEY (TISSUE AND SUBFRACTIONS) AFTER INJECTION OF 100 μg .
LEAD PER RAT: VALUES EXPRESSED IN COUNTS PER MINUTE $\times 10^4$ (PER CENT OF INJECTED DOSE)

Time (hours)	Liver							
	Homogenate	Nuclei	Washing liquid	Mitochondria	Washing liquid	Microsome	Soluble fraction	Recovery %
1	17.7	5.84	4.72	1.23	0.24	3.61	5.76	121.0
6	13.8	4.28	3.92	1.14	0.22	2.36	4.24	116.3
24	7.7	1.92	1.79	0.98	0.20	1.98	2.08	114.5
72	3.32	0.57	0.55	0.62	0.13	0.99	0.87	112.3
144	1.59	0.25	0.23	0.30	0.05	0.48	0.32	100.2
216	0.95	0.15	0.12	0.19	0.04	0.29	0.21	105.2

Time (hours)	Kidney							
	Homogenate	Nuclei	Washing liquid	Mitochondria	Washing liquid	Microsome	Soluble fraction	Recovery %
1	20.1	5.16	5.14	2.41	0.48	3.47	6.12	113.0
6	18.3	4.18	4.12	3.42	0.66	2.88	4.88	109.7
24	12.7	3.66	3.70	3.40	0.62	1.22	2.31	117.5
72	5.75	1.64	1.52	1.51	0.31	0.47	1.14	114.6
144	2.81	0.74	0.76	0.68	0.16	0.18	0.47	106.5
216	1.64	0.43	0.45	0.37	0.07	0.11	0.34	107.3

important variations occurred in the relative radioactivities in the microsomes and in the soluble fractions. At later times the relative radioactivities in all intracellular fractions became almost constant, i.e., all lost radioactivity at the same fractional rate as the liver as a whole, indicating the setting up of an equilibrium state.

In the kidney (Fig. 2), during the first 24 hours after injection, we observed increases of the relative radioactivities in the nuclear and the mitochondrial fractions, a decrease in the microsomes, and little change in the soluble fraction. After 24 hours the distribution of lead inside the kidney cells reached an equilibrium state, as indicated by relative radioactivities which remained almost constant up to nine days.

Removal of ^{210}Pb from mitochondria

As reported under Methods, during the isolation of fractions the nuclei and mitochondria were washed twice with 0.25 M sucrose. The washings removed about 50% and 20% of the radioactivity from the nuclear and mitochondrial fractions respectively.

Mitochondria from the liver and kidneys of 16 rats injected with ^{210}Pb were washed with sucrose, E.D.T.A., and D.T.P.A. solutions (see under Methods). Table 2 shows that 80.0 to 87.6% and 80.2 to 86.2% of radioactivity remained after two washes in liver and in kidney mitochondria respectively. The high proportion of radioactivity which remained bound to the mitochondria after repeated

TABLE 2
PERCENTAGE OF ^{210}Pb IN THE MITOCHONDRIAL
FRACTIONS AFTER WASHING AND IN THE
WASHING MEDIA

Washing medium	Sample	Liver %	Kidney %
Sucrose, 0.25 M	Mitochondria	82.4	80.2
	First wash	20.3	18.4
	Second wash	4.1	2.1
E.D.T.A. (mM) in sucrose, 0.25 M	Mitochondria	87.6	82.0
	First wash	15.3	12.4
	Second wash	0.0	2.1
D.T.P.A. (mM) in sucrose, 0.25 M	Mitochondria	80.0	86.2
	First wash	14.5	15.1
	Second wash	0.0	0.0

Figures refer to 1 mg. of mitochondria or 1 ml. of washing medium as percentages of the radioactivity in the unwashed mitochondrial fraction.

washing shows that the binding was remarkably strong.

Discussion

Lead poisoning is characterized by a disorder in haemoglobin synthesis, as shown by increases in haem levels and in the urinary excretion of some

haem precursors (Schmid, Hanson, and Schwartz, 1952; Tishkoff, Granville, Rosen, and Dameshek, 1958; Sano, 1958; Danieli, Gajdos-Török, and Gajdos, 1961; Pecora, Fati, Molé, Balletta, and Daniele, 1963; Crepet and Rubino, 1956; Rubino, 1961).

It is well established that heavy metals can combine with a large variety of organic molecules by interaction with ligands such as sulphhydryl, carboxyl, amino, imidazolyl or phosphoryl groups (Passow, Rothstein, and Clarkson, 1961). We think that, because of these interactions, lead may behave as an inhibitor of various enzymes involved in haem biosynthesis. It is therefore interesting that recent studies have established that some important reactions involved in haem biosynthesis take place in the mitochondria—decarboxylation and oxidation of coproporphyrin III to protoporphyrin IX; incorporation of ferrous iron into protoporphyrin IX with formation of haem; and, probably, condensation of δ -aminolaevulinic acid to porphobilinogen (Dresdel, 1955; Sano, 1958; Minakami, Yoneyama, and Yoshikawa, 1958; Eriksen, 1960; Lascelles, 1964; Pilet, 1964; Policard, 1964). The findings reported in this paper show that, within a short time after injection of lead, the metal is incorporated in all intracellular fractions obtained by differential centrifugation of liver and kidney homogenates.

Some variations in the relative amounts of ^{210}Pb in the intracellular fractions were observed up to 72 hours after injection, indicating different rates of removal of lead from each fraction. These variations rule out any major redistribution of lead *in vitro* after homogenization, and suggest that the differences in concentration inside the cell probably depend upon the nature and the stability of lead binding to intracellular structures. In both liver and kidneys the ^{210}Pb present in the mitochondria decreased more slowly than in the other fractions and in the whole homogenate.

From 72 hours to nine days from injection the relative amounts of ^{210}Pb in the fractions remained constant, suggesting that a steady state had been reached. The slowness with which this equilibrium is reached may depend either upon a strict compartmentation between the different phases in which the lead is distributed inside the cells or upon a slow transformation of the molecular species of the metal in the cell.

The failure to remove ^{210}Pb from mitochondria by washing suggests that the binding of lead within the mitochondrion is very strong. This seems to be in agreement with the hypothesis of direct interference by lead with some steps in haem biosynthesis (mainly occurring in the mitochondria), probably due to an enzymatic block of δ -aminolaevulinatidehydratase and haem synthetase.

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