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AN EFFECT OF CADMIUM ON HETEROLYSOSOME FORMATION AND FUNCTION IN MICE

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Abstract—The effects of cadmium acetate injections on heterolysosome formation and on the digestion of ^{131}I-labeled formaldehyde-treated bovine albumin in these particles from the kidneys and livers of mice have been studied. Cadmium inhibited proteolysis in liver particles 2 hr after intraperitoneal injections at LD_{50} doses (4·3 mg Cd/kg), and the effect became progressively more pronounced up to 20 hr. At this time, kidney particles were also affected but to a lesser degree. Mice surviving the cadmium injections recovered their capacity to degrade intralysosomal protein. The most pronounced effect of cadmium was on the uptake of labeled protein into subcellular particles, particularly in the liver at 24 hr after injection. Cadmium ion also caused the disruption of subcellular osmotically active particles (heterolysosomes) containing injected protein when added to particulate suspensions centrifuged from kidney and liver homogenates. Injections of cadmium acetate inhibited proteolysis in isolated liver heterolysosomes prepared 1 hr after intravenous injections of a relatively large dose of nonradioactive formaldehyde-treated albumin. This suggested that the metal prevented recovery of the capacity for normal intralysosomal proteolysis, perhaps by interfering with primary lysosome formation.

Cadmium, like many toxic heavy metals, produces a variety of pathological effects in various organs upon injection into experimental animals or on accidental ingestion by man [1]. Furthermore, the metal has been shown to be carcinogenic [2, 3]. After intravenous injection, cadmium salts initially concentrate in the livers of rats [4, 5] and then kidney levels become higher than liver levels. Repeated doses also result in highest levels in the liver [6, 7]. Injection of cadmium salts into animals causes kidney damage [7]. The metal interferes with liver function and causes morphological alterations in liver at relatively high doses (see Reference 8 for a comprehensive review of this subject).

The accumulation and retention of cadmium in the liver and kidneys of animals may have effects on lysosome function in these organs. Assay of the capacity for uptake and degradation of intravenously injected denatured (formaldehyde-treated) ^{125}I-labeled albumin in heterolysosomes provides a means for testing the effect of various agents on lysosomes or on lysosome function in the livers and kidneys of experimental animals [9]. We have used this technique to determine possible involvement of lysosomes in the toxic effects of mercuric ions [10], fungal toxins [11] and carbon tetrachloride [12]. In the present study, the technique has been applied to acute cadmium toxicity in mice.

MATERIALS AND METHODS

Adult albino Swiss–Webster mice (30–35 g) propagated in the animal facilities of this institution were used in these studies.

Crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was labeled with carrier-free ^{125}I-iodide according to the method of Bocci [13]. Treatment of the labeled protein with formaldehyde at pH 10 was performed as previously described [9, 14]. This treatment is necessary to induce uptake into heterolysosomes of mouse liver and kidney [15]. The labeled, formaldehyde-treated protein had a specific activity of $6{\cdot}25 \times 10^6$ cpm/mg, and contained 10 mg protein/ml. Animals were injected intravenously (tail vein) with 0·1 ml (1 mg) and sacrificed 30 min later by removal of the livers and kidneys under ether anesthesia. Tissue homogenizations and preparation of particulate suspensions containing heterolysosomes were performed as previously described [9, 10]. Proteolysis of particle-bound labeled protein in the heterolysosomes was performed by measuring the conversion of trichloroacetic acid-insoluble radioactivity to the acid-soluble form during incubation of the suspensions at 35° in 0·25 M sucrose containing 0·05 M mercaptoethanol and buffered with 0·025 M Tris acetate, pH 5, as previously described [9, 10].

The proportion of particle-bound radioactivity present in osmotically active form in particulate suspensions was estimated by diluting a sample in 9 vol. of 0·02 M Tris–acetate buffer, pH 7·2 [9, 10]. Radioactivity remaining in the supernatant after centrifugation was calculated as per cent of the total after a correction for radioactivity released by a smiliar dilution and centrifugation in cold 0·25 M sucrose. Distribution of radioactive protein in subcellular particulate fractions was performed as follows: Mouse livers and kidneys were removed 30 min after injections of labeled albumin under ether anesthesia and homogenized in 20 ml (liver) or 10 ml (kidneys) of ice-cold 0·25 M sucrose for 1 min in motor-driven glass–Teflon homogenizers (A. H. Thomas, Co.). Samples of these homogenates (1 ml) were diluted to 3 ml with water and counted in a Packard gamma well counter. The remaining homogenates were first centrifuged for 10 min at 1000 *g*. The supernatant solutions were carefully decanted and the pellets were resuspended in 5 ml of cold 0·25 M sucrose and washed once. The combined supernatants were then centrifuged at

30,000 g for 10 min and the resulting pellets were similarly washed. The combined supernatants from these centrifugations were then centrifuged for 1 hr at 40,000 g; these pellets were not washed. All pellets were suspended and adjusted to 3 ml in distilled water and counted.

Crude mouse liver cathepsin was prepared from 12 livers by homogenizing the organs one at a time in 10 ml of cold 0·25 M sucrose. The combined homogenates were centrifuged at 500 g for 5 min and the resulting pellet was discarded. The supernatant fraction was centrifuged at 40,000 g for 20 min to sediment lysosomes; these particles were washed once in 40 ml of cold 0·25 M sucrose by centrifugation at 40,000 g for 20 min. The pellet was then suspended in 6 ml of 0·01 M Tris–acetate buffer, pH 8·0, and dialyzed against 1 liter of cold distilled water for 4 hr to break the lysosomes. The suspension was then centrifuged at 40,000 g for 20 min and the resulting clear supernatant was used as the enzyme. This preparation contained 15·8 mg protein/ml assayed according to Lowry *et al.* [16].

Acid phosphatase activities in kidney and liver homogenates were assayed in duplicate with 0·05 M glycerol phosphate in volumes of 1 ml essentially according to Gianetto and de Duve [17]. Incubations were carried out at 37° for 5 min in the presence and absence of 0·1% Triton X-100. Reactions were stopped with 4 ml of 10% tricholoroacetic acid. The samples were chilled on ice, centrifuged for 10 min at 10,000 g in a refrigerated centrifuge, and phosphate assays were performed according to Fiske and Subbarow [18] on the supernatants after decantation. Phosphate contents in zero-time samples, in which homogenates were added after trichloroacetic acid, were subtracted from the 5-min samples. Proteins in the homogenates were assayed according to Gornall *et al.* [19].

Cadmium acetate was dissolved in 0·85% NaCl, and the appropriate volume for a given dosage (as Cd) was injected into mice according to the weight of the animals. Control animals were injected with 0·85% NaCl.

RESULTS

The LD_{50} of intraperitoneally injected cadmium acetate, determined according to Weil [20], in the mouse strain used in this study was 4·3 mg Cd/kg. This estimate was based on the number of surviving animals after 7 days, although all the deaths occurred within 2 days.

The effect of an LD_{50} dose of cadmium acetate 2 hr before sacrifice on intralysosomal proteolysis of formaldehyde-treated ^{125}I-labeled albumin in mouse liver and kidney particulate suspensions is shown in Fig. 1. The greatest effect was on liver heterolysosomes; the kidney particles were not significantly affected. The effect of cadmium dosage on intralysosomal proteolysis and on the relative proportion of the radioactivity in the 30,000 g particles releasable by osmotic shock is shown in Table 1. These data show that the effect of cadmium in liver particles occurred at doses around the LD_{50}. Higher levels were required to elicit an effect on kidney heterolysosomes. Cadmium decreased the relative proportion of labeled

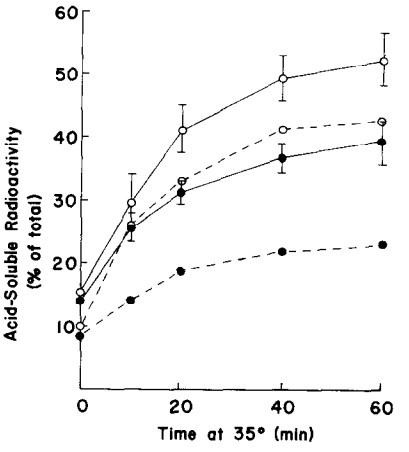

Fig. 1. Effects of intraperitoneal injections of cadmium acetate (4·5 mg/kg) on proteolysis of ^{125}I-labeled formaldehyde-treated bovine albumin in heterolysosomes from liver (●) and kidney (○). Controls, solid lines; cadmium-injected, dashed lines. Cadmium was injected 1·5 hr before labeled albumin and the animals were sacrificed 30 min later. Particulate material (500–30,000 g) centrifuged from 0·25 M sucrose homogenates was suspended in 12 ml of 0·25 M sucrose buffered with 0·025 M Tris–acetate, pH 5·0, and containing 0·05 M mercaptoethanol, and incubated at 35°. At the times indicated, 2-ml samples were removed and added to 2 ml of cold 10% trichloroacetic acid. These samples were counted, centrifuged and the supernatants counted to estimate the acid-soluble radioactivity. Mice were injected with 1 mg ($6·25 \times 10^6$ cpm) of labeled albumin, and the incubated suspensions from the cadmium-injected animals contained 64,038 cpm (kidney) and 66,833 cpm (liver). Radioactivity in the control suspensions ranged from about 40,000–50,000 cpm (kidney) to 75,000–90,000 cpm (liver). Vertical lines on the control graph refer to standard deviations.

protein in osmotically active particles about 30 per cent, but at the highest dose level (22 mg/kg) there was nearly total inhibition of proteolytic activity in liver heterolysosomes.

The effect of time after injection of cadmium acetate is shown in Table 2. The most severe effects were noted on liver particles at 12–20 hr after injections; at 20 hr, kidney heterolysosomes were also affected. Animals surviving the effects of cadmium injections also recovered from the effects on heterolysosomes (about 46 hr after injection).

From the data shown in Tables 1 and 2, it is not possible to determine if the primary effect of cadmium was on the complex series of membrane movements involved in endocytosis of labeled protein and heterolysosome formation, or if the cation affected the activity of proteolytic enzymes within heterolysosomes. Reduction in osmotically active particles in the suspensions suggests that heterolysosome formation may have been affected, but this would not adequately explain the effect on intralysosomal proteolysis. Even when measured in terms of the osmotically active radioactivity rather than as per cent of the total particle-bound radioactivity, significantly lower proteolytic activity figures were obtained, suggesting that cadmium may have had effects on enzyme activity within the particles. Figure 2 shows that, indeed, cadmium did inhibit the degradation of labeled protein in a crude lysosomal enzyme preparation.

Table 1. Effect of cadmium acetate dose (intraperitoneal injections) after 2 hr on uptake of ^{125}I-labeled albumin in osmotically active 30,000 *g* mouse kidney and liver heterolysosomes and on proteolysis in these particles*

Cd dose (mg/kg)	No. of experiments	Osmotically releasable radioactivity (%) Kidney	Liver	Intralysosomal proteolysis (% in 40 min) Kidney	Liver
1	2	78	60	34·1	19·4
2	2	76	66	32·1	21·9
3	5	78 ± 1·6	60 ± 0·9	30·3 ± 2·2	20·3 ± 1·2
4·5	6	80 ± 1·0	48 ± 2·0	29·2 ± 1·1	13·5 ± 1·4
6	5	75 ± 3·5	44 ± 2·6	31·9 ± 2·1	13·0 ± 1·5
8	5	72 ± 1·8	41 ± 3·7	24·3 ± 1·2	10·3 ± 2·0
10	2	69	46	19·1	15·6
22	1	53	45	9·8	2·8
Controls	5	79 ± 1·9	62 ± 3·4	33·8 ± 0·4	22·8 ± 0·6

* Osmotically releasable radioactivity refers to the per cent of the particle-bound (500–30,000 g) radioactivity released into the medium after suspension in a ×9 volume of 0·01 M Tris–acetate buffer, pH 7·2. Proteolysis refers to the per cent of the particle-bound radioactivity converted to a trichloroacetic acid-soluble form during 40 min of incubation at 35° (see Fig. 1). Plus–minus figures are standard errors of the mean of the number of experiments shown in column 2. The control values for proteolysis are the same as those shown in Fig. 1.

Cadmium acetate injections also had some effects on the uptake of labeled protein in the kidney and liver at the same doses which affected proteolysis in isolated heterolysosomes (Table 3). Uptake in particulate fractions was also reduced in these animals, particularly at 24 hr after injection (Table 4). This effect was more pronounced in the liver than in the kidneys. The proportion of the total organ radioactivity was not affected in the 40,000 *g* fraction, however; if calculated in terms of the total particle-bound radioactivity, the 40,000 *g* fraction contained more than 30 per cent of the total in the liver particles 24 hr after injection of cadmium compared with 13 per cent in the controls. This suggests that cadmium may have affected the formation of heterolysosomes from endocytosis vesicles. These vesicles are relatively small and they aggregate to form phagosomes or heterolysosomes within cells [21].

Cadmium chloride inhibited intralysosomal proteolysis when added to suspensions of these particles. Table 5 shows that 10^{-3} M cadmium chloride inhibited digestion about 65 per cent in kidney suspensions, but somewhat higher concentrations were required to inhibit the process significantly in liver particles. Cadmium acetate, however, had no effect, even at concentrations exceeding 10^{-2} M, and cadmium chloride also had no effect in the presence of acetate. Further experiments showed that this was due to the formation of an insoluble complex between cadmium ion, acetate and mercaptoethanol. The inhibitory effect of cadmium ion was discovered to be due to disruption of heterolysosomes in these experiments. This was demonstrated by measuring the quantity of osmotically releasable labeled protein in the suspensions during incubation. These results are shown in Table 6. The effect of cadmium on particle disruption appeared to be an all-or-none phenomenon, since 10^{-3} M concentration did not always cause disruption of liver particles. Table 6 shows that only in two of the five experiments performed were liver particles broken, but 10^{-3} M cadmium caused increased breakage in all the experiments with kidney particles. Previous studies have shown that breakage of heterolysosomes completely inhibits proteolysis during incubation [14].

In view of the data shown in Table 6 suggesting

Table 2. Effect of time after injection of cadmium acetate (4·5 mg/kg) on uptake of ^{125}I-labeled albumin in osmotically active 30,000 *g* mouse kidney and liver heterolysosomes and on proteolysis in these particles

Time (hr)	No. of experiments	Osmotically releasable radioactivity (%) Kidney	Liver	Intralysosomal proteolysis (% in 40 min) Kidney	Liver
0*	4	82 ± 5·8	56 ± 3·7	31·2 ± 1·2	20·0 ± 1·4
1	4	80 ± 5·6	58 ± 2·1	27·6 ± 2·0	20·0 ± 0·7
1·5	4	76 ± 2·7	51 ± 2·1	30·0 ± 1·4	17·0 ± 2·1
2	6	80 ± 1·0	48 ± 2·0	29·2 ± 1·1	13·5 ± 1·4
4	4	73 ± 2·0	51 ± 4·6	35·5 ± 2·4	14·5 ± 1·4
12	5	76 ± 3·6	31 ± 2·7	29·1 ± 1·8	8·6 ± 1·0
20	4	51 ± 8·2	27 ± 6·0	19·3 ± 5·5	6·7 ± 1·6
46	4	83 ± 6·4	56 ± 1·9	38·6 ± 1·3	19·8 ± 1·1

* In these experiments, cadmium acetate was injected intravenously with the labeled albumin. Osmotically releasable radioactivity and intralysosomal proteolysis figures are described in the footnote to Table 1 and in Materials and Methods. Controls are shown in Table 1 and Fig. 1. Cadmium acetate was injected intraperitoneally, 4·5 mg/kg as Cd.

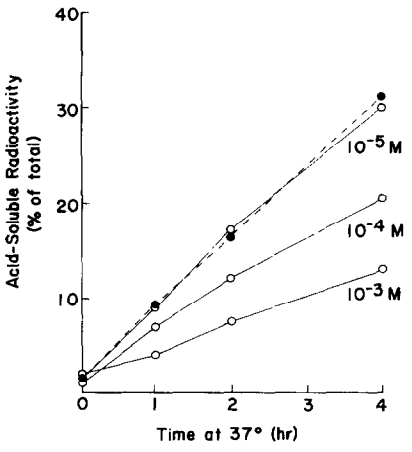

Fig. 2. Effect of cadmium acetate on proteolysis of ^{125}I-albumin by a crude mouse liver cathepsin preparation. Crude cathepsin (3·2 mg protein) was incubated at 37° with 3 mg of ^{125}I-albumin (147,214 cpm) in a total volume of 1 ml of 0·1 M Tris–acetate buffer, pH 5·0, 0·05 M mercaptoethanol, and the concentrations of cadmium acetate shown on the graph. At the times indicated, 0·1-ml samples were removed and added to 2 ml of ice-cold 10% trichloroacetic acid. These were counted, centrifuged, and the supernatants were decanted and counted to determine the per cent of the protein hydrolyzed. Control (no cadmium), dotted lines and filled circles.

that cadmium may cause disruption of heterolysosomes *in vivo*, free and bound and total acid phosphatase activities were determined in liver homogenates from control and cadmium-treated mice. No significant differences in ratios of free to bound acid phosphatase activities were noted at cadmium acetate doses of 8·0 mg Cd/kg at 4 hr after injection or at 4·5 mg/kg 24 hr after injection (Table 7). However, total liver acid phosphatase activity (per g tissue) was reduced significantly ($P < 0·05$) 24 hr after cadmium injections. This reduction appeared to be due to a depletion in total liver protein, since differences in specific activity from the controls were not statistically significant. It would appear, therefore, that acid phosphatase activity in the liver was reduced because total liver protein was depleted.

Cadmium may act to inhibit primary lysosome turnover resulting in decreased numbers of these particles available for fusion with phagosomes. This could produce heterolysosomes with lowered cathep-

Table 5. Effects of cadmium chloride on intralysosomal proteolysis*

$CdCl_2$ concn (M)	Digestion (% during 40 min at 35°) Liver	Kidney
10^{-2}	2·1	1·3
5×10^{-3}	3·4	2·6
(5×10^{-3})	2·7	2·5
10^{-2}	3·8	1·6
5×10^{-3}	17·3	10·9
10^{-3}	21·1	10·5
Control	22·8 ± 1·3	33·8 ± 0·8

* Proteolysis of particle-bound ^{125}I-labeled albumin in heterolysosomes was measured as described in Materials and Methods and Fig. 1. The suspensions contained 0·25 M sucrose, 0·05 M mercaptoethanol and the concentrations of cadmium chloride shown in the first column in a volume of 12 ml. Control values (five experiments) are shown plus and minus the standard deviations of the means.

Table 3. Effect of intraperitoneal injections of cadmium acetate on content of intravenously injected ^{125}I-labeled albumin (formaldehyde-treated) in mouse kidneys and liver at 30 min*

Cd dose (mg/kg)	Time after Cd infection (hr)	Per cent of injected dose/g tissue Kidney	Liver
	Control	18·1 ± 2·4	12·6 ± 0·9
4·5	4	20·0	9·1
4·5	24	10·2	7·7
8·0	4	11·4	9·8

* Plus minus figures are standard deviations of the means of four experiments. Data for cadmium-injected animals represent one experiment in each case.

Table 4. Effect of intraperitoneally injected cadmium acetate on uptake of intravenously injected ^{125}I-labeled albumin in subcellular particulate fractions of mouse liver and kidney 30 min after injection*

Cd dose (mg/kg)	Time after Cd injection (hr)	Per cent of total organ radioactivity 1000 *g* Kidney	Liver	30,000 *g* Kidney	Liver	40,000 *g* Kidney	Liver
	Control	11·9 ± 0·8	13·9 ± 1·6	25·5 ± 2·1	23·4 ± 1·8	3·2 ± 0·8	5·7 ± 1·1
4·5	4	12·5	11·3	24·0	17·2	3·3	5·6
4·5	24	4·9	3·3	10·2	3·0	6·1	3·2
8·0	4	12·2	6·2	25·6	9·0	3·1	4·5

* Tissues were fractionated as described in Materials and Methods. Mice were injected with 1 mg ($6·25 \times 10^6$ cpm) and sacrificed 30 min later. Plus–minus figures for the controls are standard deviations of the means of four experiments. Data for cadmium-injected animals represent one experiment in each case.

Table 6. Effect of cadmium chloride on breakage of mouse liver and kidney heterolysosomes *in vitro**

$CdCl_2$ concn (M)	Osmotically releasable radioactivity (% of total) Liver 0 min	Liver 10 min	Liver 20 min	Kidney 0 min	Kidney 10 min	Kidney 20 min
10^{-3}	58	40	33	70	4	5
10^{-3}	56	39	34	65	8	3
10^{-3}	39	41	6	48	10	5
10^{-3}	28	15	10	61	17	8
10^{-3}	52	42	38			
5×10^{-3}	23	0·1	1·3	19	3	4
5×10^{-3}	5·2	3·9	2·3	9	9	8
5×10^{-3}	13	1·7	1·3	27	0·2	3·1
Control	49 ± 6	36 ± 4	34 ± 6	60 ± 9	32 ± 5	24 ± 6

* Breakage of heterolysosomes was estimated as described in Materials and Methods. Plus–minus figures for the controls are standard deviations of the means of four experiments.

sin contents and consequently decreased rates of proteolysis, and it may also account for the 2-hr delay in the effect of cadmium noted in Table 2. To test this possibility, cadmium-treated and control mice were injected intravenously with relatively large doses (2 mg) of unlabeled, formaldehyde-denatured albumin. This quantity of protein should deplete existing primary lysosomes in the liver and kidneys. One hr after this initial injection, a time interval in which cadmium alone had no effect (Table 2), the animals were injected intravenously with the usual dose of ^{125}I-labeled formaldehyde-treated albumin and sacrificed 30 min later for assay of proteolytic activity in isolated liver and kidney heterolysosomes. If cadmium inhibited the formation of new primary lysosomes during the interval before injection of labeled protein, the digestion rates should be decreased in heterolysosomes from cadmium-treated animals. Table 8 shows that the metal inhibited proteolysis significantly (about 37 per cent, $P < 0.01$) with respect to the controls, and kidney heterolysosomes were inhibited much less (16 per cent, $P = 0.05$–0.1). The results sug-

Table 7. Per cent bound and total acid phosphatase activities in kidneys and livers from cadmium-injected and control mice*

Conditions	Acid phosphatase activity (% bound) Kidney	(% bound) Liver	(μmoles β-glycerophosphate hydrolyzed/min) (per g tissue) Kidney	(per g tissue) Liver	(per mg protein × 10^3) Kidney	(per mg protein × 10^3) Liver
Control	24·1 ± 8·1	33·5 ± 4·5	1·00 ± 0·04	1·52 ± 0·03	6·8 ± 0·4	6·3 ± 0·2
8 mg Cd/kg, 4 hr	27·5 ± 4·4	21·1 ± 5·0	1·28 ± 0·27	1·42 ± 0·18	7·0 ± 1·3	6·2 ± 0·7
4·5 mg Cd/kg, 24 hr	20·5 ± 3·2	31·7 ± 4·4	0·83 ± 0·09	1·10 ± 0·21	4·8 ± 0·5	5·2 ± 0·1

* Values are shown with standard errors of means of at least four experiments. All data for cadmium-injected animals are statistically nonsignificant ($P > 0.05$), except total acid phosphatase activity (per g) in the liver ($P < 0.05$).

Table 8. Effects of cadmium acetate and cycloheximide injections on recovery of the capacity for intralysosomal proteolysis following injections of unlabeled, formaldehyde-denatured albumin*

Conditions	N	Intralysosomal proteolysis (% in 40 min) Kidney	Liver
Control	6	26·2 ± 1·9	19·0 ± 1·0
4·5 mg Cd/kg (I.P.); 2·0 mg albumin	5	21·0 ± 1·4 ($P = 0.05$–0.1)	11·8 ± 1·3 ($P < 0.01$)
50 mg/kg cycloheximide (i.p.); 2·0 mg albumin	2	40·3	21·8

* Mice were injected intraperitoneally (i.p.) with 4·5 mg Cd/kg or 50 mg/kg cycloheximide, and then intravenously with 2 mg (0·1 ml) formaldehyde-treated bovine serum albumin. One hr later, the animals received 1 mg of ^{125}I-labeled formaldehyde-treated albumin. They were sacrificed 30 min after the latter injection, and proteolysis was measured in isolated subcellular particles as described in Materials and Methods. N = number of experiments. The data are shown plus and minus the standard error of the mean.

gest that cadmium prevented the recovery of the capacity for normal heterolysosome formation in the liver and, to a much lesser extent, in the kidneys. Cycloheximide, a potent inhibiter of protein synthesis, did not produce the same results, suggesting that this early effect of cadmium was not due to an inhibition of protein synthesis.

DISCUSSION

Results of experiments described in this study show clearly that cadmium interfered with heterolysosome formation and function in mice. Attempts have been made to determine which of the series of events involved in the uptake and degradation of labeled, denatured albumin was most affected by cadmium. Experiments performed *in vitro* show that cadmium caused disruption of heterolysosomes and inhibited cathepsin activity. However, the concentrations of metal required to break heterolysosomes and to inhibit proteolysis were perhaps of little physiological significance unless cadmium was concentrated in either primary lysosomes or heterolysosomes. Injections of cadmium acetate intravenously with labeled albumin caused only a 10 per cent inhibition of uptake of label into osmotically releasable form and produced no inhibition of proteolysis in isolated heterolysosomes, suggesting that the metal did not inhibit cathepsin activities directly nor did it cause heterolysosome disruption *in vivo*. Normal ratios of free to bound acid phosphatase in liver and kidney homogenates from cadmium-injected animals also suggest that the metal did not cause significant disruption of primary lysosomes or heterolysosomes *in vivo*.

Some of the evidence presented in this study suggests that cadmium affected heterolysosome formation. This is based on the observation that a decreased quantity of radioactivity was associated with particulate material in liver homogenates from mice injected with a lethal dose of cadmium (8 mg/kg) or 24 hr after 4·5 mg/kg (Table 4). The effects were not apparent in kidney homogenates until 24 hr after injection, even at the lethal dose. These results are consistent with the observation that cadmium first becomes concentrated in the liver and later in the kidneys [4, 5]. A decreased proportion of osmotically releasable radioactivity in liver homogenates at 20 hr after injection, but not at 2–4 hr (Table 2), also suggests damage to the heterolysosome-forming process, perhaps related to decreased protein levels at this time.

Results of experiments illustrated in Table 8 suggest that cadmium may have acted to inhibit normal rates of primary lysosome formation in the liver. Since injections of cycloheximide did not produce results similar to those obtained with cadmium, inhibition of recovery of the capacity to degrade labeled albumin by cadmium injections did not appear to be due to a general inhibition of protein synthesis. A more likely hypothesis, therefore, is that cadmium interfered with the packaging of primary lysosomes. Although the same results might have been expected if cadmium inhibited fusions of lysosomes with phagosomes, this does not appear likely, since simultaneous intravenous injections of the metal with labeled protein had no measurable effects on proteolysis (Table 2). If the primary effect of cadmium was to interfere with the packaging of lysosomes, early effects might not be expected, since liver cells should contain normal quantitites of primary lysosomes and an interval of time would be required to deplete the supply. Inhibition of primary lysosome formation alone, however, does not appear to be sufficient to explain the other effects observed in this study, unless cadmium had some general effect on membrane systems. For example, the decrease in particle-associated radioactivity (Table 4) and the reduction in osmotically releasable radioactivity at more than 2 hr after cadmium injections (Table 2), may be due to some time-dependent interaction with membranes. A time interval may be required to allow for accumulation of cadmium ions in the membranes. Alternately, perhaps these effects of cadmium may have been brought about as a consequence of lysosome depletion. Further studies are necessary before these questions can be satisfactorily answered.

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REFERENCES

1. D. F. Flick, H. F. Kraybill and J. M. Dimitroff, *Envir. Res.* **4**, 71 (1971).
2. S. A. Gunn, T. C. Gould and W. A. D. Anderson, *Proc. Soc. exp. Biol. Med.* **115**, 653 (1964).
3. S. A. Gunn, T. C. Gould and W. A. D. Anderson, *Archs Path.* **83**, 493 (1967).
4. C. F. Decker, R. U. Byerrum and C. A. Hoppert, *Archs Biochem. Biophys.* **66**, 140 (1957).
5. S. A. Gunn and T. C. Gould, *Proc. Soc. exp. Biol. Med.* **96**, 820 (1957).
6. L. Friberg, *Acta pharmac. tox.* **11**, 68 (1955).
7. B. Axelsson and M. Piscator, *Archs envir. Hlth.* **12**, 360 (1966).
8. L. Friberg, M. Piscator and G. Nordberg, *Cadmium in the Environment*, p. 79. C. R. C. Press, Cleveland, Ohio (1971).
9. J. L. Mego, in *Lysosomes in Biology and Pathology* (Ed. J. T. Dingle), Vol. 3, p. 527. North Holland, Amsterdam (1973).
10. J. L. Mego and Judith Barnes, *Biochem. Pharmac.* **22**, 373 (1973).
11. J. L. Mego and A. W. Hayes, *Biochem. Pharmac.* **22**, 3275 (1973).
12. J. L. Mego and Judith A. Cain, *Biochim. biophys. Acta* **297**, 343 (1973).
13. V. Bocci, *Ital. J. Biochem.* **18**, 346 (1969).
14. J. L. Mego, F. Bertini and J. D. McQueen, *J. Cell Biol.* **32**, 699 (1967).
15. J. L. Mego, *Biochem. J.* **122**, 445 (1971).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. T. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. R. Gianetto and C. de Duve, *Biochem. J.* **59**, 433 (1955).
18. C. H. Fiske and Y. Subbarow, *J. biol. Chem.* **66**, 375 (1925).
19. A. G. Gornall, C. J. Bardawell and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
20. C. S. Weil, *Biometrics* **8**, 249 (1952).
21. A. B. Novikoff, in *The Cell, Biochemistry, Physiology, Morphology* (Eds. J. Brachet and A. L. Mirsky), Vol. II, p. 458. Academic Press, New York (1961).