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Effects of Concurrent Administration of Lead, Cadmium, and Arsenic in the Rat

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Humans are exposed to a number of toxic elements in the environment; however, most experiments with laboratory animals investigate only one toxic element. To determine if concomitant exposure to lead (Pb), cadmium (Cd), and/or arsenic (As) modified the changes produced by any one metal in various parameters of toxicity, 168 male, Sprague-Dawley, young adult rats were fed nutritionally adequate diets to which had been added 0 or 200 ppm Pb as Pb acetate, or 50 ppm Cd as Cd chloride, or 50 ppm As as sodium arsenate or arsanilic acid in a factorial design for a period of 10 weeks.

At these concentrations, Cd and As reduced weight gain even when differences in food intake were taken into account; administration of both Cd and As depressed weight gain more than did either metal alone. Pb did not adversely affect food consumption or weight gain. Increased numbers of red blood cells (RBCs) were observed following administration of Pb, Cd, or As; usually more cells were observed when two or three metals were administered, compared to individual metals. Despite increasing numbers of circulating RBCs, hemoglobin and hematocrit were reduced, especially with the Pb-Cd combination and the Cd-arsanilic acid combination. Specific effects of Pb on heme synthesis were observed, including increased urinary excretion of δ-aminolevulinic acid; this increase was reduced by the presence of dietary cadmium.

Analyses of blood showed values for the laboratory rat within normal ranges for blood urea nitrogen, creatinine, cholesterol, calcium, albumin, total protein, and bilirubin. Uric acid was increased by Pb, with little modification by dietary Cd or As content. Serum glutamate-oxalate transaminase activity was reduced by As. Serum alkaline phosphatase was greatly reduced by either As or Cd but not Pb. Combinations of As and Cd did not further reduce the activity of this enzyme. Kidney weight and kidney weight/body weight ratios were increased by Pb alone, with no effects of Cd or As alone or as interactions. Liver weight/body weight ratios were reduced in animals fed 50 ppm dietary Cd. Kidney histology shows predominantly Pb effects, namely, intranuclear inclusion bodies and cloudy swelling. Ultrastructural evaluation of kidneys from Pb-treated animals disclosed nuclear inclusion bodies of the usual morphology and mitochondrial swelling. Concurrent administration of Cd greatly minimized Pb effects on the kidney under conditions of this experiment. Liver histology suggests an increased rate of cell turnover with either As compound, but few specific changes.

Introduction

Human populations are seldom exposed to only one toxic element in the environment. While a great deal of research utilizing experimental animals has been carried out to study the effects of metals, the great majority of this work has involved administration of one toxic metal. The current experiment was undertaken to determine if concurrent administration of lead, cadmium, and arsenic, changed the severity or type of effect produced by the individual metals, i.e., if interactive effects occurred.

Changes in the renal, hematopoietic, and hepatic systems were of special interest because Pb (*1*), Cd (*2*), and As (*3*) each have effects on these systems. For example, Pb, Cd, and As each affect specific steps in heme and porphyrin synthesis or metabolism which may not be rate-limiting under conditions of exposure to individual metals. However, their combined effect might produce anemia as shown by decreased hemoglobin concentration or hematocrit.

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Materials and Methods

One hundred and sixty-eight, male, albino, Sprague-Dawley, young, adult rats were fed nutritionally adequate, casein-based purified diets (*4*) for a period of 10 weeks. There were 14 animals per group. The diets contained background or high levels of Cd, Pb, or As and were arranged in a 2 × 2 × 2 factorial design (Table 1). The background levels were less than 20 ppb for Cd and As and less than 50 ppb for Pb. Lead was added at the level of 200 ppm in the form of Pb acetate. Cd was added as $CdCl_2$ at 50 ppm Cd and As as sodium arsenate

Table 1. Experimental design, showing number of animals per group.[a]

	<0.05 ppm As		50 ppm As	
	<0.05 ppm Pb	200 ppm Pb	<0.05 ppm Pb	200 ppm Pb
<0.02 ppm Cd	14	14	14	14
50 ppm Cd	14	14	14	14

[a]As as arsenilic acid or sodium arsenate; Pb as lead acetate; Cd as cadmium chloride.

(Inorg As) in one set of diets and as arsanilic acid (Org As) in another set of diets. Analyses of the diets showed that the actual metal content was within 10% of these calculated values. The purpose of testing these two forms of As was to determine if differences in tissue concentrations or toxicity due to chemical form of As would occur at the end of the 10-week period. Tissue concentrations of the metals are not yet available and will be reported later. Concentrations of these metals in brain, bone, liver, and kidney will be measured by plasma emission spectroscopy which will determine concentrations of approximately 20 trace elements. This type of analysis will provide considerable information on interactions between various essential and toxic metals and may provide insight on the mechanisms of toxicity. Some of the parameters measured in this study were initial body weight, final body weight, food consumption, blood pressure (systolic), hemoglobin, hematocrit, and red blood count, porphyrin intermediates, clinical chemistries (blood), kidney and liver weights, light and electron microscopy of liver and kidney, blood lead concentration, blood, brain, kidney, and liver concentrations of As, Cd, and Pb. Generally, they are standard clinical measurements used in assessment of toxicity. Hemoglobin was measured by a cyanohemoglobin technique (*5*), and blood urea nitrogen, creatinine, cholesterol, calcium, albumin, total protein, bilirubin, uric acid, alkaline phosphatase and glutamate-oxalate transaminase (SGOT) by the respective methods cited (*6–15*).

Urinary δ-aminolevulinic acid (dALA) was measured by the technique reported by Davis and Andelman (*16*).

Fixation and processing of liver and kidney tissue for histological and ultrastructural examination were conducted by previously described methods and instrumentation (*17*).

Choice of the rat as the experimental animal in this study was based on its usefulness for investigations of the effects of Pb, Cd, and As. The rat will readily consume a purified diet in which the concentrations of the metal can be closely controlled and the nutritional requirements of the rat are well known. These were considered to be important factors in an experiment of this type in which metal interactions are of interest. Although the rat may have an unusual distribution of tissue arsenic (*3*) this difference was considered of smaller importance in a longer term toxicity study than in short term metabolic experiments.

Concentrations of Pb (*4*), Cd (*17*), and As (*18, 19*) to be incorporated into the diet were based on information from previous investigations. Concentrations were selected which would produce slight to moderate toxicity, that is, tissue accumulation of the metal with demonstrable morphologic and biochemical changes. The data reported are the results of statistical evaluation using the analysis of variance technique. Levels of significance are shown in each table. In the tables the p value for a main effect or an interaction is reported beside the treatment. This does not mean that the number beside the significance level differs from control but that the overall effect is significant at the magnitude specified in the footnote.

Results

The quantity of food ingested was not significantly affected by the level of Pb or Cd in the diet (Table 2), but was reduced by addition of 50 ppm of either form of As to the diet. Weight gain was reduced more by both As and Cd than by Pb. Because the weight reduction observed in animals fed the As diets could be due to reduced food intake, utilization of feed or efficiency of utilization of feed was calculated. Food efficiency utilization was reduced when either Cd or As were fed at the concentrations used in these diets (Table 2). The combined effects of Cd and As were to reduce the food utilization even more than that which occurred with either metal alone.

Administration of Pb, Cd, or As resulted in an increase in the number of circulating red blood cells (Table 3). Combinations of metals usually produced

Table 2. Food consumption, weight gain, and food efficiency ratio in rats fed varying amounts of Pb, Cd, and As.[a]

Treatment	Food consumption, g	Body wt. gain, g	Ratio Food consumption/ weight gain
Control	1271 ± 28[b]	210 ± 7[b]	6.09 ± 0.13[b]
Pb	1232 ± 28	184 ± 6	6.77 ± 0.19
Cd	1217 ± 25	173 ± 7[c]	7.14 ± 0.28
Inorg. Ay	1098 ± 26[d]	164 ± 6[d]	6.70 ± 0.32
Org. As	1119 ± 22[d]	154 ± 6[d]	7.47 ± 0.38
Pb × Cd	1263 ± 21[e]	175 ± 6	7.23 ± 0.27
Pb × Inorg. As	1107 ± 31	180 ± 10	6.32 ± 0.39
Pb × Org. As	1068 ± 35	157 ± 9	6.92 ± 0.28
Cd × Inorg. As	1060 ± 29	140 ± 9[f]	7.83 ± 0.42[f]
Cd × Org. As	1083 ± 32	147 ± 7[f]	7.61 ± 0.36[f]
Pb × Cd × Inorg. As	1115 ± 22	138 ± 6[f]	8.19 ± 0.34
Pb × Cd × Org. As	1126 ± 17	156 ± 6[f]	7.27 ± 0.22

[a]Significance levels may represent an increase or decrease from control values.
[b]Mean ± 1 SEM.
[c]$p < 0.001$
[d]$p < 0.0001$
[e]$p < 0.01$
[f]$p < 0.05$

an increase in the number of circulating red blood cells above that produced by either metal alone. Pb or Cd alone did not result in significant reductions in hemoglobin or hematocrit, however, inorganic As alone reduced both hemoglobin and hematocrit. The greatest decreases in hemoglobin and hematocrit were seen with the combination of Pb and Cd and with Cd and organic As (Table 3). The number of peripheral white blood cells was decreased by Cd but not significantly affected by Pb and As. Pb effects on heme synthesis can be seen by measuring the urinary excretion of dALA. Urinary dALA excretion was greatly increased by Pb, however, the magnitude of this increase was decreased by the presence of Cd (Table 4). Similar effects of Pb and Cd on blood Pb concentration can be observed (Table 4). Tissue Pb concentrations (kidney, liver, and bone) are needed to confirm the probable reduction of body burden of Pb in the presence of dietary Cd.

Analysis of blood showed values within the normal range for the laboratory rat for: blood urea nitrogen, creatinine, cholesterol, calcium, albumin, total protein and bilirubin. Serum uric acid concentration was increased by Pb (Table 5). Serum alkaline phosphatase activity was decreased by both Cd and As, but was not affected by Pb (Table 5). The combination of Cd and As resulted in even greater reductions of alkaline phosphatase activity than that resulting from either metal alone. Alkaline phosphatase activity is derived from a number of different isoenzymes. The specific isoenzyme(s) affected by Cd or As were not determined in the current study. SGOT activity was greatly reduced by administration of As alone.

Both kidney weight and the kidney weight to body weight ratio were increased by elevated levels of Pb in the diet (Table 6). Cd and As were without influence on these parameters (Table 6). The liver weight/body weight ratio was decreased by dietary Cd but not Pb or As. The effects of these metals were also evaluated by light and electron microscopy.

Liver sections examined by light microscopy from animals on the lead and cadmium treatment specimens were indistinguishable from controls except that some mild parenchymal cell swelling was noted in animals exposed to either form of arsenic. Renal changes characterized by cloudy swelling of

Table 3. Hematology values for rats fed varying amounts of Pb, Cd, and As.[a]

Treatment	RBC × 10^6	Hemoglobin, g	Hematocrit	WBC × 10^{3b}
Control	6.81 ± 0.15[b]	15.2 ± 0.3[b]	43.5 ± 0.7	6.39 ± 0.42
Pb	7.36 ± 0.26[c]	15.7 ± 0.6[d]	43.9 ± 0.7[c]	6.20 ± 0.39
Cd	8.27 ± 0.23[e]	15.1 ± 0.2[d]	41.8 ± 0.6[d]	4.95 ± 0.67[c]
Inorg. As	7.73 ± 0.11[d]	14.5 ± 0.3[d]	40.0 ± 0.7[c]	6.44 ± 0.54
Org. As	7.04 ± 0.12[d]	15.7 ± 0.2[d]	43.5 ± 0.6[c]	6.13 ± 0.34
Pb × Cd	7.57 ± 0.25[e]	14.3 ± 0.3[c]	39.7 ± 0.8	5.74 ± 0.77
Pb × Inorg. As	7.97 ± 0.13[e]	15.3 ± 0.2[d]	42.8 ± 0.5[e]	7.39 ± 0.45
Pb × Org. As	8.46 ± 0.19[e]	17.3 ± 0.2[e]	46.0 ± 1.2[e]	5.32 ± 0.40
Cd × Inorg. As	7.93 ± 0.17[e]	15.3 ± 0.3[e]	42.0 ± 0.8[e]	5.58 ± 0.46
Cd × Org. As	8.01 ± 0.16[e]	14.5 ± 0.3[e]	39.6 ± 0.6[e]	6.04 ± 0.25
Pb × Cd × Inorg. As	7.51 ± 0.26	15.6 ± 0.3	42.5 ± 0.4	5.85 ± 0.72
Pb × Cd × Org. As	8.58 ± 0.22	15.6 ± 0.2	43.6 ± 0.6	5.79 ± 0.65

[a]Significance levels may represent an increase or decrease from control values.
[b]Mean ± 1 SEM.
[c]$p < 0.05$
[d]$p < 0.001$
[e]$p < 0.0001$

Table 4. Urinary δaminolevulinic acid (dALA) excretion and blood lead concentration of rats fed varying amounts of Pb, Cd, and As.[a]

Treatment	Urinary dALA, mg/24 hr	Blood Pb, mg/dl
Control	46.2 ± 7.1[b]	7.4 ± 1.0[b]
Pb	115.5 ± 12.7[c]	44.9 ± 1.7[c]
Cd	46.8 ± 5.1[d]	6.2 ± 1.2[d]
Inorg. As	41.8 ± 7.3	5.3 ± 1.1
Org. As	35.8 ± 7.5	3.2 ± 0.9
Pb × Cd	98.3 ± 9.4[c]	31.6 ± 2.8[e]
Pb × Inorg. As	136.7 ± 5.1	37.0 ± 1.8[f]
Pb × Org. As	103.6 ± 9.9	42.1 ± 2.4[f]
Cd × Inorg. As	51.1 ± 8.2[d]	5.7 ± 1.0[d]
Cd × Org. As	68.5 ± 11.3[d]	6.4 ± 1.7[d]
Pb × Cd × Inorg. As	85.5 ± 11.0	34.3 ± 2.9
Pb × Cd × Org. As	96.7 ± 9.8	40.5 ± 1.8

[a]Significance levels may represent an increase or decrease from control values.
[b]Mean ± 1 SEM.
[c]$p < 0.0001$.
[d]$p < 0.01$.
[e]$p < 0.001$.
[f]$p < 0.05$.

Table 5. Serum uric acid, alkaline phosphatase activity, and serum glutamate oxalate transaminase (SGOT) activity in rats fed varying amounts of Pb, Cd, and As.[a]

Treatment	Uric acid, mu/ml	Alkaline phosphatase, mu/ml	SGOT, mu/ml
Control	1.72 ± 0.13[b]	201 ± 12[b]	247 ± 24[b]
Pb	3.33 ± 0.86[c]	195 ± 11	240 ± 29[d]
Cd	2.02 ± 0.08	134 ± 6[e]	245 ± 21
Inorg. As	1.55 ± 0.09	127 ± 12[e]	159 ± 8[c]
Org. As	1.54 ± 0.09	135 ± 7[e]	174 ± 9[c]
Pb × Cd	1.91 ± 0.18	147 ± 11	203 ± 13
Pb × Inorg. As	1.63 ± 0.14	136 ± 11	175 ± 13[f]
Pb × Org. As	2.42 ± 0.17	143 ± 12	329 ± 46[f]
Cd × Inorg. As	1.92 ± 0.17[d]	120 ± 10[f]	211 ± 15[d]
Cd × Org. As	1.91 ± 0.13[d]	117 ± 5[f]	204 ± 9[d]
Pb × Cd × Inorg. As	2.51 ± 0.60	128 ± 12	238 ± 18
Pb × Cd × Org. As	2.34 ± 0.25	131 ± 8	263 ± 31

[a]Significance levels may represent an increase or decrease from control values
[b]Mean ± 1SEM.
[c]$p < 0.01$.
[d]$p < 0.05$.
[e]$p < 0.0001$.
[f]$p < 0.001$.

Table 6. Renal and hepatic weights and ratios to body weight in rats fed varying amounts of Pb, Cd, and As.[a]

Treatment	Kidney weight, g	Ratio Kidney wt. / Body wt. × 10^{-3}	Liver weight, g	Ratio Liver wt. / Body wt. × 10^{-3}
Control	1.333 ± 0.040[b]	2.95 ± 0.06[b]	14.36 ± 0.46[b]	31.74 ± 0.79[b]
Pb	1.343 ± 0.057	3.21 ± 0.10[c]	13.61 ± 0.42	32.61 ± 0.71
Cd	1.186 ± 0.037	2.88 ± 0.08	12.32 ± 0.20	30.01 ± 0.60[c]
Inorg. As	1.232 ± 0.033	3.10 ± 0.06	12.39 ± 0.30	31.31 ± 0.50
Org. As	1.196 ± 0.028	3.07 ± 0.08	11.89 ± 0.34	30.79 ± 0.72
Pb × Cd	1.320 ± 0.065	3.20 ± 0.17	12.47 ± 0.40	29.97 ± 0.85
Pb × Inorg. As	1.348 ± 0.051	3.25 ± 0.08	13.33 ± 0.58	32.13 ± 0.96
Pb × Org. As	1.275 ± 0.019	3.27 ± 0.10	12.44 ± 0.54	31.90 ± 0.91
Cd × Inorg. As	1.111 ± 0.042	3.12 ± 0.06	11.02 ± 0.45	30.48 ± 0.53
Cd × Org. As	1.202 ± 0.044	3.15 ± 0.14	11.48 ± 0.34	29.68 ± 0.45
Pb × Cd × Inorg. As	1.235 ± 0.027	3.24 ± 0.08	11.96 ± 0.32	30.85 ± 0.88
Pb × Cd × Org. As	1.214 ± 0.027	3.20 ± 0.09	11.51 ± 0.24	30.04 ± 0.60

[a]Significance levels may represent an increase or decrease from control values.
[b]Mean ± 1 SEM.
[c]$p < 0.001$.
[d]$p < 0.0001$.

proximal tubule cells and intranuclear inclusion bodies (Fig. 1) were observed in animals given diets containing lead with the notable exception of those animals concomitantly exposed to cadmium (Table 7). These animals had few intranuclear inclusions and relatively little cloudy swelling. Morphological changes in kidneys of animals exposed to combinations of cadmium or either arsenical were relatively slight.

Discussion

The purpose of this study was to evaluate the combined biological effects of Pb, Cd, and As in the rat. Significant interactions between all three metals were relatively infrequent. The most consistent interactions were between Pb and Cd, between Cd and As. Generally, the presence of other metals reduced the magnitude of the Pb effect. Reduction in efficiency of conversion of food energy into body weight gain was the most sensitive of the parameters measured in this study for detecting interactions of two or three metals. Reduced efficiency of food conversion may be an indication of impaired absorption of nutrients from the gastrointestinal tract or of a metabolic defect at the cellular level. Measurements of cellular utilization of carbohydrates in relation to oxidative phosphorylation and genera-

FIGURE 1. Electron micrograph of a renal proximal tubule cell from a rat exposed to dietary lead. Note large fibrillar intranuclear inclusion body. × 16,758.

Table 7. Relative incidence of lead intranuclear inclusion bodies in rats fed diets containing lead, cadmium, inorganic or organic arsenic.

	Incidence[a]
Control	0/13
Pb	10/13
Cd	0/13
Inorg. As	0/13
Org As	0/13
Pb × Cd	0/13
Pb × Inorg. As	10/13
Pb × Org. As	11/13
Cd × Inorg. As	0/13
Cd × Org. As	0/13
Pb × Cd × Inorg. As	0/13
Pb × Cd × Org As	2/13

[a]Number of rats with inclusions over number examined.

tion of high energy phosphate compounds would be of interest. Pb (*1*), As (*3*), and Cd (*2*) are all known to interfere with these processes.

From the morphological and biochemical data currently available it appears that there may be lower Pb absorption or retention at the higher level of Cd intake. This can be confirmed only when analyses of tissue Pb concentrations are completed. However, if Cd reduces Pb absorption, it is of interest to consider further a possible mechanism for this change. Recent evidence indicates that vitamin D increases gastrointestinal absorption of Pb when added to the diet of rats deficient in vitamin D (Dr. Hector DeLuca, Department of Biochemistry, University of Wisconsin, FDA contract report). Feldman and Cousins (*20*) report that incubation of Cd with kidney homogenates and isolated mitochondria from vitamin D deficient chicks decreased formation of 1,25-dihydroxycholecalciferol from 25-hydroxycholecalciferol. Chicks fed diets containing 50 ppm Cd showed depressed production of 1,25-dihydroxycholecalciferol in kidney mitochondria.

Another mechanism which may reduce Pb absorption at high levels of dietary Cd is damage to the absorptive surface of the gastrointestinal tract. Richardson and Fox (*21*) report gross, microscopic, and ultrastructural lesions in the proximal small intestine of Japanese quail fed a diet containing 75 mg Cd/kg. The villi were short and thick; the lamina propria had a dense cellular infiltrate; and microvilli on absorptive villi were markedly shortened. Such changes are similar to lesions occurring in some malabsorption syndromes in humans.

The possible mechanisms through which Cd may reduce body burden of Pb are of interest with regard to establishment of safe levels of exposure to toxic compounds in humans and animals. Reduction of body burden of a toxic substance, in this example Pb, may be achieved through a mechanism which is generally deleterious to the health of the organism. Reduction of ability to absorb the toxic compound Pb would be accompanied by reduced ability to absorb nutritionally required elements as well.

Many of the biochemical and hematological parameters reported here have been investigated in epidemiological or clinical studies of human populations. These parameters, however, give only a clinical endpoint picture of a complex metabolic situation and further studies are in process to elucidate the biochemical mechanisms for these phenomena in experimental animals. Rats fed higher levels of dietary Pb showed significantly increased serum uric acid concentrations. Concentrations of urate above 3 mg/dl are unusual in the rat. Whether this is a reflection of impaired renal excretion of urate by the kidney or impaired metabolism of urate by uric acid oxidase in the liver or kidney is not clear. Gouty changes have been described in humans having elevated body burdens of Pb (*1*). The specific isoenzyme(s) involved in the observed reduction of alkaline phosphatase activity by Cd and As in this study are not known and will be examined in future investigations. Varying degrees of inhibition of different isoenzymes by a metal have been observed, Kshirsagar (*22*) reported reduced kidney, liver and intestinal alkaline phosphatase activity, but increased bone alkaline phosphatase activity in rats fed diets containing 2% stable strontium. Reduction in SGOT activity was observed in animals fed high levels of As. Administration of other metals, for example, mercury (*23*) have resulted in an increase in SGOT, which generally reflects tissue damage. Reduction of activity by dietary As may indicate inhibition of the enzyme by As and further studies are needed to examine this possibility.

The metabolic complexities of multi-element exposure are also illustrated by hematological findings in this study. All animals maintained fairly normal hemoglobin concentrations even though excretion of intermediates in porphyrin and heme synthesis increased several fold. There is a substantial reserve capacity for formation of hemoglobin which is reflected in maintenance of effectively normal hemoglobin concentrations despite two to threefold increases in excretion of intermediate products. Certainly not all heme synthesized goes to the formation of hemoglobin. Maintenance of this heme function, however, may have priority over some other uses for heme such as heme-containing enzymes. It may also be that the tissue or organ systems synthesizing hemoglobin, such as bone marrow, concentrate lower levels of the metals than cells synthesizing heme for other compounds. Ab-

sence of an effect in an end product such as hemoglobin does not constitute an absence of metabolic effect. Biochemical studies reported at this meeting (*24–26*) examine these other effects of As more thoroughly. This experiment probably has its greatest usefulness in suggesting directions for further research and the need for more specific parameters to assess toxicity. Careful control of nutritional conditions in studies of this type is clearly essential to interpretation of toxicity data.

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