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Studies on the Evaluation of the Toxicity of Various Salts of Lead, Manganese, Platinum, and Palladium

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Preliminary studies have been conducted on various parameters in order to assess the possible and relative toxicities of a number of metallic salts. Upon oral administration in lethal-dose experiments, two soluble Pt^{4+} salts were more toxic than the other salts tested. Following intraperitoneal injection in lethal-dose experiments, $PbCl_2$ was less toxic than several of the soluble or partially soluble salts of Pt^{4+}, Pd^{2+}, and Mn^{2+}. An intake of a total of approximately 250 mg of Pt^{4+} per rat in the drinking fluid over a 30-day interval did not affect the activities of aniline hydroxylase and aminopyrine demethylase in rat liver microsomes. In rats receiving soluble Pt^{4+} salts in the drinking fluid, the highest concentration of Pt was found in the kidney and an appreciable concentration was found in the liver.

Introduction

Preliminary studies have been conducted on various parameters in order to assess the possible and relative toxicities of a number of metallic salts. The chloride, sulfate, and oxide salts of lead, manganese, platinum, and palladium were studied, since it is considered that some of these salts may be included in automotive emission products.

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Materials and Methods

All experimental studies were conducted with male Sprague-Dawley rats. The animals were received at 3–3.5 weeks of age and were maintained for 1–1.5 weeks before use. The mean body weights were usually 100–110 g when the rats were used for the lethal-dose experiments or started on the diets.

In the lethal dose experiments, the salts were administered orally (via stomach tube) or intraperitoneally. The rats were observed through a 14-day observation period. In the completed experiments, the LD_{50} values were calculated by the method of Litchfield and Wilcoxon (*1*).

In the diet experiments, four rats were maintained per cage. The metallic salt under study was dissolved in the drinking fluid. Animals consumed feed and drinking fluid *ad libitum*. Analyses for metals were performed on samples from three lots of feed (Purina Laboratory Chow). The feed contained (mean ± standard deviation): 56 ± 5 mg Mn/kg feed and 0.99 ± 0.07 mg Pb/kg feed; the analyses of the three lots for platinum were 0.09, <0.02, and <0.02 mg Pt/kg feed. Measurements were made of the body weights of individual rats and feed and fluid consumption per cage of four rats at 7-day intervals during the course of each diet experiment.

At the termination of the dietary experiments, samples of liver were used for the isolation of microsomes. Aniline hydroxylase was measured by the method of Imai et al. (*2*), modified by the addition of $HgCl_2$ (*3*). Aminopyrine demethylase was measured by the formation of formaldehyde (Nash reaction) (*4*).

The analyses of the rat tissues for platinum, lead, and manganese were carried out by Yoakum, Stewart, and Sterrett (*5*) of Stewart Laboratories, Inc. by an emission spectrochemical method.

Results and Discussion

In Table 1 are presented, for various salts, the preliminary data on the LD_{50} values, the doses lethal to 50% of the rats following oral administration or intraperitoneal injection. Upon oral administration, the toxicities of the salts are in the following decreasing order (expressed on a molar basis): $PtCl_4$, $Pt(SO_4)_2 \cdot 4H_2O$ > $PdCl_2 \cdot 2H_2O$, $RuCl_3$ > $MnCl_2 \cdot 4H_2O$, $PdSO_4$, $PbCl_2$, $PtCl_2$ > PtO_2 > MnO_2, PdO. Thus, upon oral administration, the two soluble Pt^{4+} salts are the most toxic of the compounds studied. As one might anticipate, the highly insoluble salts are least toxic and include $PtCl_2$ (Pt^{2+}) and oxides of platinum, manganese, and palladium. The relative order of toxicities may be modified somewhat by the period of observation. Although the soluble Pt^{4+} salts kill early, usually within the first 1–2 days following oral administration, the palladium salts and $PbCl_2$ often kill 4–10 days after administration.

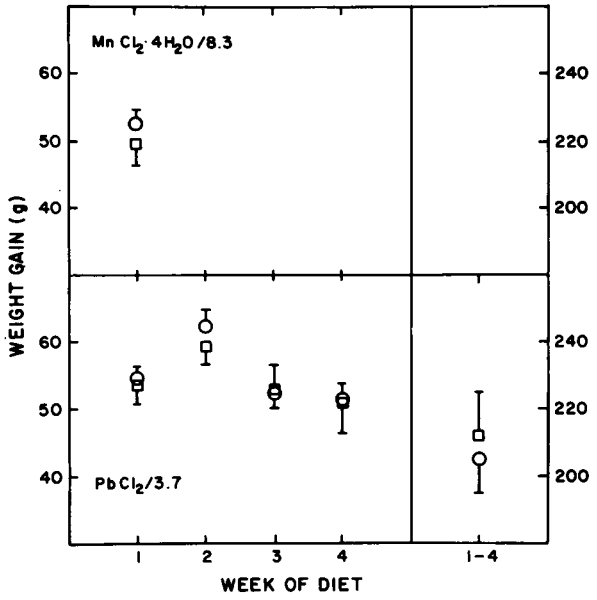
Table 1. Acute toxicities of various metallic salts.

Compound	LD_{50}, mmole/kg	
	Oral	Intraperitoneal
$MnCl_2 \cdot 4H_2O$	7.5[a] (7.0–8.1)	0.70[a] (0.61–0.80)
MnO_2	≫40	—
$PbCl_2$	>7	>4.5
$PdCl_2 \cdot 2H_2O$	2.7[a] (2.2– 3.4)	0.4 –0.6
PdO	>40	—
$PdSO_4$	>7	>0.6
$PtCl_2$	>5	≫0.9
$PtCl_4$	<1.9	≪1.2
PtO_2	>15	—
$Pt(SO_4)_2 \cdot 4H_2O$	2.2[a] (1.6–3.1)	0.68[a] (0.60–0.76)
$RuCl_3$	2.8–3.5	—

[a] Evaluated by the method of Litchfield and Wilcoxon (*1*); the ranges of the 95% confidence limits are given in parentheses.

The LD_{50} doses following intraperitoneal injection are also presented in Table 1. The relative order of the LD_{50} values are, on a molar basis: $PdCl_2 \cdot 2H_2O$ > $Pt(SO_4)_2 \cdot 4H_2O$, $MnCl_2 \cdot 4H_2O$, $PdSO_4$, $PtCl_4$ > $PbCl_2$, $PtCl_2$. The most toxic salt was $PdCl_2 \cdot 2H_2O$. $PbCl_2$ was less toxic following intraperitoneal injection than the soluble Pt^{4+} salts and $MnCl_2 \cdot 4H_2O$.

In the process of preparing rats for subsequent biochemical experiments, measurements were made at 7-day intervals of the weight gain by individual animals and the feed and drinking fluid consumption per cage of four rats. Usually each control rat gained 50–60 g per week during each of the first 4 weeks on the diets. The weight gain by rats which consumed 8.3*mM* $MnCl_2 \cdot 4H_2O$ (one week) or 3.7*mM* $PbCl_2$ (four weeks) did not differ from the weight gain by control rats (Fig. *1A*). Furthermore, when the drinking fluid was a saturated solution of $PtCl_2$ or of $PdCl_2 \cdot 2H_2O$, no statistically different weight changes were observed, although the $PdCl_2 \cdot 2H_2O$ appeared



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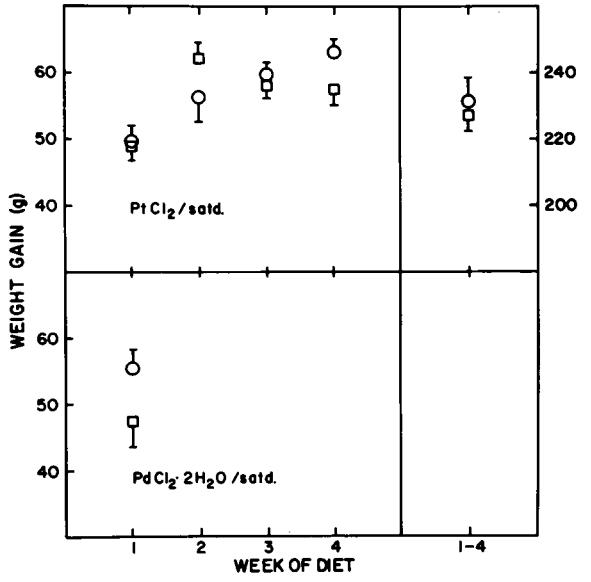

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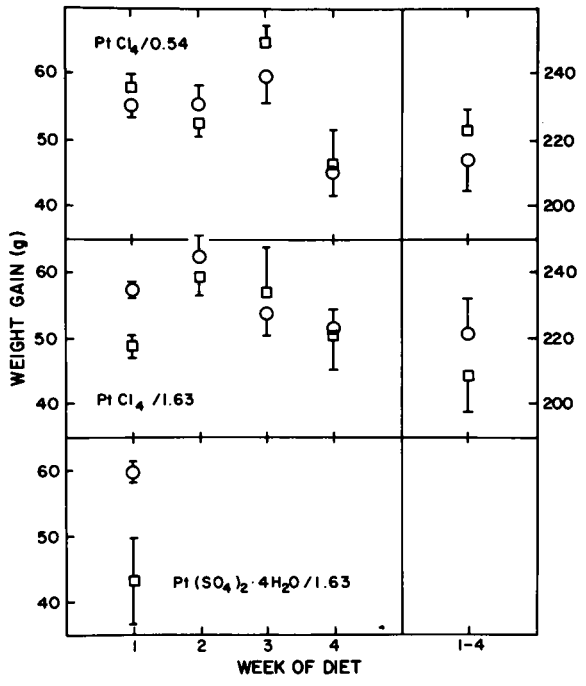

C

FIGURE 1. Weight gain (g/week/rat or g/4 weeks/rat) receiving various salts in the drinking fluid. (*A*) $MnCl_2 \cdot 4H_2O$, 8.3*mM* or 1640 mg of salt/l.; $PbCl_2$, 3.7*mM* or 1022 mg of salt/l.; (*B*) $PtCl_2$, saturated solution; $PdCl_2 \cdot 2H_2O$, saturated solution of the first aqueous extraction of the salt; (*C*) $PtCl_4$, 0.54*mM* or 183 mg of salt/l.; $PtCl_4$, 1.63*mM* or 550 mg of salt/l.; $Pt(SO_4)_2 \cdot 4H_2O$, 1.63*mM* or 750 mg of salt/l. Each point is the mean of a minimum of 8 male rats (usually 12–16 rats): (O) control; (□) metal-treated animals; vertical bars are standard deviations.

to cause some decrease (Fig. *1B*). The saturated solution of $PtCl_2$ contained only a trace of Pt (14 μg Pt/l.); the saturated solution of $PdCl_2 \cdot 4H_2O$ has not been analyzed yet for Pd.

A soluble salt of Pt^{4+}, when added to the drinking fluid as 0.54*mM* $PtCl_4$, did not affect the weight gains for the four weekly or the total interval (Fig. *1C*). When the Pt^{4+} concentration was increased 3-fold with either 1.63*mM* $PtCl_4$ or $Pt(SO_4)_2 \cdot 4H_2O$, the weight gains of the metal-treated rats was significantly less than the gains of the control rats during the first week on the diet. However, 1.63*mM* $PtCl_4$ did not decrease the weight gains during the second, third or fourth weeks. The decreases of 20% in weight gain during the first week were parallel to the decreases in feed consumption and fluid consumption which were also decreased by 20%.

The organ weights, expressed as the percentage of the body weight, of control and metal-treated rats are given in Table 2. $PbCl_2$ (3.67*mM*) did not significantly alter the organ weights through a 30–31 day diet; the total lead intake was approximately 750 mg of lead per rat for the entire interval. When essen-

Table 2. Effect of various salts in the drinking fluid on tissue weights. [a]

Drinking fluid		Duration, days	No. of rats	Body weight, g	Organ weights, % of body weight [b, c]				
Salt	Concn, *mM*				Liver	Kidney	Spleen	Heart	Testes
Controls		29–31	8	309	3.41	0.92	0.28	0.32	1.04
$PbCl_2$	3.67	29–31	8	309	3.47	0.98	0.30	0.33	1.03
Controls		90–91	4	508	2.64	0.68	0.19	0.25	0.75
$PbCl_2$	3.60	90–91	4	456 (90)	2.96 (112)	0.80 (117)*	0.24 (129)†	0.27	0.76
Controls		8	8	136	3.70	1.07	0.51	0.36	1.13
$PdCl_2 \cdot 2H_2O$	Satd.	8	8	132	3.76	1.06	0.58 (114)	0.37	1.21
Controls		29–30	8	297	3.52	0.86	0.43	0.31	1.06
$PtCl_4$	0.54	29–30	8	285	3.52	0.91	0.41	0.33	1.07
Controls		8	12	152	3.72	1.07	0.58	0.38	1.08
$PtCl_4$	1.63	8	12	145	3.72	1.13	0.57	0.39	1.11
Controls		29	12	306	3.27	0.85	0.27	0.31	1.03
$PtCl_4$	1.63	29	12	296	3.29	0.92 (108)*	0.28	0.32	1.13 (110)
Controls		8–9	8	173	3.90	1.01	0.56	0.39	1.11
$Pt(SO_4)_2 \cdot 4H_2O$	1.63	8–9	8	149 (86)	3.69	1.05	0.54	0.37	1.21 (109)

[a] Since livers were used for isolation of microsomes, rats were fasted overnight (12–15.5 hr before collection of tissues; drinking fluid provided during fasting.

[b] Where mean of tissues from metal-treated rats is outside of the range of 92.0–108.0% of the mean of control rats, the percentage is given in parentheses.

[c] Statistical analysis (Student's *t*-test): *, $P<0.05$ †, $0.05<P<0.10$; no marking is indicated where $P>0.10$.

tially the same diet was continued for approximately 90 days, the kidneys were enlarged. These data are consistent with prior reports (*6*) and with our preliminary data which showed kidney enlargement in rats which received one-half this concentration over a 90-day interval. When $PdCl_2 \cdot 2H_2O$ was extracted with water to dissolve the readily soluble material and that solution used as the drinking fluid, no significant changes were observed in the organ weights over an 8-day interval.

The dietary administration of $PtCl_4$ did not affect any of the five organ weights when the $PtCl_4$ was included in the drinking fluid for approximately 30 days at 0.5*mM* or for 8 days at 1.6*mM*. Likewise, if 1.6*mM* $Pt(SO_4)_2 \cdot 4H_2O$ was given as drinking fluid for 8–9 days, there were no significant changes in organ weights. The total intake of platinum per rat was approximately 60 mg for each of these three Pt^{4+} diets. In contrast, if 1.6*mM* $PtCl_4$ is administered for approximately 30 days (total intake of approximately 250 mg of Pt per rat), the kidney weight was increased 6–10% in each of the three experiments.

At the termination of the dietary experiments, samples of liver were used for the isolation of microsomes. Measurements were made of the weight of microsomal protein isolated per gram of liver and the activities of two microsomal enzymes: aniline hydroxylase and aminopyrine demethylase.

None of the dietary treatments consistently affected the level of microsomal protein isolated per gram of liver (Table 3). The hepatic activities of aniline hydroxylase or aminopyrine demethylase are not significantly depressed when rats were maintained for only 30 days on 3.7*mM* $PbCl_2$ in the drinking water. The use of a saturated aqueous solution of $PdCl_2 \cdot 2H_2O$ as the drinking fluid appears to decrease the activities of both aniline hydroxylase and aminopyrine demethylase. Under various concentration and duration schedules, the addition of the soluble chloride (or sulfate) salt of Pt_4+ to the drinking fluid did not alter the activity of either microsomal enzyme.

Table 3. Effect of dietary salts on the activities of microsomal activities of rat liver.

Drinking fluid		Duration, days	(Treated/control)×100[a]		
Salt	Conc, *mM*		Microsomal protein[b]	Aniline hydroxylase[c]	Aminopyrine demethylase[d]
$PbCl_2$	3.67	31	107	79†	87
		30	101	99	95
$PbCl_2$	3.60	91	75	71	86
$PdCl_2 \cdot 2H_2O$	Satd.	8	96	67*	66**
		8	113†	77†	90*
$PtCl_4$	0.54	29	98	106	109
		30	98	94	103
$PtCl_4$	1.63	8	105	107	104
		8	99	121	125
		8	100	79*	88
$PtCl_4$	1.63	29	95	108	101
		29	94	92	100
		30	107	93	99

[a] Statistical analysis (*t*-test: **, $P<0.01$; *, $P<0.05$; †$0.05<P<0.10$; no marking if $P>0.10$. Each value is a comparison of the mean of four control values and values from four metal-treated rats.

[b] In control rats, approximately 43 mg of microsomal protein was obtained per gram of liver.

[c] Aniline hydroxylase: control values were approximately 18 mμmole of *p*-aminophenol produced/mg protein/20 min incubation.

[d] Aminopyrine demethylase: control values were approximately 70 mμmole of formaldehyde produced/mg protein/10 min incubation.

Analyses for lead, manganese, and platinum were conducted by Yoakum, Stewart, and Sterrett (*5*). In a series of rats treated for 90–91 days, the control rats ingested approximately 0.15 g of manganese (from the solid feed). The tissue concentration of Mn was 1.4 and 1.0 μg Mn/g wet tissue in the liver and kidney, respectively. In Mn-treated rats, which received 8.3*mM* $MnCl_2 \cdot 4H_2O$ as the drinking fluid and ingested approximately 2.3 g of Mn per rat during the 90–91 day interval, the concentration of Mn was somewhat increased, namely 2.8 and 1.6 μg Mn/g of wet tissue in the liver and kidney, respectively. The Mn concentration in spleen, heart, testes and blood was not increased in the tissues of Mn-treated rats.

A second group of rats received 3.6*mM* $PbCl_2$ in the drinking water for 90–91 days and ingested approximately 3 g of lead per rat during the interval; control rats ingested < 0.01 g of Pb in the solid feed during the same interval. Kidney showed a marked accumulation of Pb (to 11.1 μg Pb/g of wet tissue) in the lead-treated rats; in the same rats the concentration in liver was 1.2 μg Pb/g of wet tissue. The corresponding levels in the control rats were approximately 0.3 μg Pb/g of wet tissue in both kidney and liver. The other tissues (spleen, heart, testes, and blood) did not exhibit appreciably higher levels of Pb in the Pb-treated rats.

Soluble Pt^{4+} salts were included in the drinking fluid of rats for 8–9 days. The approximate total Pt intake (mg Pt per rat) and data on the tissue concentration of Pt in various tissues are presented in Table 4. Although the Pt concentrations in tissues of untreated control rats often attain levels measurable by the technique used by Stewart Laboratories, Inc., the levels are low and are generally less than 0.1 μg Pt/g of wet tissue. For the higher levels of Pt^{4+} intake in the Pt-treated rats, the highest tissue concentrations of Pt occurred in the kidney and ranged from 4.5 to 5 μg Pt/g of wet tissue. High levels, ranging from 0.7 to 2.5 μg Pt/g, also occurred in the liver. In contrast, brain showed only a very low level of Pt which may reflect a contribution from the blood. Separate experiments were conducted on the tissue concentrations of Pt in rats which received a saturated solution of $PtCl_2$ as the drinking fluid for 30–31 days. In the $PtCl_2$-treated rats, the mean Pt concentration for liver, kidney, and spleen was < 0.08 μg Pt/g of wet tissue.

In Table 5 are presented the Pt concentrations of tissues removed from rats which had survived for the 14-day observation period in lethal-dose experiments. The doses of $Pt(SO_4)_2 \cdot 4H_2O$ administered by both the oral and intraperitoneal routes were approximately 90% of the LD_{50} values by the respective routes. During the two-week observation period, the rats gained weight at a rate from one-third to three-fourths the rate of the control rats. In the orally treated rats,

Table 4. Pt content of tissues of rats maintained on drinking fluid containing Pt salts.

	Control	$Pt(SO_4)_2 \cdot 4H_2O$		$PtCl_4$
Pt salt concn, mg Pt/l.	—	106	319	319
Duration of diet, days	—	8	9	8
Total Pt intake, mg Pt/rat	<0.01	26	80	60
Tissue concentration of Pt, μg Pt/g wet tissue [a]				
Liver		0.07	0.85	2.2
	<0.02±0.02	(0.04–0.09)	(0.73–0.97)	(2.0–2.5)
Kidney			4.6	
	<0.23±0.45	0.26±0.05	(4.5–4.7)	4.8±0.5
Spleen		0.02	0.13	0.24
	<0.08±0.08	(0.01–0.02)		
Heart	<0.02±0.01	0.02	0.25	—
Testes	<0.014±0.010	0.04±0.05	—	—
Brain	—	—	0.015±0.002	—
Blood		0.05	0.22	0.23
	0.10 ±0.13		(0.09–0.36)	(0.19–0.27)

[a] Values for control rats are those from diet experiments after approximately 8 or 30 days; 5–7 values for blood, spleen, and heart, 13–16 values for liver, kidney, and testes; standard deviation (±) is given for means with at least four values; ranges are indicated in parentheses for means of two values.

Table 5. Pt concentration in rat tissues following the administration of single high doses of $Pt(SO_4)_2 \cdot 4H_2O$.

	Controls (oral)	$Pt(SO_4)_2 \cdot 4H_2O$	
		Oral	Intraperitoneal
Dose of Pt, mg Pt/kg	—	382	113
Tissue Concentration of Pt, μg Pt/g wet weight of tissue			
Liver	<0.01	2.3	34
	(0.004–0.006)	(1.2–3.5)	(30–38)
Kidney	<0.008	16	37
	(0.004–0.004)	(13–19)	(28–46)
Spleen	<0.013	3.3	16
	(0.007–0.011)	(2.3–4.2)	(12–20)
Heart	0.02	0.8	3.0
Testes	0.011	0.5	1.2
	(0.009–0.013)	(0.4–0.6)	(0.9–1.5)
Brain	0.01	0.10	0.6
		(0.07–0.14)	(0.07–1.1)
Blood	<0.008	3.3	1.0

[a] Range of four values for control liver, kidney, and spleen, and range of 2–3 values of all other tissues are given in parentheses. Control values are the mean values of Pt concentration in two rats which received orally NaCl.

the highest concentration of Pt occurred in the kidney (approximately 16 μg Pt/g), and appreciable levels of Pt also occurred in liver and spleen (range, 1–4 μg Pt/g of wet tissue). In the intraperitoneally dosed rats, the kidney, liver, and spleen showed very high levels of Pt in the range of 10–40 μg Pt/g of wet tissue.

In a comparable lethal dose experiment, rats were treated orally with a dose of $MnCl_2 \cdot 4H_2O$ equivalent to 100% of the oral LD_{50} value, and the tissues were analyzed in surviving rats at the end of the 14-day observation period. In contrast to the finding with the Pt salt, the oral administration of a single, large but nonlethal dose of $MnCl_2 \cdot$

Table 6. Mn concentration in rat tissues following the oral administration of a single large dose of $MnCl_2 \cdot 4H_2O$.

	Controls	$MnCl_2 \cdot 4H_2O$
Dose of Mn, mg Mn/kg	—	416
Tissue concentration of Mn, μg Mn/g wet weight of tissue*		
Liver	1.60±0.87	1.9 (1.3–2.5)
Kidney	0.75±0.50	1.3 (1.0–1.5)
Spleen	1.46±1.99	1.3 (1.1–1.5)
Heart	0.55±0.35	0.7
Testes	0.44±0.35	0.5 (0.4–0.5)
Brain	0.3	0.03
Blood	0.86±0.44	0.4 (0.2–0.6)

* Control values are from rats treated orally with NaCl, and rats on diet experiments for approximately 8 or 30 days. Means ± standard deviations are given for 6–7 samples of spleen, heart and blood and for 13–18 samples of liver, kidney and testes from control rats; ranges are given in parentheses where two values are available from Mn-treated rats.

$4H_2O$ to rats did not result in the retention after 14 days of excess concentrations of Mn in any of the tissues analyzed (Table 6). Due to low levels of absorption and/or a high capacity for excretion of the Mn, the tissue Mn levels of the experimental rats were approximately equal to the levels found in control rats.

These studies show that in rats treated with soluble Pt^{4+} salts, appreciable levels of the metal can be found in the kidney, liver, and spleen. Further studies will be necessary to determine the effects of the Pt and other metals on various biochemical reactions.

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