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Concurrent Exposure to Lead, Manganese, and Cadmium and Their Distribution to Various Brain Regions, Liver, Kidney, and Testis of Growing Rats

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Abstract. Growing rats were exposed to 5 mg/L Pb, *ad libitum* in drinking water, and administered low or high doses of Mn and Cd intraperitoneal (i.p.) for 30 days. Some groups of animals were also administered combinations of Pb + Mn and Pb + Cd in an identical manner. Analysis of Pb, Mn, and Cd in tissue samples showed the expected dose-dependent accumulation when the metal was administered singly. However, combined treatment produced different types of metal shift in different tissues. Enhanced accumulation of all three metals in the brain, Mn in liver, Pb in kidney and Cd in testis and kidney after combined exposure may make target organs vulnerable to the toxic effects of metals, even when encountered at low concentrations. Further, the decreased levels of blood Pb after combined treatment with Cd or Mn suggests that the significance of blood Pb level as a diagnostic aid for Pb toxicity in coexposed conditions may not be of much value. Changes in the metallic distribution within the tissues after coexposure may be the result of a competition between the administered metals for common binding sites.

Several reports during the past decade have revealed that some populations at high risk for lead (Pb) toxicity are overexposed to other metals through environmental pollution (Creason *et al.* 1975; Landrigan *et al.* 1975; Dorn *et al.* 1976). Children residing near ore smelters are exposed to lead, zinc, copper, cadmium (Cd), and arsenic. Co-exposure to Pb and zinc in experimental animals produced zinc toxicity inspite of high concentrations of Pb in the body tissues (Chisolm 1980). Simultaneous exposure to Pb and manganese (Mn) cannot be excluded on the basis of the findings of increased blood Mn with increasing blood Pb in young children and occupationally exposed male workers (Delves *et al.* 1973; Zielhuis *et al.* 1978). Manganese has also been found to influence the metabolism of Cd in experimental animals (Nordberg 1978). These observations point clearly to the need for a better understanding of the mechanism of interactions of metals in the biological system to predict possible health hazards in humans (Shukla and Singhal 1984). A knowledge of the tissue distribution of various metals after combined exposure may be of great significance in providing a clue to such an understanding. This communication reports the effect of the interaction of Pb, Mn, and Cd on their levels in brain regions, liver, kidney, testis and blood of the rat.

Materials and Methods

Animals and Treatment

Weanling male albino rats, weighing 50 ± 5 g were housed in stainless steel cages in an air-conditioned room with regular 12-hr cycles of light and darkness. The animals had free access to the pellet diet (Hindustan Lever Laboratory Animal Feeds, India) and tap water. The average consumption of drinking water was 30 ml/rat/day. The animals were divided into 10 groups of thirty animals each and were treated with different metals for 30 days. Doses and route of administration to the different groups of rats are given in Table 1.

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Table 1. The doses and route of administration of metals in different groups of animals

Groups	Treatment solution	Doses[a]	Route
I	Physiological saline	0.5 ml	Intraperitoneal[b]
II	Lead acetate	Pb^{2+} 5 mg/l *ad libitum*	Drinking water[c]
III	Manganese chloride	Mn^{2+} 1 mg/kg	Intraperitoneal
IV	Manganese chloride	Mn^{2+} 4 mg/kg	Intraperitoneal
V	Cadmium chloride	Cd^{2+} 0.1 mg/kg	Intraperitoneal
VI	Cadmium chloride	Cd^{2+} 0.4 mg/kg	Intraperitoneal
VII	Lead acetate +	Pb^{2+} 5 mg/l *ad libitum*	Drinking water
	Manganese chloride	Mn^{2+} 1 mg/kg	Intraperitoneal
VIII	Lead acetate +	Pb^{2+} 5 mg/l *ad libitum*	Drinking water
	Manganese chloride	Mn^{2+} 4 mg/kg	Intraperitoneal
IX	Lead acetate +	Pb^{2+} 5 mg/l *ad libitum*	Drinking water
	Cadmium chloride	Cd^{2+} 0.1 mg/kg	Intraperitoneal
X	Lead acetate +	Pb^{2+} 5 mg/l *ad libitum*	Drinking water
	Cadmium chloride	Cd^{2+} 0.4 mg/kg	Intraperitoneal

[a] Treatment solutions were prepared fresh daily
[b] In all cases of intraperitoneal (i.p.) administration, a volume of 0.5 ml was used
[c] The average consumption of Pb containing drinking water was 30 ml/rat/day in all groups

Experimental Procedures and Metal Analysis

After 30 days, 18 animals from each group were randomly selected and killed by cervical dislocation. Blood was collected through cardiac puncture, using a heparinized syringe in heparinized tubes. The brain, liver, kidneys, and testes were dissected out and rinsed in a physiological saline. Brains were dissected into different regions according to the method of Glowinski and Iverson (1966). After blotting on an ash free Whatman filter paper, the tissues were weighed and stored at −20°C for metal analysis. The blood and different brain regions obtained from three rats were pooled to make one sample. Dry weights of the tissues were determined by keeping the samples in a hot air oven maintained at 110°C until a constant weight was obtained.

Measurements of Mn, Cd, and Pb in different tissues and blood were made with a Perkin Elmer Model 500 double beam Atomic Absorption Spectrophotometer equipped with a boling burner and null read out accessory (Shukla *et al.* 1976; Skogerboe *et al.* 1979).

Statistical Analysis

Results were statistically evaluated by one-way analysis of variance (ANOVA). For those effects found to be significant beyond the 0.05 level of probability, a Duncan's Multiple range test was employed.

Chemicals

Lead acetate, cadmium chloride, and manganese chloride were of AnalR grade of the British Drug House. Other chemicals used in the present study were of the purest available grade.

Results

Data in Table 2 show maximum accumulation of Pb in the region of hippocampus followed by corpus striatum, midbrain, hypothalamus, cerebral cortex, pons-medulla, and cerebellum after administration of Pb (5 mg/L in drinking water) to the rats. Administration of Cd alone for 30 days in high (0.4 mg/kg/day) and low (0.1 mg/kg/day) doses did not affect the content of Pb in the brain. However, the Mn high dose (4 mg/kg/day) increased the contents of Pb in the cerebral cortex. Administration of Pb with Mn or Cd in high and low doses of the latter two metals significantly increased the accumulation of Pb in various brain regions compared to that observed after the administration of Pb alone. However, the preference of regions of maximum accumulation remained the same. No distinct dose-relationship was noticed in the accumulation of Pb, except for an increased contents in corpus striatum and midbrain regions after combined exposure with the high dose of Mn compared to the low dose (1 mg/kg/day).

Table 3 shows that administration of Pb alone did not influence the distribution and accumulation of Mn in various regions of the brain. Administration of Mn produced a significant increase in its accumulation in hypothalamus followed by corpus striatum, midbrain, pons-medulla, hippocampus, cerebellum, and cerebral cortex. Accumulation of Mn was dose-dependent. Cd administration alone increased significantly the accumulation of Mn in hypothalamus, midbrain and hippocampus; there was no correlation between this increase and the dose of Cd administered. Co-administration of Pb and Mn significantly increased the contents of Mn in various brain regions; the increase was significantly higher compared to that observed after the administration of Mn (1 mg/kg/day) alone only in

Table 2. Regional Pb (ng/g dry tissue) distribution after Pb, Mn, and Cd treatment alone and in combination after 30 days exposure. Values in parenthesis denote statistical significance (One-way analysis of variance followed by Duncan's Multiple Range Test, P = 0.05)

Treatment	Cerebellum	Cerebral cortex	Corpus striatum	Hippocampus	Hypothalamus	Midbrain	Pons-medulla
Saline	112 ± 5.0[a]	170 ± 8.5	241 ± 15.5	349 ± 16.6	198 ± 10.1	306 ± 11.5	128 ± 9.5
Pb[b]	157 ± 6.4 (140[c])	278 ± 12.6 (164[c])	777 ± 37.6 (322[c])	1174 ± 57.6 (336[c])	355 ± 29.4 (179[c])	473 ± 21.3 (155[c])	220 ± 17.3 (172[c])
Mn (L)[d]	120 ± 6.5	150 ± 9.3	261 ± 15.0	313 ± 29.4	228 ± 17.9	279 ± 18.4	142 ± 9.2
Mn (H)[e]	126 ± 6.8	191 ± 11.9 (112[c])	263 ± 16.9	374 ± 15.3	217 ± 8.4	345 ± 22.1	132 ± 9.8
Cd (L)[f]	121 ± 9.1	166 ± 10.1	268 ± 14.9	359 ± 25.6	201 ± 14.9	319 ± 19.1	120 ± 7.7
Cd (H)[g]	129 ± 6.8	182 ± 15.0	233 ± 20.6	477 ± 23.2	227 ± 17.7	346 ± 26.4	140 ± 12.1
Pb[b] + Mn (L)[d]	194 ± 11.3 (173[c], 124[h])	303 ± 11.3 (178[c])	920 ± 45.2 (382[c], 118[h])	1654 ± 71.6 (474[c], 141[h])	343 ± 16.4 (173[c])	612 ± 41.6 (200[c], 129[h])	246 ± 22.4 (192[c])
Pb[b] + Mn (H)[e]	204 ± 12.3 (182[c], 130[h])	356 ± 14.5 (209[c], 128[h])	1097 ± 69.6 (455[c], 119[i])	1628 ± 94.2 (466[c], 139[h])	363 ± 15.9 (183[c])	817 ± 32.3 (267[c], 133[i])	244 ± 13.3 (190[c])
Pb[b] + Cd (L)[f]	236 ± 13.0 (211[c], 150[h])	275 ± 10.9 (162[c])	840 ± 31.6 (349[c])	1311 ± 83.6 (376[c])	390 ± 29.0 (197[c])	556 ± 33.6 (182[c], 118[h])	242 ± 11.2 (189[c])
Pb[b] + Cd (H)[g]	256 ± 12.1 (229[c], 163[h])	299 ± 11.6 (176[c])	869 ± 38.8 (361[c])	1351 ± 105.0 (378[c])	409 ± 25.0 (207[c])	478 ± 34.0 (156[c])	241 ± 15.1 (188[c])

[a] All values represent the mean ± SEM of 6 samples per group; one sample was prepared by pooling tissue from three rats
[b] 5 mg/L in drinking water, *ad libitum*
[c] The percentage compared to saline control as 100%
[d] 1 mg/kg/day, i.p.
[e] 4 mg/kg/day, i.p.
[f] 0.1 mg/kg/day, i.p.
[g] 0.4 mg/kg/day, i.p.
[h] The percentage compared to the Pb-treated group as 100%
[i] The percentage compared to the Pb + Mn (L) treated group as 100%

Table 3. Regional Mn (ng/g dry tissue) distribution after Pb, Mn, and Cd treatment alone and in combination after 30 days exposure Value in parenthesis denote statistical significance (One way analysis of variance followed by Duncan's Multiple Range Test, P = 0.05)

Treatment	Cerebellum	Cerebral cortex	Corpus striatum	Hippocampus	Hypothalamus	Midbrain	Pons-medulla
Saline	1480 ± 94[a]	1705 ± 75	2187 ± 75	1750 ± 58	2177 ± 97	1958 ± 103	1868 ± 72
Pb[b]	1608 ± 135	1521 ± 81	2632 ± 146	1771 ± 56	2252 ± 112	2011 ± 60	1843 ± 100
Mn (L)[d]	2188 ± 105 (148[c])	2173 ± 96 (127[c])	3688 ± 143 (169[c])	2476 ± 131 (141[c])	3965 ± 136 (182[c])	2879 ± 145 (147[c])	2482 ± 115 (133[c])
Mn (H)[e]	2875 ± 113 (194[c], 131[h])	3046 ± 136 (179[c], 140[h])	4343 ± 189 (199[c], 118[h])	3722 ± 217 (213[c], 150[h])	4473 ± 114 (205[c], 113[h])	3930 ± 99 (201[c], 137[h])	3147 ± 112 (168[c], 127[h])
Cd (L)[f]	1614 ± 91	1720 ± 106	2221 ± 120	2130 ± 100 (122[c])	3116 ± 168 (143[c])	2597 ± 87 (133[c])	1799 ± 93
Cd (H)[g]	1620 ± 87	1956 ± 104	2281 ± 107	2225 ± 121 (127[c])	3206 ± 147 (147[c])	2473 ± 123 (126[c])	1901 ± 91
Pb + Mn (L)[d]	2358 ± 151 (159[c])	225 ± 119 (132[c])	4205 ± 218 (192[c], 114[h])	2513 ± 69 (144[c])	4148 ± 88 (191[c])	3392 ± 105 (173[c], 118[h])	2640 ± 68 (141[c])
Pb + Mn (H)[e]	2988 ± 123 (202[c])	3136 ± 163 (184[c])	4534 ± 212 (207[c])	3681 ± 44 (210[c])	4600 ± 138 (211[c])	4014 ± 96 (205[c])	3339 ± 166 (179[c])
Pb + Cd (L)[f]	1505 ± 75	1713 ± 58	2162 ± 111	1381 ± 61 (79[c])	2366 ± 106	2022 ± 102	1803 ± 68
Pb + Cd (H)[g]	1327 ± 104	1611 ± 78	2242 ± 125	1468 ± 68	2299 ± 161	1935 ± 87	1768 ± 77

[a] All values represent the mean ± SEM of 6 samples per group; one sample was prepared by pooling tissue from 3 rats
[b] 5 mg/L in drinking water *ad libitum*
[c] The percentage compared to saline control as 100%
[d] 1 mg/kg/day, i.p.
[e] 4 mg/kg/day, i.p.
[f] 0.1 mg/kg/day, i.p.
[g] 0.4 mg/kg/day, i.p.
[h] The percentage compared to the Pb treated group as 100%

Table 4. Regional Cd (ng/g dry tissue) distribution after Pb, Mn, and Cd treatment alone and in combination after 30 days exposure. Values in parenthesis denote statistical significance (One way analysis of variance followed by Duncan's Multiple Range Test, P = 0.05)

Treatment	Cerebellum	Cerebral cortex	Corpus striatum	Hippocampus	Hypothalamus	Midbrain	Pons-medulla
Saline	135 ± 7.4[a]	165 ± 9.9	192 ± 13.4	148 ± 13.1	188 ± 7.2	125 ± 7.9	164 ± 5.7
Pb[b]	136 ± 4.9	160 ± 12.6	154 ± 12.0 (80[c])	144 ± 10.8	192 ± 9.0	119 ± 5.3	166 ± 13.1
Mn (L)[d]	153 ± 10.5	168 ± 10.6	156 ± 13.0	139 ± 8.4	172 ± 10.5	126 ± 6.7	179 ± 14.3
Mn (H)[e]	132 ± 8.8	171 ± 8.2	157 ± 10.5	161 ± 10.0	178 ± 7.3	137 ± 11.3	169 ± 12.0
Cd (L)[f]	174 ± 11.5 (129[c])	224 ± 9.1 (136[c])	186 ± 12.5	180 ± 9.3	275 ± 10.7 (146[c])	176 ± 9.0 (141[c])	222 ± 13.3 (135[c])
Cd (H)[g]	202 ± 10.3 (150[c])	254 ± 11.9 (154[c])	233 ± 7.9 (121[c])	200 ± 8.6 (135[c])	288 ± 11.8 (153[c])	206 ± 7.5 (165[c])	268 ± 10.6 (163[c])
Pb + Mn (L)[d]	119 ± 7.6	156 ± 10.0	131 + 15.3 (68[c])	165 ± 10.9	172 ± 11.3	108 ± 4.3	186 ± 8.2
Pb + Mn (H)[e]	158 ± 11.0	165 ± 11.7	157 ± 12.4	149 ± 7.5	178 ± 12.7	110 ± 5.5	161 ± 11.5
Pb + Cd (L)[f]	247 ± 13.7 (183[c], 142[h])	286 ± 8.4 (173[c], 128[h])	229 ± 8.8 (119[c], 123[h])	183 ± 9.9 (124[c])	274 ± 18.5 (146[c])	224 ± 9.2 (179[c], 127[h])	290 ± 17.4 (177[c], 131[h])
Pb + Cd (H)[g]	270 ± 10.7 (200[c], 134[i])	352 ± 18.7 (213[c], 139[i])	273 ± 8.4 (142[c], 117[i])	217 ± 11.9 (147[c])	302 ± 21.8 (161[c])	289 ± 11.4 (231[c], 140[i])	381 ± 10.9 (232[c], 142[i])

[a] All values represent mean ± SEM of 6 samples per group; one sample was prepared by pooling tissue from 3 rats
[b] 5 mg/L in drinking water *ad libitum*
[c] The percentage compared to saline control as 100%
[d] 1 mg/kg/day, i.p.
[e] 4 mg/kg/day, i.p.
[f] 0.1 mg/kg/day, i.p.
[g] 0.4 mg/kg/day, i.p.
[h] The percentage compared to the Cd (L) treated group as 100%
[i] The percentage compared to the Cd (H) treated group as 100%

corpus striatum and midbrain. Administration of Pb together with Cd decreased Mn content in hippocampus compared to that seen in saline treated rats.

Data in Table 4 show that administration of Pb alone significantly decreased the contents of Cd in corpus striatum compared to that observed in saline treated rats. Mn administration in low and high doses had no effect on Cd distribution in the brain. While administration of the low dose of Cd significantly increased its accumulation in hypothalamus, cerebral cortex, pons-medulla, midbrain and cerebellum, a dose of 0.4 mg/kg/day of Cd further increased its accumulation in these regions and also increased accumulation in the corpus striatum and hippocampus. Co-administration of Pb and the low dose of Mn significantly decreased the concentration of Cd in corpus striatum. Except in the hypothalamus at low Cd doses, Cd together with Pb increased the accumulation of Cd in all regions with a magnitude greater than that observed after Cd exposure alone. The dose-related response in increasing the accumulation was noticed in all regions except hippocampus and hypothalamus.

Table 5 shows the levels of metals in liver, kidney, and testis of rats after Pb, Mn, and Cd exposure alone and after co-administration. Administration of Pb and Mn alone did not affect the concentration of any other metal except to increase their own concentration. Concentrations of Mn after Cd administration (0.1 mg/kg/day) were significantly increased in liver and decreased in kidney and testis. The higher dose of Cd decreased the concentration of testicular Mn also. Co-administration of Pb with the low dose of Mn significantly decreased the levels of Pb in liver compared to Pb exposure alone. A combination of Pb and the high dose of Mn decreased Pb in liver and increased Pb in kidney. The magnitude of decrease in hepatic Pb was found to be significantly greater in rats co-exposed with the high dose of Mn compared to the low dose. Testicular Pb levels observed after Pb administration did not change significantly after co-exposure with Mn. An increased accumulation of hepatic Mn was observed after Pb and low dose of Mn exposure compared to the low dose of Mn alone. Administration of the low or high dose of Cd with Pb decreased the levels of Pb in kidney and testis; however, Cd administration increased levels of Pb in liver compared to the Pb treatment alone. Further, the magnitude of decrease in testis was significantly greater after combined treatment of Pb

Table 5. Levels of metals (ng/g dry tissue) in liver, kidney and testis after Pb, Mn, and Cd treatment alone and in combination after 30 days exposure. Values in parenthesis denote statistical significance (One way analysis of variance followed by Duncan's Multiple Range Test, P = 0.05)

	Liver			Kidney			Testis		
Treatment	Pb	Mn	Cd	Pb	Mn	Cd	Pb	Mn	Cd
Control	752 ± 37[a]	3052 ± 104	434 ± 30	993 ± 109	2502 ± 104	495 ± 31	168 ± 18	1134 ± 76	393 ± 29
Pb[b]	4414 ± 225 (587[c])	3186 ± 149	399 ± 25	9821 ± 535 (990[c])	2510 ± 144	497 ± 27	387 ± 28 (230[c])	1174 ± 64	367 ± 21
Mn (L)[d]	786 ± 46	6649 ± 299 (218[c])	446 ± 20	1015 ± 94	4524 ± 192 (180[c])	485 ± 24	152 ± 12	1996 ± 58 (176[c])	378 ± 25
Mn (H)[e]	870 ± 35	8498 ± 503 (278[c])	485 ± 35	1066 ± 76	5232 ± 191 (209[c])	479 ± 24	154 ± 14	2169 ± 90 (191[c])	403 ± 28
Cd (L)[f]	802 ± 53	4897 ± 236 (160[c])	5601 ± 431 (1290[c])	958 ± 87	1919 ± 128 (77[c])	5200 ± 328 (1951[c])	166 ± 12	1091 ± 74	637 ± 36 (162[c])
Cd (H)[g]	883 ± 80	4854 ± 208 (59[c])	7932 ± 287 (1828[c])	1180 ± 74	1829 ± 127 (73[c])	7243 ± 337 (1463[c])	173 ± 11	845 ± 48 (75[c])	953 ± 39 (242[c])
Pb + Mn (L)[d]	3456 ± 132 (460[c], 78[h])	8278 ± 298 (271[c], 125[i])	442 ± 28	11176 ± 875 (1125[c])	3759 ± 225 (150[c], 83[i])	542 ± 34	345 ± 21 (205[c])	1815 ± 79 (160[c])	390 ± 18
Pb + Mn (H)[e]	3031 ± 257 (403[c], 88[l])	8674 ± 350 (284[c])	501 ± 32	11879 ± 798 (1197[c], 121[h])	4982 ± 183 (199[c])	512 ± 32	315 ± 12 (188[c])	2343 ± 136 (207[c])	406 ± 26
Pb + Cd (L)[f]	5223 ± 309 (695[c])	3253 ± 229	5122 ± 319 (1180[c])	8051 ± 405 (811[c], 82[h])	2346 ± 152	7536 ± 333 (1522[c], 145[j])	272 ± 18 (160[c], 70[h])	846 ± 47 (75[c], 72[c])	765 ± 44 (195[c], 120[j])
Pb + Cd (H)[g]	6109 ± 423 (812[c], 138[h])	2864 ± 203	7480 ± 389 (1723[c])	7174 ± 389 (722[c], 73[h])	1502 ± 122 (60[c])	11784 ± 677 (2381[c], 163[k])	149 ± 16 (39[h], 55[m])	733 ± 63 (65[c])	1050 ± 49 (267[c], 110[k])

[a] All values represent mean ± SEM of 6 samples per group; one sample was prepared by pooling tissue from 3 rats
[b] 5 mg/L in drinking water *ad libitum*
[c] The percentage compared to saline control as 100%
[d] 1 mg/kg/day, i.p.
[e] 4 mg/kg/day, i.p.
[f] 0.1 mg/kg/day, i.p.
[g] 0.4 mg/kg/day, i.p.
[h] The percentage compared to Pb treated group as 100%
[i] The percentage compared to Mn (L) as 100%
[j] The percentage compared to Cd (L) as 100%
[k] The percentage compared to Cd (H) as 100%
[l] The percentage compared to Pb + Mn (L) as 100%
[m] The percentage compared to Pb + Cd (L) as 100%

and the high dose of Cd compared to exposure with Pb and the low dose of Cd. Administration of Pb with the low or high Cd dose significantly increased the Cd accumulation in kidney and testis compared to the Cd alone high treatment. Levels of hepatic Cd obtained after low or high dose of Cd exposure remained unaltered by co-administration of Pb.

Table 6 shows blood Pb levels after different treatment schedules. Administration of Pb alone produced a five-fold increase in the blood Pb level. The magnitude of this increase was drastically decreased in a dose-dependent manner when co-exposed with either low or high doses of Mn or Cd.

Table 6. Blood Pb (μg/100 ml) after metal treatment alone and in combination after 30 days exposure. Values in parenthesis denote statistical significance (One-way analysis of variance followed by Duncan's Multiple Range Test, P = 0.05)

Treatment	Blood Pb
Saline	4.954 ± 0.277[a]
Pb[b]	24.762 ± 2.404 (500[c])
Mn (L)[d]	4.560 ± 0.190
Mn (H)[e]	4.500 ± 0.228
Cd (L)[f]	4.502 ± 0.216
Cd (H)[g]	3.378 ± 0.269
Pb + Mn (L)[d]	19.706 ± 1.109 (80[h], 432[i])
Pb + Mn (H)[e]	16.962 ± 1.079 (68[h], 377[j])
Pb + Cd (L)[f]	15.109 ± 0.973 (61[h], 336[k])
Pb + Cd (H)[g]	11.038 ± 0.856 (45[h], 327[l])

[a] All values represent mean ± SEM of 6 samples per group; one sample was prepared by pooling blood of 3 animals
[b] 5 mg/L in drinking water *ad libitum*
[c] The percentage compared to saline control as 100%
[d] 1 mg/kg/day, i.p.
[e] 4 mg/kg/day, i.p.
[f] 0.1 mg/kg/day, i.p.
[g] 0.4 mg/kg/day, i.p.
[h] The percentage compared to Pb treated group as 100%
[i] The percentage compared to Mn (L) as 100%
[j] The percentage compared to Mn (H) as 100%
[k] The percentage compared to Cd (L) as 100%
[l] The percentage compared to Cd (H) as 100%

Discussion

In the present study, Pb (5 mg/L) exposure to rats for 30 days increased Pb levels in all brain regions with maximum accumulation in the corpus striatum and hippocampus. These results are in accordance with the report of Kishi *et al.* (1983), who showed maximum accumulation of Pb in these regions after oral Pb administration. Co-exposure of Mn and Pb further increased accumulation of Pb in different brain regions without significant correlation between Pb accumulation and doses of Mn. Increased Pb accumulation in the whole brain of adult animals coexposed to Pb intraperitoneally and Mn orally has also been reported by Chandra *et al.* (1981). It has been suggested that the presence of excess Mn in the brain increased the affinity of brain tissue to bind Pb (Kalia *et al.* 1984). Apparently, the marked increase in the Pb level after Pb and Mn co-exposure is a region specific effect and is not dependent on age of the animal and route of metal administration. Similar to Mn, Cd also increased the level of Pb in certain brain regions of animals co-exposed to Pb and Cd; however, the mechanism of this increase is not clear. Since brain Pb levels have been correlated with the severity of Pb neurotoxicity (Collins 1983), the observation of a marked increase in Pb levels in some brain regions after combined exposure with Mn or Cd may produce pronounced central nervous system dysfunction compared to Pb exposure alone.

Levels of Mn increased in all brain regions after Mn exposure and this increase was dose-dependent. However, corpus striatum and hypothalamus showed high affinity for Mn accumulation, which may be responsible for the reported pathological and biochemical alterations specifically in these regions (Gruenstein and Papova 1929; Cotzias 1958; Neff *et al.* 1969; Chandra and Shukla 1981; Shukla and Chandra 1981). The Mn levels in the corpus striatum and midbrain regions after low dose Mn treatment were further increased significantly in the rats co-exposed with 5 mg/L Pb. Behavioral abberations in the form of increased spontaneous motor activity of rats and mice exposed to an excess of Mn have been correlated with enhanced striatal dopamine turnover (Chandra *et al.* 1979; Shukla and Chandra 1981). Results of the present study showed that co-exposure of animals to even very low doses of Mn + Pb selectively raised the striatal Mn and Pb concentrations, indicating the possibility of serious brain dysfunctions after coexposure to even subclinical levels of these two metals through a polluted environment. Further studies are necessary to understand the role of altered Mn levels of certain brain regions after Cd exposure alone in producing Cd-induced behavioral alterations in growing animals (Rastogi *et al.* 1977; Chandra *et al.* 1985).

Cadmium exposure increased Cd levels in various brain regions. However, there was no uniform correlation between the dose of Cd and its accumulation, except for Cd levels in striatal and hippocampal regions, which remained unaltered after

low doses of Cd exposure and increased significantly after high dose Cd exposure. This indicated regional affinity only at the low dose of Cd. The treatment of Cd + Pb further increased Cd levels in various brain regions. Administration of Cd to adults or neonates produces a variety of neurochemical (Ribas-Ozonas *et al.* 1974; Hrdina *et al.* 1976; Rastogi *et al.* 1977; Shukla and Chandra 1982) and behavioral changes (Smith *et al.* 1983; Chandra *et al.* 1985). Furthermore, marked abnormalities in neonates were reported to be due to higher levels of Cd accumulation in the younger brain (Wong and Klaassen 1981; Shukla *et al.* 1976). The observed increase in Cd accumulation after administration of Pb + Cd indicates that co-exposure may enhance the toxic effect of Cd on the central nervous system. Observation of the marked elevation in metallic contents of various brain regions after co-administration could be the result of diversion of metals from certain other organs to brain tissue. However, the possibility of a damaged blood-barrier for these metals also exists after administration of multimetals. The mechanism causing decreased striatal Cd levels after administration of Pb alone or Pb with the low-dose exposure of Mn is not presently understood. Both Pb and Mn are maximally concentrated in this region after their individual exposure.

Administration of Pb, Mn, or Cd elevates their levels in liver, kidney, and testis in a dose-dependent manner. Our observations of maximum accumulation of Pb and Cd in kidney and Mn in liver are in accordance with previous reports (Fleischer *et al.* 1974; Barry 1975; Chandra and Srivastava 1978). Co-exposure of Pb together with Mn decreased Pb and increased Mn contents in the liver. However, the similar combination increased Pb and decreased Mn in renal tissue. There is a possibility that both Pb and Mn compete for certain common metal binding sites and, depending on their affinity to a particular tissue, one metal occupies more sites than the other; thus, producing a decrease or increase in the metallic contents (Magos and Webb 1978). Co-exposure of Pb and Cd decreased Pb and increased Cd contents in kidney and testis compared to respective metal exposure alone. While hepatic Pb accumulation was further increased after administration of Pb together with the high dose of Cd, levels of Cd remained unaltered in the liver of co-exposed animals. Comparative higher accumulation of Pb in liver and heart and Cd in kidney have been reported after combined feeding of the two metals in drinking water (Kopp *et al.* 1983). However, these workers did not find a decline in kidney Pb levels. Although testis is one of the target tissues of Cd toxicity in experimental animals, the Cd levels are not as high as in liver and kidney (Scott *et al.* 1974). It is possible that testicular and renal binding sites bind preferentially with Cd compared to Pb, thus resulting in decreased Pb and increased Cd contents in these tissues.

The reported synergistic effect of Pb and Cd in altering testicular and prostatic functions (Fahim and Khare 1980) could be the result of increased levels of testicular Cd after combined exposure. Observation of the decrease in Mn contents of testis and kidney after combined treatment of Pb and Cd could be the result of a greater accumulation of administered metals in these organs. However, the increase in tissue Mn levels after Cd treatment remains unexplained. The higher magnitude of accumulation of Mn in liver, Pb in kidney, Cd in testis and kidney after combined exposure is of great significance in view of well documented reports on hepatotoxicity of Mn (Mehrotra 1962; Jonderko and Szczurck 1970), nephrotoxicity of Pb (Morgan *et al.* 1966; Inglish *et al.* 1978), testicular (Parizek and Zahor 1956; Lee and Dixon 1973) and nephrotoxicity (Friberg 1950; Axelsson *et al.* 1968) of Cd.

Blood Pb levels have been well correlated with the severity of Pb toxicity in humans and animals (Hernberg 1980). The present study showed a decrease in blood Pb levels after administration of Pb together with Cd or Mn compared to Pb exposure alone. Decreased blood Pb levels have also been reported in rats co-administered Pb and Cd intraperitoneally by other workers also (Fahim and Khare 1980). This suggests that blood Pb levels may not be of much diagnostic significance for Pb toxicity in co-exposed conditions.

In conclusion, the interaction of metals may alter tissue distribution, which may be responsible for producing adverse health effects due to co-exposure at low levels of metals. However, the mechanism of such interactions remains to be studied.

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