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Subchronic Oral Toxicity of Zinc in Rats

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Zinc in the environment originates from natural and man-made sources. Worldwide man-made emissions of zinc originate mainly from primary zinc metal production (31%), wood combustion (24%), waste incineration (12%) and iron and steel production (11%) (Nriagu 1979; Léonard et al. 1986). Drinking water contains about 10 µg/l of zinc, but concentrations as high as 2 mg/l have been found when the water had passed through zinc pipes (Schiller and Boyle 1985; Léonard et al. 1986).

It is well known that zinc has important biological functions. Clinical manifestations in zinc-deficient animals include growth retardation, testicular atrophy, skin changes, and poor appetite (Prasad 1979; Prasad 1985). On the other hand, high levels of dietary zinc have been shown to induce copper defficiency in rats and to interfere with the metabolism of calcium and iron (Campbell and Mills 1979).

Little is known on the oral toxicity of zinc in mammals. However, some toxic effects in human subjects (Kumar 1976; Prasad 1979), rodents (Walters and Roe 1965; Lee and Matrone 1969) and sheep (Campbell and Mills 1979) have been reported. In order to extend the information about the oral toxicity of zinc, a semichronic toxicity study of zinc acetate in rats has been carried out in this paper.

MATERIALS AND METHODS

A total of forty female Sprague-Dawley rats (70-90 g, obtained from Interfauna Ibérica, Barcelona, Spain) were exposed to zinc acetate dihydrate (Merck, Darmstadt, FRG) in the drinking water at levels of 0, 160, 320 and 640 mg/kg body weight/day continously for three months. Solutions were prepared weekly to achieve a constant intake. Sugar was added to reduce the aversive effect of the zinc in the water. Similar quantities of sugar were also added to the drinking water of control animals in order to make comparable the results. Food (Panlab diet, Barcelona, Spain) and water consumption were freely available.

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The animals were placed in individual metabolism cages. Body weights of all rats were recorded prior to treatment and weekly throughout the study. Food and water consumption, and the volume of urine and the weight of excreted feces were measured daily. at the end of the experiment, blood samples were collected from the tail vein. Hematocrit and hemoglobin determinations, as well as the concentration of glucose, GOT, GPT, ALP, urea and creatinine in plasma were measured according to clinical chemistry methods described earlier (Domingo et al. 1985).

Table 1. Nutritional parameters[a] in rats receiving zinc acetate at three different doses[b] for three months

	1st 2 weeks	2nd 2 weeks	3rd 2 weeks
Food (g)			
Control	273,4±65,7	286,5±45,6	309,7±21,6
160	171,6±39,4	297,5±34,8	311,7±38,5
320	318,9±82,9	253,5±33,5	280,0±47,4
640	295,5±80,5	237,8±54,3	290,8±23,1
Weight gain (ΔP in g)			
Control	18,7±6,5	51,6±10,2	54,4±10,7
160	30,0±9,7	48,1±11,2	45,0±15,6
320	24,5±8,6	37,9±16,7	34,3±11,7
640	20,6±7,7	32,7±10,9	47,1±5,3
Water (ml)			
Control	500,6±115,0	483,4±86,0	476,3±71,2
160	367,5±106,4	424,7±57,1	433,1±99,8
320	278,6±193,8	359,4±25,6	388,7±53,1
640	90,0±34,0*	219,6±41,3*	350,5±83,5*
Urine (ml)			
Control	76,9±19,4	149,7±41,3	104,0±46,8
160	36,1±11,0	60,0±30,8	80,8±24,1
320	25,1±6,5	46,4±23,8*	58,4±17,0
640	11,3±8,0*	27,5±17,8*	46,0±10,8*
Feces (g)			
Control	42,2±10,2	61,3±9,7	65,6±11,3
160	33,8±10,6	50,3±14,3	62,1±13,7
320	30,2±12,7	43,6±14,7	45,3±15,4
640	31,7±11,0	37,6±10,4	46,8±5,4

[a]Results are expressed as arithmetic means ± S.E.
[b]Doses are expressed in mg/kg/day
*P<0,05, **P<0,01 by the Student's t test.

After three months of the treatment the animals were sacrificed and the weight of the brain, heart, lungs, spleen, liver and kidneys measured. Histological examinations (paraffin slices,

hematoxilin-eosin) of spleen, liver, heart, lungs and kidneys were performed in three rats of each group.

Table 1 (continuation). Nutritional parameters[a] in rats receiving zinc acetate at three different doses[b] for three months.

	4th 2 weeks	5th 2 weeks	6th 2 weeks
Food (g)			
Control	313,5±50,5	250,0±46,2	275,8±43,8
160	329,5±50,4	263,6±51,8	307,6±36,9
320	280,6±54,6	226,2±30,0	249,6±39,6
640	251,6±43,7	158,6±54,1	163,1±71,0
Weight gain (ΔP in g)			
Control	24,7±11,2	24,1±5,4	-9,2±6,4
160	18,4±7,2	17,8±8,1	2,5±2,0
320	22,1±8,7	21,8±14,6	-4,6±3,5
640	7,5±5,0	7,3±6,1	-15,8±11,8
Water (ml)			
Control	576,9±44,2	411,6±91,0	392,2±72,9
160	371,1±51,8**	250,9±21,4	338,4±71,9
320	364,3±40,1**	251,6±56,4	229,3±29,7
640	198,3±15,0**	143,3±27,2*	180,7±27,8*
Urine (ml)			
Control	157,5±45,4	133,1±46,3	140,6±37,6
160	54,5±17,9	59,6±10,3	52,5±20,5
320	43,9±7,3*	49,8±18,9	34,3±13,8*
640	31,2±13,0*	41,1±13,1*	30,6±17,5*
Feces (g)			
Control	62,7±17,5	47,7±3,7	42,7±10,3
160	52,1±7,9	45,8±8,5	49,5±19,0
320	54,0±9,1	41,1±12,6	34,4±7,5
640	41,7±7,3	29,1±8,1	20,9±14,0

[a]Results are expressed as arithmetic means ± S.E.
[b]Doses are expressed in mg/kg/day
*P<0,05, **P<0,01 by the Student's t test.

Zinc concentrations in brain, liver, kidneys, spleen, heart, lungs, bone (femur), abdominal muscle and blood were determined by atomic absorption spectrophotometry (Perkin-Elmer 4000) after digestion as previously described (Domingo et al. 1985).

The significance of the differences in the results was determined by the Student's t test. A difference was considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

During the total 3-month treatment period some outstanding changes were found as regards to appearance, mortality and behaviour between the rats receiving the higher doses of zinc acetate (640 mg/kg/day) and the controls. Treated rats showed a tendency towards apathy and two animals in this group died throughout the study. Food consumption as well as the amount of feces excreted were about equal in all groups. There was no effect on weight gain caused by treatment (Table 1, $P>0.05$).

Table 2. Fresh weights of organs from rats receiving zinc acetate in their drinking water for three months*.

	Dose (mg/kg/day)			
	0	160	320	640
Brain	1,88±0,11	1,83±0,12	1,74±0,15	1,72±0,24
Heart	0,86±0,10	0,76±0,13	0,84±0,09	0,66±0,17
Lungs	1,44±0,29	1,51±0,37	1,39±0,20	1,03±0,14
Spleen	0,48±0,24	0,46±0,11	0,51±0,15	0,29±0,24
Liver	8,56±0,75	8,38±1,32	7,50±1,29	5,02±1,97
Kidneys	1,81±0,17	1,71±0,11	1,73±0,21	1,61±0,46

*Results are presented as arithmetic means. No significant differences could be detected by the Student's t test.

Table 3. Hematological and clinical chemical parameters* in rats dosed with zinc acetate for three months.

	Dose (mg/kg/day)			
	0	160	320	640
Hematocrit (%)	46,7±4,9	41,0±12,7	49,5±3,5	39,0±11,8
Hemoglobin (g/100 ml)	12,2±0,7	14,5±1,0	14,1±2,7	10,3±1,9
Glucose (mg/100 ml)	163,5±9,9	142,3±29,1	156,3±29,1	142,3±8,5
GOT (U/l)	118,9±22,1	96,6±47,5	113,2±25,5	141,6±8,1
GPT (U/l)	40,1±10,9	50,4±15,1	51,9±7,0	42,1±6,1
ALP (U/l)	196,9±37,9	227,0±19,8	228,4±28,5	236,7±38,7
Urea (mg/100 ml)	39,5±5,2	40,5±11,4	35,5±3,1	59,2±4,2**
Creatinine (mg/100 ml)	0,7±0,21	1,7±1,22	0,6±0,24	4,4±0,70**

*Results are expressed as arithmetic means ± S.E. **$P<0,01$ by the Student's t test.

With regard to the other nutritional parameters measured, the drinking water ingested and the volume of urine excreted were always significantly lower for the 640 mg/kg/day group.

Nevertheless, the animals receiving 160 and 320 mg/kg/day did not show significant differences in any of the nutritional parameters studied. No treatment-related effects on organ weights or organ/body weight ratios were observed during the study (Table 2, P>0,05).

Table 4. Zinc concentrations* in organs and tissues of rats receiving zinc acetate for three months.

	Dose (mg/kg/day)			
	0	160	320	640
Brain	9,5±1,9	9,4±3,2	13,5±1,8	15,4±2,8
Liver	20,3±5,3	24,7±5,7	47,6±8,4*	59,9±7,0**
Kidneys	16,0±1,09	20,6±4,9	41,1±4,9**	38,3±3,2**
Spleen	15,6±2,05	15,9±3,4	22,0±3,6	36,4±6,0*
Heart	10,5±1,7	11,9±1,8	16,8±1,1*	21,9±2,5**
Lungs	12,7±2,4	14,2±3,6	20,2±1,3	20,7±2,8
Bone	92,3±21,9	127,3±39,7	326,3±47,6**	330,6±58,6**
Muscle	14,5±3,2	13,9±1,6	18,0±1,3	30,0±9,1
Blood	3,4±2,0	4,2±1,7	16,3±2,3**	21,1±3,0***

*Zinc concentrations are expressed as µg/g fresh weight. *P<0,05, **P<0,01, ***P<0,001 by the Student's t test.

Table 3 summarizes the hematological and plasma analyses carried out in the treated and control animals after three months of treatment. No significant differences were found in the mean values of hematocrit and hemoglobin. The concentrations of glucose, GOT, GPT and ALP in plasma were within the normal range for treated and untreated animals. However, the concentrations of urea and creatinine and plasma were significantly higher for the animals receiving 640 mg/kg/day zinc acetate than for the control rats.

The concentrations of zinc in tissues of rats given zinc acetate are shown in Table 4. The highest concentrations were found in bone, liver and kidneys as well as in blood. A significant relationship between dose received and tissue concentrations can be seen.

The most severe histological lesions were observed in kidneys. These lesions consisted in glomerular Bowman's capsule with flattened epithelial cells (Fig. 1) and proximal convoluted tubules with desquamation of tubular epithelial cells and picnotic nucleus (Fig. 2).

The above is a report on the results of a subchronic test in rats with zinc for a period of three months. During this treatment period the animals were continously given zinc acetate at concentrations of 160, 320 and 640 mg/kg/day which correspond approximately with 1/5, 1/2.5 and 1/1.25 of the acute oral LD_{50} of zinc acetate previously determined (Colomina et al. 1987).

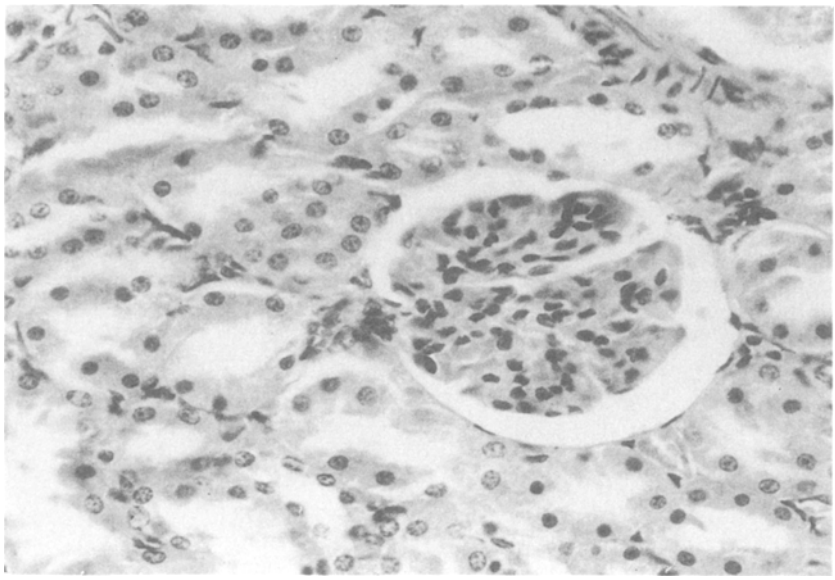

Figure 1. Renal section from a Sprague-Dawley rat which received 640 mg/kg/day of zinc acetate in the drinking water for 3 months.

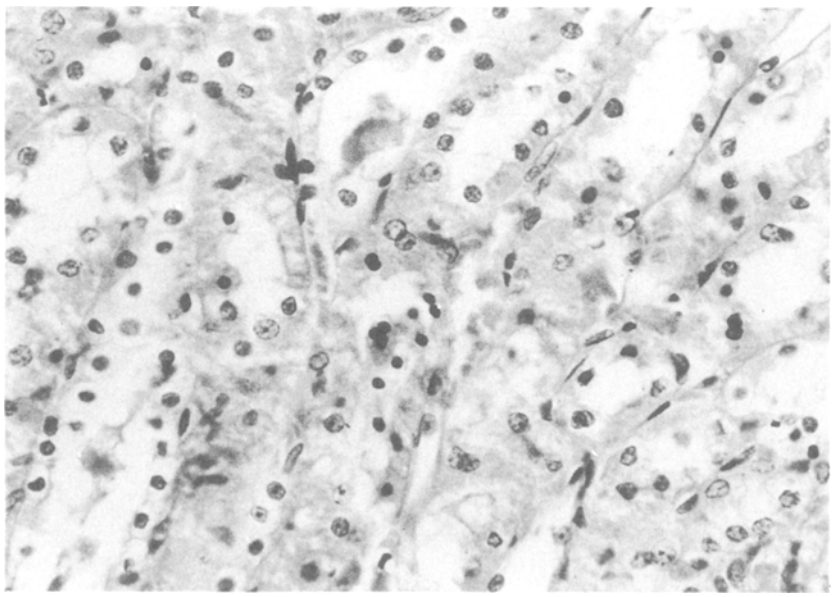

Figure 2. Renal tubules from a Sprague-Dawley rat which received 640 mg/kg/day of zinc acetate in the drinking water for 3 months.

These concentrations did not affect weight gain, nutrition or organ weights. Nor were significant differences in the values of hematocrit and hemoglobin between the treated and the control rats.

Long-term feeding to rodents of very large amounts of zinc salts were reported to result in growth retardation, anemia and metabolic effects (Léonard et al. 1986). These disorders were described to be most likely due to a lowered uptake of food and/or a competition with other essential elements such as iron (Lee and Matrone 1969; Léonard et al. 1986). Our results have not corroborated these changes. On the other hand, the clinical chemistry did not indicate that zinc administered for 3 months had any effect on the liver which was confirmed by the histopathological study. Nevertheless, the concentrations of urea and creatinine in plasma for the 640 mg/kg/day group were significantly increased compared with the control group. This fact suggests a remarkable effect on the renal function which has been corroborated by the histopathological study of the kidneys. The significant decreases in the volume of urine excreted for these animals could also suggest a disorder on the renal function. Notwithstanding, it seems that these decreases agree with a significant lower ingestion of water in this group.

With regard to the concentrations of zinc in the organs and tissues analyzed, these were especially significant in bone, kidneys, liver and blood. These results are in accordance with previous findings (Kang et al. 1977).

Zinc has been considered a relatively non-toxic element, but toxicity has been reported to arise in sheep and cattle when dietary concentrations exceed 900-1000 mg/kg (Campbell and Mills 1979). In rats, ingestion of 0.5% to 1.0% of diet as zinc has been reported to result in reduced growth and anemia (Prasad 1979). Anemia had also been reported in mice (Walters and Roe 1965). Our study has not corroborated these effects. Nevertheless, we have observed a significant renal disorder which had not been previously described.

However, our results have demonstrated a toxicological no-observable-effect level (NOEL) for zinc acetate of 160 mg/kg body weight/day in female rats. Assuming that 40% of the dietary zinc is biologically available, the recommended zinc intake for adults would fall between 5 and 15 mg/day (Rabbani et al. 1986). Therefore, the possibility of oral zinc intoxication in the human would be unusually low.

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