Blood and Bone Marrow Effects of Feeding Zinc Sulfate to Rats and Dogs*

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Because of its emetic properties zinc sulfate has been proposed for inclusion in barbiturate preparations to prevent deaths due to overdosage of these hypnotics. Since this would lead to chronic ingestion of zinc sulfate in persons receiving prolonged barbiturate medication, we have studied the chronic toxicity of this emetic. Rats were fed zinc sulfate ($ZnSO_4.7H_2O$) incorporated in ground commercial Purina Laboratory Chow at 1,000, 500, 100, and 0 parts per million for twenty-one months. Four of six dogs all maintained on Purina Chow received zinc sulfate by capsule for seventy weeks. The initial daily dose for dogs was 200 mg./Kg. Emesis forced a change of dosage to 100 mg./Kg. at seven weeks, and again in three of four dogs at thirty-two weeks to 50 mg./Kg. The other dog was changed because of emesis to 50 mg./Kg. after three weeks on the 100 mg/Kg. level. Effects observed in rats were microcytosis, coupled with polychromasia in some cases, and hyperchromia in others, and a hypochromic anemia in dogs. The blood effect noted in rats was reversed despite continued zinc feeding. The average myeloid-erythroid ratio of rat bone marrow smears taken at the termination of the experiment ranged from 1.16 to 1.35 for the various groups of zinc animals and read 2.14 for the untreated controls. Dog bone marrow also studied at the termination of that experiment was slightly hyperplastic. Although no differential marrow count was made, the myeloid-erythroid ratio appeared unchanged.

ZINC SULFATE is used medicinally as an emetic, and this has led to the suggestion by Miskimon and Miskimon (1) that it be added in small amounts to barbiturate preparations so as to cause vomiting when these drugs are taken in overdosage either accidentally or with suicidal intent. A corollary of the use of this drug combination would be the ingestion and retention of zinc by the person using reasonable daily amounts of barbiturates. When confronted with this latter possibility we felt it desirable to study the chronic effects of feeding zinc sulfate to laboratory animals.

Smith and Larson (2) fed zinc carbonate to rats for five weeks at a level providing 1.0 per cent zinc and observed a microcytic hypochromic anemia together with retardation of growth. The hemoglobin deficiency failed to develop when a mixture of iron, copper, and cobalt were fed in the anemia-producing diet, while dietary liver produced significant growth response in the retarded animals. Sadasivan (3) fed zinc oxide to rats at 0.5 and 1.0 per cent for fifteen days, and found it to exert a lipotropic effect. Further findings were failure to grow normally and retardation in development and mineralization of bone. At the 1.0 per cent level of zinc oxide feeding Sadasivan (4) observed rats to excrete increased amounts of nitrogen through urine and feces. At 0.5 per cent dietary zinc oxide urinary excretion of phosphorus and sulfur decreased, while urinary excretion of uric acid and creatinine increased. Fecal excretion of phosphorus and sulfur increased. Sutton and Nelson (5) fed rats zinc carbonate at levels corresponding to 0.10, 0.50, and 1.0 per cent zinc. The 1.0 per cent level was found overwhelmingly toxic while impairment of reproduction and decrease in hemoglobin concentration occurred at 0.5 per cent. Hegsted and co-workers (6) have reviewed the literature pertaining to zinc. Drinker, *et al.* (7), reported negative results for production of anemia after administering zinc oxide to dogs and cats. Doses fed to dogs were 1,000 and 500 milligrams per animal for feeding periods of fifteen, and three to nineteen weeks, respectively. Doses ranging from 600–1,000 milligrams were poorly tolerated by cats, and the maximum time such levels were fed was twenty-one weeks. The longest period of feeding at any level was that fed to 1 cat and measured 175 milligrams for eight weeks, then 354 milligrams for forty-five weeks. Drinker, *et al.* (8), fed zinc to rats in daily doses of 0.5 to 34.4 milligrams for from thirty-five to fifty-three weeks. Zinc was fed as the oxide, acetate, citrate, and malate. Blood studies made prior to autopsy failed to reveal abnormalities.

EXPERIMENTAL RESULTS

A.—Four groups of eight Osborne Mendel weanling rats (4 males and 4 females) were fed *ad libitum* a basal diet of ground commercial Purina Chow containing 1,000, 500, 100, and 0 p. p. m. of zinc sulfate ($ZnSO_4.7H_2O$). The rats were individually housed in wire mesh cages. Food consumption and animal weight were recorded weekly. Eight complete blood counts were made which were confined to the

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first three and last five months of the experiment. A study of bone marrow smears was made at the termination of the experiment.

During the approximately twenty-one-month feeding period, food intake and weights of the rats fed zinc varied within normal limits. Hemoglobin values, red cell, white cell, and differential counts remained normal throughout the experiment. However, inspection of smears showed the occurrence of microcytosis, coupled with polychromasia in some cases and hyperchromia in others. These blood changes occurred at all levels of feeding, and were first observed at the 1,000 p. p. m. level on the resumption of blood studies at the sixteenth month. One month later this change was noted at the 500 and 100 p. p. m. levels of feeding. Despite the continuation of these rats on the zinc-containing diets the red cells subsequently returned to normal size. Counts (500 cells each) of the bone marrow smears taken at autopsy revealed the average myeloid-erythroid ratio (M/E) of rats fed the basic diet to be 2.14 and of the rats fed all levels of zinc sulfate to vary from 1.16 to 1.35. The bone marrow data are given in Table I.

At autopsy, there were no gross pathological changes attributable to zinc, with one possible exception. The kidneys of the male rats on 500 and 1,000 p. p. m. of zinc sulfate were larger and more granular than those of 100 p. p. m. or the controls. Unfortunately, the kidneys were not weighed. The kidneys of the females were only rarely enlarged or granular, and there was no difference between groups. Liver weights, when expressed as percentage of body weight, showed no difference between groups. All 32 rats were to at least some extent examined microscopically. Six animals given 1,000 p. p. m. zinc sulfate and 6 controls, evenly divided by sex, were examined in detail, while for the remaining 20 animals sections were limited to liver, kidney, testis (if male), and in 8 instances bone and bone marrow. Only the kidney and bone marrow showed microscopic changes which could be attributed to zinc. In the bone marrow sections the cellularity of the marrow was alike for all groups, and within normal limits, but the M/E ratios appeared altered, as was better shown by the actual counts of smears. The kidneys of the males, both control and treated, commonly showed at least a slight degree of the spontaneous nephritis (sometimes called nephrosis) which is frequently seen in older rats. The condition is described and illustrated by, among others, Saxton and Kimball (9). Among all the females there was but one in which this condition was present in as much as a moderate degree. Among the males, however, there were 6 with the condition in moderate degree, and 5 with it in severe degree. All the latter 5 animals were in the 500 and 1,000 p. p. m. groups, suggesting, if not proving, that zinc increased the severity of the normally occurring nephritis or nephrosis in males.

B.—The toxicity of zinc sulfate to dogs was also determined. A litter of 6 Dalmatian puppies, ten weeks old, was selected for the experiment. Four of the 6 dogs were given zinc sulfate by capsule at a daily level of 200 mg./Kg. to start. Complete blood counts were taken every two weeks at the start of the experiment and at longer intervals thereafter. No counts were taken from the eighth to the thirtieth week of the experiment because of the absence of a technician. After seven weeks of zinc sulfate administration at this level most of the dogs began to vomit consistently after their doses, and the daily dosage level was reduced to 100 mg./Kg. After three weeks on this dose, dog No. 5 began to vomit again, and its dose was reduced to 50 mg./Kg. daily. After thirty-two weeks on 100 mg./Kg. daily, the other 3 experimental dogs began to vomit fairly consistently after dosage and their daily dose was likewise reduced to 50 mg./Kg. At this time dog No. 6 was losing weight and appeared to be in bad physical condition, though all other dogs showed normal appetite and growth throughout experimentation. This dog was sacrificed *in extremis* one week later. After seventy

TABLE I.—BONE MARROW COUNTS—ZINC SULFATE

1,000 P. P. M.				500 P. P. M.			
Animal Number	Total Myeloid, %	Total Erythroid, %	M/E Ratio	Animal Number	Total Myeloid, %	Total Erythroid, %	M/E Ratio
5605	49	44	1.11	5613	50	38	1.32
5606	48	41	1.17	5614	51	41	1.24
5607	51	40	1.27	5615	48	45	1.07
5608	49	43	1.14	5616	49	36	1.33
5609	55	45	1.22	5617	50	44	1.14
5610	44	46	0.96	5618	47	40	1.17
5611	50	48	1.04	5619	49	47	1.04
5612	58	40	1.42	5620	46	46	1.00
Av.	50.5	43.0	1.17	Av.	48.7	42.1	1.16
100 P. P. M.				Control			
Animal Number	Total Myeloid, %	Total Erythroid, %	M/E Ratio	Animal Number	Total Myeloid, %	Total Erythroid, %	M/E Ratio
5621	52	40	1.30	5629	60	28	2.14
5622	60	33	1.52	5631	58	29	2.00
5623	57	40	1.43	5632	63	29	2.17
5624	53	40	1.33	5633	67	21	3.19
5625	54	37	1.73	5634	64	28	2.28
5626	45	46	1.00	5635	64	34	1.88
5627	49	45	1.09	5636	54	38	1.42
5628	53	37	1.43				
Av.	52.8	39.8	1.35[a]	Av.	61.4	29.6	2.14

[a] Probability <0.01.

weeks the experiment was terminated and all surviving dogs were sacrificed.

Blood studies of the dogs receiving prolonged daily doses of zinc sulfate showed that although all 6 dogs had, because of their young age, low hemoglobin values at the start of the experiment, they soon attained normal adult hemoglobin values. However, the 4 experimental dogs all eventually developed hypochromic anemia, in that the hemoglobin values declined while the red cell counts remained at normal levels (Table II). No significant effects on the white blood count, total or differential, were noted. Histopathological examination of from 20 to 25 tissues of each of these dogs revealed the only change attributable to zinc to be in the bone marrows, which were uniformly slightly hyperplastic as compared with the controls.

TABLE II.—THE EFFECT ON THE BLOOD HEMOGLOBIN OF ZINC SULFATE ADMINISTRATION TO DOGS

	Hemoglobin—Gm. per 100 Cc. of Blood					
	Untreated		Treated			
Weeks	Dog No. 1	Dog No. 2	Dog No. 3	Dog No. 4	Dog No. 5	Dog No. 6
0	10.0	12.0	10.0	10.0	8.5	10.5
3	10.5	11.0	11.5	11.5	10.5	11.5
5	12.2	12.0	14.0	13.0	13.0	11.9
7	13.0	12.0	11.0	13.0	11.0	11.5
31	14.0	14.0	12.0	13.5	12.8	12.5
39	13.5	15.0	13.5	13.5	11.2	9.0[a]
53	14.0	15.0	12.5	14.0	13.0	...
61	15.0	14.0	10.8	11.0	12.0	...
70	14.0	14.0	10.5	10.0	9.0	...

[a] This value was taken before the dog was sacrificed after 33 weeks on experiment.

Marrow counts were not done on the dogs, but by inspection of sections and smears the M/E ratio did not appear altered.

C.—It was believed desirable to attempt to confirm the results reported by the Miskimons that zinc sulfate is effective in preventing poisoning by fatal doses of pentobarbital. We determined that 8 Gm. of zinc sulfate consistently produced vomiting in twenty to forty-five minutes in dogs concomitantly receiving 450 Gm. of horse meat. Then, to a group of 4 dogs, 8 Gm. of zinc sulfate and 4 Gm. of pentobarbital were administered along with 450 Gm. of horsemeat. This experiment was repeated, omitting the horsemeat. Whether or not the administration of the zinc sulfate-pentobarbital combination was accompanied by the feeding of horsemeat, all 4 of the dogs exhibited emesis in twenty to forty-five minutes. Only 1 of the 4 exhibited sedation, this lasting in both experiments from fourteen to eighteen hours. The other 3 dogs showed no effects from the pentobarbital administration. However, when the dogs were given the same dose of pentobarbital alone, all 4 succumbed within thirty hours. These results indicate that zinc sulfate is effective in preventing poisoning by fatal doses of pentobarbital.

DISCUSSION

Because of the relative leveling off of the growth of rats at about the sixteenth week, the amounts of zinc sulfate subsequently ingested measured about 4–6 mg./Kg. daily, at the 100 p. p. m. level. To correlate this and human consumption it is felt that the ingestion by a human being of a single overdose of 10 or even fewer therapeutic doses (usually 100 milligrams each) of a barbiturate, could easily have serious consequences. The human emetic dose of zinc sulfate is commonly accepted as being from 1 to 2 Gm. The Miskimons found emesis to occur in dogs with 0.3- to 0.5-Gm. doses of zinc sulfate given in single capsules. An increase in the amount of zinc sulfate was found necessary when the dose was divided into several capsules. These investigators also found that the usual dose of zinc sulfate required for emesis in dogs was considerably increased by the simultaneous administration of pentobarbital. It seems probable therefore that each hypnotic capsule intended for human use might have to contain several hundred milligrams of the emetic. The daily intake of zinc sulfate per kilogram of body weight in humans would then be comparable to that which was found to produce adverse effects in rats. It is felt, therefore, that this emetic should not be taken over prolonged periods in amounts that might be included in reasonable barbiturate dosage.

SUMMARY

Zinc sulfate was fed to weanling rats at 1,000, 500, and 100 p.p.m., and to dogs at 200 mg./Kg./day initially, then 100 mg./Kg., and finally 50 mg./Kg. The dose in dogs had to be reduced because of emetic effects. The effects resulting in the rat were microcytosis coupled in some cases with polychromasia and in others with hyperchromia. No reduction of red cell numbers or increase in hemoglobin values occurred. Hypochromic anemia was observed in the dog. The blood changes observed in the rat returned to normal despite continued zinc feeding. The rat bone marrow showed a myeloid-erythroid ratio average of 1.16 to 1.35 in the various groups of zinc animals in contrast to the value of 2.14 found in the untreated controls, all taken on 500 cell counts. The intake of zinc sulfate at the lowest level of rat feeding was approximately 4–6 mg./Kg. daily for the last seventy-seven weeks of feeding. The dog bone marrows showed slight hyperplasia as compared to the controls, with no change in the myeloid-erythroid ratio apparent on inspection.

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