this 'peak' is reached in 3–5 min., and the optimal 'alumina' plasma and serum dilutions are 1 : 5 and 1 : 10 respectively, as recommended by Biggs and Douglas.

When frozen, the concentrated saline suspension remains stable for at least twelve months ; it may be thawed and re-frozen repeatedly without loss of activity. The exact dilution for use should be determined for each batch of extract.

This modification was evolved in the process of work[2] on an extract for therapeutic use in thrombocytopenic states. It has proved entirely satisfactory in the diagnosis of hæmophilia and Christmas disease.

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Influence of Ethylenediamine-tetra-acetic Acid on the Excretion of Zinc by the Rat

The administration of ethylenediamine-tetra-acetic acid (versene, sequestrene) causes increased urinary excretion of calcium, iron, lead and other metallic ions[1]. We became interested in this chelating agent as a possible means for reducing zinc stores and thus hastening the onset of deficiency in rats maintained on a diet low in zinc. Information on the effect of ethylenediamine-tetra-acetic acid on zinc metabolism was also required in planning an investigation of the excretion of recently absorbed zinc-65. Stability constants indicate[2] that zinc forms one of the most stable complexes with ethylenediamine-tetra-acetic acid, but we are not aware of any previous experiments to test its action on body stores of zinc, apart from a brief reference to unpublished work by Mager[3].

The present report concerns a preliminary investigation of the effect of ethylenediamine-tetra-acetic acid on the urinary excretion of zinc in rats. The calcium disodium salt of ethylenediamine-tetra-acetic acid was injected intraperitoneally into young adult male rats in doses of 100 mgm. per kgm. daily for three days. In experiment 1, four rats were kept in a stainless steel metabolism cage and the pooled urine collected each day for 18 hr. after the injection, and once for the following 6 hr. A control sample was collected on the day before injections began. During the control period and the first test period, the rats received distilled water but no food. Thereafter, a synthetic diet (modified from Day and McCollum[4]) containing less than 0·5 μgm. zinc per gm. was supplied continuously. In experiment 2, the animals received

Table 1. Influence of Ethylenediamine-tetra-acetic Acid on Urinary Excretion of Zinc

Sample	Collection time (hr.)	Mean urine zinc (μgm. per rat) Exp. 1	Exp. 2
Control	18	2·2	3·8
EDTA, 1st day	18	68·5	37·1
EDTA, 2nd day	18	69·5	61·0
Remainder of 2nd day	6	1·8	—
EDTA, 3rd day	18	155·2	75·7

Controls : Exp. 1 : Urine from four rats collected for one 18-hr. period. Exp. 2 : Urine from eight rats collected for three 18-hr. periods.
Tests. Exp. 1 : 100 mgm. per kgm. $CaNa_2EDTA$ per day—1st day in 5 per cent, 2nd and 3rd days in 2·5 per cent solution ; Exp. 2 : 100 mgm. per kgm. $CaNa_2EDTA$ per day in 0·83 per cent solution.

Table 2. Effect of Ethylenediamine-tetra-acetic Acid on Zinc Content of Ileum and Pancreas

Treatment and diet since weaning	Ileum No. of rats	Ileum Mean Zn (μgm. per gm.)	Ileum S.E. of mean	Pancreas No. of rats	Pancreas Mean Zn (μgm. per gm.)	Pancreas S.E. of mean
Controls						
Zinc-deficient	10	19·7	±1·8	6	18·5	±1·2
Deficient + 100 μgm. zinc per day	14	37·2	±2·6	12	25·7	±1·4
Fox chow	13	38·7	±1·7	10	23·9	±0·7
EDTA injection						
Fox chow ; zinc-deficient for 3 days	4	29·7	±2·9	4	18·1	±3·1

Controls are mean values from unpublished experiments.

Purina Fox Chow (75 μgm. zinc per gm.) during the daily 6-hr. feeding period and were kept in the metabolism cages, with water but no food, for the 18-hr. collection period. Two groups of four rats each were used. Control samples were collected for three days previous to the injection of ethylenediamine-tetra-acetic acid. Results of urine zinc analyses by the method of Vallee and Gibson[5] are given in Table 1.

Intraperitoneal injection of the calcium disodium salt greatly increased urinary excretion of zinc. Very little zinc is normally excreted in the urine, and the range of values observed in experiment 2 was 1·6–5·9 μgm. zinc per 18 hr. Zinc mobilization occurred immediately after injection of ethylenediamine-tetra-acetic acid, and from the one determination for the final 6-hr. period in experiment 1, it seems that little excess zinc was excreted after the 18 hr. following injection.

After the last collection period, the following tissues from experiment 1 were analysed for zinc : stomach, ileum, pancreas, kidney, testis, epididymis, dorsolateral prostate, bone (tibia) and blood. All tissues except ileum and pancreas gave results within the normal range for rats fed on Purina Fox Chow—the diet of these rats before the experiment began. Ileum zinc approached that found in rats maintained for 5–8 weeks on the zinc-deficient diet (Table 2), while pancreas zinc in three out of four cases was equal to that found in such zinc-deficient rats. The reduced zinc concentration in pancreas and ileum was not unexpected, since zinc excretion normally occurs via the pancreas[6] and alimentary canal. The reduced intake and increased urinary excretion would decrease the need for this excretion route and thus lower the concentration of transient zinc.

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