PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 240–244 (1980)

Short-Term Zinc Deficiency and Hemostasis in the Rat (40755)[1]

P. R. GORDON AND B. L. O'DELL

Department of Biochemistry, University of Missouri, Columbia 65211

Zinc deficiency in animals results in diverse pathological signs including retarded growth, alopecia, dermatitis, skeletal anomalies, loss of appetite, and reproductive failure (1). The pregnant zinc-deficient rat exhibits prolonged difficult parturition associated with depressed body temperature, low blood pressure, and excessive blood loss (2, 3). Tail wounds in zinc-depleted postparous females also result in greater blood loss than normal (3). Pregnant rats treated near term with aspirin exhibit signs highly analogous to those observed in zinc deficiency (3). Aspirin and other prostaglandin inhibitors also give rise to a bleeding tendency in rats. (4, 5).

Some signs of zinc deficiency occur shortly after removal of zinc from the diet. A dramatic decrease in plasma zinc concentration occurs in rats within hours after they consume a low-zinc diet (6) and food intake is decreased within 4 days or less (7). Abnormalities occur in preimplantation rat embryos after only 4 days of low-zinc intake by the dam (8). These pathological signs of zinc deficiency appear to be associated with low levels of extracellular zinc because they occur before there is a general decrease in the concentration of soft tissue zinc (6, 9, 10). In fact many tissues, such as liver, muscle, and brain show little change in zinc concentration even after induction of severe pathology.

The purpose of this study was to determine whether or not zinc deficiency causes a bleeding tendency in young male rats as it does in the reproducing females, and, if so, to determine if the pathology is associated with the extracellular zinc concentration. The rate of appearance of the defect and of its reversal were used to ascertain the compartment of zinc responsible.

Materials and methods. Immature male rats, weighing 100±20 g, were produced in the departmental colony of Wistar-derived albino rats. They were housed in an environmentally controlled room on a 12-hr light–dark cycle and had access to deionized water and food *ad libitum*. The composition of the basal diet is given in Table I. This diet contained 0.5±0.1 ppm zinc, and the control diet was the basal supplemented with 100 ppm zinc, added as $ZnCO_3$. Zinc was determined by atomic absorption spectrophotometry after dry ashing the samples.

Saline bleeding time was determined by the method of Copley and Lalich (13). Rats were anesthetized with sodium pentobarbital (50 mg/kg body wt.) and the tip of the tail transected 2 mm from tip by use of a sharp scalpel. The tail was then submerged in saline at 37° and allowed to bleed freely. Bleeding ceased and resumed several times and the accumulative bleeding time was recorded. Bleeding was considered stopped when it did not resume for 1 min. Blood loss during this period was measured gravimetrically.

Whole blood clotting time was determined by drawing blood from a tail wound into a 10-μl glass capillary tube. The tube was broken at 15-sec intervals and the endpoint was the first observation of fibrous strands. To evaluate the status of specific coagulation factors, prothrombin time, and activated partial thromboplastin time were determined by the use of commercial kits.[2] For this purpose blood was collected in 3.8% sodium citrate, using one part citrate to nine parts of blood.

For zinc determinations blood was collected by cardiac puncture in a heparinized plastic syringe. Plasma was prepared immediately by centrifugation and diluted 1:4 with distilled water. Its zinc content was determined by use of a Perkin–Elmer

[1] Contribution of the Missouri Agricultural Experiment Station, Journal Series No. 8394. Supported in part by Public Health Service Grant HL 11614 and the Graduate Nutrition Area.

[2] General Diagnostics, Morris Plains, N.J.

0037-9727/80/020240-05$01.00/0

TABLE I. COMPOSITION OF BASAL DIET

Ingredient	Percentage
Soybean protein (EDTA)[a]	20.0
DL-Methionine	0.2
Glucose hydrate	66.7
Corn oil	5.0
Mineral premix[b]	5.0
$CaCO_3$	1.0
Vitamin premix[c]	2.0
Choline Cl	0.1

[a] Promine, Central Soya, Chicago, Illinois. The protein was extracted twice with 0.5% ethylenediaminetetraacetate and reprecipitated twice to remove EDTA. (11).

[b] The mineral premix (12) supplied, as percentage of diet, Ca, 0.74; P. O., 57; K, 0.40; Na, 0.14; Cl, 0.22; Mg, 0.07 and, as ppm, Fe, 50; Mn, 80; Cu, 8.0; Cr, 2.0; I, 0.3; Se, 0.1.

[c] The vitamin premix supplies as mg/kg, thiamin–HCl, 8; riboflavin, 8; pyridoxine–HCl, 8; niacin, 16; Ca-pantothenate, 20; biotin, 0.2; folacin, 2; cyanocobalamin, 0.05; menadione, 1; retinyl acetate, 1.7 (5000 IU of A); cholecalciferol, 0.025 (1000 IU of D); α-tocopherol, 45 (50 IU of E).

Model 303 atomic absorption spectrophotometer (14).

Experiment 1. A group of 54 rats was fed the control diet for 2 days after which one-half received the low-zinc basal diet. Bleeding time and blood loss measurements were made on rats from each group 2, 4, 6, and 8 days later.

Experiment 2. A group of 16 rats was fed the control diet for 2 days and then fasted for 48 hr. One-half was then fed the control and one-half the basal diet for 28 hr. They were again fasted for 8 hr and measurements made.

Experiment 3. The conditions for this experiment were similar to those of experiment 1 except that all measurements were made 7 days after feeding the basal diet. On Day 7 the rats were given an intragastric dose (100 μg/kg body wt.) of zinc as zinc acetate and measurement made 2, 4, 6, and 8 hr later. The effect of a lower dose (10 μg/kg) of zinc was also determined after 4 hr.

Experiment 4. This experiment was designed to evaluate the effect of low dietary zinc on blood coagulation. Groups of rats were fed as in experiment 1 and blood collected 7 days after feeding the basal diet.

Results. The results of experiment 1, summarized in Fig. 1, show that young male rats fed a low-zinc diet exhibit a bleeding tendency analogous to that previously observed in postparous females (3). The defect occurred within a short period of time after the animals consumed the zinc-deficient diet. Plasma zinc was decreased approximately 50% ($P < 0.01$) within 2 days and continued to decrease slowly through Day 4 (Fig. 1A). Both bleeding time (Fig. 1B) and blood loss (Fig. 1C) increased as the plasma zinc concentration decreased, bleeding time reaching statistical significance ($P < 0.05$) on Day 4 and blood loss on Day 6.

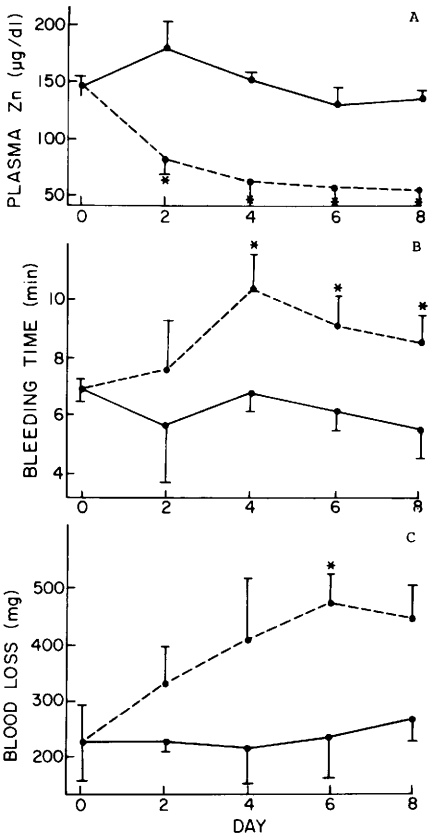

FIG. 1. Relationship of plasma zinc concentration (A), bleeding time (B), and blood loss (C). Rats were fed the low-zinc basal diet (O– –O) or the control diet (O——O) for approximately 1 week and four to six measurements made at each point. Bars indicate the standard error of the mean (SEM) and asterisks, statistical significance at $P < 0.05$.

In view of the rapid induction of bleeding tendency indicated by the first experiment, experiment 2 was designed to test an even shorter period of feeding the low-zinc diet. The results are presented in Table II. Under the conditions of this experiment, 48 hr of fast followed by 28 hr of *ad libitum* food consumption, the bleeding time of the control group was less than the usual *ad libitum*-fed control value. Nevertheless, the value for the low-zinc group was significantly higher than that of the control ($P < 0.01$). Plasma zinc concentration was also depressed by the short-term zinc deprivation.

Experiment 3 was designed to determine the rate of reversal of the hemostatic disorder by zinc repletion and thus to gain insight as to the zinc compartment involved in the mechanism. The results, depicted in Fig. 2, show that a gavage of zinc increased the plasma zinc concentration within 2 hr. The effect was more dramatic in deficient rats than in controls. The concentrations in both groups dropped after 2 hr and reached a level near the initial control value by 8 hr. The bleeding time of the originally deficient group had returned to normal by 4 hr, but the value for the control group was greatly prolonged at that time (Fig. 2B). The deficient group maintained normal bleeding times throughout the 8-hr experiment and the controls decreased toward the normal value. Blood loss followed the same trend as bleeding time, the basal group being depressed below normal at 2 hr and both returning to normal at 6 and 8 hr (Fig. 2C). The effect of a smaller (10 μg/kg) intragastric dose of zinc is shown in Table III. After 4 hr this dose raised the plasma zinc of the deficient group to 88 μg/dl and restored the bleeding time and blood loss to normal values. It had no significant effect on plasma zinc or bleeding tendency in the control group fed 100 ppm zinc.

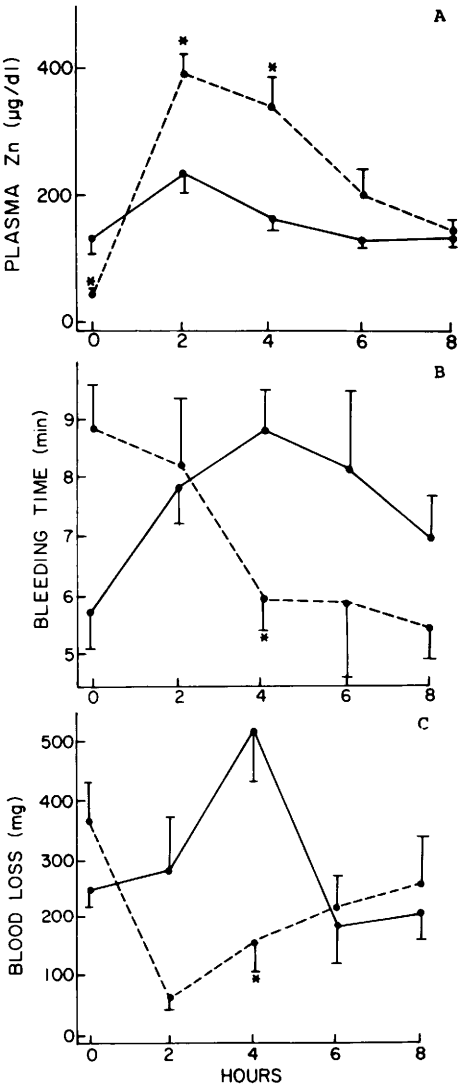

FIG. 2. Rate of reversal of bleeding tendency by oral zinc. Rats were fed the basal (O– –O) or control (O——O) diet for 7 days and then given an intragastric dose (100 μg/kg) of zinc. There were four to six measurements per point and bars indicate the SEM. The zero hour values are the 8-day value from experiment 1.

TABLE II. EFFECT OF 1-DAY CONSUMPTION OF A LOW-ZINC DIET ON BLEEDING TIME[a]

Dietary zinc (ppm)	Plasma zinc (μg/dl)	Bleeding time (min)
0	74 ± 7.5 (5)[b]	6.4 ± 0.4 (9)
100	130 ± 4.4 (7)	4.6 ± 0.4 (7)
$P<$	0.001	0.01

[a] Rats fasted 48 hr, refed 28 hr, and fasted 8 hr before sampling.
[b] Number of samples.

The results of experiment 4, designed to determine whether or not a coagulation defect is responsible for the bleeding ten-

TABLE III. REVERSAL OF BLEEDING TENDENCY 4 hr AFTER AN INTRAGASTRIC DOSE OF ZINC

Dietary Zn supplement (ppm)	Intragastric dose (μg/kg)	Plasma zinc (μg/dl)	Bleeding time (min)	Blood loss (mg)
0[a]	0	60 ± 7	8.8 ± 0.9	389 ± 62
0[b]	10	88 ± 7	6.0 ± 1.0	182 ± 91
100[a]	0	137 ± 6	5.8 ± 0.8	264 ± 33
100[b]	10	145 ± 10	6.8 ± 0.7	183 ± 68

[a] These values from Day 8 of experiment 1 are included for direct comparison. All values are means ± SEM.
[b] Six animals fed the basal diet for 7 days and six fed the control diet were given an oral dose (10 μg/kg) of zinc and measurements made after 4 hr.

dency, revealed no significant differences. Rats fed the basal and control diets had mean clotting times of 3.3±0 and 3.3±0.1 min (n=4), prothrombin times of 12.5±0.4 and 12.7±0.4 sec (n=5), and activated partial thromboplastin times of 18.4±1 and 17.5±0.3 sec (n=4), respectively.

Discussion. In confirmation of the observations of Dreosti *et al.* (6), plasma zinc concentrations decreased sharply and rapidly after consumption of the low-zinc diet. It is also significant that an oral dose of zinc elevated plasma zinc within 2 hr and that the magnitude of the increase was greater in rats previously fed the zinc-deficient diet. The latter observation was to be expected in as much as zinc-deficient animals absorb zinc more efficiently than controls (15). The magnitude of the response was not expected and the data suggest that intracellular zinc was released as result of the intragastric dose or zinc was not promptly removed from the plasma space. When the plasma zinc concentration dropped, an increased bleeding time and blood loss followed shortly. Restoration of the plasma zinc levels in deficient animals eliminated the bleeding tendency within 4 hr.

These results show that the bleeding tendency in zinc deficiency is not unique to the female rat during parturition. It occurs in immature male rats and relates closely to the level of plasma zinc concentration. Thus, the bleeding tendency is a sign analogous to loss of appetite in that it develops rapidly and is easily and promptly reversed. The fact that this pathology correlates closely with plasma zinc and responds rapidly to therapy suggests that it is a function of the extracellular rather than intracellular zinc concentration.

The mechanism by which zinc deficiency causes increased bleeding time and blood loss is not known but it does not appear to be a defect in coagulation. Zinc deficiency in the reproducing female rat results in low blood pressure. This would be consistent with a dilation of the capillary beds, but preliminary experiments, not reported here, show no evidence of vascular malfunction in the cremaster muscle of zinc-deficient male rats. Extracellular zinc concentration could affect platelet aggregation. Chvapil *et al.* (16) observed that *in vitro* addition of zinc to washed dog platelets at a level above the normal plasma concentration inhibits aggregation. This effect of excess zinc may explain the prolonged bleeding time observed in control rats given the high dose of intragastric zinc in this study. An abnormally low level of plasma zinc may also impair platelet aggregation so that for normal hemostasis the tolerance for plasma zinc concentration is narrow and critical.

Summary. Immature male rats fed a soybean protein-based diet containing less than 1 ppm of zinc, showed a 50% reduction of plasma zinc within 2 days and an increased saline bleeding time within 4 days. Administration of a single dose (10 μg/kg body wt.) of zinc intragastrically restored bleeding time to normal within 4 hr and this was associated with increased plasma zinc concentration. A larger dose (100 μg/kg) also restored the bleeding time of deficient rats to normal, but increased the bleeding time of controls fed adequate zinc. This pharmacological effect in controls was largely

dissipated after 8 hr but the repleted animals maintained the normal bleeding time during this period. Thus, consumption of a low-zinc diet rapidly induces a bleeding tendency which is closely related to plasma zinc concentration. It is postulated that extracellular zinc plays a significant role in hemostasis.

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Received September 5, 1979. P.S.E.B.M. 1980, Vol. 163.