Experimental zinc deficiency in guinea-pigs: clinical signs and some haematological studies

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(*Received 9 January 1985 – Accepted 7 March 1985*)

1. Thirty-three male, weanling guinea-pigs were divided into two groups. One group was fed on a zinc-deficient (ZnD) diet (1·25 mg Zn/kg diet) for 45 d and then divided into two subgroups: subgroup 1 continued on diet ZnD while subgroup 2 was fed on a Zn-repleted diet (100 mg Zn/kg diet) for 15 d. The second group was fed on a Zn-adequate diet (50 mg Zn/kg diet) for 60 d.

2. Zn deficiency could be produced within 21 d as evidenced by clinical signs and reduction in serum Zn concentration.

3. Clinical signs exhibited were listlessness, scabby lesions on skin near the foot pads, mild alopecia and a reduction in body-weight gain. No effect was observed on food intake.

4. Significant decreases in packed cell volume and total leucocyte count from 48 d onwards accompanied by absolute lymphocytopenia and relative neutrophilia were observed.

5. Supplementation of Zn in the Zn-repleted group resulted in marked, though incomplete, improvement with regard to serum Zn concentration, clinical signs and haematological changes.

Zinc has been shown to be an essential element for mammals and plays an indispensable role in a number of body functions. Natural cases of its deficiency in man and domestic animals have been encountered throughout the world (Underwood, 1977). McBean *et al.* (1972) and others (Alberts *et al.* 1977; Hsieh & Navia, 1980; Gordon & O'Dell, 1983) have reported clinical signs of Zn deficiency in guinea-pigs experimentally fed on a Zn-deficient diet up to 20 d. The only report (Gordon & O'Dell, 1983) traced in the literature regarding haematological observations in Zn-deficient guinea-pigs revealed no effect on packed cell volume (PCV). The present work was, therefore, undertaken to produce Zn deficiency in guinea-pigs and to study the clinical signs and some haematological indices. An attempt was also made to examine the response to Zn repletion with respect to the previously mentioned indices.

MATERIALS AND METHODS

Thirty-three male albino guinea-pigs (20-d-old) of an English breed, weighing about 170–275 g were obtained from the Disease-Free Small Animal House, Haryana Agricultural University, Hissar and divided into two main groups containing nineteen and fourteen animals with mean body-weights of 215·6 (SE 7·18) and 204·83 (SE 3·91) g respectively. These were fed *ad lib.* on a semi-synthetic basal diet (Hsieh & Navia, 1980). The diet contained egg albumin powder, sucrose, starch, potassium acetate, agar, salt mixture, cellulose, choline chloride, magnesium oxide, vitamin mixture and arachis oil. Based on the high requirement of vitamin C by guinea-pigs, ascorbic acid was added at the rate of 2 g/kg diet, as reported by Navia & Lopez (1973). To avoid loss of nutrients during storage, particularly ascorbic acid and other vitamins, the diets were prepared in different lots at fortnightly intervals and kept under refrigeration in closed plastic containers as suggested by Navia & Lopez (1973).

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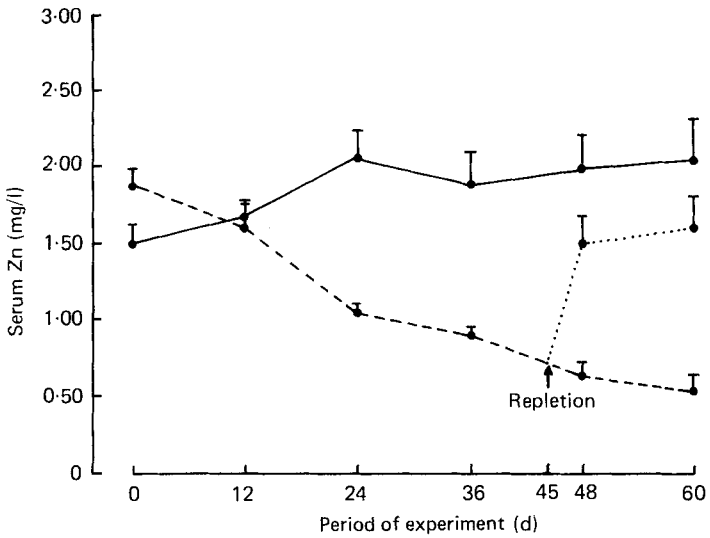

Fig. 1. Serum zinc concentration (mg/l) of Zn-deficient (---), Zn-replete (. . .) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars.

Animals of one group were fed on a Zn-deficient (ZnD) diet containing 1·25 (SE 0·12) mg Zn/kg for 45 d and then divided into two subgroups: animals of subgroup 1 (ten animals) were maintained on diet ZnD, while animals of subgroup 2 (nine animals) were fed on a Zn-repleted (ZnR) diet (100 mg Zn/kg) for 15 d. All fourteen animals of the second group were fed on a Zn-adequate control diet (50 mg Zn/kg). All animals were kept individually in plastic cages and provided with double-distilled water *ad lib.* To avoid coprophagy, perforated acrylic sheets were fixed at the bottom of cages. Just before the start of the experiment, the cages, feeding dishes and water bottles were rinsed in EDTA solution (10 g/l; Zn-chelating agent) and then thoroughly washed with double-distilled water.

The animals were closely observed daily for clinical signs and weighed at weekly intervals up to the end of the experiment (60 d). Blood samples were taken from the heart at the start of the experiment and subsequently at 12-d intervals, and placed separately in vials containing EDTA (10 g/l) and in sterilized tubes for serum separation. For differential leucocyte counts (DLC), smears were prepared from fresh blood and stained with Giemsa's stain.

Serum Zn concentration was estimated by atomic absorption spectrophotometry. PCV and total leucocyte counts (TLC) were determined using a microhaematocrit and improved Neubaur haemocytometer methods respectively (Schalm *et al.* 1975).

The statistical significance was assessed using Student's *t* test.

RESULTS

Serum Zn concentration

Mean serum Zn concentrations of each group are illustrated in Fig. 1.

A statistically significant decrease ($P < 0·01$) was observed from 24 d onwards in serum Zn levels of the group given diet ZnD as compared with the control group. The group given diet ZnR showed a rapid increase in serum Zn concentration within 3 d of repletion. Later, the rate of increase in Zn concentraion appeared to be very moderate.

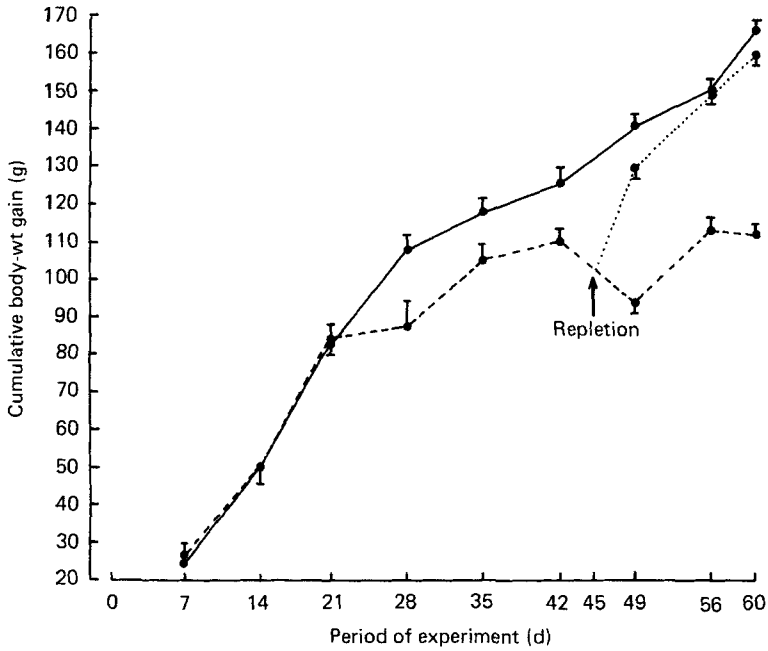

Fig. 2. Body-weight gain (g) of Zn-deficient (---), Zn-replete (. . .) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars.

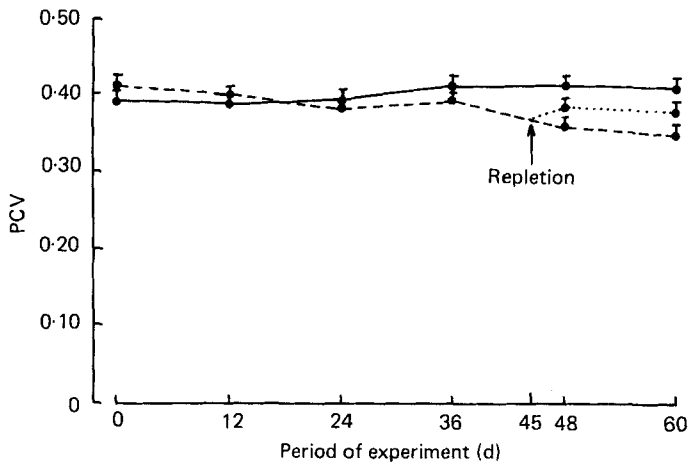

Fig. 3. Effect of zinc on packed cell volume (PCV) of Zn-deficient (---), Zn-replete (. . .) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars.

Clinical signs, growth and feeding response

In guinea-pigs given diet ZnD, no clinical sign was observed up to the 2nd week of the experiment. During the 3rd week, the animals appeared listless and disinclined to move. Subsequently, scabby lesions appeared on the skin near the foot pads; there was mild alopecia and the animals were seen to lick the cages. None of the guinea-pigs in the control group showed any untoward clinical sign.

Average body-weight gains of each experimental group are shown in Fig. 2. A

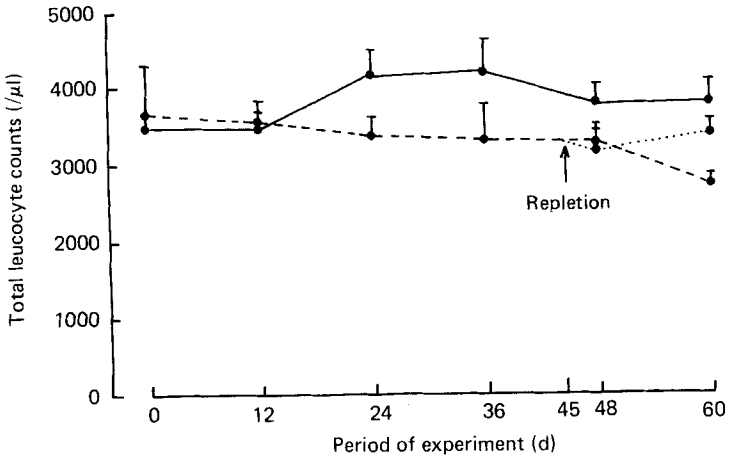

Fig. 4. Effect of zinc on total leucocyte counts of Zn-deficient (---), Zn-replete (. . .), and control (—) guinea-pigs. Values are means with their standard errors represented by vertical bars.

statistically-significant decrease ($P < 0{\cdot}05$) in body-weight gain in group ZnD as compared with the control group was observed from week 4 onwards. This difference was highly significant ($P < 0{\cdot}01$) from week 6 of the experiment. At the end of the experiment, the average body-weight gain (g) of group ZnD was 112·8 (SE 2·00) while that of the control group was 167·00 (SE 2·24).

No significant difference in food consumption in the different groups of guinea-pigs was observed during the experiment. Average food intake was about 15–25 g/d per guinea-pig.

Following 15 d of Zn supplementation, guinea-pigs given diet ZnR showed a marked improvement in clinical signs and body-weight gain.

Haematological observations

PCV. The mean PCV values for each of the experimental groups are shown in Fig. 3. The decrease in PCV in group ZnD as compared with the control group was statistically significant ($P < 0{\cdot}01$) from day 48 onwards. Following 15 d of Zn supplementation, an increase in mean PCV values was observed in group ZnR when compared with values for group ZnD.

TLC. Mean TLC values for each experimental group are shown in Fig. 4. The mean TLC of group ZnD decreased within 24 d of the experiment as compared with the controls but the difference was statistically significant ($P < 0{\cdot}01$) on day 60 only. Following 15 d of Zn supplementaion, the mean TLC in group ZnR (3400 (SE 167·93)/μl) was higher than that of group ZnD (2710 (SE 90·82)/μl) but was still less than that of the control group (3779 (SE 319·00)/μl).

DLC and absolute leucocyte count. Mean DLC values for each experimental group are given in Table 1 and absolute values of lymphocytes and neutrophils, calculated on the basis of TLC and DLC, are shown in Fig. 5.

In group ZnD, a continuous increase in mean percentage neutrophils and a decrease in mean percentage lymphocytes was observed from day 24 onwards. A statistically significant difference ($P < 0{\cdot}05$) between the groups, however, was found from day 36 onwards.

From Fig. 5, it is clear that there was a continuous decrease in absolute lymphocyte counts of group ZnD from the start of the experiment, except on day 48 when it was relatively higher. Absolute neutrophil counts in group ZnD were similar at different stages of the experiment except on days 36 and 48 when values were relatively higher. In group ZnR

Table 1. *Differential leucocyte count (per 100 cells) of zinc-deficient, Zn-repleted and control guinea-pigs*†

(Mean values with their standard errors, no. of animals in parentheses)

		Differential leucocyte count (per 100 cells)											
Period of experiment (d) ...		0		12		24		36		48		60	
Group	Leucocytes	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	Neutrophils	28·67	2·15	27·57	2·05	31·42	1·37	32·08	1·86	32·54	2·64	30·17	3·10
	Lymphocytes	70·33	2·25	71·07	2·73	68·67	2·73	66·67	1·68	66·36	2·80	69·33	3·21
	Monocytes	0·50	0·06	0·57	2·00	0·25	1·25	0·66	0·30	0·54	0·10	0·33	0·23
	Eosinophils	0·50	0·70	0·60	0·05	0·58	0·05	0·58	0·20	0·54	0·38	0·16	0·25
	Basophils	0·00	—	0·07	0·05	0·00	—	0·00	—	0·00	—	0·00	—
		(14)		(14)		(13)		(12)		(12)		(12)	
Zn-deficient	Neutrophils	27·80	1·52	26·27	2·14	32·63	2·34	49·00*	2·70	43·87*	2·89	42·23*	3·49
	Lymphocytes	70·93	2·50	72·33	0·98	65·63	2·44	50·01*	2·69	55·25*	2·79	55·80*	3·34
	Monocytes	0·60	0·05	0·72	1·20	1·42	0·32	0·65	0·17	0·50	0·28	0·40	0·27
	Eosinophils	0·67	0·15	0·67	0·03	0·31	0·15	1·23	0·27	0·37	0·19	0·40	0·27
	Basophils	0·00	—	0·00	—	0·00	—	0·11	0·20	0·00	—	0·00	—
		(19)		(19)		(19)		(19)		(10)		(10)	
Zn-replete‡	Neutrophils	—		—		—		—		32·22	1·90	32·00	1·09
	Lymphocytes	—		—		—		—		66·78	1·88	62·00	1·05
	Monocytes	—		—		—		—		0·33	0·17	0·50	0·37
	Eosinophils	—		—		—		—		0·67	0·30	0·50	0·27
	Basophils	—		—		—		—		0·00		0·00	
										(9)		(9)	

Mean value significantly different from that of the control value: $*P < 0·05$.

† For details of treatments, see p. 421.

‡ Repleted after 45 d of depletion.

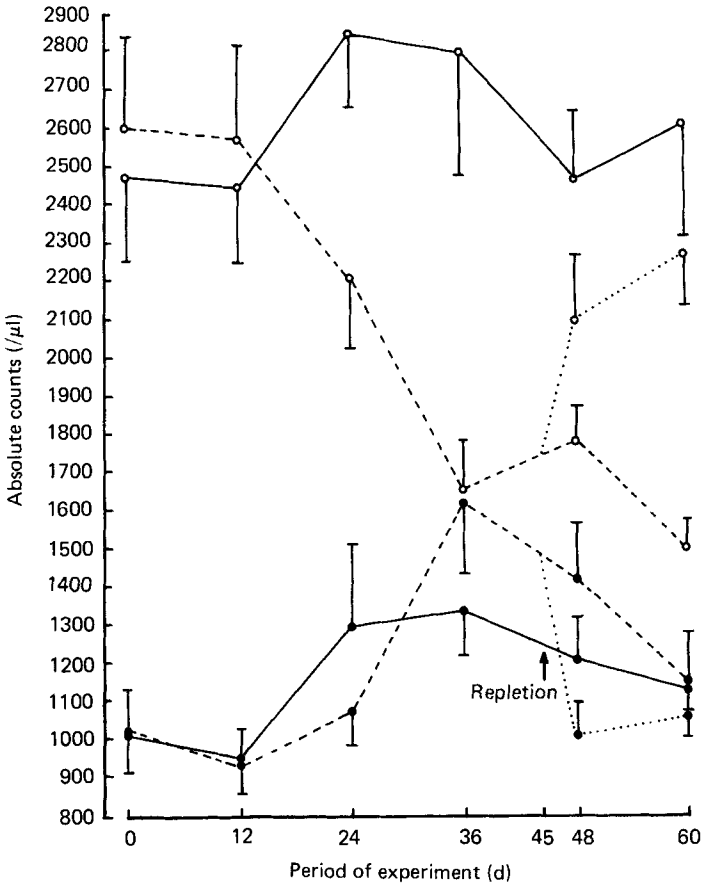

Fig. 5. Effect of zinc on absolute lymphocytes (○) and neutrophils (●) of Zn-deficient (---), Zn-replete (. . .) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars.

a marked improvement in percentage and absolute neutrophils counts and lymphocytes values was noticed within 3 d of repletion. There was no significant difference in monocyte, eosinophil and basophil counts between the three groups (ZnD, ZnR and controls) throughout the experiment.

DISCUSSION

Zn deficiency could be produced experimentally within 21 d in the guinea-pigs given diet ZnD, as evidenced by clinical signs and the reduction in the serum Zn level. Clinical signs observed included listlessness, scabby lesions on the skin near foot pads, mild alopecia and licking habits. McBean *et al.* (1972), Alberts *et al.* (1977) and Gordon & O'Dell (1983) also reported similar signs in guinea-pigs within about 20 d of feeding them with the Zn-deficient diet.

A reduction in body-weight gain in group ZnD was observed from week 4 onwards. McBean *et al.* (1972) did not find any significant reduction in weight gains in guinea-pigs given a Zn-deficient diet containing 1·2 mg Zn/kg diet. Hsieh & Navia (1980), however, reported a reduction in body-weight gain in Zn-deficient guinea-pigs after 2 weeks on the

experimental diet. This could be explained by their use of a diet containing less than 1 mg Zn/kg diet whereas, in the present study, the level of Zn in the diet was 1·25 mg/kg. Growth retardation due to Zn-deficiency might be due to a decrease in the activity of thymidine kinase (*EC* 2.7.1.21) (Prasad & Oberleas, 1974) or DNA nucleotidyltransferase (*EC* 2.7.7.7) (Smith & Gawthorne, 1975). Zn is known to play an important role in cell division directly, especially in the G1 phase; Zn deficiency may thus lead to decreased growth (Riordan, 1976).

No significant difference in food consumption in the present study was observed in guinea-pigs of the various experimental groups. More or less similar findings were reported by McBean *et al.* (1972) who found that food consumption of Zn-deficient guinea-pigs was identical to that of the controls over the 20 d of their experiment. Gordon & O'Dell (1983) reported that guinea-pigs weighing about 350 g when given a Zn-deficient diet did not show any reduction in food consumption, whereas guinea-pigs weighing less than 200 g showed a reduction in food intake but, on the basis of metabolic body-weight, it was neither significantly nor consistently depressed.

A significant decrease in PCV in group ZnD was evident from 48 d onwards. One of the possible causes for the decrease in PCV as reported by many other workers (Grant & Root, 1952; Gordon *et al.* 1968; Benjamin, 1978) may be testicular atrophy and degeneration, a consistent feature of Zn deficiency (Prasad & Oberleas, 1976; Mason *et al.* 1982). Splenomegaly, as observed in Zn deficiency by Prasad (1980) in human patients and by Gupta (1984) in guinea-pigs, may also have attributed to this reduction since sequestration of erythrocytes in an enlarged spleen results in a fall in PCV (Hess *et al.* 1976). Hypothyroidism as observed in the Zn-deficient guinea-pigs (Gupta, 1984) may also have played a role in the reduction in PCV values, since Morley *et al.* (1980) reported that in rats, Zn deficiency resulted in decreased levels of triiodothyronine and thyroxine, the hormones responsible for decreased PCV (Grant & Root, 1952; Tudhope & Wilson, 1969; Benjamin, 1978). Gordon & O'Dell (1983), during 18-d experimental studies in guinea-pigs, reported that Zn deficiency had no effect on PCV.

TLC in the present study decreased in group ZnD within 24 d of the experiment but the decrease was statistically significant ($P < 0{\cdot}01$) on day 60 only. A significant increase in percentage neutrophils and decrease in percentage lymphocyte counts in group ZnD were evident from day 36 onwards. With regard to their absolute counts, a decrease in lymphocyte count was observed only for group ZnD. The absolute neutrophil counts were similar throughout the experiment except on days 36 and 48 when the values were relatively higher. Since there was a decrease in absolute lymphocyte counts and almost no change in absolute neutrophil counts, it could be inferred that the decrease in TLC found in group ZnD was mainly due to the decrease in the absolute lymphocyte count. One of the reasons for lymphocytopenia observed in the present study may be hyperadrenocorticism. Depasquale-Jardieu & Fraker (1979) reported that Zn deficiency caused an increase in plasma corticosterone level which in turn destroyed thymic lymphocytes.The splenomegaly observed (Gupta, 1984) might have also resulted in some degree of lymphocytopenia. Lymphocytopenia due to Zn deficiency has also been reported in rats (Macapinlac *et al.* 1966).

In group ZnR, the serum Zn concentration increased rapidly within 3 d of repletion. Alleviation in clinical signs, partial resumption in body-weight gain and marked improvement in PCV and TLC were noticed. Complete recovery in percentage and absolute values of neutrophils and lymphocytes was noticed after 3 d of repletion. Our findings relating to Zn repletion, with respect to clinical manifestations, are similar to those of Quarterman & Humphries (1983), who also reported rapid healing of all skin lesions and resumption of growth within 8 d of Zn supplementation (20 mg Zn/kg diet) to Zn-deficient guinea-pigs.

R.P.G. acknowledges the award of Junior Research Fellowship by the Indian Council of Agricultural Research, New Delhi.

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Printed in Great Britain