Effect of experimental zinc deficiency and repletion on sodium, potassium, copper and iron concentrations in guinea-pigs

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Zinc, sodium, potassium, copper and iron concentrations were analysed in serum and tissues of guinea-pigs fed on a diet containing 1·25 mg Zn/kg diet over a period of 60 d. The response of the Zn-deficient (ZnD) animals to Zn supplementation (100 mg Zn/kg diet) was also studied for 15 d. Serum studies in the ZnD group revealed significant decreases in the concentrations of Zn and Na from 24 d, and increases in the concentrations of Fe and K from 36 and 48 d onwards respectively; an increase in Cu was seen on day 60 only. Zn deficiency caused significant reductions in Na, K and Zn and increases in Cu and Fe contents of liver and kidney. In testis, significant decreases were noted only in Zn, K and Fe contents. Zn supplementation of the previously ZnD group resulted in marked improvements in serum and tissue mineral levels. However, hepatic Cu and Fe and renal K did not appear to respond appreciably.

Minerals: Zinc deficiency: Guinea-pigs

Zinc deficiency in man and domestic animals has been reported throughout the world. There have been a number of studies on Zn deficiency in laboratory animals, but limited information is available on the interaction of Zn with other elements during Zn deficiency ((US) National Research Council, 1979). Most of the studies conducted on Zn interactions are related to high dietary Zn levels with copper and iron only. Recently, an interaction of Zn with sodium and potassium in the brain of Zn-deficient (ZnD) rats has been described (Wallwork *et al.* 1983). The object of the present investigation was, therefore, to study the serum and tissue levels of Na, K, Cu and Fe in experimental ZnD and Zn-repleted (ZnR) guinea-pigs.

MATERIALS AND METHODS

Experimental studies on Zn deficiency were conducted using two groups of individually housed, male albino guinea-pigs, aged 21 d. The first group (nineteen animals) received a ZnD diet (1·25 mg Zn/kg) and the second group (fourteen animals) a diet adequate in Zn (50 mg Zn/kg). The first group was divided into two subgroups, one of ten animals which received the ZnD diet throughout and another of nine animals which received a ZnR diet (100 mg Zn/kg) after 45 d. Details of the animals used and treatments given have already been described (Gupta *et al.* 1985). The results presented here were obtained from the same animals.

Blood samples were taken from the heart at the start of the experiment, and thereafter at 12-d intervals, and placed in sterilized tubes for serum separation. On day 60, all animals were killed and the liver, kidney and testis were removed, weighed and stored at −20° until required for analysis. Zn, Cu and Fe concentrations in serum and tissues were determined by atomic absorption spectrophotometry after wet ashing with a perchloric–nitric acid mixture (Horwitz, 1965), and Na and K by flame photometry (Wootton, 1974).

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The results of serum analysis were subjected to a two-way analysis of variance, variation being apportioned to treatment and time intervals, and those of tissues to one-way analysis of variance for treatments (Snedecor & Cochran, 1967). Individual means were compared for statistical significance using least significant difference.

RESULTS

Food intake

Mean food intakes for the animals in each experimental group are shown in Fig. 1. No significant difference was observed in the values in any of the groups.

Clinical signs

Appearance of clinical signs in guinea-pigs given a ZnD diet has been reported earlier (Gupta *et al.* 1985).

Serum studies

Zn concentration. Mean serum Zn concentrations of each group are given in Fig. 2. An overall significant decrease ($P < 0{\cdot}01$) in serum Zn concentration was observed in the ZnD group compared with the control group. The interaction between treatments and time intervals was found to be significant ($P < 0{\cdot}01$). The significant difference between the groups was observed from day 24 onwards. The group given the ZnR diet showed a rapid increase in serum Zn concentration within 3 d of repletion.

Na concentration. Mean serum Na concentrations for each group are given in Fig. 3(*a*). Overall, serum Na concentration of the ZnD group was significantly ($P < 0{\cdot}01$) lower than that of the control group. The interaction between treatment and time intervals was significantly different ($P < 0{\cdot}05$) from day 24. The Na concentration in the ZnR group returned almost to that of the control group within 3 d of Zn repletion.

K concentration. Mean serum K concentrations for each group are presented in Fig. 3(*b*). There was an overall significant ($P < 0{\cdot}01$) increase in K concentration in the ZnD group compared with the control group. Interaction between treatment and time intervals was significant ($P < 0{\cdot}01$) from day 24 onwards. Following Zn repletion for 15 d, almost complete recovery was noticed in the serum K values of the ZnR group compared with values for the ZnD and control groups.

Cu concentration. Mean serum Cu concentrations for each group are given in Fig. 3(*c*). Though there was a significant ($P < 0{\cdot}05$) increase overall in the serum Cu concentration in the ZnD group compared with the control group, the interaction with time interval was not significant. However, when mean values at different time intervals were compared, a significant increase ($P < 0{\cdot}05$) in Cu level of the ZnD group was observed on day 60 only. The guinea-pigs of the ZnR group showed a marked recovery in Cu levels when compared with the corresponding control value on day 15 of Zn repletion.

Fe concentration. Mean Fe concentrations for each experimental group are shown in Fig. 3(*d*). A significant increase ($P < 0{\cdot}01$) in Fe concentration was observed in the ZnD group compared with the control group from day 36 onwards. Following 15 d of Zn repletion, guinea-pigs exhibited a marked change in serum Fe concentration.

Tissue studies

The average weights of liver, kidney and testis along with the mean values of Zn, Na, K, Cu and Fe are given in Table 1. While a significant difference ($P < 0{\cdot}01$) among the experimental groups was observed only in the absolute weight of testis, there was no significant difference in weights of the tissues when expressed as a proportion of the body-weight.

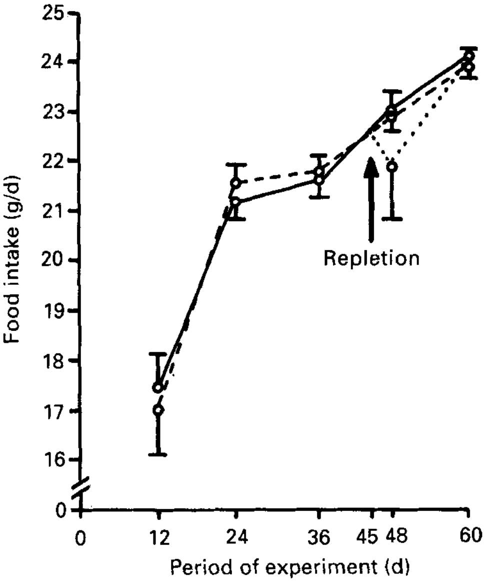

Fig. 1. Feed intake of zinc-deficient (---), Zn-repleted (...) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* (1985).

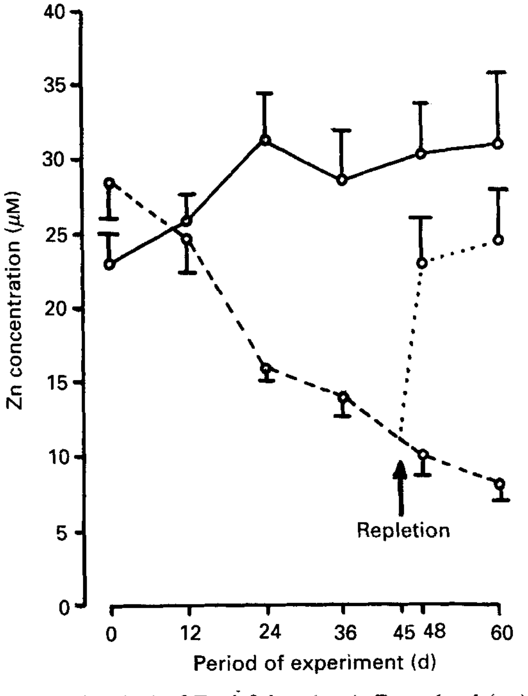

Fig. 2. Serum zinc concentration (μM) of Zn-deficient (---), Zn-repleted (...) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* (1985).

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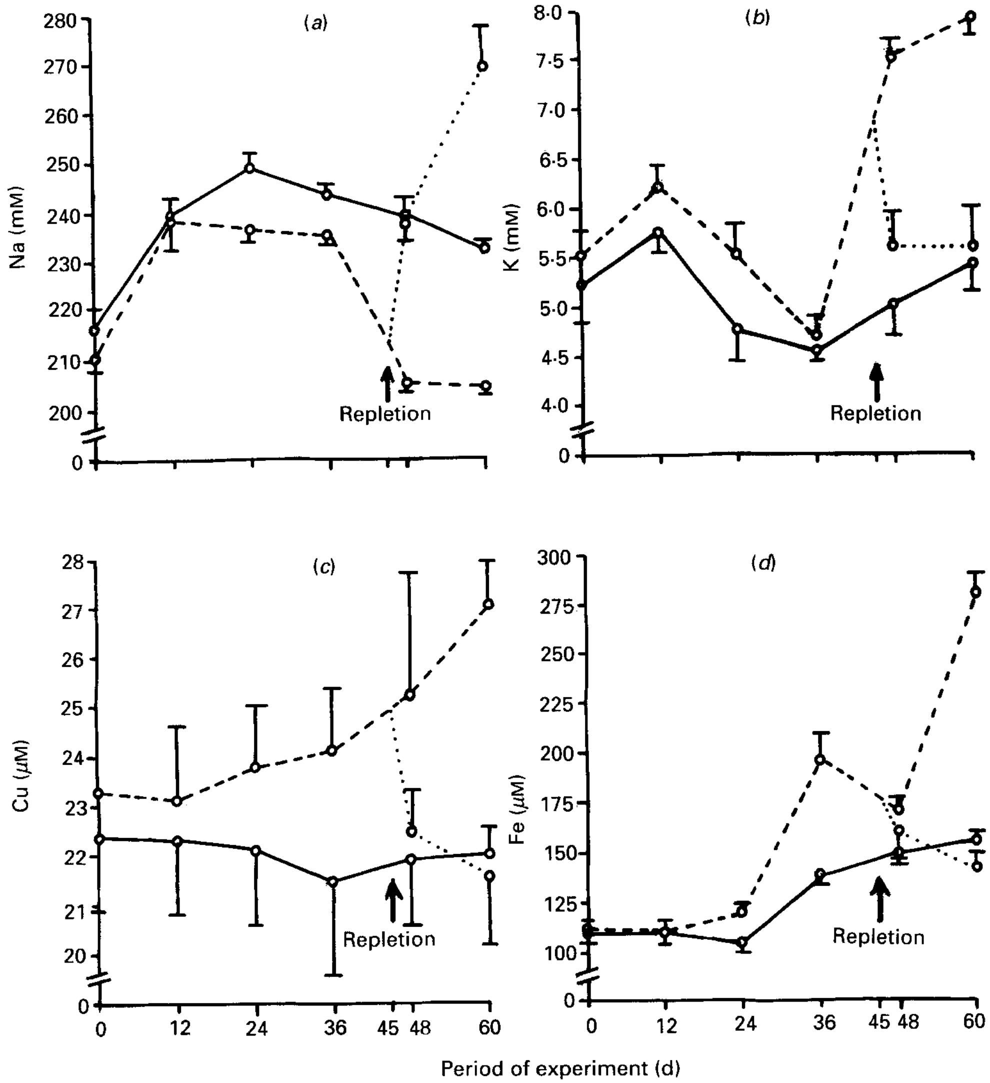

Fig. 3. Serum concentrations of sodium (*a*), potassium (*b*), copper (*c*) and iron (*d*) in zinc-deficient (– – –), Zn-repleted (...) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* (1985).

Significant decreases in Na concentrations of liver and kidney were observed in the ZnD group when compared with the controls. In the ZnR group, a marked improvement in Na concentration was noticed within 15 d of repletion. Significantly ($P < 0.01$) lowered values of K contents were observed only in kidney and testis of the ZnD group when compared with the control group. The guinea-pigs of the ZnR group showed a rapid increase in the K content of testis only following Zn repletion. Although the Cu contents in liver, kidney and testis of the ZnD group were higher than those of the control group, the differences were significant ($P < 0.01$) only for the liver and kidney. After 15 d of Zn repletion, decreases in Cu content of these tissues were observed but Cu concentration in liver was still significantly higher ($P < 0.01$) in comparison with that of the controls. Hepatic and renal Fe concentrations in the ZnD group were found to have increased ($P < 0.01$), whereas

Table 1. *Average organ weights (g) and sodium and potassium (mg/g fresh tissue), zinc, copper and iron (mg/kg fresh tissue) concentrations of Zn-deficient (ZnD), Zn-repleted* (ZnR) and control guinea-pigs† killed at day 60 of the experiment*

(Mean values for no. of animals shown in parentheses)

	Liver				Kidney				Testis			
Dietary groups...	Control (12)	ZnD (10)	ZnR (9)	Pooled SE	Control (12)	ZnD (10)	ZnR (9)	Pooled SE	Control (12)	ZnD (10)	ZnR (9)	Pooled SE
Absolute organ wt	4·09^{a}	3·96^{a}	3·84^{a}	0·41	0·80^{a}	0·83^{a}	0·82^{a}	0·10	0·61^{a}	0·50^{b}	0·52^{b}	0·07
Relative organ wt (per kg body-wt)	11·1^{a}	11·9^{a}	11·0^{a}	1·3	2·2^{a}	2·5^{a}	2·3^{a}	0·5	1·7^{a}	1·5^{a}	1·5^{a}	0·3
Na	2·78^{a}	1·66^{b}	2·72^{a}	0·80	7·51^{a}	5·19^{b}	8·44^{a}	2·59	3·38^{a}	2·60^{a}	4·46^{a}	1·66
K	5·96^{a}	5·34^{a}	5·52^{a}	1·33	11·56^{a}	5·34^{b}	6·16^{b}	1·97	9·82^{a}	6·13^{b}	10·06^{a}	2·45
Zn	17·35^{a}	9·43^{b}	15·53^{a}	5·87	20·00^{a}	10·17^{b}	18·05^{a}	4·68	27·36^{a}	9·46^{b}	18·98^{c}	8·03
Cu	3·24^{a}	8·61^{b}	6·17^{b}	2·35	3·22^{a}	6·65^{b}	3·33^{a}	1·97	1·33^{a}	1·50^{a}	1·40^{a}	0·36
Fe	337·50^{a}	611·51^{b}	405·13^{c}	65·57	94·16^{a}	177·77^{b}	96·79^{a}	32·62	128·99^{a}	19·09^{b}	130·43^{a}	33·94

^{a,b,c} In each row means with unlike superscript letters were significantly different: a *v.* c $P < 0{\cdot}05$; b *v.* a or c $P < 0{\cdot}01$.

* Repleted on 45th day of depletion.

† For details of treatment, see Gupta *et al.* (1985).

in the testis Fe concentration decreased significantly ($P < 0.01$). The differences in Fe values of the kidney and testis between the ZnR and control groups were not significant, but hepatic Fe concentration in the ZnR group was still higher, although at a lower level of significance ($P < 0.05$), indicating slight improvement.

DISCUSSION

The functional roles (growth, bone formation, brain development, reproduction, immune mechanism, membrane stability and wound healing) of Zn are fairly well understood (Nariagu, 1980), but its interaction with other elements when in the deficient state is not well-documented. Anorexia is generally accepted as inevitable during Zn deficiency and pair-feeding with Zn-adequate controls is considered desirable. However, in the present study food intakes by the animals in different groups were almost identical. Similarly, Mc Bean *et al.* (1972) and Gordon & O'Dell (1983) did not notice any effect of Zn deficiency on food intake in guinea-pigs. Thus the results reported in the present study suggest that the primary cause of the alterations noticed in the minerals is Zn deficiency *per se* unaccompanied by reduced food intake.

There was a significant decrease in the serum Na concentration of the ZnD group from day 24 onwards. A significant decrease in Na concentration was also noticed in liver and kidney. The decrease in serum Na concentrations was parallel to the serum Zn levels which were also significantly lower from day 24 onwards. The only report (Wallwork *et al.* 1983) traced in the literature also revealed a decrease in Na concentration in the brain of ZnD rats. The mechanism involved in Na depletion during experimental Zn deficiency has to be investigated. However, medullary hyperplasia and atrophy of zona glomerulosa as observed in the adrenal glands of ZnD guinea-pigs (Gupta *et al.* 1988) and angiotensin II deficiency (Reeves & O'Dell, 1986) might have contributed to hyponatraemia, since these changes have been reported to impair Na re-absorption from renal tubules due to impairment of synthesis and secretion of mineral corticoids (Duncan *et al.* 1951; Forbes, 1962; Jubb *et al.* 1985).

Serum K concentration in the ZnD group increased significantly from day 48 onwards. This was accompanied by a decrease in its concentration in kidney and testis. Widdowson & Dickerson (1964) reported a decrease in the K content of the testis during its atrophy and degeneration, a consistent feature of Zn deficiency. Moreover, an increase in catecholamine secretion (Wallwork *et al.* 1982) or adrenal medullary hyperplasia (Gupta *et al.* 1988) observed during Zn deficiency might have caused liberation of K from tissues (D'Silva, 1937; Todd & Vick, 1971; Kaufman & Papper, 1983) into the plasma, thus leading to a fall in the K level of tissues. This release of intracellular K into the circulation might be one of the important factors in the hyperkalaemia observed in ZnD guinea-pigs. Atrophy of the zona glomerulosa of the adrenal cortex, as observed in ZnD guinea-pigs (Gupta *et al.* 1988), might also have contributed to hyperkalaemia since hypoaldosteronism has been reported to impair renal tubular K secretion resulting in a rise in serum K levels (Kaufman & Papper, 1983; Jubb *et al.* 1985).

Serum Cu in the present study increased in the ZnD group within 24 d of the experiment, but the increase was statistically significant ($P < 0.05$) on day 60 only. Concurrently, there were appreciable increases in the Cu contents of liver and kidney. A similar inverse interrelation between Zn and Cu has also been reported in rats (Murthy *et al.* 1974; Burch *et al.* 1975; Roth & Kirchgessner, 1977; Kirchgessner *et al.* 1979). This may be due to an increase in Cu absorption (Schwarz & Kirchgessner, 1974) or Cu-binding proteins (Bremner & Marshall, 1974*a*, *b*), or a decrease in faecal and urinary Cu excretion (Gandhi, 1982). Moreover, Speckhard *et al.* (1977) reported the replacement of intrinsic Zn ions of

E. coli RNA polymerase with Cu in vivo in a low-Zn medium without alteration in cell morphology, growth rate and yield. Hypothyroidism and thyroid atrophy, as observed in Zn deficiency by Morley *et al.* (1980) in rats and Gupta *et al.* (1988) in guinea-pigs, might also have contributed to the increase in hepatic and serum Cu contents since Gubler *et al.* (1952) and Evans *et al.* (1970) reported that in rats, thyroid insufficiency resulted in accumulation of hepatic Cu because of decreased biliary Cu excretion, a major pathway for hepatic Cu removal.

The significant increase in serum, liver and kidney Fe concentration observed in the ZnD group in the present study has also been documented in rats (Roth & Kirchgessner, 1977; Reinstein *et al.* 1984). The mechanism underlying the effect of Zn on Fe metabolism during Zn deficiency is not known. O'Nell-Cutting *et al.* (1981) reported that the mechanism by which high levels of dietary Zn resulted in depletion of hepatic Fe stores remains unexplained, since they found no interference of Zn with either intestinal Fe absorption or with cellular uptake of Fe from circulating transferrin and storage as ferritin.

Zn repletion to previously ZnD guinea-pigs resulted in a rapid improvement in serum and tissue concentrations of the previously mentioned minerals within 15 d. However, hepatic Cu and Fe and renal K did not appear to respond appreciably.

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