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Experimental zinc deficiency in guinea-pigs: biochemical changes

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1. Zinc deficiency was produced experimentally in guinea-pigs fed on a diet containing 1·25 mg Zn/kg diet over a period of 60 d. In addition, the response of the Zn-deficient (ZnD) animals to Zn supplementation was studied for 15 d.

2. In the ZnD group a significant reduction was found in serum Zn and protein concentrations and in alkaline phosphatase (*EC* 3.1.3.1; AP) activity from day 24 onwards.

3. Paper electrophoretic studies on serum revealed a significant decrease in relative values, as well as absolute values, of albumin and γ-globulin and an increase in β-globulin.

4. Albumin:globulin increased on day 24 but decreased significantly from day 48 onwards.

5. The kidney and testis of the ZnD group showed a reduction in Zn and protein contents, and AP activity.

6. Zn supplementation of the previously ZnD group resulted in marked although incomplete improvement in the biochemical indices studied.

Zinc has been shown to be an essential element for mammals and plays an indispensable role in a number of body functions. Naturally occurring cases of Zn deficiency in man and domestic animals have been encountered throughout the world (Underwood, 1977). There have been a number of studies of the biochemical changes associated with Zn deficiency in laboratory animals but there have been no reports of quantitative estimations of serum protein fractions. Moreover, of the biochemical changes associated with experimentally-produced Zn deficiency in guinea-pigs, only alkaline phosphatase (*EC* 3.1.3.1; AP) activity has been studied (Alberts *et al.* 1977; Hsieh & Navia, 1980). The present work was, therefore, undertaken to study biochemical changes in experimentally produced Zn-deficient guinea-pigs. A study was also made of the response to Zn repletion of the previously-mentioned changes.

MATERIALS AND METHODS

Experimental studies on Zn deficiency were conducted using two groups of male albino guinea-pigs. The first (nineteen animals) received a Zn-deficient (ZnD) diet, the second (fourteen animals) a diet adequate in Zn (50 mg Zn/kg). The first group was divided into two sub-groups, one of ten animals which received the ZnD diet throughout and another of nine animals which received a Zn-repleted (ZnR) diet (100 mg Zn/kg) after45 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 subsequently 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 and stored at −20° until analysed. Serum and tissue samples were analysed for Zn by atomic absorption spectrophotometry and for AP activity by the Sigma method (Coles, 1967). Total protein content in the serum was estimated by the Biuret method (Wootton, 1974) and in tissues by the method of Lowry *et al.* (1951), and serum protein fractions by a paper electrophoretic technique (Huisman, 1963).

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Table 1. *Feed intake (g/d) of zinc-deficient, Zn-repleted and control guinea-pigs**

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

	Feed intake (g/d)									
Period of experiment (d) ...	12		24		36		48		60	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	17·44 (14)	0·74	21·22 (13)	0·36	21·64 (12)	0·33	23·05 (12)	0·33	24·07 (12)	0·12
Zn deficient	17·01 (19)	1·23	21·70 (19)	0·33	21·84 (19)	0·27	22·97 (10)	0·40	23·94 (10)	0·19
Zn repleted†	—		—		—		21·85 (9)	1·07	24·08 (9)	0·14

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

† Repletion after 45 d of depletion.

RESULTS

Feed intake

Mean feed intakes for the animals in each experimental group are given in Table 1. There was no significant difference in values for each group.

Serum studies

Zn concentration. Values for serum Zn concentration for each experimental group have been published (Gupta *et al.* 1985).

Protein concentration. Mean total serum protein concentrations for each group are given in Fig. 1. Total serum protein levels of the ZnD group were significantly lower ($P < 0·05$) than those of the control group from day 24 onwards. Total serum proteins in the ZnR group increased rapidly within 3 d of Zn repletion and almost reached control values by day 15 of repletion.

Protein fractions. Mean percentage contents of individual serum proteins (albumin and α-, β- and γ-globulins) for each of the experimental groups are shown in Table 2 and absolute values are shown in Fig. 2. In the ZnD group, there were statistically significant decreases ($P < 0·01$) in γ-globulin and albumin contents and increases ($P < 0·05$) in α- and β-globulin contents when these were compared on a percentage basis with control values. There was a decrease in absolute values of albumin and γ-globulin in the ZnD group from day 24 onwards and an increase in β-globulin from day 36 (Fig. 2). There was no apparent difference in absolute values for α-globulin between the ZnD and control groups throughout the experiment. The ZnR guinea-pigs showed a marked recovery in absolute values for individual serum proteins when compared with the corresponding control values.

Albumin:globulin (A:G). The decrease in A:G in the ZnD group on day 12 was not significant but there was a significant increase on day 24 ($P < 0·05$) when compared with the control value (Fig. 3). The subsequent decrease in A:G in the ZnD group was significant ($P < 0·01$) from day 48 onwards. After 15 d of Zn repletion, there was an almost complete recovery in serum A:G in the ZnR group when compared with values for the ZnD and control groups.

AP activity. Mean serum AP activities for each group are given in Table 3. There was a significant decrease ($P < 0·05$) in the serum AP activity in the ZnD group when compared

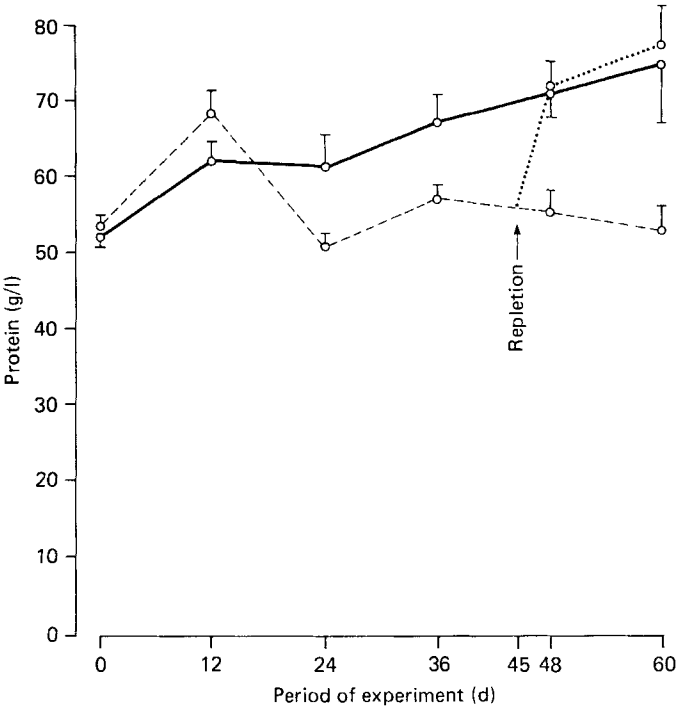

Fig. 1. Total serum protein concentration (g/l) of zinc-deficient (– – –), Zn-repleted (· · ·) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* (1985).

with the control group from day 24 onwards. After 15 d of Zn repletion, there was a considerable recovery in serum AP activity.

Tissue studies

Values for Zn and protein concentrations and AP activities of kidney, testis and liver for each experimental group are given in Table 4. There was a significant decrease ($P < 0{\cdot}01$) in the Zn contents of kidney, testis and liver from the ZnD group compared with the control group. There was a recovery in the mean Zn concentration after 15 d of Zn supplementation. Although kidney, testis and liver protein contents for the ZnD group were lower than those for the control group, the difference was significant ($P < 0{\cdot}01$) only for the kidney and testis. After 15 d of Zn repletion, a recovery in protein content was observed only in the kidney. There was a decrease in AP activities of the kidney and testis but the difference was significant ($P < 0{\cdot}01$) only for kidney. AP activities of the kidney and testis in the ZnR group were lower than those of the control group but higher than the corresponding values for the ZnD group, indicating restoration of AP activity.

DISCUSSION

In the present study, feed intakes for the different groups were almost identical. There was a significant decrease in the serum Zn concentration of the ZnD group from day 24 onwards and a significant decrease in the Zn contents of the kidney, testis and liver. Kirchgessner & Pallauf (1972) reported that in rats the Zn contents of various tissues were related to

Table 2. *Mean percentage composition of serum protein fractions of zinc-deficient, Zn-repleted and control guinea-pigs*†

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

Protein fractions	Period of experiment (d)											
	0		12		24		36		48		60	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
						Control group						
Albumin	68·70	1·53	69·16	1·91	67·51	2·04	69·67	1·54	68·13	1·18	64·44	1·09
Globulin:												
α	15·66	2·67	15·90	1·07	17·35	1·00	16·68	0·61	15·82	0·63	18·10	1·35
β	6·44	0·73	5.94	0·31	6·49	0·74	6·10	0·73	7·57	0·65	8·26	0·76
γ	9·20	0·86	9·00	0·95	8·65	1·20	7·55	0·92	8·48	0·50	9·20	0·74
	(14)		(14)		(13)		(12)		(12)		(12)	
						Zn-deficient group						
Albumin	67·41	0·94	65·82	1·53	72·02	1·18	68·29	1·16	61·33**	0·89	59·98**	0·57
Globulin:												
α	16·51	0·44	16·43	0·51	16·79	0·77	17·90	1·16	22·98*	0·52	23·00*	0·72
β	7·83	0·38	9·68	0·87	6·87	0·45	9·96*	0·61	13·90**	0·67	13·26**	0·57
γ	8·25	0·63	8·07	0·24	4·32**	0·52	3·85**	0·60	3·79**	0·37	3·76**	0·82
	(19)		(19)		(19)		(19)		(10)		(10)	
						Zn-repleted group‡						
Albumin	—	—	—	–	—	—	—	—	67·46	0·35	64·58	1·92
Globulin:												
α	—	—	—	—	—	—	—	—	18·86*	1·08	20·56	1·22
β	—	—	—	—	—	—	—	—	8·81	0·91	10·27	1·32
γ	—	—	—	—	—	—	—	—	4·87**	0·84	4·59*	1·58
									(9)		(9)	

Mean values were significantly different from those of the control group: * $P < 0·05$, ** $P < 0·01$.

† For details of treatments, see Gupta *et al.* 1985.

‡ Repletion after 45 d of depletion.

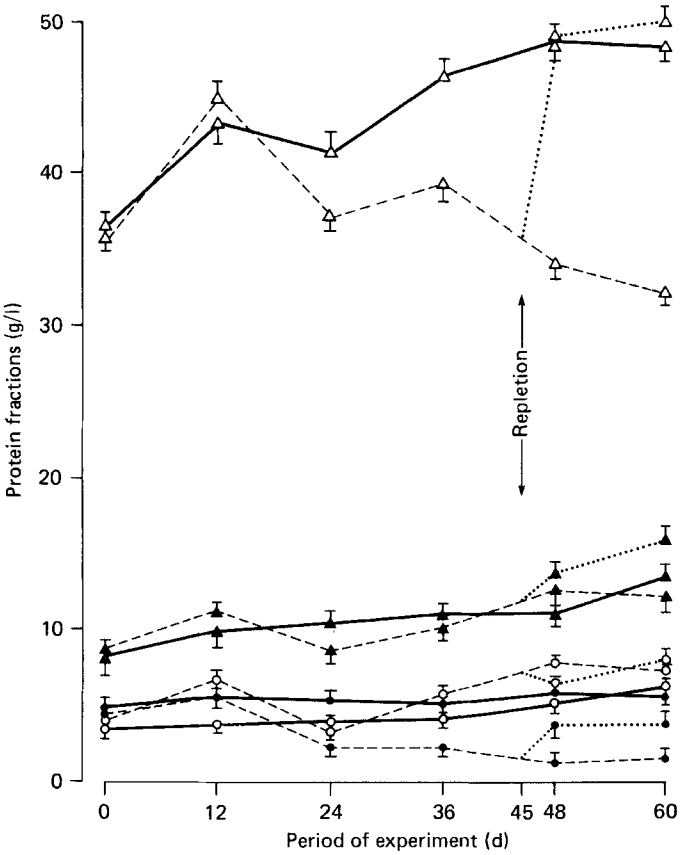

Fig. 2. Serum protein fractions (g/l): albumin (△), α-globulin (▲), β-globulin (○) and γ-globulin (●) of zinc-deficient (---), Zn-repleted (···) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* (1985).

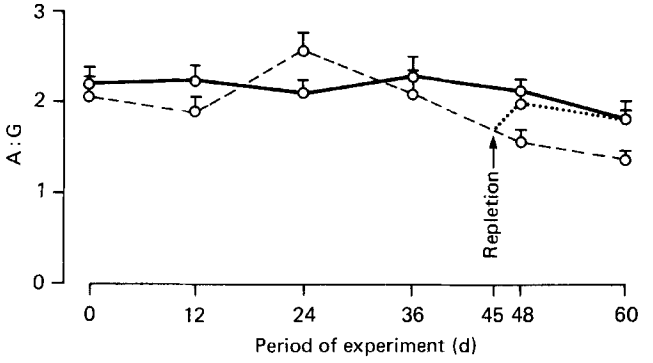

Fig. 3. Serum albumin: globulin of zinc-deficient (---), Zn-repleted (···) and control (——) guinea-pigs. Values are means with their standard errors represented by vertical bars. For details of treatments, see Gupta *et al.* 1985.

Table 3. *Serum alkaline phosphatase* (EC *3.1.3.1*) *activity* (*Sigma units/ml*) *of zinc-deficient, Zn-repleted and control guinea-pigs*†

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

	Period of experiment (d)											
	0		12		24		36		48		60	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	9·34 (14)	0·40	10·51 (14)	0·50	11·46 (13)	0·90	8·76 (12)	0·62	7·51 (12)	0·60	5·82 (12)	0·50
Zn deficient	10·07 (19)	0·28	10·74 (19)	0·68	8·40* (19)	0·40	6·68* (19)	0·54	4·87* (10)	0·88	3·38* (10)	0·62
Zn repleted‡	—	—	—	—	—	—	—	—	6·13 (9)	0·53	4·41 (9)	0·87

Mean values were significantly different from those of the control group: * $P < 0·05$.
† For details of treatments, see Gupta *et al.* (1985).
‡ Repletion after 45 d of depletion.

Table 4. *Mean zinc* (*mg/kg*) *and protein* (*mg/g*) *concentrations and alkaline phosphatase* (EC *3.1.3.1; AP*) *activity* (*Sigma units/g*) *of Zn-deficient, Zn-repleted and control guinea-pigs*† *killed after 60 d of experiment*

(Mean values with their standard errors)

		Kidney						Testis						Liver					
		Zinc		Protein		AP activity		Zinc		Protein		AP activity		Zinc		Protein		AP activity	
Group	No. of animals	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	12	20·00	2·57	79·29	1·80	181·00	24·26	27·36	3·92	51·44	1·25	183.97	18·88	17·35	2·24	70·59	4·07	3·72	0·50
Zn deficient	10	10·17**	0·95	67·02**	1·96	93·40**	10·22	9·46**	0·78	41·59**	1·48	128·40	15·93	9·43**	0·65	64·12	1·86	3·68	0·50
Zn repleted‡	9	18·05	2·93	75·69	2·06	135·57	14·53	18·98	2·75	41·71**	1·50	133·83	41·21	15·53	1·60	68·20	1·96	4·48	0·79

Mean values were significantly different from those of the control group: ** $P < 0·01$.
† For details of treatments, see Gupta *et al.* (1985).
‡ Repletion after 45 d of depletion.

the dietary Zn level. Moreover, only certain tissues showed a significant decrease in their Zn content during Zn deficiency, because of the difference in binding affinities of tissue protein for Zn (Prasad & Oberleas, 1976). Our results are similar to those reported by McBean *et al.* (1972) and Quarterman & Humphries (1983) for guinea-pigs.

A significant decrease in total serum protein in the ZnD group when compared with the control group was observed from day 24 onwards. Although the kidney, testis and liver of the ZnD group had lower protein contents than the control group, the difference was significant ($P < 0{\cdot}01$) only for the kidney and testis. Paper electrophoretic studies on the serum revealed a significant decrease in the percentage levels of γ-globulin and albumin from days 24 and 48 onwards respectively and an increase in α- and β-globulins from days 36 and 48 onwards respectively. With regard to their absolute values, an appreciable decrease in albumin and γ-globulin was observed from day 24, with an increase in β-globulin from day 36 onwards. Values for total serum protein content and absolute values for individual serum proteins indicate a decrease in total serum protein for the ZnD group, due mainly to hypoalbuminaemia and hypo-γ-globulinaemia. Hypoalbuminaemia and hypo-γ-globulinaemia observed in the present study may have been associated with hyperadrenocorticism (Luetscher, 1947; Lewis, 1950), a condition associated with Zn deficiency (Quarterman, 1972; Gupta, 1984). The increase in β-globulin may be attributed to hypothyroidism (Lewis, 1950). The lower protein values observed in the present study may be due to impaired protein synthesis since Zn deficiency impairs the utilization of amino acids in protein synthesis (Hsu *et al.* 1969, 1970). A decrease in total protein content has been reported in plasma (Tao & Hurley, 1971) and various tissues (Macapinlac *et al.* 1966; Sandstead *et al.* 1971) of Zn-deficient rats. Tao & Hurley (1971) suggested that changes in plasma protein might be due either to impairment of protein synthesis or an increase in protein breakdown.

Serum A:G in the ZnD group was increased significantly on day 24 and decreased thereafter; a statistically significant decrease was obtained on days 48 and 60. The increased A:G on day 24 may be due to a decrease in γ-globulin. The subsequent decrease in A:G may be attributed to hypoalbuminaemia and an increase in α- and β-globulin levels.

There was a significant decrease in serum AP activity in the ZnD group from day 24 onwards. In kidney and testis too there was an appreciable decrease in AP activity. The decrease in AP activity may be due to the fact that Zn is essential for the catalytic function and structural stability of the enzyme (Simpson & Vallee, 1968). Roth & Kirchgessner (1974) reported decreased plasma AP activity in Zn-deficient rats even before any sign of lowered food intake or reduced rate of growth was observed. From this they concluded that the loss in AP activity was directly attributed to Zn deficiency. A decrease in AP activity in the plasma and tissue of Zn-deficient guinea-pigs has been reported by Alberts *et al.* (1977) and Hsieh & Navia (1980). Similar observations have been reported in rats (Prasad & Oberleas, 1971; Adeniyi & Heaton, 1980).

In the ZnR group, serum Zn concentration showed a rapid increase within 3 d of repletion. The Zn contents of the kidney, testis and liver increased also but did not reach the levels of the control group. Although the protein concentrations of the serum, kidney and liver showed marked recovery the testis did not appear to respond appreciably. All serum protein fractions showed marked recovery in their values. Roth & Kirchgessner (1974) reported that AP activity returned almost to the level of that in control rats within 3 d of Zn repletion.

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