Increased retention of orally administered zinc and raised blood cell zinc concentrations in iron-deficient rats

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1. Rats were made severely iron-deficient by feeding with Fe-deficient diets, which contained adequate amounts of zinc.

2. Ten days after an oral dose of 250 μg ^{65}Zn the Fe-deficient rats had retained 17 % whereas control rats had retained only 8 % of the dose.

3. When unlabelled Zn was given intravenously to the Fe-deficient and control rats there was a reduction in retention of the orally administered ^{65}Zn. This effect was not produced when Fe was given before the oral dose of ^{65}Zn.

4. Total body Zn, whole blood Zn and plasma Zn concentrations of the rats were measured by atomic absorption spectrophotometry. Both the Fe-deficient rats and the control rats had the same total body Zn calculated as a proportion of body-weight. The plasma Zn concentrations were normal in all the animals. Blood cell Zn concentrations in the Fe-deficient animals were raised.

5. The blood cell Zn concentration was directly related to the reticulocyte count in all the rats.

6. It was concluded that Fe and Zn are absorbed by different metabolic pathways, and that there is an increased turnover of Zn in the Fe-deficient rats, possibly related to the short life-span and increased Zn concentration of Fe-deficient rat erythrocytes. In the rat abnormalities of Zn concentration can only be considered significant if Fe status is also known.

A group of young men with retarded growth and poor sexual development was described by Prasad and his colleagues (Prasad, 1966; Sandstead, Prasad, Schulert, Farid, Miale, Bassilly & Darby, 1967). The men had low concentrations of plasma Zn and alkaline phosphatase and cleared injected ^{65}Zn rapidly from the blood. Consequently they were thought to be Zn-deficient. But well developed young men living in the same area had these abnormalities of Zn metabolism (Coble, VanReen, Schulert, Koshakji, Farid & Davis, 1966). As iron deficiency was prevalent in this area, Coble *et al.* postulated that the changes in Zn metabolism might be due to Fe deficiency. However, this was not investigated, and the effects of Fe therapy were not studied.

For this reason, rats with Fe deficiency anaemia were studied to see if Fe deficiency alone could produce increased Zn turnover and whether Zn and Fe shared a common metabolic pathway in the intestine. When Fe-deficient rats were found to have high levels of blood cell Zn, further investigations were made to study the cause of this finding.

EXPERIMENTAL

Animals. Female weanling albino Wistar strain rats were obtained from the Ministry of Defence, Allington Farm, Porton Down, Salisbury. They were housed in Perspex

cages with aluminium floors and tops, with up to ten rats per cage. In the second series of experiments litter-mate pairs were used, one of each pair being made Fe-deficient and the other acting as a control. Animals were 3–4 months old and had been fed on their special diets for at least 2 months, before dosing with Zn.

Diets. The diets were made up and given as described by McCall, Newman, O'Brien, Valberg & Witts (1962). The diets consisted of spray-dried skim milk, sucrose, salt mixture, choline dihydrogen citrate, lard with arachis oil and vitamins. This diet contained only 1–3 mg Fe/kg and the animals developed a severe Fe-deficiency anaemia in 2 months. The control diet had added ferric ammonium citrate, 240 mg/kg diet; the animals fed on this diet developed normally and had no evidence of Fe deficiency (Table 2). The Zn content of both diets was found to be 29 mg/kg diet. Drinking water was glass-distilled and, with the food, was available *ad lib.*

Oral dosing with ^{65}Zn. An aqueous solution was made up from anhydrous $ZnCl_2$ and $^{65}ZnCl_2$ (The Radiochemical Centre, Amersham, Buckinghamshire) so that 0·2 ml contained 250 μg Zn and gave 100 disintegrations/sec when counted in a scintillation counter: 0·2 ml of this solution was given by gastric tube under light ether anaesthesia. Rats were not deprived of water or food as starvation has been found to decrease the absorption of ^{65}Zn (Orr, 1967).

Solutions for intravenous injections. The Zn solution contained 80 μg Zn/ml as $ZnCl_2$, in isotonic saline. The Fe solution contained 50 μg Fe/ml as ferric ammonium citrate in isotonic saline. Isotonic saline was used as the control. The volume given was 1 ml in each instance.

Counting and estimation of ^{65}Zn *retention.* Each rat was placed in a waxed carton and counted in a plastic phosphorwell scintillation counter (Warner & Oliver, 1962). The mean of two 100 sec counts was taken as the initial count, C_i, and after 10 days as the final count, C_f. Background counts, B_i and B_f, and ^{65}Zn standard counts, S_i and S_f were taken at the same times. The percentage of Zn retained at 10 days was calculated by the formula

$$100 \times \frac{C_f - B_f}{C_i - B_i} \times \frac{S_i - B_i}{S_f - B_f}.$$

Determination of Zn. Whole blood and plasma Zn concentrations were determined by atomic absorption spectrophotometry (Piper & Higgins, 1967). Blood cell Zn was calculated from the formula

$$\mu g \text{ Zn/ml packed cells} = \frac{1}{H}\left[(x-y)+\frac{Hy}{100}\right],$$

where $x = \mu$g Zn/100 ml whole blood, $y = \mu$g Zn/100 ml plasma and $H =$ haematocrit.

Whole body Zn was measured after acid digestion with nitric and sulphuric acids, by atomic absorption spectrophotometry. Blanks and standards were measured in the same way.

Haematological procedures. Blood was taken by aortic puncture for routine haematological procedures. Reticulocytes were counted after supravital staining with 0·6 %

(w/v) brilliant cresyl blue on a microscopic slide. Haematocrits were measured with a micro-haematocrit centrifuge.

Expt 1. Six Fe-deficient and seven control rats were given 250 μg ^{65}Zn in 0·2 ml water by stomach tube. The total body radioactivity of the rats was measured immediately after dosing and at 10 days, and ^{65}Zn retention was estimated.

Expt 2. The effect of three previous intravenous injections of 150 μg Fe, or 240 μg Zn or saline on the retention of 250 μg of orally administered ^{65}Zn was studied in sixteen Fe-deficient and eighteen control rats. Injections were made 21, 18 and 6 h before the oral dose of ^{65}Zn. The rats were then dosed with ^{65}Zn and counted as in Expt 1, and ^{65}Zn retention at 10 days was estimated. At the end of the experiment determinations of Zn contents and routine haematological investigations were made for these rats, and for six rats total body Zn was also measured.

RESULTS

Retention of oral ^{65}Zn by Fe-deficient and control rats. The Fe-deficient rats retained more than twice as much ^{65}Zn as the control rats ($P < 0.001$) (Table 1).

Table 1. *Percentage of an oral dose of ^{65}zinc retained at 10 days by iron-deficient and control rats, with weights and haematological measurements*

(Mean values and standard deviations: the number of animals in each group is given in parentheses)

	Fe-deficient group	Control group
^{65}Zn retained (%)	19·0 ± 3·4 (6)	7·8 ± 3·1 (7)
Rat weight (g)	132 ± 19	163 ± 25
Haemoglobin (g/100 ml)	5·9 ± 0·8	14·3 ± 0·4
Haematocrit (%)	24 ± 3·0	43 ± 1·4

Effect of intravenous Zn, Fe and saline on the retention of oral ^{65}Zn. As in the first experiment, there was a marked increase in the retention of ^{65}Zn by the Fe-deficient rats. Intravenous injection of Zn depressed the retention of orally administered ^{65}Zn in both control and Fe-deficient rats ($P < 0.001$) but intravenous injection of Fe and saline had no effect on the ^{65}Zn retention (Table 2).

Total body Zn and blood Zn. Despite the increased retention of ^{65}Zn by the Fe-deficient rats, total body Zn was similar in the Fe-deficient and normal rats (Table 2). Whole blood Zn concentrations in the Fe-deficient rats were 60% of the levels found in the control rats. The rats which had been given intravenous Fe had higher plasma Zn concentrations than the other rats. There was more Zn per ml packed blood cells in the Fe-deficient rats than in the control rats. The increased blood cell Zn concentrations (x) were found to correlate with the increased reticulocyte percentage (y) in the Fe-deficient rats and were related by the regression equation

$$y = 2.89\,x - 41.38 \quad \text{(Fig. 1)}.$$

Table 2. *Zinc retention after 10 days and blood characteristics in groups of Fe-deficient and control rats given either saline, Zn or Fe intravenously at 21, 18 and 6 h before an oral dose of ^{65}Zn*

Group	Zn retained (% of dose)*	Whole body Zn mg/rat	Whole body Zn mg/g rat	Blood Zn* μg/100 ml blood	Blood Zn* μg/100 ml plasma	Blood Zn* μg/ml packed cells (calculated)	Rat wt* (g)	Haemoglobin* (g/100 ml)	Haematocrit* (%)	Reticulocytes* (%)	Leucocyte count* (cells/mm³)
Saline:											
Fe-deficient	17·5 ±4·9 (4)	6·1 (2)	0·034 (2)	376 ±30·6 (4)	107 ±22 (4)	26·7 ±2·2 (4)	178 ±14 (4)	2·6 ±0·4 (4)	10·5 ±0·7 (4)	37·0 ±3·4 (4)	9870 ±3800 (4)
Control	8·8 ±0·3 (5)	7·4 (1)	0·036 (1)	711 ±66 (4)	103 ±24 (3)	14·4 ±1·7 (4)	231 ±25 (5)	15·7 ±1·3 (5)	44·0 ±2·0 (5)	8·4 ±4·4 (5)	8160 ±3900 (5)
Zinc:											
Fe-deficient	8·8 ±1·6 (6)	6·2 (2)	0·034 (2)	497 ±135 (6)	112 ±27 (6)	29·9 ±2·9 (6)	191 ±21 (6)	3·1 ±0·5 (6)	12·8 ±2·8 (6)	37 ±7·5 (6)	8150 ±2400 (6)
Control	4·1 ±2·6 (6)	7·2 (1)	0·030 (1)	814 ±126 (5)	127 ±23 (6)	16·8 ±2·5 (6)	240 ±19 (6)	15·1 ±0·4 (6)	44·0 ±2·1 (6)	5 ±1·9 (6)	6220 ±2200 (6)
Iron:											
Fe-deficient	13·7 ±2·8 (6)	—	—	637 ±153 (5)	150 ±15 (3)	33·3 ±7·3 (4)	165 ±23 (6)	3·7 ±0·7 (5)	14·0 ±3·4 (5)	71 ±20·0 (5)	5560 ±1700 (5)
Control	7·0 ±2·6 (7)	—	—	951 ±77 (7)	157 ±42 (6)	18·8 ±2·0 (7)	205 ±14 (7)	16·4 ±1·0 (7)	46·0 ±3·2 (7)	5 ±3·0 (7)	6930 ±1500 (7)

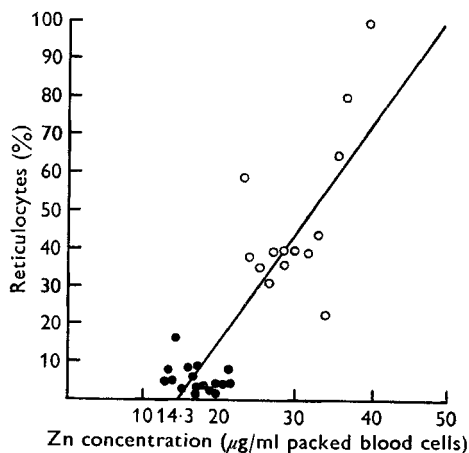* Mean values and standard deviations. Numbers of rats in each group are given in parentheses.

Fig. 1. Blood cell zinc concentrations and reticulocyte levels in normal rats (●——●) and in iron-deficient rats (○——○). There is a direct relationship.

DISCUSSION

The first finding was that Fe-deficient rats retain more ^{65}Zn than control rats. Care was taken to exclude Zn deficiency in the Fe-deficient rats as a possible cause for the increased ^{65}Zn retention (Furchner & Richmond, 1962), by showing that dietary Zn concentrations were normal, both by calculation and measurements on sample. Also, at the end of the second experiment, total body Zn was similar in rats in both the Fe-deficient and control groups. Vitamin D deficiency has also been reported by Becker & Hoekstra (1966) to produce increased Zn absorption in rats, but in our experiment both diets contained vitamin D in equal amounts from the same source.

A possible cause for the increased ^{65}Zn retention of Fe-deficient rats may be the rapid turnover of duodenal mucosal cells which is induced by Fe deficiency (Charlton, Jacobs, Torrance & Bothwell, 1965). Charlton *et al.* (1965) considered that the rapid turnover of absorptive cells could be the cause of the increased Fe absorption in Fe-deficient animals. This link between Zn and Fe absorption is strengthened by the finding that Zn, like Fe, is absorbed mainly in the duodenum of the rat (Van Campen & Mitchell, 1965), and the duodenal mucosa contains more Zn than other parts of the intestine (Sahagian, Harding-Barlow & Mitchell Perry, 1966).

The second finding was that intravenous Zn will decrease the retention of ^{65}Zn. This occurred both in Fe-deficient and control rats and is similar to the effect of intravenous Fe on ^{59}Fe retention found by Charlton *et al.* (1965), who considered that intravenous Fe may saturate absorptive duodenal cells and so prevent Fe uptake there. Intravenous Fe did not affect the retention of ^{65}Zn significantly, hence the absorption of Zn must involve at least one different step from the absorption of Fe.

The third finding was that Fe-deficient blood cells in the rat contain more Zn than normal and that this relates to the very high reticulocyte counts which are found in rats with Fe deficiency. This abnormality of blood cell Zn content has not been found in other species, except the rabbit, in which moderately raised Zn concentration in

red blood cells of macrocytic rabbits have been reported by Valberg, Card, Paulson & Szivek (1967). Fe-deficiency anaemia in the rat produces a very rapid turnover of some erythrocytes (McKee, Wasson & Heyssel, 1968) and this may play some part in the increased retention of Zn in Fe-deficient rats, despite normal total body concentrations. The plasma Zn concentrations were raised in the Fe-deficient and control rats which had been given Fe intravenously 10 days before. There is no simple explanation for this finding.

These results in the rat show that any analysis of Zn metabolism should be questioned if strict attention is not paid to Fe status. A change in Fe concentration in the diet alone of rats produced changes in Zn retention and distribution. If these findings are relevant to other species then considerable doubt should remain about assertions that abnormalities of Zn metabolism cause disorders in which Fe status is also abnormal. The men described by Sanstead *et al.* (1967) may have been showing features of Fe deficiency alone, and the findings of abnormal Zn metabolism may have been caused by the Fe deficiency which has been shown here in the rat to affect Zn metabolism secondarily. From this study in rats, it is clear that Fe and Zn metabolism are closely related and that, when abnormalities of Zn metabolism are found, Fe status must also be taken into account.

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