

Zinc absorption in the rat

BY M. J. JACKSON, D. A. JONES AND R. H. T. EDWARDS

*Department of Human Metabolism, University College London School of Medicine,
University Street, London WC1E 6JJ*

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1. A method of studying zinc absorption in rats has been developed in which binding of radioactive Zn to the intestinal mucosa and absorption into the carcass was determined at different times after administration by stomach tube.
2. This technique has been used to evaluate different hypotheses concerning the control of Zn absorption and to examine the processes by which this occurs.
3. The proportion of radioactive Zn absorbed into the carcass was found to be dependent on the Zn status of the animals but that found within the small intestinal wall was independent of this, indicating the existence of two mechanisms of Zn absorption.
4. One of these two mechanisms has been shown to be induced by a low dietary Zn content while the other was shown to be insensitive to this. This latter mechanism predominated in rats of normal dietary Zn status and a study of the characteristics of this process indicated that the quantity of Zn absorbed was proportional to the dietary Zn content over the normal range of intake. This implies that normally Zn homeostasis in rats is achieved through variations in Zn excretion. The additional mechanism of Zn absorption only becomes fully active at levels of dietary Zn below 0.24 $\mu\text{mol/g}$ diet.

Zinc is an essential trace element for man having been shown to be necessary for the normal function of a large number of enzymes (Parisi & Vallee, 1969). A deficiency of Zn has been shown to cause a severe pathological state in man, both in the genetically-determined disorder acrodermatitis enteropathica (Moynahan, 1974) and in some patients undergoing total parenteral nutrition (Kay *et al.* 1976). Acrodermatitis enteropathica is thought to be caused by a defective absorption of Zn (Lombeck *et al.* 1975), although the processes by which Zn is normally absorbed are the subject of some controversy.

Hurley *et al.* (1977) have proposed that in neonatal rats and humans, normal mechanisms of Zn absorption are immature and a low-molecular-weight binding ligand from maternal milk facilitates Zn absorption until normal mechanisms mature. This group of workers claim to have isolated the ligand and have found it to be citrate (Lönnerdal *et al.* 1979).

Evans *et al.* (1975) have proposed a mechanism of Zn absorption in rats in which a low-molecular-weight binding ligand of pancreatic origin binds to Zn within the intestinal lumen, this complex then being transported through the microvilli into the epithelial cells. Within the epithelial cells the Zn is thought to bind to ligands on the basolateral plasma membrane from where it is transferred to albumin in the portal circulation. Evans and co-workers have attempted to characterize the Zn-binding ligands of pancreatic origin and have recently described it as picolinic acid (Evans & Johnson, 1979). Evans *et al.* (1975) have also claimed that it is the amount of Zn-free albumin available at the basolateral plasma membrane of the intestinal mucosal cell which regulates the absorption of Zn.

An alternative mechanism for the control of Zn absorption is that of Cousins (1979) who proposed that control is achieved by production of the protein metallothionein within the mucosal cells. Furthermore Richards & Cousins (1975) have postulated that an increase in the circulating plasma Zn level leads to an increased production of metallothionein in the liver and in the intestinal mucosa. This protein is then thought to sequester unrequired newly-absorbed Zn and prevent its transfer to plasma albumin. Since the life of metallo-

thionein is thought to be longer than that of the cells of the mucosal epithelium the sequestered Zn would then be lost from the mucosa by desquamation. If this hypothesis is true then Zn-sufficient animals given oral doses of Zn would be expected to show an increased mucosal Zn content due to the presence of metallothionein. This would subsequently be lost to the gut lumen as mucosal cell desquamation occurred. If Zn absorption is controlled by altering the amount of Zn transported into the mucosal cells (e.g. control achieved by the amount of available binding ligand) then neither an increased mucosal Zn content nor re-excretion of Zn from the mucosa into the lumen should be seen in Zn-sufficient animals. Similarly if Zn absorption is controlled by changes in the amount of Zn transported from the mucosa to the blood stream (e.g. control achieved by the amount of Zn-free albumin available at the basolateral plasma membrane of the mucosal cells) then no re-excretion of Zn from the mucosa into the lumen should be seen.

In order to investigate these possible controlling mechanisms and to obtain further information on the mechanisms of Zn absorption, the distribution of radioactivity was examined at different times following an oral dose of ^{65}Zn . The effects of the dietary Zn level and of various substances on Zn absorption have also been examined and a new hypothesis for the mechanism of Zn absorption in rats is proposed.

METHODS

Groups of four or five male Wistar rats (100–200 g) were maintained on diets of different Zn content for at least 2 weeks before the experiment. The diet used was essentially that used by Suda *et al.* (1970). Appropriate precautions were taken during its preparation to avoid external contamination and different amounts of zinc sulphate were added to the mineral mixture to produce the required level of Zn in the diet. The Zn-depleted diet (Zn content 0.06 $\mu\text{mol/g}$ diet) was found to produce a moderate but significant Zn-depletion in the rats (see plasma Zn levels in Table 1). In order to ensure that the stomach and small intestine were relatively free of digesta the animals were fasted for 24 h before and throughout the experiment (up to 42 h total time).

Each animal received 0.25 μmol zinc chloride containing 4 μCi ^{65}Zn (The Radiochemical Centre, Amersham, Bucks.) in 0.5 ml distilled water by stomach tube and was then placed in an individual small-animal metabolism cage for a specified period. During this period they were allowed free access to distilled water and the total urine and faeces excreted were collected.

At specified intervals following administration of the oral ^{65}Zn load the animals were killed by exsanguination under diethyl ether anaesthesia, the entire gut was immediately removed, cleaned of extraneous tissue and cut into the following segments: stomach, six segments of small intestine and the caecum plus large intestine. The gut contents were then washed out by flushing each segment with 1–2 ml cold isotonic saline (9 g sodium chloride/l).

The various lumen washings, the individual gut wall segments, the rat carcass and the collected faeces and urine were placed in separate silica pots, dried and then ashed at 450° for 48 h. The ash was then dissolved in dilute hydrochloric acid and the ^{65}Zn content determined using a gamma counter. The Zn content of these solutions was also determined by atomic absorption spectroscopy. The plasma taken at the time of death was also analysed for Zn.

The ^{65}Zn contents of the small intestinal wall and the carcass of the animal are expressed as percentages of the total radioactivity recovered from the rat, i.e. as percentages of the sum of the ^{65}Zn found in the carcass, the gut wall, the gut-lumen washing, the urine and the faeces. Any ^{65}Zn present in the stomach lumen was considered to be unavailable for absorption and was not included in the calculations.

The role of certain binding ligands on Zn absorption was investigated by examining the

effect of diiodoquin, picolinic acid and citrate on ^{65}Zn absorption in normal rats. Diiodoquin (250 μmol) (Sigma Chemical Co. Ltd) was given in an emulsion with the oral dose of ^{65}Zn ; picolinic acid (0.7 μmol) (Sigma Chemical Co. Ltd) was dissolved in the test dose of ^{65}Zn which was administered in the usual manner and trisodium citrate (0.75 μmol) (BDH Chemicals Ltd) was given together with 0.25 μmol zinc citrate (Pfaltz & Bauer Inc.) containing 4 μCi ^{65}Zn .

The possibility that intraluminal binding ligands control the extent of Zn absorption was examined by oral administration of the contents of the small intestinal lumen from one group of rats to another group of different dietary status. At 1 h after administration of the standard oral ^{65}Zn dose the contents of the first 300 mm of the small intestinal lumen of a group of Zn-depleted rats were flushed out with a minimum amount of isotonic saline. The ^{65}Zn content of the carcass and the amount bound to the small intestinal wall was then measured in the usual manner. Portions (0.5 ml) of the duodenal washings to which 0.25 μmol zinc chloride and 4 μCi ^{65}Zn had been added, were then given orally to each of a group of normal rats. These normal rats were then killed 4 h later and the ^{65}Zn binding and absorption estimated.

This experiment was then repeated, but this time the proximal lumen washings from normal rats were orally administered to a group of Zn-depleted rats.

The effect of copper on Zn absorption was examined by administration of 3 μmol copper nitrate with the oral ^{65}Zn to groups of normal and Zn-depleted rats.

The significance of the various results was assessed using Student's *t* test, significance values are given in the appropriate place in the text. *P* values greater than 0.05 were regarded as non-significant.

RESULTS

The technique chosen to study Zn absorption, although complex, has proved to be remarkably reproducible; this is because the results are expressed as a percentage of the recovered ^{65}Zn excluding the stomach contents. At short periods of time following the ^{65}Zn dose substantial portions of the dose were still present in the stomach lumen and therefore not available for absorption (Methfessel & Spencer, 1973). This was particularly true in the Zn-depleted rats where the stomach appeared to contain a large amount of scaly material, presumably the result of oesophageal parakeratosis. If allowance were not made for this substantially aberrant results would be obtained.

Time-course of Zn absorption in normal and Zn-depleted rats

Groups of Zn-depleted rats (dietary Zn level 0.06 $\mu\text{mol/g}$) or normal rats (dietary Zn level 0.46 $\mu\text{mol/g}$) were given oral ^{65}Zn by stomach tube and killed 0.5, 1, 4 or 18 h later. The percentage recovery of the ^{65}Zn within the small intestinal wall and within the carcass of both groups of rats is shown in Fig. 1(a). The sum of the ^{65}Zn found in the small intestinal wall and that within the carcass (i.e. the total ^{65}Zn absorbed) is shown in Fig. 1(b). Preliminary experiments demonstrated that, of the ^{65}Zn bound to the small intestinal wall, the major part (> 80%) was found within the mucosal layer.

The proportion of ^{65}Zn bound to the small intestinal wall of both normal and Zn-depleted rats decreased throughout the period of study (Fig. 1(a)), while that in the carcass increased. The amount of ^{65}Zn found within the carcass of Zn-depleted rats was significantly ($P < 0.001$) higher than that within the carcass of normal rats at all time intervals studied, but the proportion found within the small intestinal wall was the same for each group of rats.

The results in Fig. 1(b) demonstrate that in Zn-depleted rats the total ^{65}Zn absorbed was significantly ($P < 0.001$) higher than that in normal rats and also that the difference between these groups cannot be accounted for by a re-excretion of ^{65}Zn from the mucosa of normal

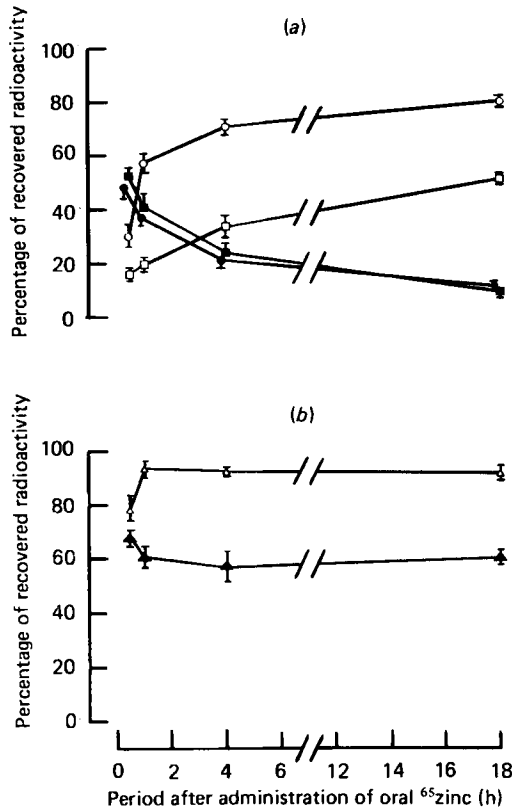


Fig. 1. Distribution of ^{65}Zn following an oral load in rats. (a), Percentage of the recovered radioactivity bound to the small intestinal wall for normal rats (■—■) and for Zn-depleted rats (●—●) and also the percentage within the carcass of normal rats (□—□) and Zn-depleted rats (○—○); (b), total percentage absorbed by normal rats (▲—▲) and Zn-depleted rats (△—△). Points are the mean values with their standard errors represented by vertical bars for four to five rats.

rats. No further absorption appears to have occurred in either group of rats after the first hour because the total amounts absorbed at 4 and 18 h do not significantly differ from those at 1 h.

In subsequent investigations animals were only killed after 4 h. At this time the absorption from the lumen was completed and there was a clear difference between the two groups of rats.

Effect of intraperitoneal injections of Zn on ^{65}Zn absorption in Zn-depleted rats

A group of Zn-depleted rats were given intraperitoneal injections of $25\ \mu\text{mol ZnSO}_4$ in $0.5\ \text{ml}$ isotonic saline 6 h before the oral ^{65}Zn load. This failed to cause any significant decrease in the absorption of ^{65}Zn compared to non-injected Zn-depleted rats (Fig. 2(a)), although it produced a large increase in the plasma Zn level (Table 1).

The experiment was repeated, but this time a group of four Zn-depleted rats was injected with $25\ \mu\text{mol ZnSO}_4$ 24 and 6 h before giving the oral ^{65}Zn load. This produced a significant decrease in the total amount of ^{65}Zn absorbed (Fig. 2(a)) without any effect on the amount bound to the small intestinal wall.

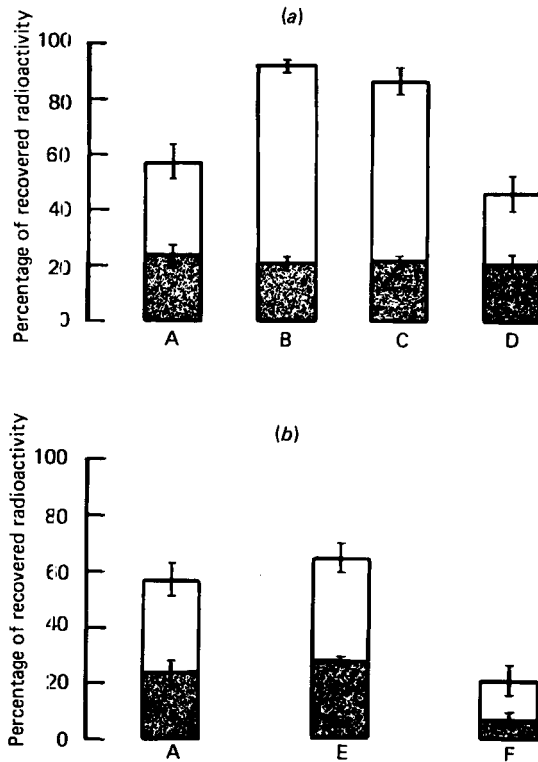


Fig. 2. Absorption of ^{65}Zn by (a) rats of differing body Zn status and (b) rats given increasing amounts of stable Zn with the oral ^{65}Zn . A, normal rats; B, Zn-depleted rats; C, Zn-depleted rats injected with zinc sulphate 6 h before the oral ^{65}Zn ; D, Zn-depleted rats injected with ZnSO_4 24 and 6 h before the oral ^{65}Zn ; E, normal rats given $1.0 \mu\text{mol ZnCl}_2$ with the ^{65}Zn ; F, normal rats given $5.0 \mu\text{mol ZnCl}_2$ with the ^{65}Zn ; ($\square + \blacksquare$), total percentage of the ^{65}Zn absorbed; (\blacksquare), proportion bound to the small intestinal wall. All values are means with their standard errors represented by vertical bars for four to five rats.

Intraluminal Zn concentration and the rate of Zn absorption

Variations in the retention of ^{65}Zn can arise for two reasons; there may be a change in the rate of Zn absorption or alternatively the rate may be unaffected but the specific radioactivity of Zn in the intestinal lumen may be changed (Evans *et al.* 1979).

To see whether changes in the intraluminal Zn concentration affected the rate of absorption of ^{65}Zn the stable Zn contents of the luminal washings were measured and the results obtained shown in Table 1. These indicate that irrespective of the site of Zn absorption (i.e. within the first 300 mm of the small intestine or throughout the small intestine) the differences between the normal and Zn-depleted rats cannot have been due to a greater dilution of the isotope in the lumen of the normal rats. Neither can the decrease in absorption seen when Zn-depleted rats were injected with ZnSO_4 24 and 6 h before giving the oral ^{65}Zn be simply attributed to dilution of the isotope with stable Zn because the group of rats injected only 6 h prior to the oral ^{65}Zn had a higher luminal Zn content and yet also had a higher ^{65}Zn absorption.

The effect of the luminal Zn content on the absorption of ^{65}Zn was also investigated by oral administration of radioactive Zn together with either 1 or $5 \mu\text{mol ZnCl}_2$ to normal rats. The results obtained are shown in Fig. 2(b) together with the results from the normal rats given the standard load of ZnCl_2 ($0.25 \mu\text{mol}$).

Table 1. Zinc content (μmol) of the plasma, small intestinal lumen of rats of differing body Zn status

	Zn content of the first 300 mm of the small intestinal lumen		Total Zn content of the small intestinal lumen		Zn content of the small intestinal wall (/g small intestinal wall)		Plasma Zn (/l)	
	Mean	Range	Mean	Range	Mean	SE	Mean	SE
Normal rats	0.09	0.04-0.15	0.31	0.21-0.50	0.37	0.04	27.7	0.5
Zn-depleted rats	0.12	0.03-0.35	0.32	0.12-0.62	0.36	0.01	21.8	1.2
Zn-depleted rats injected with ZnSO_4 :								
6 h before oral ^{65}Zn	0.61	0.04-1.74	1.08	0.32-1.88	0.65	0.06	79.4	10.1
24 and 6 h before oral ^{65}Zn	0.27	0.08-0.74	0.89	0.69-1.42	0.67	0.04	107.8	20.1
Normal rats given ZnCl_2 with ^{65}Zn :								
1.0 μmol	0.14	0.06-0.29	0.38	0.18-0.53	0.31	0.06	26.6	2.9
5.0 μmol	0.09	0.03-0.18	0.68	0.16-1.99	0.31	0.05	27.8	0.2

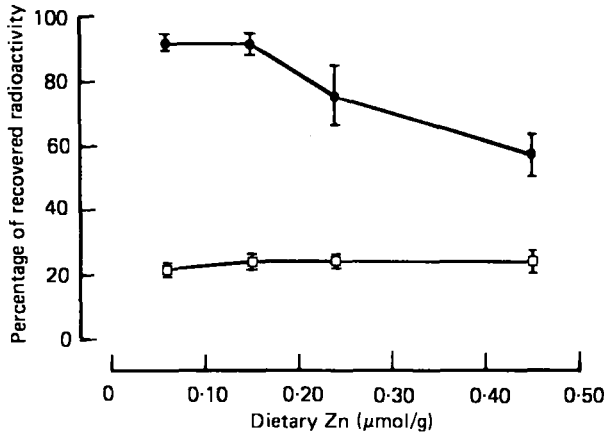


Fig. 3. Absorption of ^{65}Zn by rats given different dietary levels of Zn. (●—●), Total percentage of ^{65}Zn absorbed; (□—□), proportion bound to the small intestinal wall. Points are mean values with their standard errors represented by vertical bars for four to five rats.

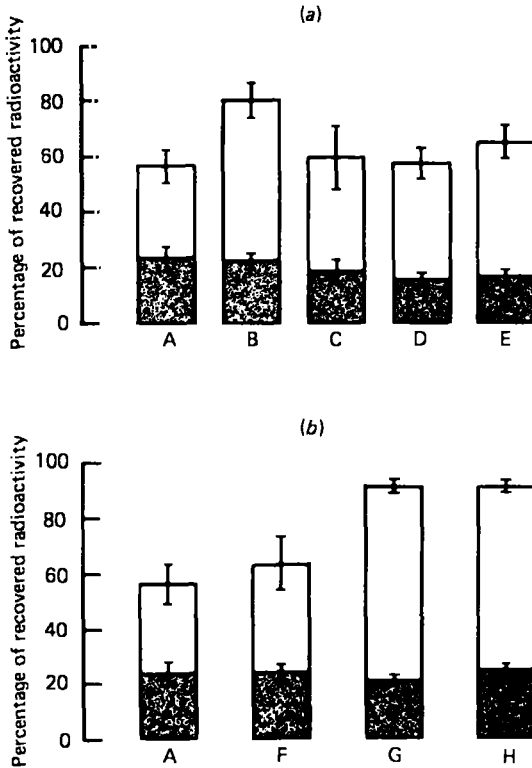


Fig. 4. Absorption of ^{65}Zn by rats given various different substances together with the ^{65}Zn . A, normal rats; B, normal rats given diiodoquin; C, normal rats given picolinic acid; D, normal rats given citrate; E, normal rats given $\text{Cu}(\text{NO}_3)_2$; F, normal rats given lumen washings from Zn-depleted rats; G, Zn-depleted rats; H, Zn-depleted rats given lumen washings from normal rats. (□ + ■). Total percentage of the ^{65}Zn absorbed; (■), proportion bound to the small intestinal wall. All values are means with their standard errors represented by vertical bars for four to five rats.

Administration of $1 \mu\text{mol ZnCl}_2$ did not significantly affect the distribution of radioactivity compared to the group given $0.25 \mu\text{mol}$, but when $5 \mu\text{mol}$ were given the percentage of the ^{65}Zn found within the small intestine wall and the carcass were both significantly reduced ($P < 0.001$). This indicates that in normal rats the percentage of Zn absorbed is independent of the luminal Zn content in the range $0.25 - 1.0 \mu\text{mol}$, but that the mechanism becomes saturated when the intraluminal content is between 1 and $5 \mu\text{mol}$. The level of Zn found in the small intestinal lumen when these animals were killed is shown in Table 1.

The Zn content of the small intestinal wall is also shown in Table 1. The content of the walls of the two groups of rats which were intraperitoneally injected with ZnSO_4 was approximately twice that of the normal and Zn-depleted rats, but there was no significant difference between the two groups of injected rats or between the normal and Zn-depleted rats. This indicates that it cannot be the Zn content of the small intestinal wall which regulates the extent of Zn absorption in the rat.

Dietary Zn content and Zn absorption

Groups of rats were fed on diets containing either $0.15 \mu\text{mol Zn/g}$ or $0.24 \mu\text{mol Zn/g}$ for two weeks; they were then given the oral dose of ^{65}Zn by stomach tube and killed 4 h later. The distribution of the recovered ^{65}Zn together with that from the groups of Zn-depleted (dietary Zn $0.06 \mu\text{mol/g}$) and normal rats (dietary Zn $0.46 \mu\text{mol/g}$) are shown in Fig. 3. The percentage of the recovered ^{65}Zn bound to the small intestinal wall did not vary with the dietary Zn level, but the proportion within the rat carcass and hence the total percentage of radioactivity absorbed, decreased as the dietary Zn level increased above $0.15 \mu\text{mol/g}$.

Various possible binding ligands and Zn absorption

The effects of various different compounds on the absorption of the test dose of ^{65}Zn by normal animals are shown in Fig. 4(a).

Oral administration of diiodoquin significantly increased ($P < 0.001$) the ^{65}Zn absorbed without affecting the amount bound to the small intestinal wall. No effect on the total ^{65}Zn absorbed occurred when either citrate or picolinic acid was administered with the oral ^{65}Zn load. Citrate caused a significant decrease ($0.02 > P > 0.01$) in the amount of ^{65}Zn bound to the small intestinal wall and slightly increased ($0.05 > P > 0.02$) the amount of ^{65}Zn transferred to the carcass of the animal. Picolinic acid did not significantly affect the distribution of the isotope.

The luminal Zn content of these groups of animals was measured and was not found to be significantly different from the normal control group of rats. Picolinic acid or diiodoquin treatment did not significantly alter the plasma Zn concentration of the normal rats, but citrate treatment appears to have significantly ($P < 0.001$) decreased this value compared to the normal group (mean (\pm SE): 23.2 ± 1.1 v. $27.7 \pm 0.5 \mu\text{mol/l}$).

Fig. 4(b) shows the results of oral administration of the contents of the proximal small intestine from one group of rats to rats of a different dietary status. In the first experiment the lumen washings from Zn-depleted rats were orally administered to a group of normal rats, but no significant effect on the ^{65}Zn absorption of the normal rats was seen. In the second experiment the lumen washings from a group of normal rats were given to Zn-depleted rats, but again no significant effects were seen. Both groups of rats from which the luminal washings were obtained absorbed the expected amount of ^{65}Zn .

Effect of Cu on Zn absorption

$\text{Cu}(\text{NO}_3)_2$ ($3 \mu\text{mol}$) was administered to groups of normal and Zn-depleted rats together with the oral dose of ^{65}Zn . It was found that this had no effect on the absorption of ^{65}Zn

by Zn-depleted rats. The total ^{65}Zn absorbed by normal rats was also unaffected by the presence of copper ions (Fig. 4(a)), but the amount of ^{65}Zn bound to the small intestinal wall was significantly less than normal ($0.05 > P > 0.02$) and the amount transferred to the carcass was slightly increased ($0.01 > P > 0.002$).

DISCUSSION

Various techniques have been used for the study of Zn absorption in rats (Davies, 1980; Evans *et al.* 1973; Methfessel & Spencer, 1973; Richards & Cousins, 1975; Van Campen, 1969), but many of these may be criticized to varying extents in that they are isolated from the normal circulation or the movement of gut contents is restricted. The system we have chosen for this study examined ^{65}Zn absorption in the whole animal and, in order to improve the reproducibility of the technique the animals were fasted for 24 h prior to administration of the isotope. It is possible that this period of fast may have had some effect on the extent of Zn absorption, but nevertheless clear differences between normal and Zn-depleted animals have been found.

Early workers found that the extent of Zn absorption in animals was small, e.g. Cotzias *et al.* (1962) found that mice absorbed between 7 and 22% of a ^{65}Zn dose and values of this order are usually quoted in review articles (Halstead *et al.* 1974; Underwood, 1977); there is now a considerable amount of information suggesting that a larger proportion of a test dose of ^{65}Zn is absorbed by experimental animals (Becker & Hoekstra, 1971). The results shown in Fig. 1(b) support this view; normal control animals absorbed approximately 60% of the ^{65}Zn and the Zn-depleted rats absorbed almost all (90%) of the dose. These values also demonstrate that there is a control of Zn absorption in rats.

Control of Zn absorption

No evidence has been found to support the Richards & Cousins (1975) hypothesis. The total level of Zn binding to the small intestinal wall was the same in Zn-depleted rats and Zn-sufficient animals. If the hypothesis were true the binding would be expected to be greater in animals with a lower absorptive capacity. There was also no evidence of excretion of mucosally-bound ^{65}Zn from the small intestinal wall into the lumen. The steady decrease in mucosal ^{65}Zn in Fig. 1(a) can all be accounted for by the transfer of ^{65}Zn from the mucosa to the carcass of the animal. The small decrease in total absorption in the normal rats which occurred between 0.5 and 1 h (Fig. 1(b)) is much too rapid to be attributed to desquamation and too small to account for the differences between the normal and Zn-depleted groups. These findings are in agreement with those of Davies (1980), who studied the fate of mucosally-bound ^{65}Zn for up to 6 h following insertion of the isotope into ligated loops of rat duodenum. He also found that all of the bound ^{65}Zn was transferred to the carcass of the animal and none lost to the lumen.

The results in Fig. 2(x) show that 6–24 h is required to decrease the rate of Zn absorption following intraperitoneal injections of ZnSO_4 . This time interval is compatible with that necessary for the de novo synthesis of 'thionein' as proposed by Cousins (1979).

Although the rate of absorption of Zn was not decreased within 6 h of injecting stable Zn into the Zn-depleted rats, the plasma Zn level of the rats was found to be very high (Table 1). It seems probable that any available binding sites on the plasma albumin would have been filled with this Zn and hence the amount of Zn-free albumin reaching the basolateral plasma membrane of the intestinal cells would have been small. According to the hypothesis of Evans *et al.* (1975) this should have resulted in reduced Zn absorption, but this did not occur.

Fig. 1(a) shows that the total amount of ^{65}Zn bound to the small intestinal wall is independent of the Zn status of the animal and that the decrease in the amount of bound

^{65}Zn with time follows the same pattern irrespective of the dietary status. These results contradict the conclusions of other authors (Evans *et al.* 1973; Evans *et al.* 1975) who have shown increased binding of ^{65}Zn to a part of the small intestinal wall concurrent with an increase in the absorption of ^{65}Zn . This may be explained by the fact that Evans and his co-workers have looked at the ^{65}Zn bound to only a small part of the duodenal wall, whereas the present results represent ^{65}Zn binding to the whole length of the small intestine.

This finding, that the percentage of ^{65}Zn bound to the gut wall at any time is constant, and independent of the total Zn absorbed, was unexpected but can be explained in two ways. The rate of absorption may be modified by changing the number or turnover of mucosal binding sites, or, alternatively, there may be two mechanisms, only one of which involves binding to the mucosa.

Although it was not possible to make reliable estimates of the extent of mucosal Zn binding at very short times (less than 0.5 h) the results in Fig. 1(a) are not compatible with an increased number of binding sites in Zn-depleted rats. The similar rate of decrease of mucosal Zn binding in the normal and Zn-depleted rats suggests that the rate of turnover of the bound Zn is the same in the two groups of rats. Consequently a change in the rate of turnover of bound Zn is unlikely to be the means whereby Zn absorption is modulated.

The amount of ^{65}Zn bound to the small intestinal wall at 0.5 h accounts for all the ^{65}Zn absorbed by normal rats (Fig. 1(a)), but Zn depleted animals appear to absorb approximately 30% more of the ^{65}Zn by a process which does not involve binding to mucosal ligands (Fig. 1(a)). Therefore it appears that there are two mechanisms of Zn absorption one of which is independent of homeostatic control involving binding of ^{65}Zn within the gut mucosa from whence it is released into the body (an obligatory absorption) and a second mechanism by which Zn is very rapidly transported into the body without remaining in the mucosa for any substantial length of time. This second controllable mechanism would need to be sited very near to the pyloric end of the small intestine as all absorption by this process is complete within 1 h of administration of the ^{65}Zn .

This hypothesis receives support from the work of Davies (1980), who has demonstrated both a slow and rapid phase of Zn absorption using loops of rat duodenum ligated *in vivo*. He also compared the kinetics of these phases and found that they represent distinct processes of Zn absorption.

Dietary Zn content and Zn absorption

In order to investigate at what dietary level the second 'high affinity' mechanism for increasing absorption becomes active ^{65}Zn absorption was studied in rats which had been maintained on different dietary levels of Zn. The proportion of the ^{65}Zn absorbed decreased at levels of dietary Zn above 0.15 $\mu\text{mol/g}$ (Fig. 3) and was still above the proportion absorbed by normal rats when the dietary Zn level was 0.24 $\mu\text{mol/g}$. The second controllable mechanism, therefore, appears to be at least partially active at levels of dietary Zn up to 0.24 $\mu\text{mol/g}$.

Intraluminal Zn content and the rate of Zn absorption

The administration of different amounts of Zn to normal rats indicated that the percentage of Zn absorbed by these animals (and hence by the mechanism involving binding to mucosal ligands) is not dependent on the luminal Zn content in the range 0.25 – 1.0 μmol , but that saturation of the process occurred when 5 μmol Zn was given. The actual amount of Zn absorbed by these animals can be calculated from the specific activity of the oral dose of ^{65}Zn ; a plot of the total amount absorbed *v.* amount administered is shown in Fig. 5. The characteristics of the absorption are consistent with a carrier-mediated process, following

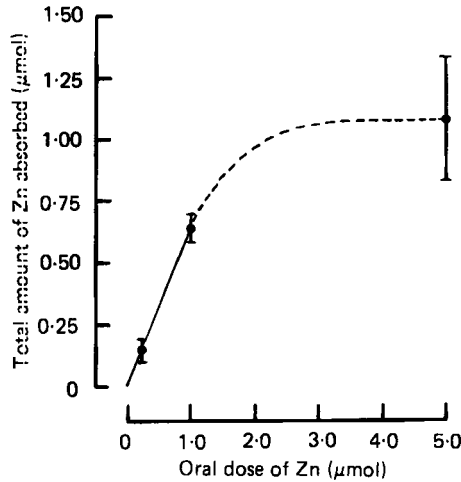


Fig. 5. Total quantity of Zn absorbed by normal rats (calculated from the specific activity of the oral dose) as a function of the oral dose of Zn. Points are mean values with their standard errors represented by vertical bars for four to five rats.

Michaelis-Menten kinetics, which has become saturated when the amount of Zn in the lumen was increased from 1 to 5 μmol .

Preliminary work has shown that the daily Zn intake of a rat maintained on a standard laboratory diet (e.g. Zn content approximately 0.5 $\mu\text{mol/g}$) is of the order of 3–4 $\mu\text{mol/d}$; it is therefore probable that there will be much less than 1 μmol Zn in the small intestinal lumen at any one time. This suggests that in normal dietary conditions the absorptive mechanism of the rat is operating on the linear portion of the curve shown in Fig. 5. This means that a rise in the Zn content of the diet would result in a proportionate rise in the amount of Zn absorbed. In order to maintain body homeostasis in these circumstances a rise in the excretion of Zn must occur. Some evidence in support of this hypothesis is that rats which have been injected intraperitoneally with ZnSO_4 show both an increased luminal Zn content and an increased Zn level in the small intestinal wall (Table 1), suggesting that an increased gastrointestinal secretion of Zn had occurred.

Weigand & Kirchgessner (1978) and Evans *et al.* (1979) have also concluded that in normal circumstances the control of body homeostasis in rats is achieved by variations in Zn excretion, but Evans *et al.* (1979) have also claimed that no control of Zn absorption occurs at any dietary Zn level. The probable reason for this apparent disagreement with the present findings is that Evans *et al.* (1979) have not studied rats maintained on a sufficiently low dietary Zn intake to induce the activation of the second 'high affinity' mechanism of Zn absorption.

Various possible Zn binding ligands and Zn absorption

Although several workers have proposed a major role for intraluminal binding ligands in Zn absorption the results of the 'cross-over' experiment shown in Fig. 4(b) demonstrate that the absorption of ^{65}Zn by normal rats was unaffected by the oral administration of duodenal lumen washings from Zn-depleted rats. Thus no evidence for intraluminal activators of Zn absorption in the lumen of Zn-depleted rats has been obtained. The results in Fig. 4(b) also show that the duodenal lumen washings from normal rats do not contain an inhibitory factor which can affect Zn absorption in Zn-depleted rats. These findings indicate that the extent of Zn absorption in rats is not controlled by intraluminal binding

ligands, but do not exclude such ligands from a role in either of the two proposed mechanisms of Zn absorption.

Both picolinic acid (Evans & Johnson, 1979) and citrate (Lönnerdal *et al.* 1979) have been implicated in normal Zn absorption processes. No effect of picolinic acid on Zn absorption in normal rats has been seen (Fig. 4(a)) while citrate was found to increase the rate of transfer of bound ^{65}Zn into the carcass of normal rats (Fig. 4(a)). These results indicate the possibility that citrate is involved in the proposed process of Zn absorption which involves binding to mucosal ligands.

A closely related compound to the chelating agent diiodoquin (i.e. 8-hydroxy-quinoline) has previously been shown to increase Zn absorption in the rat (Weisman & Knudsen, 1979) and large oral doses of diiodoquin are effective in the treatment of the genetically-determined human Zn deficiency disorder acrodermatitis enteropathica (Dillaha *et al.* 1953). Fig. 4(a) shows that a large dose of diiodoquin increases Zn absorption in normal rats without any effect on Zn binding to the small intestinal wall; this is consistent with an effect on only one of the two proposed mechanisms of Zn absorption (i.e. the rapid controllable mechanism).

As intraluminal binding ligands do not appear to control the second high affinity mechanism of Zn absorption an alternative regulatory system must exist. No information concerning the nature of this system has been obtained, but the existence of inducible Zn-binding ligands on the surface of the mucosal cells in the proximal small intestine would be compatible with a controlling role in this process.

Effect of Cu on Zn absorption

Van Campen (1969) found that $3\ \mu\text{mol Cu}(\text{NO}_3)_2$ inhibited the transfer of ^{65}Zn from in vivo duodenal sacs into the rat carcass, but increased the accumulation of ^{65}Zn in the intestinal mucosa. Fig. 4(a) shows that in normal rats the opposite effect was seen after administration of $3\ \mu\text{mol Cu}(\text{NO}_3)_2$ with the oral ^{65}Zn . The amount of ^{65}Zn bound to the small intestinal wall was decreased, but that in the carcass was increased, indicating that Cu had increased the transfer of ^{65}Zn from the small intestinal mucosa into the body.

The reason for this discrepancy is not clear but the relatively large amount of $\text{Cu}(\text{NO}_3)_2$ ($3\ \mu\text{mol}$) which Van Campen (1969) injected into small (70 mm) ligated segments of duodenum may have exerted a toxic effect on the absorptive processes. No effect of Ca was found on ^{65}Zn absorption Zn-depleted rats.

In summary, firm evidence has been obtained for a dual mechanism of Zn absorption in rats. In normal rats a relatively slow, obligatory, carrier-mediated mechanism predominates and the absorption of Zn is therefore proportional to the dietary intake of Zn. This implies that, under normal circumstances, control of body Zn levels must be achieved by regulation of the amount of Zn excreted. When the level of Zn in the diet is low the rat responds by increasing the proportion of Zn absorbed. This does not occur by increasing the mucosal binding of Zn but rather a second 'high affinity' mechanism of Zn absorption appears to be activated. This second mechanism is fully activated at levels of dietary Zn up to $0.15\ \mu\text{mol/g}$ and is partially activated at dietary levels of at least $0.24\ \mu\text{mol/g}$.

The relevance of these findings in rats to the maintenance of zinc homeostasis in man is difficult to evaluate. We have previously demonstrated the existence of Zn homeostasis in man (Jackson *et al.* 1980) and have shown that one of the responses to an increased dietary Zn level is an increased gastrointestinal secretion of Zn. This is entirely in accord with the existence of an obligatory, carrier-mediated mechanism of Zn absorption similar to that found in rats. It is not known whether there is a second high affinity mechanism in man, but if it does exist and it becomes active as the dietary Zn level falls only slightly below normal (as the present findings suggest it does in the rat), then it may play a major role in the maintenance of Zn homeostasis.

It is of interest to speculate what the defect in acrodermatitis enteropathica may be if these two proposed mechanisms exist in man. Acrodermatitis enteropathica is a disease characterized by Zn deficiency (Moynahan, 1974) which appears to be caused by a defective absorption of Zn (Lombeck *et al.* 1975) and which is curable by either oral Zn supplements or large oral doses of diiodoquin (Dillaha *et al.* 1953). These characteristics could be produced by a defect in only one of the two proposed mechanisms, i.e. the high affinity, controllable process, if it plays a major role in the normal maintenance of Zn homeostasis. In this situation Zn deficiency would result during the first occasion when the dietary Zn level became lower than normal and the patient would be unable to respond by increasing the proportion of the dietary Zn absorbed. The Zn deficiency could then only be repleted by increasing the dietary Zn content sufficiently to enable the carrier-mediated mechanism to absorb extra Zn (i.e. by giving Zn supplements) or by stimulating the defective high affinity mechanism by giving diiodoquin.

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