The effect of pregnancy and lactation on the absorption of zinc and lysine by the rat duodenum *in situ*

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1. The absorption of zinc by the duodenum of the rat was greatly enhanced at late stages of pregnancy and during lactation.
2. During pregnancy no increase in lysine uptake could be demonstrated, but during lactation, when further increases in Zn absorption occurred, uptake of lysine was increased.
3. The increased absorption of Zn at different stages of pregnancy and lactation appeared to be related to the demand made by the developing foetuses and post-natal offspring.

The absorption of zinc by animals of different species has been investigated by many workers with conflicting results. Becker & Hoekstra (1971) cite ten references to studies of Zn absorption in rats which indicate that from 5 to 98 % of the administered dose is absorbed or retained. Wide variations in Zn absorption were also reported for humans, pigs and cattle. These authors have discussed the many factors which may have a bearing on these widely disparate results. Among these are the age of the animal under test, dietary Zn level, availability of Zn in the diets used and the effects of agonists or antagonists, such as natural or synthetic chelating agents or competing mineral elements, on the ability of the gut to absorb and transport the metal.

Our earlier finding that the rate of deposition of Zn in the pregnant or lactating rat and its offspring (Williams, Davies & McDonald, 1977) is much greater than that which occurs in normal rapidly-growing animals, suggested that this effect may be associated with increased absorption of Zn by the maternal gut. This paper describes the results of an experiment designed to investigate the effects of differing stages of pregnancy and lactation on Zn absorption by the rat duodenum.

EXPERIMENTAL

Animals and diets

The management of the animals was the same as in our previous study (Williams *et al.* 1977).

Zn and lysine absorption

Techniques for the isolation and preparation of duodenal loops in situ and measurements of Zn absorption were as described by Davies & Nightingale (1975), except in this experiment studies were made to determine whether any effects on Zn absorption might be accompanied by changes in amino acid absorption. Simultaneous doses of Zn and [^{3}H]lysine were therefore injected, i.e. 5 μg Zn labelled with 0·5 μCi ^{65}Zn and 20 μmol L-lysine labelled with 1·0 μCi L-[4,5-^{3}H]lysine monohydrochloride (specific activity 5 μCi/mmol; Radiochemical Centre, Amersham, Bucks) in 1 ml of 0·15 M-NaCl solution, into the duodenal loops. The animals were killed 15 min later. The assay of unabsorbed ^{65}Zn activity of the loops was carried out as previously described (Davies & Nightingale, 1975). [^{3}H]lysine was assayed on a 1 ml sample of the trichloroacetic acid supernate of the loop contents. This was treated with 1 ml of 0·35 M-$ZnSO_4$ followed by 1 ml 0·5 M-NaOH

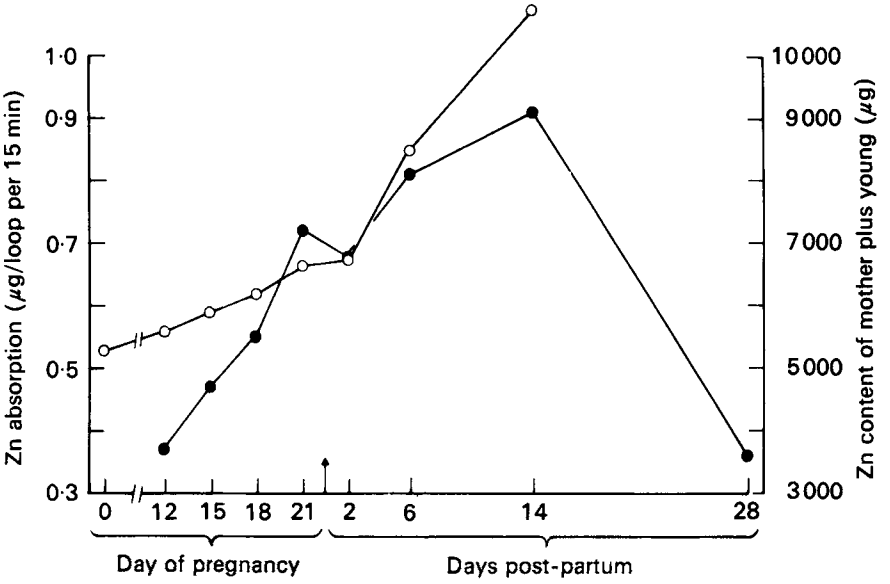

Fig. 1. The effects of pregnancy and lactation in rats on the rate of absorption of Zn by isolated duodenal loops (●—●), together with the rates of accretion of Zn in mothers and young (○—○) (from the results of Williams, Davies & McDonald, (1977)).

to co-precipitate ^{65}Zn activity with $Zn(OH)_2$ and to precipitate bile pigments. After centrifugation, 0·1 ml of the supernate, rendered free of ^{65}Zn activity and coloured quenching agents by the Zn–alkali treatment, was added to 10 ml of Bray's solution (Bray, 1960), and counted for residual [^{3}H]lysine activity in a Packard Tri-Carb scintillation counter (Packard Instruments Ltd, Caversham, Berks).

Expression of results

'Zn absorption' from loop to carcase was measured as the difference between injected activity and that recovered from the loop contents and the loop tissue. Mucosal uptake of lysine refers to the loss of the injected [^{3}H]lysine activity from the loop contents. All results were converted to either μg Zn absorbed or bound or μmol lysine taken up as calculated from the respective initial specific activities.

The duodenal loops were oven-dried at 110° for 12 h to enable Zn absorption and mucosal uptake of lysine to be expressed on a tissue dry matter basis.

RESULTS

The stages of pregnancy and lactation at which Zn absorption by the duodenum was measured were the same as those at which Zn accretion in mother and young had been determined in our previous study (Williams *et al.* 1977). In addition, in the present experiment a group of animals was also examined at 28 d post-partum, by which time the young had been weaned and lactation had ceased. The changes in duodenal Zn absorption expressed both as Zn absorbed per loop and Zn absorbed per g dry weight of duodenal tissue are shown in Table 1. By the 21st d of pregnancy the amount of Zn absorbed per loop had doubled. In addition, the rate of absorption per loop continued to rise to the 14th d of lactation. By the 28th d post-partum, Zn absorption by the duodenal loops had declined to the control values. These results are also shown in Fig. 1 together with the

Table 1. *Rates of Zn absorption by isolated duodenal loops in situ at different stages of pregnancy and lactation together with values for non-pregnant control rats*

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

		Zn absorption			
		μg Zn absorbed/loop per 15 min		μg Zn absorbed/g dry wt per 15 min	
	Day of pregnancy or equivalent in non-pregnant controls	Mean	SE	Mean	SE
Pregnancy					
Controls	18	0·40	0·05 (5)	1·44	0·19 (6)
Pregnant	12	0·37	0·02 (5)	1·16	0·04 (5)
	15	0·47	0·06 (4)	1·60	0·25 (4)
	18	0·55	0·05 (6)**	1·55	0·09 (6)
	21	0·72	0·05 (5)***	2·43	0·22 (5)***
Lactation					
	Day post-partum or equivalent in non-pregnant controls				
Controls	6	0·39	0·05 (5)	1·40	0·22 (6)
Lactating	2	0·67	0·07 (5)***	2·07	0·19 (5)
	6	0·81	0·14 (5)***	2·32	0·43 (5)
	14	0·91	0·11 (5)***	2·17	0·41 (5)
	28	0·36	0·04 (6)†††	1·13	0·13 (6)†
All non-pregnant controls (see below)		0·38	0·03 (13)	1·38	0·12 (13)

For statistical treatment, the non-pregnant animals tested on the equivalent of the 18th d of pregnancy and those tested on the 6th d post-partum were combined with three further individual rats (two on 15th d and one on 21st d of pregnancy) which were found on examination to be non-pregnant, to give the mean values for all non-pregnant controls. All comparisons were made by Student's *t* test. For specific comparisons between all non-pregnant controls, and pregnant or lactating animals: ***$P < 0·001$, **$P < 0·01$; and for comparisons between 14th d post-partum and 28th d post-partum: †††$P < 0·001$, †$P < 0·05$

superimposed curve of Zn accretion in the maternal–offspring complex determined in our previous study (Williams *et al.* 1977).

From the 12th to the 21st d of pregnancy, the trend in the increasing rate of absorption of Zn by the duodenal loop could be expressed by the equation:

$$y = 0·038d - 0·009 \ (t\ 5·28;\ P < 0·001),$$

where y is μg Zn absorbed/loop per 15 min and d is the day of pregnancy. Significant differences in the rate of absorption as compared with non-pregnant controls had not, however, occurred before the 18th d of pregnancy.

Expression of the results in terms of Zn absorbed per loop (i.e. per unit length) of duodenum can be considered a measure of Zn absorptive capacity. Zinc absorption was also calculated as Zn absorbed/g dry weight as a measure of the efficiency of the Zn absorptive process. These results, also shown in Table 1, indicate that at the later stages of pregnancy the efficiency of Zn absorption is increased. However, in contrast to those results expressed on the basis of absorption per unit of loop length, no further increases were found on the 6th and 14th d post-partum.

In this experiment it seemed advisable to investigate whether any increase in ^{65}Zn absorption observed was a reflection of a general increase in nutrient absorption. Accordingly studies were made of the mucosal uptake of lysine, at the same time as measurement

Table 2. *Rates of uptake of lysine by isolated duodenal loops in situ at different stages of pregnancy and lactation together with values for non-pregnant control rats*

		lysine uptake			
		μmol/loop per 15 min		μmol/g dry wt per 15 min	
	Day of pregnancy or equivalent in non-pregnant controls	Mean	SE	Mean	SE
Pregnancy					
Controls	18	12·5	0·21 (6)	44·3	2·41 (6)
Pregnant	12	12·6	0·20 (5)	40·1	1·32 (5)
	15	12·5	0·40 (4)	42·0	2·80 (4)
	18	13·1	0·20 (6)	37·9	1·80 (6)
	21	11·6	0·60 (5)	40·6	3·01 (5)
All pregnant		12·5	0·19 (20)	41·9	1·18 (20)
Lactation					
	Days post-partum or equivalent in non-pregnant controls				
Controls	6	12·7	0·24 (5)	46·1	3·21 (5)
Lactating	2	14·4	0·21 (5)***	45·4	1·61 (5)
	6	14·5	0·22 (5)***	41·7	0·73 (5)
	14	15·7	0·31 (5)***	36·5	2·82 (5)*
	28	13·2	0·33 (6)†††	40·9	1·12 (6)
All non-pregnant controls (see below)		12·5	0·19 (13)	45·8	1·67 (13)

For statistical treatment, the non-pregnant animals tested on the 18th d of pregnancy and those tested on the 6th d post-partum were combined with three further individual rats (two on the 15th d and one on the 21st d of pregnancy) which were found on examination to be non-pregnant. All comparisons were made by Student's *t* test. For specific comparisons between all non-pregnant controls and pregnant or lactating animals: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; and for comparisons between the 14th d post-partum and 28th d post-partum: †††$P < 0.001$.

of Zn absorption, as an indicator of uptake of non-metallic nutrients. The results expressed both on a loop and dry weight basis are shown in Table 2.

No detectable increase in lysine uptake occurred throughout pregnancy when expressed in either manner. In lactating animals, however, from the 2nd to the 14th d post-partum, a progressive increase from 15 to 26 % occurred in lysine uptake by the duodenal loops as compared with non-pregnant controls. The efficiency of uptake when calculated on a dry weight basis was not affected. By the 28th d post-partum, by which time the young had been fully weaned, the mucosal uptake of lysine by the duodenal loops was no longer significantly different from that in unmated, non-lactating rats.

DISCUSSION

The relationship between changes in rates of Zn absorption and its retention in pregnancy and lactation

The absorption of Zn by the duodenum of the rat was markedly influenced by the stage of pregnancy and lactation. Within the limits imposed by comparisons between the present experiments and those carried out earlier (Williams *et al.* 1977), it seemed justifiable to assume that some relationship existed between demand for Zn by the developing foetuses or young, which at the later stages of pregnancy and in early lactation was very high, and the capacity of the gut to adapt to that demand.

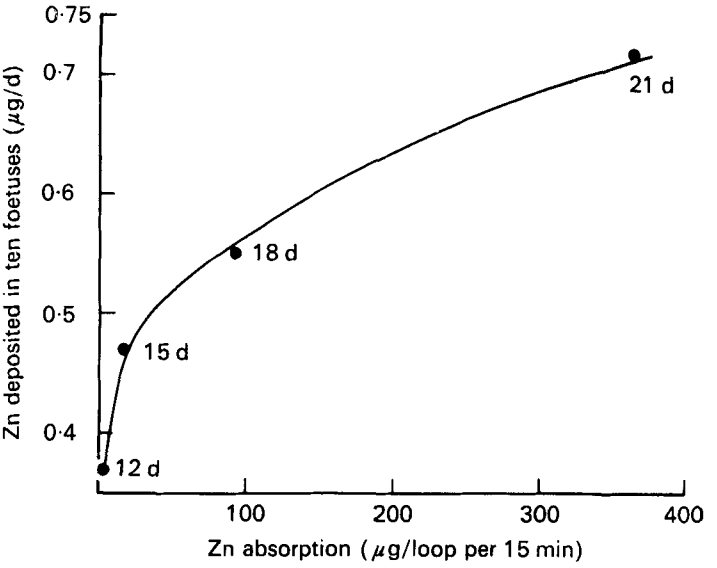

Fig. 2. Relationship between the mean rates of Zn absorption by isolated duodenal loops in pregnant rats and the instantaneous rates of Zn accretion (μg Zn/d) by ten foetuses (from the results of Williams, *et al.* (1977) at the equivalent stages of pregnancy).

If such a relationship were to exist it would suggest, firstly, that retention of Zn during the first half of pregnancy was a reflection of maternal growth only, since the rate of Zn absorption by duodenal loops on the 12th d of pregnancy was the same as in non-pregnant controls (Table 2). Secondly, it would suggest that the increased retention of Zn by the maternal–offspring complex during the latter half of pregnancy could be accounted for almost entirely by deposition of Zn in the foetuses. Both of these assumptions appear to be valid since the average daily accumulation of Zn by the maternal bodies from 0 to 12 d of pregnancy (23·5 μg) and 12 to 21 d (21·6 μg) was little different from that of non-pregnant control rats throughout the 36 d period corresponding to pregnancy and lactation (30·7 μg) (see Williams *et al.* 1977, Table 1).

A plot of the instantaneous rates of Zn deposition in foetal rats taken from our previous study (Williams *et al.* 1977, Table 5) and the rates of Zn absorption by duodenal loops of pregnant rats at equivalent stages of pregnancy, is shown in Fig. 2. The curvilinear relationship obtained can be described by the equation:

$$y = 0{\cdot}28 + 0{\cdot}069 \log_e x \; (r\ 0{\cdot}97,\ P < 0{\cdot}025),$$

where y is rate of Zn absorption by duodenal loops and x is instantaneous daily rate of Zn deposition in ten foetuses.

Assuming that the rates of Zn absorption as measured by the duodenal loop technique were proportional to daily absorption of dietary Zn, these results indicate that during the latter half of pregnancy the retention of absorbed Zn increased. It would seem, therefore, that the large increase in demand for Zn by the developing foetuses was satisfied not only by an increased absorption of Zn but also by a relative decrease in the proportion of absorbed Zn that was excreted.

Although, as shown in Fig. 1, the increasing rate of Zn accumulation during lactation in the maternal–offspring complex coincided with further increases in the capacity of the duodenal loops to absorb Zn, no correlation between the rates of Zn retention and absorption was demonstrable. The reasons for this probably lie in changes in the mechanisms

involved in the increased rates of Zn absorption by duodenal loops during lactation. Thus, during pregnancy, the increased capacity of the intestine can be accounted for by an increased efficiency of the absorptive process. However, the further increases observed during lactation probably occur as a consequence of the mucosal hypertrophy and an increased mucosal mass per unit length of intestine as reported by Boyne, Fell & Robb (1966). These conclusions are supported by our findings that no significant changes were noted in the dry weights of the duodenal loops during pregnancy ((mean ± SE) non-pregnant controls 0·28 ± 0·01 g (13) and 21 d pregnant rats 0·29 ± 0·01 g (5)), whereas a 57 % increase in dry weight of the loops ($P < 0·001$) was found on the 14th d of lactation (0·44 ± 0·03 g (5)). In addition, during lactation the increasing autonomy of the young rats in eliminating, at an undetermined rate, any excess Zn ingested would make estimates of the total demands for Zn by the mother plus young impossible to quantify accurately. Nevertheless, when considered together, the results of this current study and those of our previous paper (Williams *et al.* 1977) strongly suggest that the increased demands for Zn by pregnant and lactating rats are met, in part, by an increased capacity of the intestine to absorb this nutrient.

Other conditions in which absorption of Zn may be affected by the demand for this nutrient include those of age of animal and the effect of dietary Zn deficiency. Zinc absorption has been shown to be higher in the young of many species compared with older animals. These examples include rats (Ballou & Thompson, 1961), cattle (Miller & Cragle, 1965) and pigs (Whiting & Bezeau, 1958). However, it must be emphasized that in the studies of rats and cattle, the younger animals were given rations of different composition from those fed to the more mature animals and it was not established how much of the difference in Zn absorption observed was due to age and growth rate and how much to alterations in dietary constituents.

Dietary restriction of Zn to rats has been shown to influence intestinal absorption of Zn both by in vitro techniques (Kirchgessner, Schwarz & Grassman, 1973) and when absorption was measured in vivo (Pallauf & Kirchgessner, 1972). Taken together with the results of this present investigation, these findings emphasize the importance of the intestine as the major site at which animals adapt to changes in demand for, or supply of, dietary Zn.

The relationship between Zn and lysine uptake by the maternal duodenum in pregnancy and lactation

Our findings that no increase in the duodenal uptake of lysine occurred during pregnancy leads us to speculate that the effects of pregnancy on the duodenal uptake and transport of Zn is not a consequence of a general increase in absorptive efficiency. If, during pregnancy, total amino acid uptake by the intestine is not increased, as was found for lysine uptake by the duodenum, it would suggest that during the late stages of pregnancy when the daily rate of accretion of tissue by the mother–offspring complex is nine times greater than in non-pregnant animals and five times greater than over the first 12 d of pregnancy (Williams *et al.* 1977), absorbed N was utilized with increased efficiency. This conclusion is supported by the observations of Naismith & Ritchie (1973) who demonstrated that pregnant rats showed a greater positive N balance than non-pregnant controls, particularly during the later stages of pregnancy. This was associated with a reduction in urinary N excretion in the pregnant animals.

Finally, our finding that on the 14th d of lactation the increases in absorption of Zn compared with 21 d pregnant rats and the uptake of lysine compared with non-pregnant animals were proportionately similar, being 25·6 and 26·4 % respectively, may as already suggested be attributable to intestinal mucosal hypertrophy as demonstrated by Boyne *et al.* (1966), with a consequent increase in mucosal mass per unit length of gut. Similar conclu-

sions regarding the intestinal absorption of leucine and glucose during pregnancy and lactation in rats were reached by Cripps & Williams (1975). These workers showed that during pregnancy leucine and glucose absorption increased, but not significantly. However, during the first half of lactation absorption of these compounds was increased and this coincided with an intestinal hypertrophy. By the 21st d of lactation both leucine and glucose absorption had fallen to the non-pregnant control values. Similarly, Penzes & Simon (1968) have reported that the absorption of methionine increased during the early stages of lactation in rats, but had returned to control levels by the 21st d of lactation. Fell, Smith & Campbell (1963) have suggested three possible causes of the hypertrophy of the alimentary canal during lactation. Firstly, that the changes are due to a 'work hypertrophy' due to the 2–3-fold increase in food consumption throughout lactation. Secondly that hormonal changes are responsible and finally that the hypertrophy could be a functional adaptation to the increased demands made on the body during lactation. Which of these explanations is correct has yet to be established. However, when considered together these observations may be indicative of a general increase in intestinal absorptive capacity during lactation which would enable a more efficient utilization of both organic and inorganic dietary nutrients.

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