Studies of zinc metabolism in pregnant and lactating rats

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1. The metabolism of ^{65}Zn administered intramuscularly (Expt 1) or enterally (Expt 2) at the beginning of pregnancy in rats given a control or marginal-Zn diet was measured. In Expt 2 a comparison was also made between pregnant and non-pregnant rats. The loss of ^{65}Zn (assumed to represent labile body Zn) was markedly reduced in animals fed on a marginal-Zn diet compared with controls, and this effect occurred very rapidly, i.e. within 48 h of introducing the marginal-Zn diet. Pregnancy itself had a much less important effect on ^{65}Zn turnover than diet. Transfer of ^{65}Zn to the litter was significantly greater in the animals fed on a marginal-Zn diet compared with controls but total Zn transfer was reduced.

2. The effect of length of time on a marginal-Zn diet on fetal growth and composition was examined. Compared with controls, fetal weight was significantly greater in litters from mothers fed on a marginal-Zn diet during the last 4, 7 or 14 d of pregnancy, but no different in litters from mothers fed on a marginal-Zn diet throughout pregnancy. There were no differences in the proportions of protein or fat in the fetuses from mothers fed on the control or marginal-Zn diets but the Zn concentration was lower in litters from mothers fed on a marginal-Zn diet during part or all of the pregnancy when compared with controls.

3. The transfer of ^{65}Zn from mothers to litters during birth and the first 3 d of lactation was measured. There were no differences in maternal or litter ^{65}Zn just before or just after birth, but within 72 h maternal ^{65}Zn had significantly decreased and litter ^{65}Zn increased. Increases in litter size were associated with greater total litter ^{65}Zn but reduced individual fetal ^{65}Zn.

4. These experiments demonstrate the importance of an adequate daily supply of Zn during pregnancy. Although there is room for adaptation to a marginal-Zn intake (by reducing Zn excretion) the maintenance of Zn homeostasis is only possible in the absence of other forms of stress, such as pregnancy, to the body. The consequence of insufficient Zn at times of rapid fetal growth on carbohydrate and lipid metabolism warrants further investigation.

There is an increasing volume of evidence that the capacity of the body to respond to diets of differing zinc levels is quite considerable. It has been clearly demonstrated that Zn-deficient rats show an enhanced uptake of ^{65}Zn (Evans *et al.* 1973) and at low dietary intakes there is 100% true absorption of Zn, all faecal Zn being comprised of obligatory endogenous losses (Weigand & Kirchgessner, 1980). Conversely, with high intakes of Zn, total body retention is stabilized by a decrease in true absorption and an increase in endogenous excretion. This intestinal adaptation also takes place in humans; for example, when omnivorous women were given a lacto-ovo-vegetarian diet rich in phytic acid for 3 weeks, they showed an accelerated uptake of pharmacological doses of Zn (Freeland-Graves *et al.* 1980). Thus there appears to be great scope for the body to adapt to changing requirements, such as the demands made in pregnancy, but the speed with which it can respond is not known. It is not clear to what extent the demands of the developing fetus can be met by endogenous sources of Zn or whether dietary Zn plays a crucial role in providing for the mother and fetus. The present study explores the response of pregnant rats to a marginal-Zn diet and the fate of the fetuses and sucking pups.

MATERIALS AND METHODS

Animals

In all experiments Wistar rats were used. They were housed individually in stainless-steel and plastic cages with gridded bottoms and fed on semi-synthetic diets *ad lib.* (for composition see p. 402). Mating was carried out by housing several newly-castrated male

rats in the same room as the females for at least 1 week in order to synchronize oestrus. The stage of oestrus was determined by taking vaginal smears daily. When most of the females were synchronized an adult male Wistar rat was put in each female's cage the day before oestrus and left until a mating plug was found. The day on which the plugs were found was designated day 0 of pregnancy.

Diets

The rats were fed on a marginal-Zn or control diet, as described previously (Fairweather-Tait *et al.* 1984), and unless otherwise stated they were given a semi-synthetic control diet. The marginal-Zn diet was made by omitting the zinc carbonate normally added to the mineral mix in the control diet. In all experiments the control diet contained 68 μg Zn/g and the marginal-Zn diet 10 μg Zn/g.

Atomic absorption spectroscopy (AAS)

Samples for Zn analysis were freeze-dried, ground to a fine powder with a pestle and mortar and subsamples ashed in silica crucibles at 480° for 48 h in a muffle furnace. The ash was dissolved in warm concentrated hydrochloric acid and made up to an appropriate volume with distilled water. The Zn content of the resultant solution was measured by flame spectroscopy using a PU 9000 AAS (Pye Unicam, Cambridge) with background correction, using standards supplied by the National Bureau of Standards (Office of Standard Reference Materials, Washington, USA).

Whole-body counting

The ^{65}Zn content of the animals was measured in a NE 8112 small-animal counter (Nuclear Enterprises, Edinburgh) as described previously (Fairweather-Tait & Wright, 1984). The counting efficiency for ^{65}Zn was approximately 25%.

Expt 1

The first experiment in the series was designed to investigate the extent to which pregnant rats can mobilize body Zn for fetal requirements when fed on a diet containing insufficient Zn during pregnancy. Female rats were mated overnight with male Wistar rats and those that had successfully mated, as evidenced by the presence of a mating plug, were injected intramuscularly with 1·0 μCi ^{65}Zn (Amersham International, Amersham, Bucks; 0·1 ml solution of zinc chloride in saline (9 g sodium chloride/l)) in the left hind limb. They were allocated to two equal groups and fed on either the control or marginal-Zn diet. Food intakes were measured, maternal weights recorded and the animals counted daily in the whole-body counter, the last count being in the morning of day 21 of pregnancy just before parturition. Immediately after the mothers had cleaned the pups and consumed the placentas, mothers and litters were counted separately in the whole-body counter.

Expt 2

The purpose of this experiment was to compare the response of pregnant and non-pregnant animals to a marginal-Zn diet in terms of mobilizing body stores. A different method of labelling endogenous Zn was used whereby the animals were allowed to absorb ^{65}Zn enterally instead of giving it by injection, to confirm that the findings in Expt 1 were not in any way connected with the mode of labelling used. At 5 d before female rats were at the appropriate point of the oestrus cycle to be mated, they were given a marginal-Zn diet for 3 d (to enhance subsequent ^{65}Zn retention). At 2 d before mating they were given a meal of the marginal-Zn diet containing approximately 1·0 μCi ^{65}Zn and, after a 3 h fast, returned to the control diet. After 2 d half of them were mated overnight with male Wistar rats, as

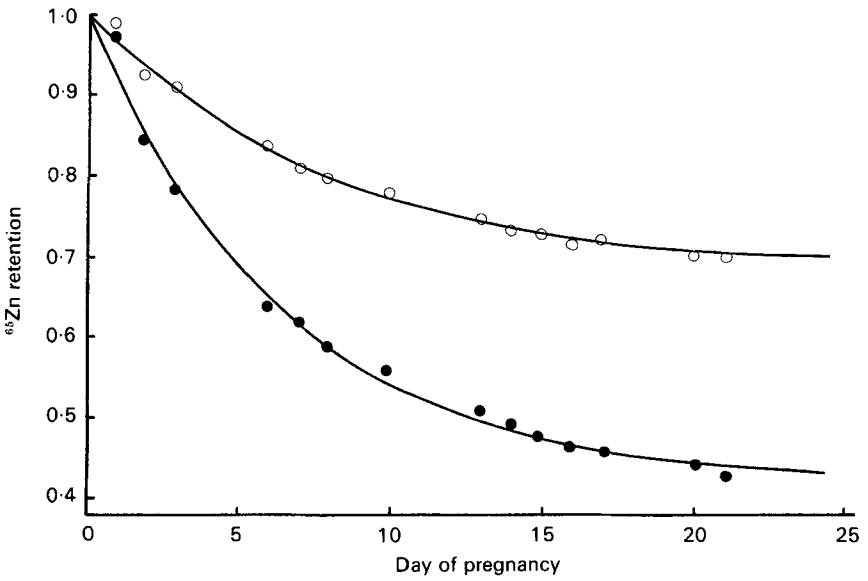

Fig. 1. Expt 1. ^{65}Zn retention over 22 d (proportion of amount present on day 0 of pregnancy) by rats injected intramuscularly with 1 μCi ^{65}Zn on day 0 of pregnancy. Solid lines describe exponential decay curves, points are mean values for rats fed on the control (68 μg Zn/g) diet (●) or marginal-Zn (10 μg Zn/g) diet (○) from day 0 of pregnancy onwards. Values were significantly different between the two groups from day 2 onwards ($P < 0{\cdot}001$).

described previously, and then allocated to the following groups: group 1, non-pregnant, control diet; group 2, non-pregnant, marginal-Zn diet; group 3, pregnant, control diet; group 4, pregnant, marginal-Zn diet. The ^{65}Zn content of the animals was monitored in the whole-body counter until day 19 of pregnancy. The animals were killed on day 20 of pregnancy under carbon dioxide. The fetuses and placentas were removed from the pregnant animals and the liver and both femurs from all the adult animals, and the ^{65}Zn and total Zn content of each measured. In every experiment the fetuses from each mother were combined and analysed collectively.

Expt 3

The effect of the length of time on a marginal-Zn diet on fetal growth and composition and Zn content, and on maternal liver Zn, was examined in the following experiment. Female rats were mated as described previously and then allocated to the following groups: group 1, control diet throughout pregnancy; group 2, marginal-Zn diet throughout pregnancy; group 3, control diet for first trimester, marginal-Zn diet in last two trimesters; group 4, control diet for first two trimesters, marginal-Zn diet in last trimester; group 5, control diet for first 17 d of pregnancy, marginal-Zn diet in last 4 d. The animals were killed under CO_2 on day 20 of pregnancy and the fetuses and maternal livers were removed and analysed for Zn.

Expt 4

The final experiment in the series was designed to study the transfer of Zn during delivery and the first few days of suckling from mother to pups. It was not considered necessary to give the animals a marginal-Zn diet before feeding the ^{65}Zn-labelled meal (to enhance ^{65}Zn uptake) since the ^{65}Zn retention by the pregnant rats in Expt 2 was more than sufficient for accurate whole-body counting.

Table 1. *Expt 1.* ^{65}*Zn content of mothers and litters immediately after birth, expressed as a proportion of the* ^{65}*Zn content of the pregnant mothers on day 0 and day 21 of pregnancy, and no. of pups and pup weight (g) in rats fed on a control or marginal-Zn diet*

(Mean values with their standard errors)

Dietary group†...	Control (*n* 13)		Marginal-Zn (*n* 12)	
	Mean	SEM	Mean	SEM
^{65}Zn (as a proportion of day 0)				
Mothers	0·379	0·005	0·597***	0·009
Litters	0·052	0·002	0·104***	0·003
^{65}Zn (as a proportion of day 21)				
Mothers	0·878	0·004	0·852***	0·005
Litters	0·120	0·005	0·149***	0·004
No. of pups	12·4	0·6	12·8	0·7
Pup wt (g)	5·4	0·2	5·1	0·1

*** $P < 0·001$.

† For details, see p. 402.

Female rats were given a ^{65}Zn-labelled meal of the control diet (1 μCi ^{65}Zn, $ZnCl_2$; Amersham International) 5 d before oestrus. They were mated at oestrus, continued on the control diet, and then randomly allocated to three groups. The first group was killed under CO_2 on day 20 of pregnancy, the fetuses removed and the ^{65}Zn content of mothers and fetuses measured separately. The second group was allowed to give birth and the mothers and pups killed before suckling and the ^{65}Zn content measured as described previously. The third group were allowed to suckle their young, killed 72 h after birth and the previously described procedure repeated.

Statistical analysis

Results from Expt 1 were analysed by *t* tests. The statistical package GENSTAT (Alvey *et al.* 1977) was used to fit an exponential decay curve to ^{65}Zn retention over 22 d by the least squares method. A separate curve was fitted for rats fed on a control or marginal-Zn diet. For each curve, two parameters were fitted, the decay constant (τ) and the proportion of ^{65}Zn lost over time (c):

$$^{65}\text{Zn retained} = 1 - c(1 - e^{-\text{day}/\tau}).$$

Results from Expt 2 were subjected to two-way analysis of variance with due allowance for differing variances where appropriate, as described previously (Fairweather-Tait *et al.* 1984). Results from Expt 3 were tested by one-way analysis of variance and where this showed a treatment effect, multiple *t* tests were performed to identify which groups differed from the controls. Results from Expt 4 were similarly treated but the litter size was included as a covariate in the analysis of variance. The number of rats per group in each experiment is given in the tables.

RESULTS

Expt 1

The initial mean weights of the mothers on day 1 of pregnancy were 241 (SE 4·2) g for the control-fed animals and 235 (SE 4·0) g for those fed on the marginal-Zn diet. The final weights on day 21 of pregnancy (just before parturition) were 373 (SE 6·9) g for the controls

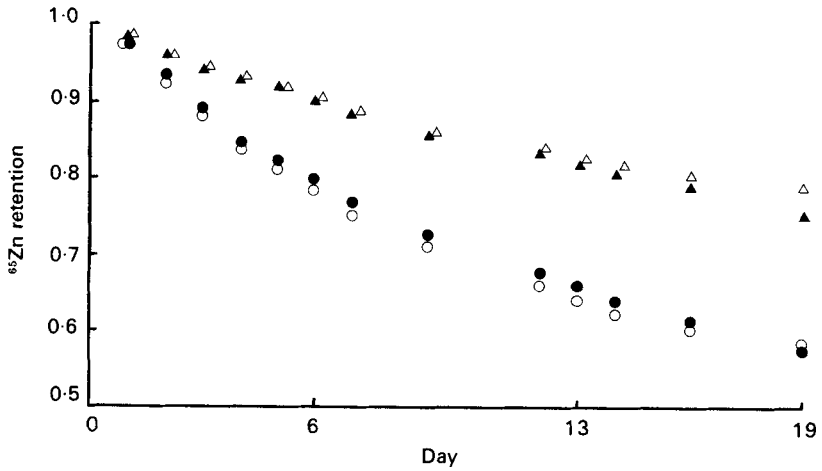

Fig. 2. Expt 2. ^{65}Zn retention over 20 d (proportion of amount present on day 0) by rats given 1 μCi ^{65}Zn 2 d before mating: (●), non-pregnant, control (68 μg Zn/g) diet; (○), pregnant, control diet; (▲), non-pregnant, marginal-Zn (10 μg Zn/g) diet; (△), pregnant, marginal-Zn diet.

Table 2. *Expt 2. ^{65}Zn retention (proportion of original amount present 23 d after oral administration), ^{65}Zn and Zn contents of livers and femurs, and liver weights (g) in 20-d pregnant and non-pregnant female rats fed on a control or marginal-Zn diet, together with significance levels*

(Mean values with their standard errors)

Dietary group†...	Control			Marginal-Zn		
	n	Mean	SEM	*n*	Mean	SEM
Non-pregnant rats	18	—	—	17	—	—
^{65}Zn retention	—	0·575	0·005	—	0·752	0·006
Wet liver wt	—	10·96	0·441	—	10·48	0·354
Total liver Zn (μg)	—	296·7	10·89	—	276·3	9·13
^{65}Zn content of livers	—	0·0345	0·0007	—	0·0465	0·001
Zn concentration of femurs (μg/g dry wt)	—	235·2	5·61	—	214·6	8·88
^{65}Zn content of femurs	—	0·036	0·0009	—	0·031	0·001
Pregnant rats	10	—	—	11	—	—
^{65}Zn retention	—	0·584	0·005	—	0·789	0·006
Wet liver wt	—	15·38	0·420	—	14·87	0·488
Total liver Zn (μg)	—	414·9	39·31	—	359·3	14·65
^{65}Zn content of livers	—	0·0341	0·00095	—	0·0425	0·00121
Zn concentration of femurs (μg/g dry wt)	—	217·5	4·87	—	221·4	9·65
^{65}Zn content of femurs	—	0·034	0·0016	—	0·029	0·0012

	Significance level (t value)		
	Diet	Pregnancy	Interaction
^{65}Zn retention	34·7***	4·2***	2·6*
Wet liver wt	1·2	10·3***	0·0
Total liver Zn (μg)	1·7	4·5***	0·8
^{65}Zn content of livers	10·2***	2·2*	1·8
Zn concentration of femurs	1·1	0·7	1·6
^{65}Zn content of femurs	4·2***	1·7	0·0

* $P < 0·05$, *** $P < 0·001$.

† For details, see p. 402.

Table 3. *Expt. 2.* ^{65}Zn *(proportion of amount present in the mother on day 19 of pregnancy) and Zn content of litters and placentas, fetal and placental weights (g) and litter size, in rats fed on a control or marginal-Zn diet*

(Mean values with their standard errors)

Dietary group†...	Control (*n* 10)		Marginal-Zn (*n* 11)	
	Mean	SEM	Mean	SEM
No. fetuses per litter	13·1	1·14	14·3	1·09
Wet fetal wt	3·26	0·08	3·43	0·07
Zn concentration of dry fetal tissue (μg/g)	176·5	2·59	161·9***	3·08
^{65}Zn content of litters	0·063	0·0044	0·084**	0·0057
Wet placental wt	0·435	0·011	0·513***	0·014
Zn concentration of dry placental tissue (μg/g)	70·4	0·79	65·1***	0·67
^{65}Zn content of placentas	0·0079	0·00056	0·0084	0·00063

** $P < 0·01$, *** $P < 0·001$.

For details, see p. 402.

and 354 (SE 8·7) g for the marginal-Zn group. The differences between the two groups were not significant, and their food intakes did not differ.

The maternal ^{65}Zn content throughout pregnancy is shown in Fig. 1; results are expressed as a proportion of the amount of ^{65}Zn injected on day 0 of pregnancy (i.e. immediately after mating). The difference between the groups was significant from day 2 onwards. Fig. 1 shows that the data are well fitted by the exponential curve, with the exception of the first measurements on day 1. Presumably this is the time required for the injected ^{65}Zn to equilibrate with the endogenous pools of Zn in the body. The proportion finally retained $(1-c)$ clearly differs according to diet: control 0·415 (SE 0·010), marginal-Zn 0·679 (SE 0·006). The decay constant (τ) also differs significantly according to diet ($P < 0·05$), but only by 20% of its mean value: control 6·39 (SE 0·33), marginal-Zn 7·79 (SE 0·38). The rats fed on a marginal-Zn diet attained equilibrium slightly more slowly. The proportion of ^{65}Zn remaining in the mothers or transferred to the pups is shown in Table 1. The ^{65}Zn content of mothers and litters (when expressed as a proportion of the amount on day 0 of pregnancy) was significantly higher in the marginal-Zn group ($P < 0·001$) compared with controls. When the proportional distribution of ^{65}Zn in the pregnant mothers on day 21 of pregnancy between mothers and newborn pups was examined, it was seen that animals fed on a marginal-Zn diet transferred a greater amount of ^{65}Zn to the litters ($P < 0·001$). There were no differences in litter size or mean pup weight from mothers fed on the control or marginal-Zn diet.

Expt 2

The retention of ^{65}Zn in pregnant and non-pregnant rats fed on the control or marginal-Zn diet is shown in Fig. 2. There was a highly significant effect of diet ($P < 0·001$) and pregnancy ($P < 0·001$) on ^{65}Zn retention at day 19 but no effect of diet on food intake or weight gain during the experimental period. As expected, there was a significant effect of pregnancy on food intake ($P < 0·001$). When the ^{65}Zn retention on day 19 was examined separately by *t* tests, there was a significant difference between the pregnant and non-pregnant animals fed on the marginal-Zn diet ($P < 0·001$) but not in those fed on the control diet

Table 4. *Expt 3. Initial (day 0) and final (day 20) maternal weights (g), no. of fetuses per litter, and wet and dry fetal weights (g) for rats fed on a control or marginal-Zn diet for differing lengths of time throughout pregnancy*

(Mean values with their standard errors)

Dietary group†...	1		2		3		4		5	
No. of litters ...	21		19		22		21		19	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial maternal wt	267	3·8	259	4·8	263	3·2	261	4·3	271	4·1
Final maternal wt	404	7·6	398	8·2	402	6·2	391	7·7	415	9·3
No. fetuses per litter	14·6	0·64	14·4	0·77	13·8	0·63	13·7	0·60	14·3	0·84
Wet fetal wt	3·38[a]	0·065	3·45[a]	0·053	3·59[b]	0·049	3·59[b]	0·046	3·63[b]	0·057
Dry fetal wt	0·452[a]	0·010	0·465[a]	0·008	0·486[b]	0·007	0·500[b]	0·007	0·503[b]	0·008

[a, b] Values in the same row with different superscript letters are significantly different ($P < 0·05$).

† Group 1, control diet throughout pregnancy; group 2, marginal-Zn diet throughout pregnancy; group 3, control diet first trimester, marginal-Zn diet last two trimesters; group 4, control diet first two trimesters, marginal-Zn diet last trimester; group 5, control diet first and second trimesters and first 3 d of third trimester, marginal-Zn diet last 4 d of third trimester.

Table 5. *Expt 3. Composition of litters from rats fed on a control or marginal-Zn diet for differing lengths of time throughout pregnancy*

(Mean values with their standard errors)

Dietary group† ...	1		2		3		4		5	
No. of litters ...	21		19		22		21		19	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Water (g/kg)	866[a]	0·8	865[a]	0·9	865[a]	0·6	861[b]	1·2	862[b]	0·5
Fat (mg/g dry wt)	82·2	1·03	81·4	0·75	82·8	0·82	81·5	0·89	83·5	0·99
Protein (mg/g dry wt)	446·3	4·43	452·2	4·93	456·6	4·75	450·4	4·52	436·8	4·34
Zn (μg/g dry wt)	124·9[a]	1·12	111·4[b]	1·14	111·2[b]	1·41	114·0[bc]	1·22	115·5[c]	1·53

[a, b, c] Values in the same row with different superscript letters are significantly different ($P < 0·05$).

† Group 1, control diet throughout pregnancy; group 2, marginal-Zn diet throughout pregnancy; group 3, control diet first trimester, marginal-Zn diet last two trimesters; group 4, control diet first two trimesters, marginal-Zn diet last trimester; group 5, control diet first and second trimesters and first 3 d of third trimester, marginal-Zn diet last 4 d of third trimester.

Table 6. *Expt 3. Weight, fat and Zn concentration of maternal livers from rats fed on a control or marginal-Zn diet for differing lengths of time throughout pregnancy*

(Mean values with their standard errors)

Dietary group† ...	1		2		3		4		5	
No. of litters ...	21		19		22		21		19	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Wet liver wt (g)	18·61[a]	0·56	17·44[ab]	0·58	16·99[b]	0·48	16·22[b]	0·64	16·44[b]	0·51
Dry liver wt (g)	5·60[a]	0·18	5·16[ab]	0·20	4·95[b]	0·16	4·64[b]	0·20	4·69[b]	0·15
Fat concentration (mg/g dry wt)	155·0[a]	4·4	173·5[ab]	10·1	—	—	185·6[b]	8·1	—	—
Zn concentration (μg/g dry wt)	75·5[a]	1·1	77·4[ab]	1·6	80·7[b]	1·2	86·5[c]	1·7	87·3[c]	1·4

[a, b, c] Values in the same row with different superscript letters are significantly different ($P < 0·05$).

† Group 1, control diet throughout pregnancy; group 2, marginal-Zn diet throughout pregnancy; group 3, control diet first trimester, marginal-Zn diet last two trimesters; group 4, control diet first two trimesters, marginal-Zn diet last trimester; group 5, control diet first and second trimesters and first 3 d of third trimester, marginal-Zn diet last 4 d of third trimester.

Table 7. *Expt 4. Maternal and litter ^{65}Zn (proportion of amount present on day 0 of pregnancy) in rats fed on the control diet throughout pregnancy*

(Mean values with their standard errors)

Dietary group† ...	1 (*n* 10)		2 (*n* 11)		3 (*n* 10)	
	Mean	SEM	Mean	SEM	Mean	SEM
^{65}Zn in mothers (proportion)	0·567[a]	0·0071	0·554[a]	0·0062	0·531[b]	0·0047
^{65}Zn in litters (proportion)	0·044[a]	0·0043	0·052[a]	0·0035	0·069[b]	0·0038

[a, b] Values in the same row with different superscript letters are significantly different ($P < 0·05$).

† Group 1, killed on day 20 of pregnancy; group 2, killed just after birth; group 3, killed 72 h after birth.

as shown in Table 2. There was a significant interaction ($P < 0·05$) between diet and pregnancy on ^{65}Zn retention on day 19. The increase in retention due to a marginal-Zn diet was 16% greater for pregnant than for non-pregnant rats. The pregnant rats had significantly larger livers ($P < 0·001$) and the total liver Zn was increased in pregnancy ($P < 0·001$) but unaffected by diet. The liver ^{65}Zn content was higher in rats fed on a marginal-Zn diet ($P < 0·001$) and slightly reduced in pregnancy ($P < 0·05$). Bone Zn was unaffected by diet or pregnancy but bone ^{65}Zn was significantly lower in rats fed on a marginal-Zn diet ($P < 0·001$). The ^{65}Zn and total Zn contents of 20-d-old fetuses and placentas are shown in Table 3. There were no differences in numbers of fetuses per litter or mean fetal weights in the control and marginal-Zn groups. However, the mean placental weight was significantly greater ($P < 0·001$) in the marginal-Zn groups. The ^{65}Zn content of the litters (but not the placentas) was significantly higher ($P < 0·01$) and the Zn concentrations of both fetal and placental tissue were lower ($P < 0·001$) in the animals fed on the marginal-Zn diet than in the controls.

Expt 3

The mean weights of the rats in some groups were slightly different at the beginning of the experiment and therefore there were some differences between the groups in food intake and weight gain. However, when the results were expressed as food conversion efficiency (weight gain divided by food eaten) these differences disappeared. Values for maternal and fetal weights and litter sizes are shown in Table 4. Analysis of variance showed no differences between the groups in initial or final maternal weights, or litter size. There was a significant effect of diet on wet and dry fetal weights ($P < 0·01$): when compared with the control-fed animals the mean fetal weights were highest in rats fed on a marginal-Zn diet during the last 4 d of pregnancy (group 5) followed by those fed on a marginal-Zn diet during the last trimester (group 4) and finally those fed on a marginal-Zn diet during the last two trimesters (group 3). There were no significant differences between fetal weights from mothers fed on a marginal-Zn diet throughout the whole of pregnancy (group 2) and those fed on the control diet (group 1).

The composition of the fetuses is shown in Table 5. There was a significant effect of diet on Zn content ($P < 0·001$), but no effect on the proportion of fat and protein in the fetuses. All mothers given a marginal-Zn diet for any length of time during pregnancy produced fetuses with a lower concentration of Zn, and those given a marginal-Zn diet at the end

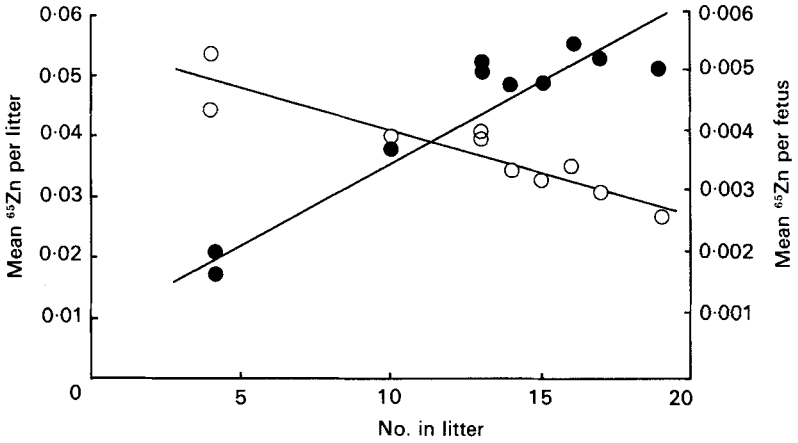

Fig. 3. Expt 4. Effect of litter size on amount of ^{65}Zn in litters and individual fetuses (proportion of ^{65}Zn present in mothers on day 0 of pregnancy): (●), mean litter ^{65}Zn; (○), mean fetal ^{65}Zn. Correlation between litter size and (a) total litter ^{65}Zn is 0·90 ($P < 0·01$), (b) individual fetal ^{65}Zn is −0·92 ($P < 0·01$).

of pregnancy (groups 4 and 5) produced fetuses with a lower water content ($P < 0·01$), although such small differences in water are unlikely to have any biological significance.

Maternal liver weights, fat content (of groups 1, 2 and 4) and Zn content are shown in Table 6. Mothers given a marginal-Zn diet during the last 2 weeks, 1 week or 4 d of pregnancy had significantly smaller livers than those fed on a control diet throughout the whole of pregnancy. There was no difference between groups in total fat content but the fat concentration was significantly higher in the group given a marginal-Zn diet during the last week of pregnancy. Total liver Zn content was no different but the Zn concentration was higher in the animals with smaller livers, i.e. groups 3, 4 and 5.

Expt 4

There were no significant differences in the numbers of pups per litter between the groups killed 24 h before birth, immediately after birth and 72 h after birth. The maternal and litter ^{65}Zn (expressed as a proportion of the amount present on day 0 of pregnancy) are shown in Table 7. There were no significant differences in maternal or litter ^{65}Zn just before or just after birth, but 72 h later the maternal ^{65}Zn had decreased and the litter ^{65}Zn had increased significantly.

The relation between the number of fetuses in the litter and the ^{65}Zn content of the litter and of each fetus was examined further. It was found that as litter size increased, total litter ^{65}Zn also increased (r 0·90, $P < 0·01$) but individual fetal ^{65}Zn fell (r −0·92, $P < 0·01$), as shown in Fig. 3. As litter size influenced ^{65}Zn retention it was included as a covariate in the analysis of variance which demonstrated a highly significant effect of time after birth ($P < 0·001$) on ^{65}Zn retention.

DISCUSSION

Zn deprivation is a risk factor for the outcome of pregnancy, causing teratogenesis (Hurley, 1981), growth retardation (McKenzie *et al.* 1975), prolonged parturition (Apgar, 1975), delivery complications (Golub *et al.* 1984) and lowered Zn status in the offspring (Fosmire *et al.* 1977). In many studies of Zn deprivation it is impossible to separate the direct effect of Zn deficiency from the secondary effect mediated by reduced food intake, which is particularly severe just before delivery (Apgar, 1975). The anorexia associated with low-Zn

diets only occurs when the dietary Zn drops to a very low level, and was not observed in any of the studies described in the present paper because the Zn concentration, although inadequate at 10 μg Zn/g diet, was high enough to prevent anorexia. Thus the effects observed were the direct result of insufficient dietary Zn, and the results of these experiments are more likely to be relevant to humans consuming marginal-Zn diets than studies where the Zn is reduced to extremely low levels of 1 μg Zn/g diet or less.

Expts 1 and 2 demonstrate the speed with which rats can reduce body Zn turnover in response to a low-Zn diet by using ^{65}Zn to label the metabolically-active pools of Zn in the body. Both pregnant and non-pregnant rats retained significantly more ^{65}Zn (whether introduced into the animal enterally or intramuscularly) within 48 h of changing their diet from control to marginal-Zn. Pregnancy itself had only a small effect on ^{65}Zn turnover, since the pregnant and non-pregnant rats exhibited similar ^{65}Zn turnover curves (Fig. 2) until nearly the end of pregnancy. Swanson *et al.* (1983) found that pregnant rats did not increase ^{70}Zn absorption compared with non-pregnant rats, when given adequate levels of Zn. However, Davies & Williams (1977) showed that Zn absorption by the duodenum was greatly enhanced at later stages of pregnancy in the rat. In the light of accumulating evidence that Zn homeostasis is controlled to a great extent by Zn secretion (Methfessel & Spencer, 1973), it is quite possible that there is enhanced Zn absorption in pregnant rats but in order to maintain a similar net Zn balance to non-pregnant rats on the same dietary Zn level, Zn secretion must also be increased. Since the ^{65}Zn pool was not diluted in the pregnant compared with the non-pregnant rats, it is likely that the major part of the secreted Zn was derived from recently absorbed dietary Zn rather than endogenous ^{65}Zn-labelled body Zn.

More ^{65}Zn was transferred to fetuses and pups of mothers fed on a marginal-Zn diet than those fed on the control diet throughout pregnancy, yet the Zn concentration of fetal and placental tissue was reduced (Expt 2). It appears that under conditions of inadequate dietary Zn supply the pregnant rat can mobilize endogenous Zn from metabolically-active pools such as the liver (Cousins & Failla, 1983) and transfer it to the fetuses. However, the pool is insufficient, even in rats fed on a Zn-adequate diet up to the first day of pregnancy, to provide as much Zn as animals fed on a Zn-adequate diet throughout pregnancy. Williams *et al.* (1977) concluded that no useful store of Zn appeared to exist in pregnant rats fed on a Zn-adequate diet. Litter size influenced the amount of Zn found in each fetus (Expt 4). Rats fed on a Zn-adequate diet were able to increase placental Zn transfer in response to larger litter size but not enough to maintain individual fetal Zn levels at a constant (maximum) value, as seen in Fig. 3. Bone ^{65}Zn was lower in rats fed on a marginal-Zn diet (Expt 2) which indicates that the transfer of mobilizable Zn to the relatively non-mobilizable pool in bone was reduced in order to preserve the Zn in metabolically active pools. There is some evidence that a portion of Zn associated with bone may be metabolically available (Brown *et al.* 1978) but, generally speaking, bone Zn is a relatively unavailable pool since mobilization is dependent on bone resorption and is thus regulated by 1,25-dihydroxycholecalciferol and parathyroid hormone. The stress of pregnancy, even when coupled with a marginal-Zn diet, is not great enough to stimulate the mobilization of bone Zn in response to increased demands.

The response of pregnant dams to a marginal-Zn diet given at different stages of pregnancy (Expt 3) is intriguing and raises many questions concerning the ability of the rat to adapt to a diet containing inadequate levels of Zn. Cerklewski (1982) demonstrated that pregnant rats can adapt over two successive pregnancies to a marginal-Zn diet. Rats were depleted of Zn by feeding a diet containing 12 μg Zn/g diet for 3 weeks before breeding, and then fed on a diet containing 8 μg Zn/g. This resulted in reduced gestational weight gain, litter size, weanling pup weight and serum Zn. The mothers were maintained on a marginal-Zn diet and the procedure repeated. The second pregnancy resulted in an increased

gestational weight gain, litter size and dam serum Zn compared with the first pregnancy. Tibia Zn increased, which again supports the finding of Expt 2 that bone Zn is not available. Cerklewski (1982) suggested that the rats had responded to the marginal-Zn diet by increasing their efficiency to absorb Zn.

The rats in Expt 3 produced significantly larger fetuses when they had been on the marginal-Zn diet for the last 4 or 7 d of pregnancy. The groups that were given the marginal-Zn diet from the start of pregnancy were no different from the control-fed animals and the earlier in pregnancy the introduction of the marginal-Zn diet the less was the effect on fetal weight. The fact that the fetuses were heavier was initially surprising considering the number of reports in the literature linking Zn-deficiency with fetal growth retardation. However, there are a few reports of birth weight in human infants being negatively correlated with maternal plasma Zn (Metcoff *et al.* 1981; Prema, 1981; McMichael *et al.* 1982). Golub *et al.* (1984) found that birth weight and maternal plasma Zn of Rhesus monkeys were negatively correlated in Zn-deficient mothers but positively correlated in controls. It should be noted that the experiments described in the present paper were designed to investigate the effect of marginal-Zn diets on the outcome of pregnancy, whereas most studies of Zn and pregnancy have used diets containing much lower levels of Zn. The reason for the increase in fetal weight coupled with a decrease in liver weight in rats given a marginal-Zn diet towards the end of pregnancy might possibly be explained in terms of altered carbohydrate and lipid metabolism. The reduction in liver weight may be due to reduced glycogen (and water) levels, as found in diabetes, and this is being examined further. The involvement of Zn in insulin production in the β-cells of the pancreas has been known for some time (Scott, 1934), although its exact role is still not clear. Zn-deficient rats have lower plasma insulin and raised free fatty acid levels (Quarterman & Florence, 1972). The reduced insulin level is associated with delayed insulin release from the pancreas rather than enzymic acceleration of insulin degradation in the liver (Hsu *et al.* 1980). The effect of maternal diabetes is usually to enhance the growth rate of the fetus (Ounsted & Ounsted, 1973) resulting in large-for-gestational-age (LGA) infants. Apart from delivery complications, LGA infants from diabetic mothers are more likely to become obese in later life (Vohr *et al.* 1980) and therefore such a condition is undesirable. Even in non-diabetic mothers many of the hormonal changes in pregnancy result in changes in carbohydrate and lipid metabolism that resemble the diabetic state, as reviewed by Williams (1978). Bearing this in mind, the findings of Expt 3 lead to the tentative supposition that the introduction of a marginal-Zn diet during the rapidly-growing phase of pregnancy, without allowing time for adaptation (i.e. increased absorption coupled with reduced excretion) induced a diabetic state in the pregnant rat and this in turn resulted in LGA pups. We are currently investigating the possible involvement of marginal-Zn intakes in the aetiology of the diabetes of pregnancy.

The transfer of Zn at birth was minimal (Expt 4) since the ^{65}Zn content of litters did not increase significantly between day 21 of pregnancy and immediately after delivery. Significant amounts of Zn are transferred during the first 14 d of lactation (Fairweather-Tait *et al.* 1984) and this process starts within the first 3 d of lactation (Table 7). The Zn content of milk declines from day 3 onwards (Kirksey *et al.* 1979) and there is no correlation between maternal Zn intake and concentration of Zn in breast milk (Vuori *et al.* 1980; Moser & Reynolds, 1983). Thus there appears to be no mechanism for transferring extra Zn via breast milk to infants of low-Zn status. This again demonstrates how vital it is for pregnant mothers to receive an adequate supply of dietary Zn throughout the whole of pregnancy.

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