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Zinc deficiency and the zinc requirements of calves and lambs

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1. The effects of changes in zinc intake on weight gain, plasma Zn concentration and the development of clinical lesions of Zn deficiency have been studied in Zn depletion and repletion studies with calves and lambs.

2. A basal diet, the principal components of which are urea, dried egg white, starch, glucose, cellulose and arachis oil has been developed for trace element deficiency studies with ruminants.

3. Weight gain ceased abruptly in both calves and lambs when either the unsupplemented basal diet was given or when Zn supplements provided only 0·05 mg Zn/kg live weight per day. Mean plasma Zn concentrations in these animals fell from pre-experiment values of between 0·8 and 1·2 μg Zn/ml to below 0·4 μg Zn/ml after 1 week on these treatments.

4. Supplements providing 0·2 mg Zn/kg live weight per day were sufficient to maintain a good rate of growth but insufficient to prevent a fall in plasma Zn.

5. Growth arrest occurring within 2 weeks and a rapid fall in plasma Zn occurring within 1 week after Zn supplements were withheld from calves and lambs that had previously received 0·7 mg Zn/kg live weight per day for 6 and 14 weeks respectively indicated that these species have only a limited capacity to store Zn in a form that can be utilized during periods of inadequate Zn intake.

6. Tentative estimates are presented of the Zn requirements of calves maintained on this type of basal diet and the influence of ration composition on Zn availability is discussed.

7. The possible value and the limitations of plasma Zn determination as an aid to the field diagnosis of Zn deficiency are considered.

Zinc deficiency has been extensively studied in non-ruminant animals. Hove, Elvehjem & Hart (1937) first described the lesions of this condition in the rat and later work showed that the deficiency could arise in pigs (Tucker & Salmon, 1955) and poultry (O'Dell, Newberne & Savage, 1958). Few studies have been made however on the nature and occurrence of this deficiency in ruminants.

The first indication that Zn deficiency could arise in grazing cattle came from Guyana where Legg & Sears (1960) found that it was responsible for outbreaks of parakeratosis in cows and yearlings and poor growth in young stock. Haaranen & Hyppola (1961) later described work carried out in Finland in which a syndrome characterized by skin lesions, poor reproductive performance and low milk production in housed dairy cattle responded to Zn therapy.

In the Netherlands, Grashuis (1963, 1964) described a syndrome in stall-fed cattle on 150 farms which he attributed to Zn deficiency. Affected animals had a poor reproductive performance, a low milk yield, developed eczematous scabs and shed hair around the muzzle, neck and tail root and developed oedematous swellings of the hind legs. The hays fed to these animals contained between 20 and 80 ppm Zn (all concentrations quoted in this paper are expressed on a dry-matter basis unless stated otherwise) and the silages used contained between 40 and 70 ppm Zn. Hartmans (1965)

reinvestigated the problem on these farms and vigorously contested Grashuis's findings claiming that the condition was probably attributable to 'poor management' and not to a deficiency of Zn.

Discrepancies between the various descriptions of the clinical lesions of presumed Zn deficiency in the ruminant, coupled with the fact that the diagnosis of deficiency by determination of the Zn content of the diet is made difficult by inadequate knowledge of Zn requirements, prompted us to study the effects of Zn depletion and repletion on calves and lambs maintained under controlled environmental conditions. The effect of different levels of Zn supplement on performance was investigated so that approximate estimates of Zn requirements could be derived. It should be appreciated at the outset that the estimates of requirement so obtained apply only to animals maintained on the semi-synthetic basal diet we have used. This diet was developed from easily obtainable materials low in trace element content so that, with little modification, it is suitable for use in studies with other trace elements. It is used by us as a standard diet for studies of trace element requirements of ruminants in which the effects of substituting components present in rations of a more practical nature are being systematically examined.

Brief reports of part of this work have been published (Mills, Quarterman, Williams & Dalgarno, 1965; Mills, Dalgarno, Williams & Quarterman, 1966).

EXPERIMENTAL

Animals and pre-experiment treatments

Dorset Horn lambs 5–6 weeks of age were used. They were bottle-fed with cow's milk for 14 days and a Zn-deficient basal diet and hay were offered *ad lib.* for the first 24 days, after which lambs were individually housed, hay was withheld and all the animals were given orally a zinc sulphate solution providing 0·7 mg Zn/kg live weight per day for 6 days before the start of the experiment. Supplementary studies on the clinical signs of Zn deficiency were carried out using Suffolk × Greyface and Cheviot lambs similarly treated before Zn depletion.

Experiments on the Zn requirements of calves were carried out with Friesian bull calves 3–6 days old at reception. Calves were offered cow's milk in polythene buckets until they were weaned 4 weeks later. The quantity of milk offered was gradually reduced from 6 to 3 pints daily during this period. Zn-deficient basal diet and hay were offered *ad lib.* for the first 3 weeks of this period, after which hay was withdrawn and drenches providing 0·7 mg Zn/kg live weight per day were given for 6 days before the start of the experiment.

Animals were housed throughout these experiments in pens constructed of wood coated with polyurethane lacquer; all metallic fittings were coated with nylon, and food and water were offered in plastic buckets.

Zn-deficient basal diet. The composition of the basal diet used in these experiments is given in Table 1. Spray-dried egg albumen was obtained from Messrs H. D. Hardie and Co., Edinburgh (Zn content 0·05–0·4 ppm), urea from Scottish Agricultural Industries (Zn content < 0·05 ppm), maize starch and glucose from Brown & Polson,

Glasgow (Zn contents 0·3–0·5 ppm and < 0·05 ppm respectively), and bleached cotton-hull fibre from Cotton Cellulose Ltd, Haslingden, Lancs, and Hercules Powder Co., London (Zn contents 0·6–2·7 ppm). Dicalcium phosphate and calcium carbonate were in the form of BP grade salts; all other salts were analytical reagent grade. Fat-soluble vitamins were in a stabilized form dispersed in gelatin–sugar–starch beadlets (Roche Products Ltd, London). Each batch of mixed diet was analysed for Zn before feeding. During the course of this series of experiments the Zn content of the diet ranged between 0·86 and 1·99 ppm; mean, 1·20 ppm. Diet was offered *ad lib.* Water purified by passage through a mixed-bed ion exchange column (Deminrolit, MB; Permutit Co., London) was provided *ad lib.*

Table 1. *Percentage composition of zinc-deficient basal diet for lambs and calves*

Dried egg albumen	6
Urea	3
Maize starch	38
Glucose	32
Arachis oil	6
Cotton-hull fibre	9
Mineral supplement*	5·4
Vitamin supplement†	0·003

* Composition of 100 g mineral mix: $CaHPO_4.2H_2O$, 40·92 g; $CaCO_3$, 4·08 g; $MgSO_4.7H_2O$, 17·12 g; $KHCO_3$, 14·26 g; NaCl, 22·97 g; $FeSO_4.7H_2O$, 0·28 g; $CuSO_4.5H_2O$, 0·04 g; $CoCl_2.6H_2O$, 0·01 g; $MnSO_4$, 0·30 g; KI, 0·23 mg.

† Composition of 100 g vitamin mix: stabilized vitamin A concentrate (325000 i.u./g), 15·9 g; stabilized cholecalciferol concentrate (100000 i.u./g), 4·8 g; stabilized α-tocopherol concentrate (100 i.u./g), 79·4 g.

Experimental treatments

The conduct of the experiments to be described was governed by frequent appraisal of the clinical condition of experimental animals during Zn depletion and repletion and by the availability of the relatively costly basal diet used. These considerations precluded the use of an inflexible experimental design that was identical for both sheep and calves. Decisions on the duration of depletion and repletion periods were taken as the experiments with each species progressed.

Unless otherwise stated, Zn supplements were given in the form of drenches of an aqueous solution of zinc sulphate containing 5 mg Zn/ml.

Sheep. Lambs were randomized by weight into four groups of four animals. Details of experimental treatments are given in Table 2. The experiment consisted of four periods: period 1 in which groups A, B and C were depleted of Zn on different low Zn intakes; period 2 in which repletion of groups A, B, and C was initiated by giving 0·3 mg Zn/kg live weight per day and during which the effects of previous treatment on the rate of repletion were examined; period 3 in which the Zn intakes were increased to achieve more rapid repletion and in which the effects of giving Zn supplements as a drench were compared with those obtained from the inclusion of Zn in the basal diet; period 4 in which Zn supplements were withheld from groups A–D and the effects of previous treatment on rate of depletion were examined. During periods 1, 2 and 3 the supplementary intake of group D was maintained at 0·7 mg/kg live

weight per day—a level which pilot experiments indicated would maintain a rapid rate of growth and normal plasma Zn concentrations (*c.* 0·8–1·2 μg/ml plasma).

It was originally intended that the depletion period (period 1) should occupy 8 weeks but the rapid decline in condition of lambs in groups A and B caused us to modify this plan and include the repletion studies described above. The comparison of the effectiveness of Zn given in solution as a drench and given as a supplement to the diet during period 3 was achieved by frequent adjustment of the Zn content of the diet fed to group B by adding appropriate quantities of zinc sulphate to ensure that the daily intake of Zn corresponded to that of group C receiving the drench. During this period the concentration of Zn in the diet of group B was increased from 11 to 16 ppm as the Zn intake of group C was raised progressively from 0·4 to 0·5 mg/kg live weight per day to achieve repletion of the plasma Zn pool (see p. 756).

Table 2. *Supplementary zinc intake (mg Zn/kg live weight day unless otherwise stated) of lambs during Zn depletion and repletion studies*

	Period 1, weeks 1–4	Period 2, weeks 5–9	Period 3, weeks 10–15	Period 4, weeks 16 and 17
Treatment group				
A	No supplement	0·3	0·4 (4 weeks) 0·5 (1 week)	No supplement
B	0·05	0·3	11–16 ppm of diet (see p. 753)	
C	0·2	0·3	0·4 (4 weeks) 0·5 (1 week)	
D	0·7	0·7	0·7	

Supplementary observations on the clinical lesions of Zn deficiency were made on four Suffolk × Greyface and four Cheviot lambs given the unsupplemented Zn-deficient basal diet for periods of up to 10 weeks.

Calves. The animals were randomized by weight into four treatment groups. Details of experimental treatments are given in Table 3. From experience gained during pilot experiments with calves, we considered that the period of depletion adopted for calves given the unsupplemented Zn-deficient basal diet should be brief and that animals so treated should be withdrawn from the experiment at an early stage for rapid repletion. Of the fourteen calves available for this experiment only two (group A) were thus allocated a treatment involving extreme depletion on the unsupplemented basal diet. The remaining twelve calves were allocated to form three groups of four animals as indicated in Table 3. During period 1 groups A, B and C were depleted using different levels of Zn supplements to determine the effect of Zn intake on rate of depletion while group D received sufficient Zn (0·7 mg/kg live weight per day) to maintain plasma Zn concentrations at or near their initial value. During period 2 the Zn intakes of groups B and C were raised to examine the effects of previous treatment on rate of repletion as judged by changes in the rate of live-weight gain and plasma Zn concentration. Zn supplements were withheld from group D during period 2 to determine whether the high Zn intake of that group during period 1

had permitted sufficient storage of Zn to protect these animals against rapid depletion when given the Zn-deficient basal diet.

Zn analyses. The Zn content of diet and plasma samples was determined by atomic absorption spectrophotometry. Samples of diet (usually about 1 g) were boiled in 8 ml conc. HNO_3 (sp. gr. 1·42), 1 ml 60% w/v perchloric acid and 0·5 ml 98% w/v H_2SO_4 until a final volume of 0·5 ml was obtained without charring of the digest. All the acids used were of 'lead-free reagent' quality (British Drug Houses, Poole) and acid 'blanks' were carried out with each series of analyses. (Care was taken to adjust heating conditions so that the volume of residual sulphuric acid in the digestion tube was reproducibly constant because the sulphate ion has a mild energy-scattering effect at a wavelength of 213·8 nm at which Zn is determined. The alternative of omitting sulphuric acid from the digestion mixture was not adopted because of the risks of explosive decomposition of the digest that arise if overheating occurs.) Digests were diluted to 10 ml with glass-distilled water and this solution was then drawn into the atomizer of the atomic absorption spectrophotometer (Model A.A.2; Hilger and Watts Ltd, London). An air–acetylene flame was used.

Blood samples were obtained from the jugular vein and collected through stainless-steel needles into heparinized tubes. Plasma was diluted 1:2 with glass-distilled water and was introduced into the atomic absorption spectrophotometer without further treatment.

Table 3. *Supplementary Zn intake (mg Zn/kg live weight day) of calves during Zn depletion and repletion studies*

Treatment group	Period 1, weeks 1–6	Period 2, weeks 7–10
A	No supplement	Removed from experiment
B	0·05	0·3
C	0·2	0·3
D	0·7	No supplement

RESULTS

Zn depletion and repletion of the lamb

Plasma Zn. Changes in the mean plasma Zn concentration of lambs during the experiment are illustrated in Fig. 1, and Table 4 gives details of the mean plasma Zn concentrations attained by each group during the last 3 weeks of periods 1 and 3.

There was an immediate and rapid fall in plasma Zn concentration in lambs of groups A, B and C during period 1 in which they received 0, 0·05 and 0·2 mg supplementary Zn/kg live weight per day respectively. The rate of fall of plasma Zn in group C was slower than that in groups A and B during the 1st week but the low values finally attained by these animals during weeks 2, 3 and 4 were not significantly different between groups. The mean plasma Zn concentration of lambs of group D (0·7 mg Zn/kg live weight) was significantly higher ($P < 0·001$) than that of groups A, B and C during weeks 2–4.

The increase in Zn intake of groups A, B and C during period 2 (0·3 mg supplementary Zn/kg live weight per day in each group) was initially reflected by an increase in plasma Zn concentration, but this increase was maintained for only 3 weeks in groups B and C and for only 2 weeks in group A before values again declined. At no time during this period were there statistically significant differences between the mean plasma Zn concentrations of groups A, B and C, which suggested that the different treatments imposed during period 1 were having no influence on plasma Zn concentrations during period 2. The plasma results for group D, plotted in Fig. 1,

Table 4. *Mean plasma Zn concentration (μg/ml) in lambs in groups A–D during Zn depletion and repletion (details of the Zn supplements given to these animals are presented in Table 2)*

Period	A	B	C	D	SE of differences: A *v.* B, C and D	SE of differences: Among B, C and D
1 (weeks 2–4)	0·35	0·38	0·46	0·92	±0·075	±0·069
3 (weeks 13–15)	0·89	0·97	0·96	1·14	±0·078	±0·072

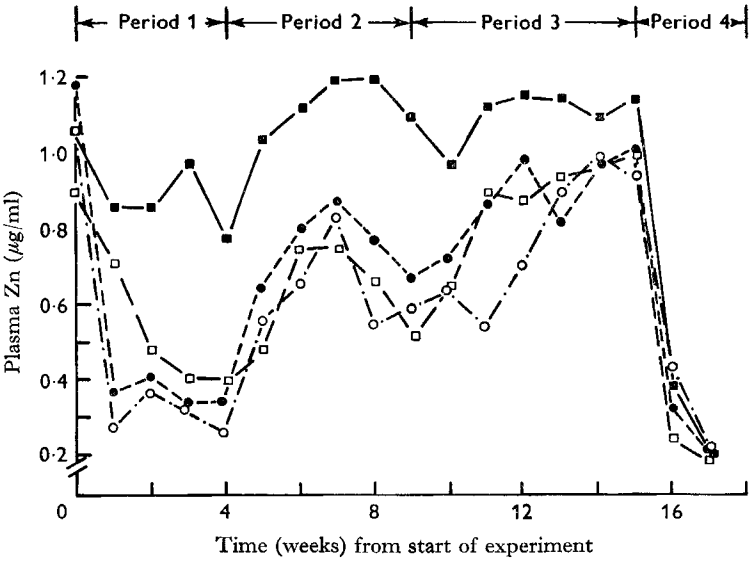


Fig. 1. Changes in group mean plasma Zn concentration of Dorset Horn lambs during Zn depletion and repletion: O—·—O, group A ●— —●, group B; □— —□, group C; ■——■, group D. Treatments during periods 1–4 were as stated in Table 2.

may suggest that a decline in plasma Zn was also taking place in animals of this group at the end of period 1. This was due, however, to an inexplicably low plasma Zn concentration (0·80 μg/ml) found in one animal of this group in the sample taken at the end of week 10 of the experiment. The plasma Zn concentration of group D was significantly greater than those of groups A, B and C throughout period 2 ($P < 0·001$).

After the Zn intakes of groups A, B and C were again increased (period 3) plasma Zn rose until, by week 12, the mean plasma concentrations for these groups were not

significantly different from that of group D that had been maintained on a high Zn intake throughout the experiment. Comparison of the results obtained for group B (Zn given as an aqueous drench) with those for group C (supplementary Zn mixed with the food) showed that the route of Zn administration had no significant effect on plasma Zn concentration.

Table 5. *Mean rates of weight gain (kg/week) of lambs in groups A–D during Zn depletion and repletion (details of the Zn supplements given to these animals are presented in Table 2)*

						SE of differences	
	A	B	C	D	SD	A *v.* B, C and D	Among B, C and D
Period							
1	0·35	0·52	1·56	1·76	±0·42	±0·32	±0·30
2	1·47	1·60	2·05	1·94	±0·27	±0·21	±0·19
3	1·37	1·60	1·57	1·65	±0·31	±0·23	±0·22
4	−0·50	−0·67	−0·30	−0·65	—	—	—

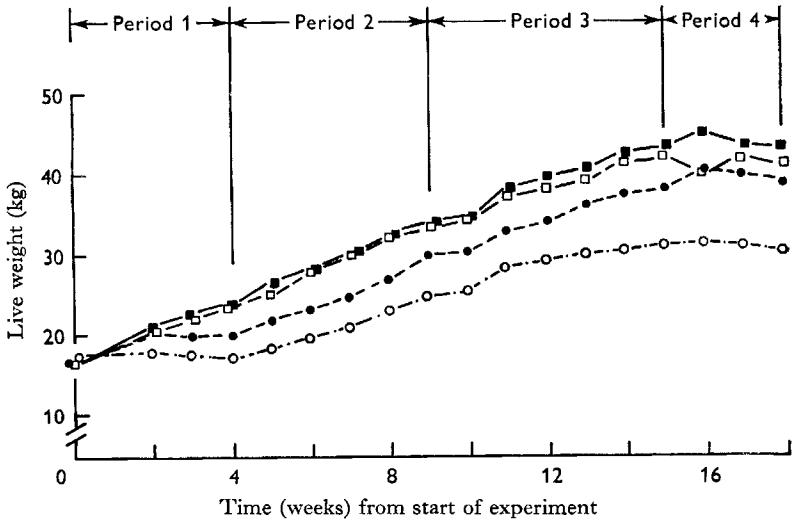


Fig. 2. Changes in live weight of Dorset Horn lambs during Zn depletion and repletion: ○—·—○, group A; ●— —●, group B; □— —□, group C; ■——■, group D. Treatments during periods 1–4 were as stated in Table 2.

There was a significant linear trend in the weekly values for plasma Zn for group D during weeks 1–14 ($P < 0·01$). Values increased from a mean concentration of 0·86 μg/ml at week 0 to 1·15 μg/ml at week 15. In addition to the linear trend, there were other significant differences of an erratic nature from one week to another ($P < 0·01$) within this group. We are unable to explain these effects.

Zn supplements were withheld from all animals from the start of period 4. Plasma Zn fell dramatically in all groups at a rate closely similar to that found in groups A and B at the start of period 1. No effects of previous treatment on the rate of decline were apparent.

Weight changes. Results obtained from weekly weighing of lambs are presented in Fig. 2; mean results for each period are given in Table 5.

During period 1 lambs of groups C and D (0·2 and 0·7 mg Zn/kg live weight per day respectively) grew significantly faster than those of groups A and B (no Zn and 0·5 mg Zn/kg live weight) ($P < 0·001$). Within 2 weeks of the start of this period lambs of groups A and B had ceased gaining weight (Fig. 2) and rapidly declined in condition.

Although lambs of groups A, B and C all received equivalent daily quantities of Zn during period 2 (0·3 mg Zn/kg live weight) the mean rate of weight gain of group C remained significantly higher than that of groups A and B ($P < 0·05$), indicating that severe depletion during period 1 was influencing later performance. It was only during the first half of period 2 that the growth of lambs of group B was retarded; their performance later in this period was not significantly different from that of groups C and D.

The effects of severe depletion of group A early in the experiment may also have had an adverse effect on the weight gain of this group during period 3 (see Table 5) but, in contrast to period 2, differences between group A and groups B, C and D were no longer statistically significant. It should also be noted that although the plasma Zn concentrations of all the animals rose during this period in response to the increased intake of Zn (see p. 753) there was no evidence of any increase in the rate of weight gain in response to this treatment. Comparison of the results for groups B and C during period 3 showed that differences in the route of Zn administration had no significant effect on rate of gain.

The growth rate of the lambs of group D receiving the same treatment throughout (0·7 mg Zn/kg live weight per day) did not deviate significantly from linearity during periods 1, 2 and 3. In no period was the rate of gain of group D significantly greater than that of group C.

Zn supplements were withheld from all the animals from the start of period 4. Weight gain ceased abruptly in all animals 1 week after the start of this period. There was no evidence that previous treatments influenced the rate of weight loss that subsequently occurred (see Fig. 2).

Gross clinical signs of Zn deficiency. All the lambs of group A (no supplementary Zn) and group B (0·05 mg Zn/kg live weight per day) started to produce excessive quantities of frothy saliva by the 12th day of period 1. This was a transitory phenomenon seen only during periods of 5–10 days in different animals. A pronounced pallor of the tongue developed in all animals of these two groups and this effect was associated with a marked increase in the bacterial flora of the mouths of these lambs, details of which will be reported elsewhere (G. Mann & R. Summers, personal communication).

By the 17th day of period 1 lambs of group A were losing hair around the mouth and eyes, and the wool became loose and was easily pulled from the skin. These changes were also apparent, but to a less marked degree, in group B at this time.

When the Zn intake of groups A and B was increased to 0·3 mg Zn/kg live weight per day during period 2 the tongues of affected animals returned to normal within 14 days and all other clinical signs of deficiency disappeared by 21 days.

Lambs of groups C and D appeared clinically normal throughout period 1. Lesions

of Zn deficiency appeared in all groups during depletion in period 4. The first signs of deficiency appeared within 10–12 days and the time of appearance of lesions bore no relation to the nature of experimental treatment in previous periods.

In supplementary experiments with Suffolk × Greyface and Cheviot lambs longer periods of depletion were employed to study the progressive development of lesions of Zn deficiency. In these animals loss of hair around mouth and eyes (Pl. 1 *a*, *b*) was later followed by the development of hyperkeratosis of the skin in these areas. After 5 weeks of depletion, hyperkeratosis of the skin of the legs developed, appearing first immediately above the coronary border of the hoof and over the carpal and tarsal joints. Severe lesions later developed on the skin overlying the metacarpus (Pl. 2 *a*, *b*). Changes in the structure of the hooves of Zn-deficient lambs became evident after 6 weeks of depletion (Pl. 2 *b*). Pronounced lateral distortion of the hoof walls took place presumably as a consequence of defective hardening of hoof keratin. It is noteworthy, however, that the rate of keratin production, as judged by the rate of hoof elongation, appeared to be unimpaired. The nature of these changes is being examined in greater detail. A similar defect in keratogenesis probably underlies the surprising observation that prolonged Zn depletion of the Cheviot lamb (a breed normally showing no evidence of horn growth) leads to the development of keratinous outgrowths or 'buds' in the position normally occupied by horns in other breeds (see Pl. 2 *c*, *d*). Zn depletion of horned breeds led to the production of soft, deformed horns lacking the typical surface striations of normal horn.

In some, but not all, of the lambs depleted for periods in excess of 6 weeks there was evidence of macroscopic changes in the nature of their wool (Pl. 3 *a*, *b*). Wool fibre emerging from the follicle during the period of low Zn intake was brittle, had no 'crimp', appeared thinner than normal and readily came away from the skin when gently pulled. No regrowth of wool took place in areas from which the fleece had been shed unless Zn supplements were given, when a resumption of fibre growth was detectable on examination of the skin 24 h after giving a single drench of a solution of zinc sulphate providing 1·0 mg Zn/kg live weight.

Zn depletion and repletion of the calf

Plasma Zn. Changes in the mean plasma Zn concentration of calves during depletion and repletion are illustrated in Fig. 3. There was a rapid decline in plasma Zn in calves of groups A, B and C during the first 2 weeks of period 1. The rate of decline in group C (receiving 0·2 mg Zn/kg live weight day) was less than that of groups A and B (no Zn and 0·05 mg Zn/kg live weight respectively) and values for group C remained at a significantly higher level than those for groups A and B throughout the rest of this period ($P < 0{\cdot}01$), overall mean values for weeks 3–6 inclusive being 0·26 μg Zn/ml for group A, 0·20 for group B and 0·40 for group C.

When the amount of supplementary Zn given to groups B and C was increased to 0·3 mg/kg live weight per day from the start of period 2 there was a rapid rise in the plasma Zn concentration in all the animals. By the end of the 3rd week of this treatment the differences in plasma Zn concentration between these two groups were no longer statistically significant (mean for group B, 0·74 μg Zn/ml and for group C,

0·79 μg Zn/ml; SE of difference = ±0·041). At the time the experiment was unavoidably terminated the plasma Zn concentrations of these groups of animals were still rising and were approaching the mean plasma Zn concentrations found in calves of group D during period 1.

Throughout period 1 the mean plasma Zn concentration of calves of group D (0·7 mg Zn/kg live weight per day) remained significantly higher than those of groups A, B or C ($P < 0·001$). There were significant weekly changes in the mean

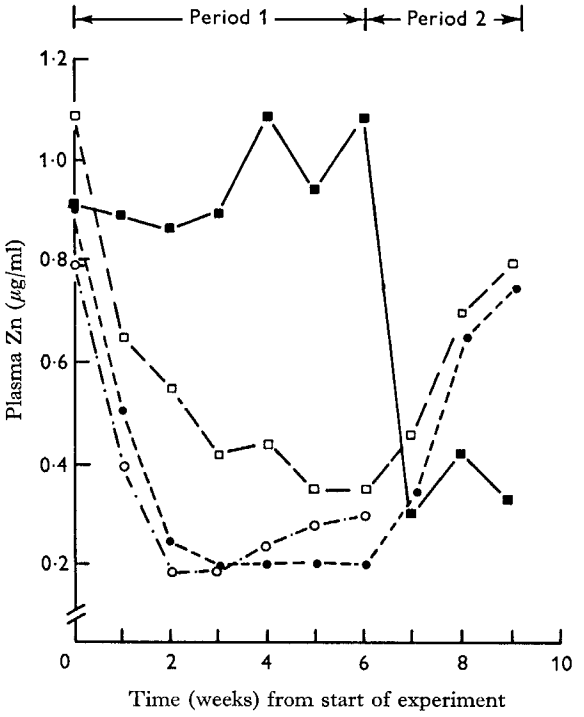


Fig. 3. Changes in group mean plasma Zn concentration of Friesian calves during Zn depletion and repletion: ○—·—○, group A; ●— —●, group B; □— —□, group C; ■——■, group D. Treatments during periods 1 and 2 were as stated in Table 3.

plasma Zn concentration of this group ($P < 0·001$, range of weekly mean values 0·86–1·21 μg Zn/ml; SE of difference of weekly mean values = ±0·043). These changes were erratic in nature and we have been unable to determine their cause. When Zn supplements were withheld from this group during period 2 there was an abrupt fall in plasma from a mean initial value of 1·16–0·25 μg Zn/ml within 1 week. This rate of fall was similar to that found in calves of group A at the start of period 1.

Weight changes. Results obtained from weekly weighing of calves are illustrated in Fig. 4; results for individual periods of the experiment are summarized in Table 6. Although mean values are presented for the performance of group A (no Zn) only two animals were allocated to this group (see p. 754) and thus results from this group could not be included in the statistical analyses of treatment effects.

Table 6. *Mean rates of weight gain (kg/day) of calves in groups A–D during Zn depletion and repletion (details of the Zn supplements given to these animals are given in Table 3)*

	A	B	C	D	SE of differences among A, B and C
Period 1					
Weeks 1 and 2	0·35	0·58	0·61	0·44	±0·10
Weeks 3–6	0·04	0·21	0·65	0·54	±0·11
Period 2					
Weeks 7 and 8	—	0·86	0·72	1·23	±0·18
Weeks 8–10	—	0·49	0·41	−0·44	±0·17

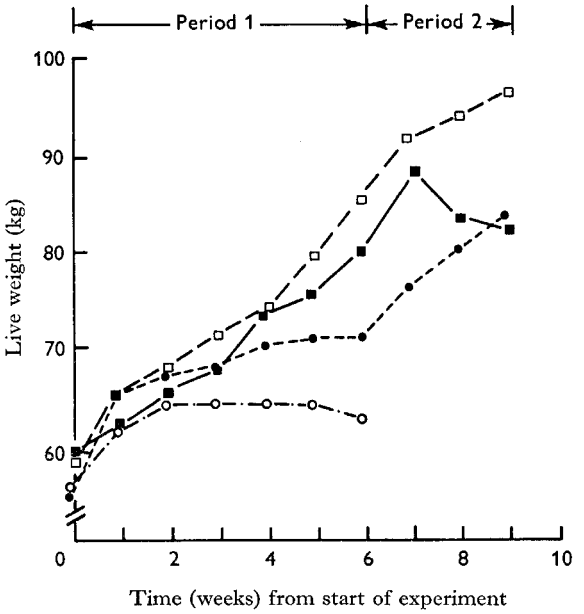


Fig. 4. Changes in live weight of Friesian calves during Zn depletion and repletion: ○—·—○, group A; ●— —●, group B; □— —□, group C; ■——■, group D. Treatments during periods 1 and 2 were as stated in Table 3.

Differences in the rates of weight gain between groups B, C and D during the first 2 weeks of period 1 were not statistically significant. During the following 4 weeks of period 1 the two calves of group A ceased gaining weight and the mean rate of gain of group B (0·05 mg Zn/kg live weight per day) during this period was significantly less than those of groups C and D receiving 0·2 and 0·7 mg Zn/kg live weight respectively ($P < 0·05$). At no time during period 1 were the differences between the rates of gain of groups C and D statistically significant.

Increasing the Zn intake of group B from the earlier level of 0·05 mg to 0·3 mg Zn/kg live weight at the start of period 2 immediately increased the rate of weight gain. From measurements of food consumption it appeared possible that at least a part of the gain during the 1st week of this period might have been caused by an increase

in digestive tract 'fill'. Changes in weight during the 1st week of this period were accordingly excluded from the statistical analysis of treatment effects. The rate of gain of group B during the remainder of period 2 was found to be significantly greater than that during period 1 ($P < 0.05$). This response did not occur in group C in which the daily Zn intake was only increased from the earlier level of 0·2 mg to 0·3 mg/kg live weight during period 2.

Calves of group D continued to gain weight for 1 week after the Zn supplement was withdrawn in period 2 but the weight of all animals in this group declined sharply thereafter.

Gross clinical signs of Zn deficiency. Calves of group A showed the first clinical signs of deficiency 16 days after the start of depletion in period 1. Deficiency signs appeared in group B after 21 days of treatment. In each instance the lesions of Zn deficiency appeared progressively in the following order: (1) excessive salivation (Pl. 4*a*), (2) loss of hair around eyes and mouth (Pl. 4*b*), (3) hyperkeratosis of the skin of the neck, heel of the mandible and inside the legs, (4) enlargement of the hock bones (Pl. 4*c*), and (5) the appearance of a continual fidgeting and stepping movement of the hind legs. The general appearance of typical calves from groups A and B at the end of period 1 is illustrated in Pl. 5*a* and 5*b* respectively in which the effects of Zn deficiency on the development of a 'staring' hair coat, hyperkeratosis of the skin and hoof changes similar to those described previously for lambs can be seen. Pl. 5*c* illustrates the superior condition of a typical calf of group C which received 0·2 mg Zn/kg live weight per day during period 1.

DISCUSSION

Probably the most outstanding feature of the experiments was the speed with which Zn deficiency developed in the lamb and the calf when rations low in Zn content were given. Figs. 1 and 3 illustrate the rapid decline in plasma Zn concentration when Zn supplements were withheld. Experiments carried out when these reported here were complete show that the greater part of the decline in plasma Zn concentration that takes place during the 1st week in which a calf or sheep of normal Zn status is given a Zn-deficient diet occurs within 36 h of the decrease in Zn intake. This situation contrasts markedly with that noted with most of the other trace metals. With these it is usual to find that a rapid fall in the trace metal content of the plasma is prevented by withdrawal of the metal from tissue stores. As these stores become depleted, so does the trace metal content of the plasma fall. Equilibrium between plasma and presumed stores is not attained with sufficient rapidity when Zn is removed from the diet to prevent a dramatic fall in plasma Zn content. From the experiments of Miller & Miller (1962) in which Zn deficiency was produced in calves by giving rations containing 3·6 ppm Zn it appears that changes in Zn concentration in whole blood were less marked than those observed in plasma Zn in the present experiments. In their experiments clinical signs of Zn deficiency developed in 11 weeks while Zn in whole blood fell from 2·7 to 1·9 μg/ml. This contrasts with the fall in plasma content from an initial value of 0·9 to 0·2 μg/ml 2 weeks later in calves of

group B that had approximately the same daily Zn intake as those of Miller & Miller (1962). These rapid changes in plasma Zn concentration in both the sheep and the calf are particularly interesting since previous studies with ruminants (Miller & Miller, 1962; Ott, Smith, Stob & Beeson, 1964) and with rats (Millar, Fischer, Elcoate & Mawson, 1958) have shown that, with the exception of the pancreas and possibly bone, the Zn content of body tissues changes but little during Zn depletion.

From our studies with lambs and calves it appears that if the plasma concentration of Zn falls below about 0·4 μg/ml and remains below this figure for more than 1 week growth is arrested and, later, clinical signs of Zn deficiency develop. In the groups of calves and lambs given 0·7 mg Zn/kg body-weight per day, group mean values for plasma Zn ranged from 0·86 to 1·20 μg/ml during the experiments in both species. Ott, Smith, Stob, Parker, Harrington & Beeson (1965) suggested that 'the optimum *serum* Zn concentration associated with maximal gains appears to be above 100 μg/100 ml'. Their conclusion from work with sheep contrasts markedly with our finding during period 1 of the experiments with calves and sheep that an intake of 0·2 mg Zn/kg body-weight per day maintained a rate of growth that was not significantly different from that attained by calves receiving 0·7 mg/kg even though the former group of animals had suffered an appreciable depletion of plasma Zn, the mean concentration during weeks 3–6 of period 1 being 0·40 μg/ml in the calves and 0·46 μg/ml during a similar time in the sheep.

These observations that good rates of growth may be maintained even when plasma Zn concentrations are low suggest that plasma Zn determinations may have only a limited value for the detection of Zn deficiency. Arising from the observations of the rapidity with which plasma Zn concentration reflects the immediately preceding Zn intake, it is our opinion that, to be of diagnostic value, plasma Zn determinations should be carried out on at least two occasions separated by an interval of 1 week or longer and that values should be below 0·4 μg/ml before Zn deficiency can be reasonably suspected on the basis of this evidence alone.

In our experiments the development of clinical lesions in calves and lambs followed the same sequence in every Zn-deficient animal. This is in contrast to the finding of Ott *et al.* (1964) that the sequence was not constant between lambs. It is possible that this difference may be a reflection on the fact that our basal low-Zn diet contained only between one-half and one-third of the Zn of the deficient basal rations of other workers, a difference which led to the more rapid and severe development of Zn deficiency in our experiments. This difference may again be reflected in the more marked defects in the development of hoof and horn keratin than have apparently been observed by others. The sequence in which lesions of hyperkeratosis developed on different parts of the body in our experimental animals was also uniform between individuals. It should however be stressed that this sequence was not identical for the lamb and the calf; nor is the sequence identical in all details with that reported by other workers who have studied the effects of Zn deficiency in these species. It is possible that these differences may be the result of differences between experiments in the conditions of management and housing of experimental animals. From experiments in which skin damage was inflicted and was shown to initiate the development

of parakeratosis in Zn-deficient calves, Miller, Morton, Pitts & Clifton (1965) have suggested that local trauma initiated by abrasion during animal handling and confinement may initiate this lesion. Our observations support this suggestion.

Our results fully support the findings of Miller & Miller (1962) and Ott *et al.* (1964, 1965) that the earliest manifestation of Zn deficiency is failure to grow. In the young animal the other signs of Zn deficiency develop later, but it has been our experience with older (6–9 months old) animals that the rate of appearance of clinical lesions of Zn deficiency is greatly reduced; indeed lesions may not appear at all. However, in these older animals, which were studied during earlier development trials, the rate of fall of plasma Zn concentration and the speed with which growth was arrested on giving a Zn-deficient diet (containing 1–2 ppm Zn) was just as dramatic as in the studies with young lambs and calves reported in detail here. It thus appears probable that the growth and development of the ruminant may in some circumstances be restricted by a deficiency of Zn without the animal exhibiting any of the characteristic clinical lesions of Zn deficiency.

Zn requirements of lambs and calves

In most of our experiments we gave Zn supplements in the form of a drench so that the daily intake of the element could be stated with greater certainty. This procedure suffers from the limitation that comparison of estimates of Zn requirements obtained from these trials with those obtained by other workers who have presented tentative estimates of requirements expressed as a dietary concentration of this element involves the assumption that the utilization of Zn salts given as an aqueous drench is the same as that observed when the same soluble Zn salts are mixed with the ration. That this assumption is valid, at least for Zn given in the form of zinc sulphate, is shown from the results obtained during period 3 of the experiments with lambs in which the same quantity of Zn was given daily in the form of a drench to lambs of group C and mixed with the diet given to group B. The performance of these two groups of animals was identical as judged from changes in plasma Zn concentration and rate of weight gain. Accordingly we consider it is valid to present estimates of 'equivalent dietary concentrations' of Zn from records of mean daily food consumption and known daily intakes of Zn given as a drench during different experimental periods.

For the purpose of these calculations the concentration of Zn in the unsupplemented basal diet was assumed to be 1·20 μg/g (see p. 753) and estimates of equivalent dietary Zn concentration were based upon the mean daily food consumption and Zn intake during the last week of each experimental period.

In the experiments with lambs, the Zn given to animals of group C, during period 1, equivalent to a dietary concentration of 7·1 ppm, was adequate to maintain a rapid rate of gain not significantly different from that of lambs of group D given three times as much Zn. As has been previously pointed out, however, the Zn intake of group C lambs did not prevent a decline in plasma Zn concentration, although not to the low concentrations found in the plasma of lambs of groups A and B that developed clinical signs of deficiency during period 1. During period 3 the increased quantity of

Zn supplement given to lambs of group C corresponded to a dietary concentration of 14·7 ppm and during this period plasma Zn concentration steadily rose to concentrations not significantly different from those found in lambs of group D. From these estimates it is suggested that with the basal diet used in these studies the Zn requirement for growth of the lamb is not greater than 7 ppm and the equivalent dietary concentration required to maintain plasma Zn levels within the normal range is approximately 15 ppm.

These estimates should be compared with that of Ott *et al.* (1965) who, from experiments with crossbred 'native' lambs, suggested that the Zn requirement for optimal weight gain was between 18 and 33 ppm. In comparing the two studies the most important difference between them appears to be that, though the total nitrogen intakes of experimental animals were very similar, we provided 60% of the nitrogen as urea, whereas Ott *et al.* (1965) used dried egg-white protein as the nitrogen source. Other aspects relating to the possible effect of diet composition on Zn availability will be considered later.

Using the same procedure for calculating equivalent dietary Zn concentration in the study with calves, we conclude that 3·1 ppm Zn is inadequate to maintain a good rate of growth and to prevent a decline in plasma Zn concentration (cf. group B, period 1). An intake of Zn corresponding to 8 ppm in the diet (group C, period 1) was insufficient to maintain a normal plasma Zn concentration and from results obtained later in the experiment (groups B and C, period 2) it appears probable that a dietary concentration between 10 and 14 ppm may be the minimum necessary to maintain the plasma Zn concentration within the normal range. Despite these observations it is noteworthy that, as with the sheep, the minimum dietary concentration required to maintain a good rate of weight increase was less than that required to maintain the concentration of Zn in the plasma. The results obtained during period 1 show that a dietary concentration of 8 ppm Zn (group C) permitted a rate of weight gain of 0·65 kg/day that was not significantly different from that obtained with approximately 25 ppm (group D). This observation agrees well with the suggestion of Miller, Clifton & Cameron (1963) that a concentration of 8·6 ppm Zn in the diet of calves is sufficient to meet requirements for growth.

The effect of ration composition on the Zn requirement of ruminants

With Zn, as with many other trace elements, it is becoming apparent that the composition of the diet may markedly influence requirements. The discrepancy between our results with sheep and those of Ott *et al.* (1965) has been commented upon and differences between the nitrogen sources used in these two studies have already been emphasized. The close agreement between our results with calves and those of Miller *et al.* (1963) and the fact that urea was used as the major nitrogen source in both the studies with cattle supports the suggestion that the form of dietary nitrogen may influence Zn utilization.

Phytic acid, particularly in association with high concentrations of calcium in the diet, has been shown to interfere with the utilization of Zn by rats (Oberleas, Muhrer & O'Dell, 1966), pigs (Oberleas, Muhrer, O'Dell & Kintner, 1961) and poultry

(O'Dell & Savage, 1960) but, from the work of Ott *et al.* (1964), it appears probable that this situation does not occur in ruminants. Pilot experiments in which we failed to produce Zn deficiency in lambs given diets containing 35% soya-bean meal (a rich source of phytic acid) and high in calcium (1·6% Ca as $CaCO_3$) support this finding of Ott *et al.* (1964). We are, however, unable to agree with their later assumption 'that organic factors have no effect on Zn availability for ruminants' (Ott *et al.* 1965) in view of the often wide discrepancies between estimates of Zn requirement based on work with semi-synthetic diets and the concentrations of Zn encountered in pastures and conserved forages in field outbreaks of Zn deficiency in ruminants.

Zn deficiency has been encountered under practical conditions with growing and mature cattle where pasture or fodder has contained 18–42 ppm Zn (Legg & Sears, 1960), 19–83 ppm Zn (Dynna & Havre, 1963) and 28–50 ppm Zn (Haaranen, 1963, values estimated from author's fig. 2), and from field survey work Haaranen (1963) has suggested that the Zn requirement of cows is approximately 45 ppm.

Two striking discrepancies emerge from these points: first, that estimates of requirement based on work with semi-synthetic diets are lower than would be suggested by field surveys of Zn deficiency and, secondly, that the Zn contents of feeding-stuff and pasture found in these field cases are similar to those found in areas where clinical Zn deficiency is unknown and growth of young stock is normal. The possible nature of dietary and environmental factors which influence the requirement of ruminants for Zn is now being investigated.

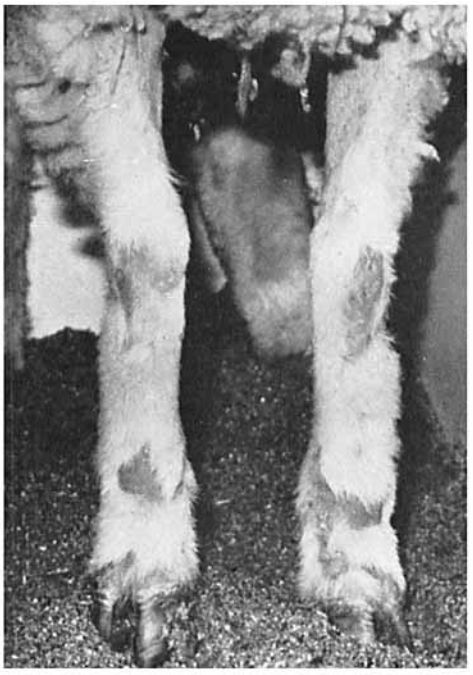
The diagnosis of Zn deficiency in the ruminant

Blackman, Miller & Morton (1967) have recently discussed the problems that exist in the differential diagnosis of Zn deficiency in ruminants and have particularly emphasized the superficial similarity of the clinical lesions of Zn deficiency and those of vitamin A deficiency, of photo-sensitization and of hyperkeratosis arising from their ingestion of traces of chlorinated hydrocarbons. While agreeing with their suggestion that the best diagnostic tool so far available is to note the response of the suspect animal to the administration of Zn supplements, we are of the opinion that the determination of plasma Zn concentrations may be of value in differential diagnosis providing the limitations of this technique are borne in mind.

Our experiments have shown that the plasma Zn concentration rapidly changes to reflect the Zn intake of the animal immediately before sampling. We have also shown that plasma Zn may fall to within the range 0·28–0·65 (mean 0·40) μg/ml in calves which, although receiving low intakes of Zn, were not sufficiently depleted for this to impair growth. A decrease in the rate of weight gain occurred only in those animals in which repeated sampling showed that the plasma Zn concentrations were consistently below about 0·3 μg/ml (the observed range of values in such animals was 0·27–0·15 μg/ml). It is less easy to draw conclusions about the Zn status of the lamb from the results for plasma Zn concentration. Plasma concentrations were in the range 0·45–0·28 (mean 0·36) μg Zn/ml in lambs suffering growth arrest or retardation as a result of a low Zn intake. Although experimental groups making satisfactory gains in

(*a*)

(*b*)



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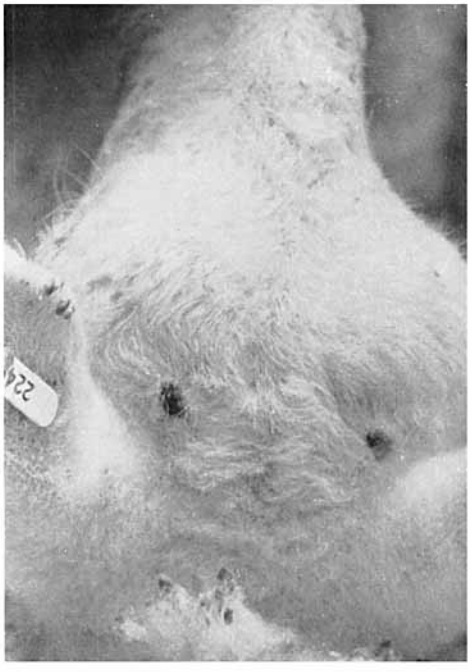

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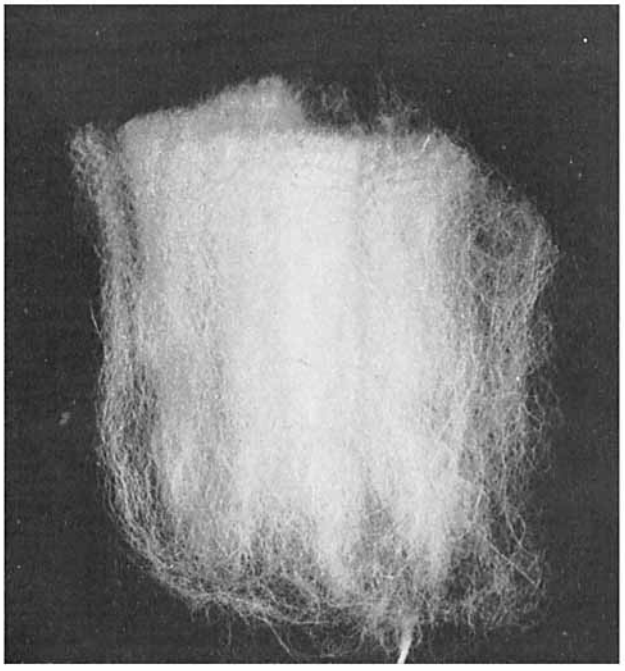

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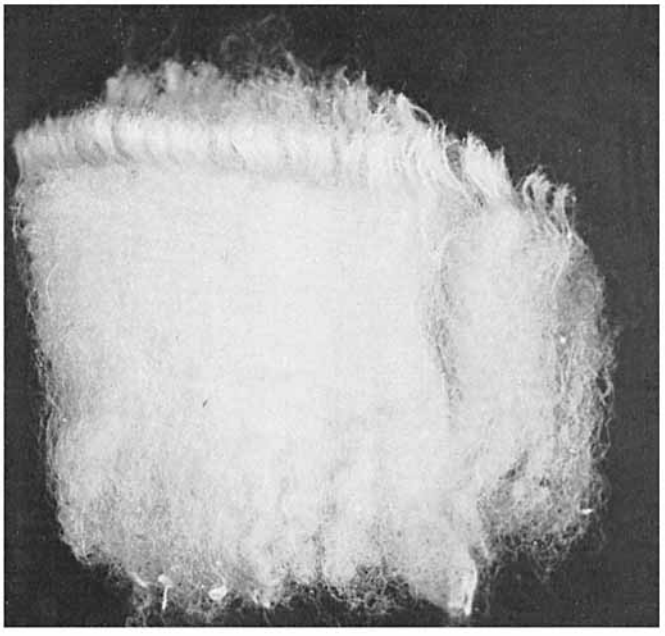

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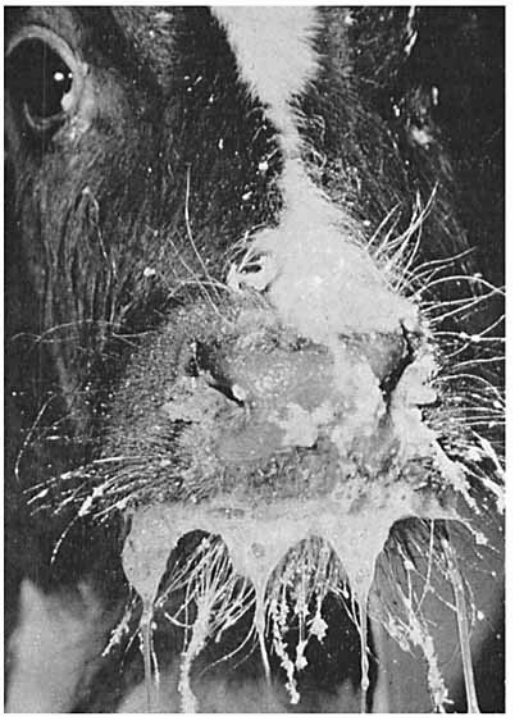

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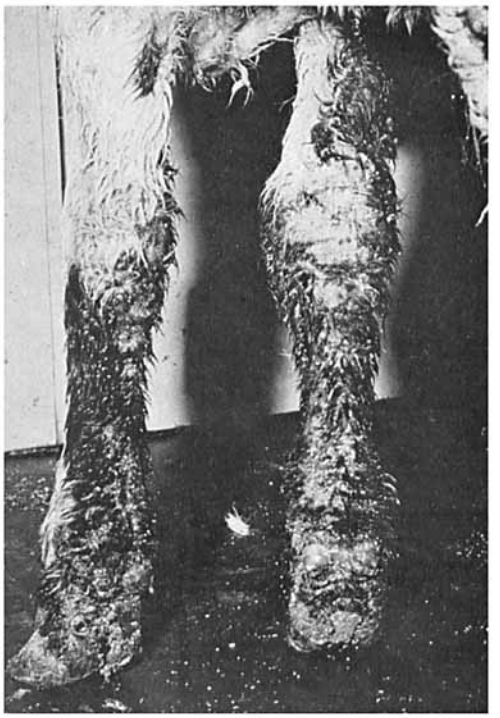

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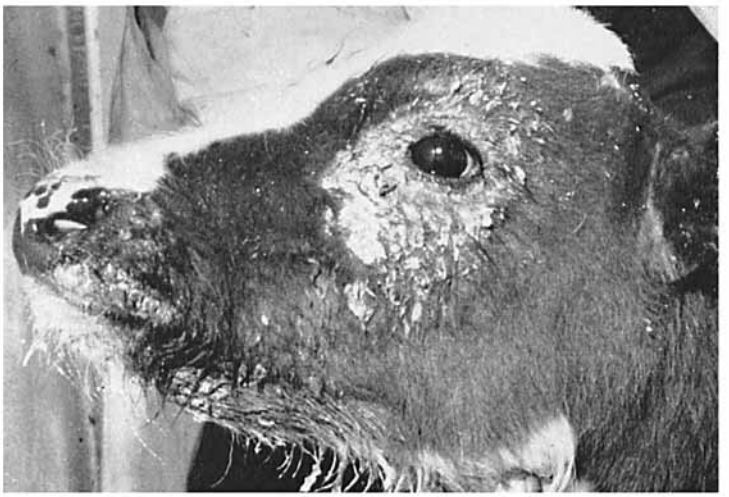

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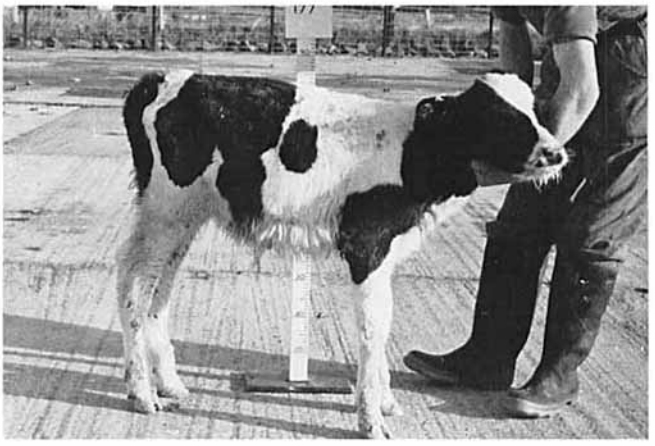

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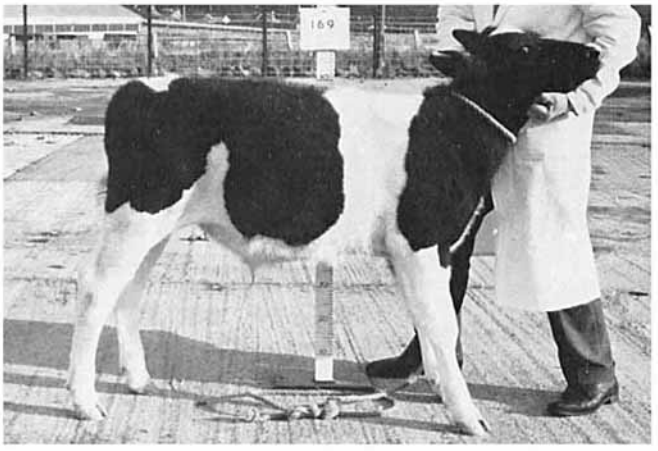

(*c*)

weight on higher Zn intakes had higher mean plasma Zn concentrations, individual animals of one of these groups (group C: mean 0·46 μg/ml) occasionally had values below 0·3 μg/ml at this time.

From these experiments it appears that plasma Zn determination may be valuable as a diagnostic aid, but only if values are repeatedly found to be below about 0·3 μg/ml plasma. Limited experience suggests that plasma Zn determinations may be of particular value with cattle and sheep older than those used in the work described above because growth arrest may be the only clinical indication of deficiency in older animals maintained for several months on rations low in Zn. The pattern of changes in plasma Zn in response to changes in Zn intake appears to be identical in both young and old animals.

We thank Mr C. Milne for his careful work in managing the experimental animals throughout these studies. Thanks are due also to Mr I. McDonald who did the statistical analyses and to Mr W. R. Humphries, Miss E. M. Mecklenburgh and Miss L. Pilling who made trace element analyses. We are also grateful to Roche Products Ltd, London, for providing stabilized vitamins for experimental diets.

EXPLANATION OF PLATES

Plate 1

Pattern of hair loss and early development of skin lesions around the eyes and mouth of a Cheviot lamb during Zn depletion: left, lambs given an unsupplemented Zn-deficient basal diet for 25 days; right, lambs that were receiving the same diet plus a drench of zinc sulphate solution providing 0·7 mg Zn/kg live weight per day.

Plate 2

(*a*) Hair loss on the legs of a lamb given the Zn-deficient diet for 5 weeks. Note that the affected areas are those subject to the greatest extension during joint movement (see p. 763).

(*b*) Hyperkeratosis of skin and hoof deformation in a lamb given the Zn-deficient basal diet for 6 weeks. (*c* and *d*) Head of a Cheviot lamb (*c*) given the unsupplemented Zn-deficient basal diet or (*d*) the diet supplemented to give a Zn intake of 0·7 mg Zn/kg live weight per day. Note formation of keratinous horn 'buds' in the Zn-deficient lamb.

Plate 3

Wool clipped (*a*) from a Cheviot lamb during Zn depletion and (*b*) from a lamb receiving a Zn supplement. Note the absence of 'crimp' and the 'stringy' nature of the fibre produced during Zn depletion.

Plate 4

(*a*) Excessive salivation, (*b*) loss of hair and early skin lesions around the eye and mouth and (*c*) enlargement of hock bones, mild skin lesions and early changes in hoof structure in a Friesian calf given the unsupplemented Zn-deficient basal diet.

Plate 5

General appearance of typical Friesian calves from (*a*) group A (no supplementary Zn), (*b*) group B (0·05 mg Zn/kg live weight per day), and (*c*) group C (0·2 mg Zn/kg live weight per day) after 6 weeks' treatment, i.e. at the end of period 1 (see p. 755). Note: skin, leg and hoof lesions and 'staring' coat of calf of group A, milder lesions in calf of group B and good clinical condition of calf of group C.

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