Effects of Zinc Deficiency on the Wool Growth, Skin and Wool Follicles of Pre-ruminant Lambs

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Abstract

Two groups of 1-month-old pre-ruminant lambs of similar mean liveweights were fed identical liquid milk-replacer diets except that the zinc contents were either 5 μg (deficient diet) or 32 μg per gram of dry matter (control diet). These diets were fed for 4 weeks, after which all the lambs received the control diet for 2 weeks.

In the lambs fed the deficient diet plasma zinc concentration decreased markedly during the first 2 weeks and skin lesions developed around their mouths. Autophagic vacuoles also developed in most follicle bulbs along with a variety of defects in the wool fibres and progressive inhibition of wool growth. Food intake and liveweight increase were not significantly depressed until the third and fourth weeks of feeding the deficient diet. During this period the wool was shed from the zinc-deficient lambs as a result of the fibres being degraded and distorted within thickened outer root sheaths in the distal (upper) parts of the follicles. In addition, the epidermis of the wool-bearing skin became slightly acanthotic and hyperkeratotic, although not parakeratotic.

When the deficient lambs were fed the control diet for 2 weeks, their food intake, liveweight gain and plasma zinc concentration increased to almost those of the control lambs, but their rate of wool growth was still low and the epidermis had not returned to normal. Compared with previous studies the findings of this study suggest that pre-ruminant lambs may be more susceptible to the effects of zinc deficiency than ruminant lambs.

Introduction

Zinc deficiency in rats, piglets and young ruminants results in depressed food intake, decreased rate of gain in liveweight and a reduced efficiency of food utilization (Follis *et al.* 1941; Miller and Miller 1960, 1962; Miller *et al.* 1964; Ott *et al.* 1964, 1965; Mills *et al.* 1967; Somers and Underwood 1969; Underwood and Somers 1969). Accompanying clinical symptoms which develop in calves, lambs, kids and piglets include dermatitis, parakeratotic scabby lesions and alopecia around the eyes and mouth and on the nose, ears, neck, ventral areas, scrotum and legs (Tucker and Salmon 1955; Leucke *et al.* 1956; Miller and Miller 1960, 1962; Miller *et al.* 1964; Ott *et al.* 1964, 1965; Mills *et al.* 1967; Underwood and Somers 1969). In extreme cases the parakeratosis in piglets (Lewis *et al.* 1956) and the alopecia in calves (Ott *et al.* 1965) and lambs (Underwood and Somers 1969) may involve all body regions. Prior to being shed, the wool of lambs may lose its crimp and become brittle (Mills *et al.* 1967). Lambs may also shed their horns (Underwood and Somers 1969). Histological changes in the skin resulting from zinc deficiency include acanthosis, hyperkeratosis and/or parakeratosis of the epidermis, extension of the rete pegs and oedema (Follis *et al.* 1941; Miller and Miller 1963; Ott *et al.* 1964). Disappearance of hair follicles has been reported in rats (Follis *et al.* 1941).

The aim of this study was to investigate the effects of zinc deficiency on skin, mature wool follicles, wool fibres and the maturation of wool follicles. Changes in food intake and liveweight gain are also reported. Pre-ruminant lambs consuming a liquid milk-replacer diet were used because they grow rapidly, and may therefore be more susceptible to zinc deficiency than older animals, and because their skin contains a proportion of immature wool follicles.

Materials and Methods

Lambs and Diets

Nine 1-month-old Merino ram lambs, ranging in liveweight from $5 \cdot 0$ to $6 \cdot 4$ kg, were allocated to two groups of similar mean liveweight. Each lamb was individually confined to a metabolism cage made of stainless steel and high-density polythene and lined with sheets of water-proof plywood. Each cage had an individual plastic feed container connected by plastic tubing to a teat in the cage.

Table 1. Dry matter composition of the zinc-deficient diet[A]

Component	%	Component	%
Sodium caseinate	$24 \cdot 5$	KCl	$0 \cdot 4$
Methionine	$0 \cdot 5$	Citric acid	$0 \cdot 6$
Butter oil	$29 \cdot 5$	Mineral and trace	
Glucose monohydrate	$39 \cdot 4$	element mix[B]	$0 \cdot 65$
$CaCO_3$	$1 \cdot 7$	Vitamin mix[C]	$0 \cdot 13$
KH_2PO_4	$1 \cdot 4$	Choline chloride	$0 \cdot 07$
Na_2HPO_4	$1 \cdot 2$		

[A] The sodium caseinate was dissolved in deionized water at 70°C and homogenized with the other components in a two-stage laboratory homogenizer (Model 15M-8BA, APV Company Ltd, U.K.) operating at a pressure of 17 000 kPa to provide a final concentration of 15% (w/w) dry matter.
[B] Mineral and trace element mix (g/kg of mix): $MgCl_2.6H_2O$, 952; KI, $0 \cdot 10$; $MnCl_2.4H_2O$, $4 \cdot 25$; $CuCl_2.2H_2O$, $2 \cdot 20$; H_2SeO_3, $0 \cdot 03$; $NaMoO_4.2H_2O$, $0 \cdot 07$; $FeSO_4.7H_2O$, $40 \cdot 9$.
[C] Vitamin mix (g/kg of mix): retinol $0 \cdot 17$, cholecalciferol $0 \cdot 01$, D-α-tocopherol $15 \cdot 6$, riboflavin $17 \cdot 0$, pyridoxine $3 \cdot 0$, cyanocobalamin $0 \cdot 015$, menadione $3 \cdot 3$, ascorbic acid $1 \cdot 6$, nicotinamide $24 \cdot 6$, D-calcium pantothenate $11 \cdot 9$, biotin $0 \cdot 3$, thiamin $2 \cdot 6$, pteroylmonoglutamic acid $0 \cdot 01$, *p*-aminobenzoic acid $0 \cdot 37$, inositol 596. The vitamin mix as described was prepared by mixing additional vitamins with Vitamix + Bioten (D.H.A. Rural, Victoria, Australia).

Five lambs were given a zinc-deficient liquid milk-replacer diet (Table 1) containing 5 μg zinc per gram of dry matter (i.e. $0 \cdot 75$ μg zinc/ml), referred to as the deficient diet. The other four were given the same diet supplemented with zinc sulfate to provide 32 μg zinc per gram of dry matter (i.e. $4 \cdot 8$ μg zinc/ml), referred to as the control diet. The lambs were allowed free access to their respective diets for two periods of 1 h each at 0930 and 1630 h, and the amounts consumed by each lamb were recorded. These diets were fed for 28 days, after which all the lambs were fed the control diet for a further 14 days. The lambs were weighed every 7 days.

Samples and Examination

Blood

Blood samples were collected weekly by venipuncture and centrifuged. The plasma was deproteinized with an equal volume of an aqueous 10% (w/v) solution of trichloroacetic acid and centrifuged. The resulting supernatant was aspirated into an atomic absorption spectrophotometer, without further treatment, for zinc determination (AA-375, Varian Tectron Pty Ltd, Melbourne, Australia.)

Wool

Wool was clipped with fine animal clippers from patches (initially 100 cm^2 in area) on the right midside of each lamb every 14 days during the 6 weeks of observation. The wool was degreased in Shell X4 solvent, oven-dried at 70°C for 12 h and weighed. Individual wool fibres were then withdrawn from the degreased samples and mounted with double-sided adhesive tape on scanning electron microscope stubs. The fibres were sputter-coated with gold and examined in an ISI Super IIIA scanning electron microscope.

Skin

A skin sample, 1 cm in diameter, was biopsied from the left midside of each lamb at fortnightly intervals. These samples were fixed in 10% (v/v) buffered formalin, dehydrated in increasing concentrations of ethanol and embedded in paraffin. Sections, 8 μm thick, were cut longitudinal to the follicles and were stained with haematoxylin and eosin for examination of the state of the epidermis and follicles and for subjective assessment of the sizes of the sebaceous and sweat glands by light microscopy.

Statistical comparisons

Statistical comparisons between groups were made using Student's *t*-test.

Table 2. Food intake, liveweight growth rates, plasma zinc concentrations and wool growth rates of the control and zinc-deficient lambs

Values given are means $\pm$ s.e.m. *, *** Significantly different from control at $P < 0.05, 0.001$ respectively

Lambs	Period (weeks)	Food intake (g dry matter/day)	Liveweight growth rate (g/day)	Plasma zinc concn (μg/ml)	Wool growth rate (mg/cm^2.day)
Control	0–2[A]	172 ± 10	120 ± 4	0·74 ± 0·08	0·79 ± 0·08
	2–4[A]	203 ± 13	169 ± 12	0·94 ± 0·03	0·99 ± 0·17
	4–6[B]	203 ± 12	132 ± 13	0·91 ± 0·06	1·17 ± 0·10
Deficient	0–2[A]	156 ± 7	112 ± 17	0·07 ± 0·01***	0·52 ± 0·04*
	2–4[A]	120 ± 6***	20 ± 9***	0·07 ± 0·01***	0***
	4–6[B]	195 ± 13	121 ± 15	0·90 ± 0·02	0·19 ± 0·07***

[A] Four-week period of zinc repletion.   [B] Two-week period of zinc repletion.

Results

Food Intake and Liveweight Gain

Food intake and liveweight gain of the lambs fed the deficient diet both became significantly ($P < 0.001$) lower than those of the control lambs during the second 2-week period (Table 2). During this period the lambs fed the deficient diet consumed a mean of 120 g dry matter per day, which was 59% of the 203 g consumed per day by the lambs fed the control diet. The resultant liveweight increase of 20 g/day was 12% of that of the control lambs (170 g/day). During the third 2-week period, when all the lambs were fed the control diet, both the food intake and liveweight gain of the previously deficient lambs improved markedly and were similar to those of the control lambs.

Plasma Zinc Concentration

Plasma zinc concentration of lambs fed the deficient diet decreased rapidly to a mean value of 0·07 μg/ml plasma during each of the first two periods (Table 2). Transfer of the deficient lambs to the control diet produced a rapid increase to a concentration of 0·90 μg/ml similar to that of the control lambs (0·91 μg/ml).
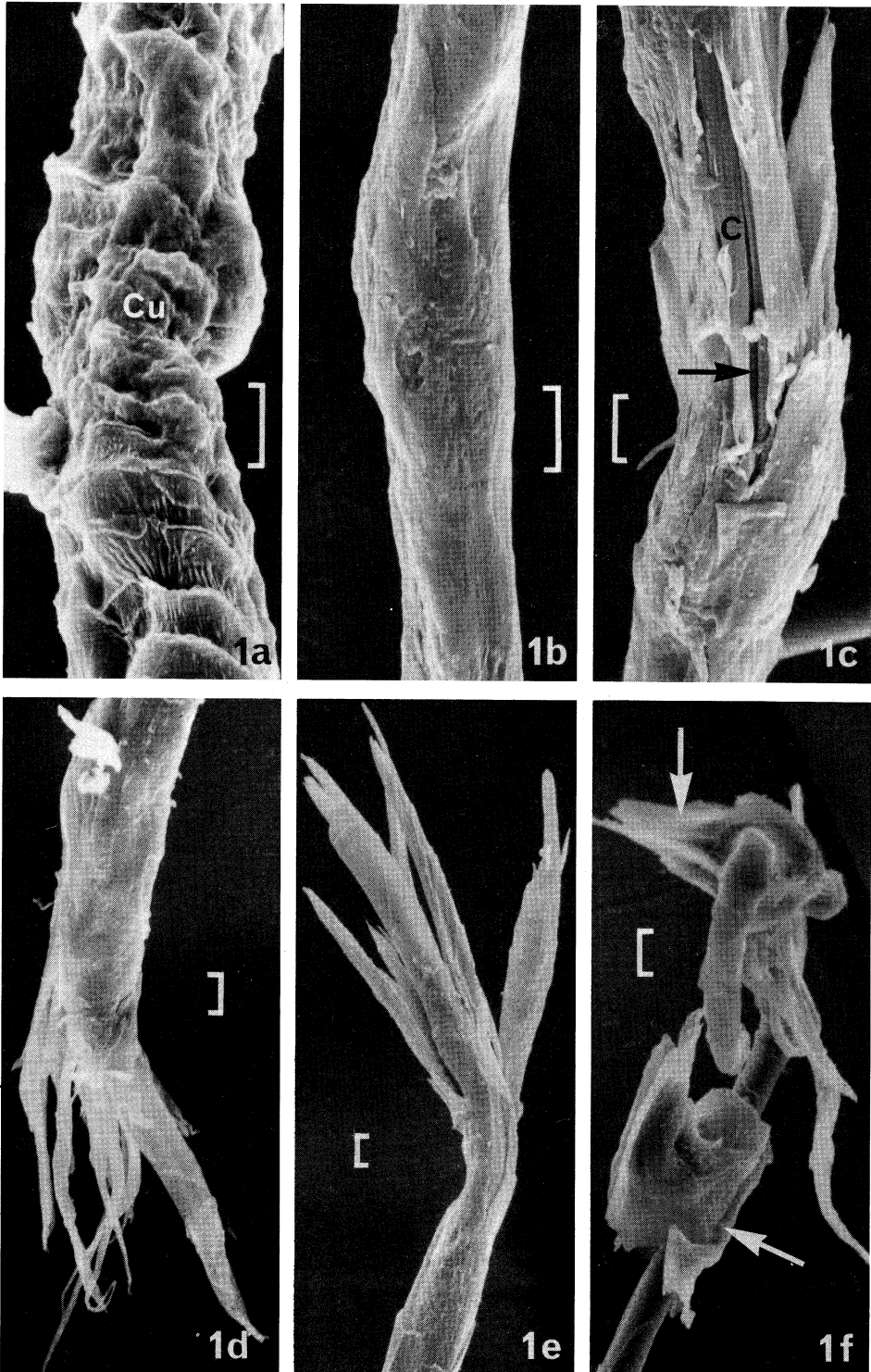
Fig. 1. Various abnormalities that developed in wool fibres during the first two weeks of zinc deficiency; (*a*) exaggerated and distorted cuticle scale pattern (*Cu*) on the surface of the fibre; (*b*) loss of cuticle scale pattern on the surface of the fibre; (*c*) separation (→) of cortical cells (*C*); (*d*) fractured basal end of a fibre that broke at a weakened zone; (*e*) frayed tip on a fibre which regrew during zinc repletion; (*f*) epithelial debris (→) encasing the distorted tip of a fibre that regrew during zinc repletion. Bar lines = 10 μm.

Wool Growth

The wool growth of $0\cdot52$ mg/cm^2. day of the lambs fed the zinc-deficient diet during the first 2 weeks was significantly less ($P < 0\cdot05$) than the $0\cdot79$ mg/cm^2. day grown by the control lambs. By the end of this first period wool growth had ceased on the deficient lambs, because no wool was harvestable by the clippers at the end of the second 2-week interval, and much of the previously grown wool had been shed. Wool growth recommenced at a low rate ($0\cdot19$ mg/cm^2. day) on the deficient lambs when they were fed the control diet.

A variety of defects developed in individual wool fibres during the first 2 weeks of zinc deficiency (Figs 1*a*–1*f*). These included an irregular and exaggerated cuticle scale pattern on some fibres (Fig. 1*a*), while others were devoid of cuticle scale pattern (Fig. 1*b*). Cortical cells were separating in portions of some fibres, as if the intercellular cementing material was missing (Fig. 1*c*). Some fibres had broken at such regions (Fig. 1*d*). In the wool which regrew when the deficient lambs were fed the control diet, many of the tips were frayed (Fig. 1*e*), while some were distorted and encased in epithelial debris (Fig. 1*f*).
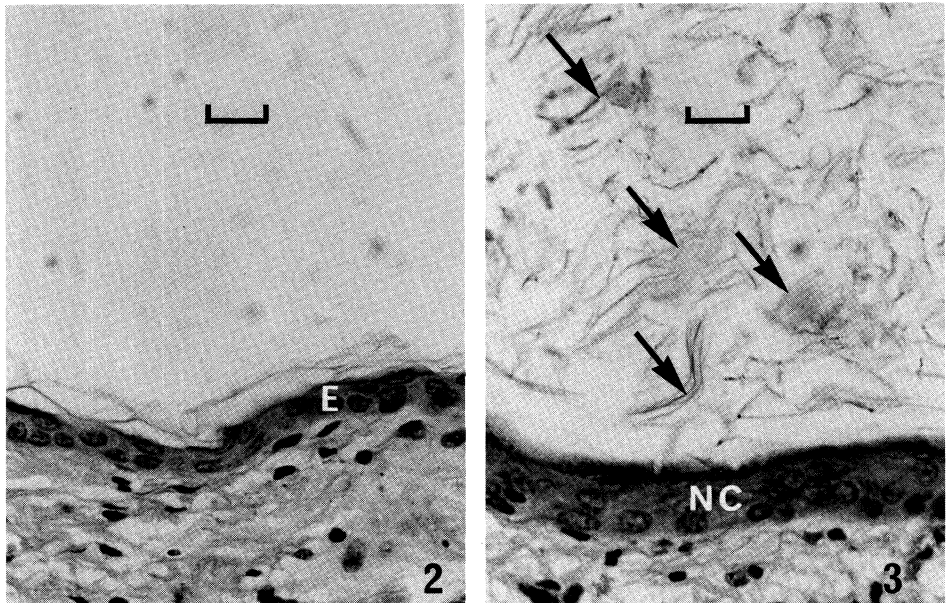

Fig. 2. Normal epidermis (*E*) of a lamb fed the control diet. Haematoxylin and eosin staining. Bar line = 20 μm.

Fig. 3. Acanthosis of the nucleated cell layers (*NC*) and hyperkeratosis and flaking of the stratum corneum (→) of the epidermis of a lamb fed the zinc-deficient diet for 4 weeks. Haematoxylin and eosin staining. Bar line = 20 μm.

Skin

The lambs fed the deficient diet developed skin lesions around the mouth within 2 weeks. However, histological changes in the epidermis of the midside skin were not detectable until the lambs had been fed the deficient diet for 4 weeks. By this time slight acanthosis of the epidermis had developed together with a moderate increase in the amount of stratum corneum, which was not parakeratotic (Fig. 3, cf. Fig. 2). These features were still evident after the deficient lambs had been fed the control
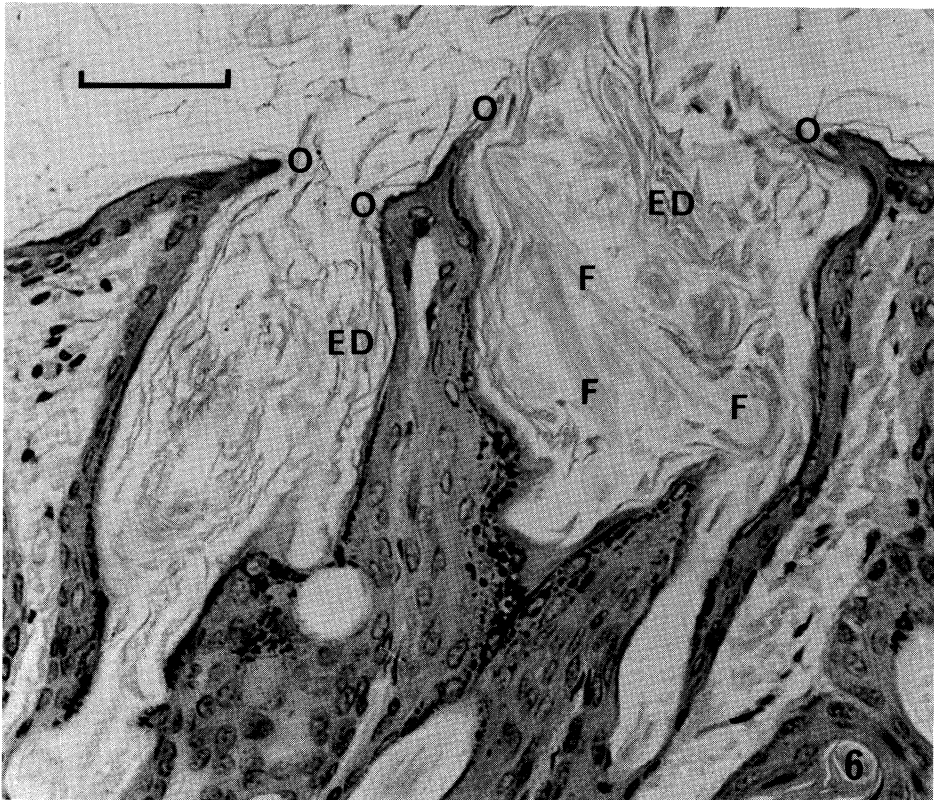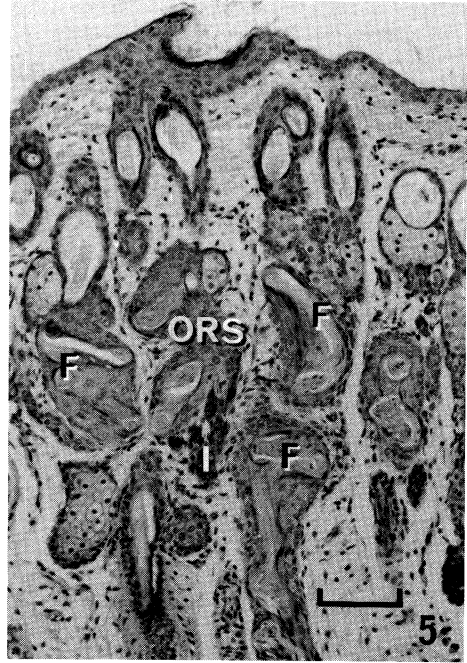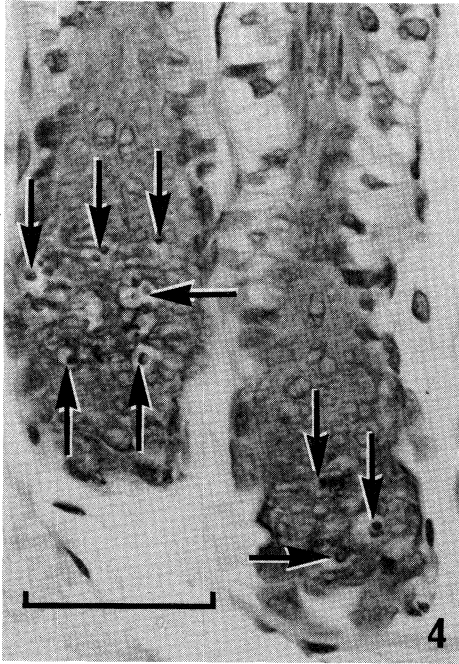
Wool Follicles

Autophagic vacuoles (or apoptotic bodies) developed in *c*. 70% of follicle bulbs of the deficient lambs by the end of the first 2 weeks (Fig. 4). Also by this time the wool fibres in the distal parts of almost all follicles in the deficient lambs were distorted and appeared to be partly degraded within thickened follicle outer root sheaths (Fig. 5). After the lambs had been fed the deficient diet for 4 weeks *c*. 90% of follicle bulbs had autophagic vacuoles and the masses of degraded fibres and epithelial debris in the distal parts of the follicles were larger. In some follicles these accumulations were being expelled through greatly enlarged follicle orifices (Fig. 6). After the deficient lambs had been fed the control diet for 2 weeks, the proportion of follicle bulbs with autophagic vacuoles had decreased to *c*. 5%, and the fibres in the distal parts of the follicles appeared to be normal, although there was still some slight thickening of the outer root sheaths above sebaceous gland level.

Immature follicles (Fig. 5) in early stages of development and not producing fibres were present in all the lambs. At the end of the first 2 weeks *c*. 50% of follicles in the deficient lambs were still immature compared with *c*. 35% in the control lambs. These respective percentages were *c*. 35% and 25% after 4 weeks and *c*. 30% and 20% after all the lambs were fed the control diet for a further 2 weeks.

Skin Glands

The sizes of the sebaceous glands did not change in the control lambs during the first 4 weeks, but increased in four of the five deficient lambs. In the subsequent 2 weeks with all lambs receiving the control diet, there was a slight increase in size of these glands in two of the control lambs and a further increase in two of the deficient lambs. The changes which occurred in sweat gland size in the deficient lambs during both regimes of feeding were similar to those in the control lambs.

Discussion

The rapid decline in plasma zinc concentration after the commencement of feeding the zinc-deficient diet is in agreement with that observed in lambs and calves by Mills *et al*. (1967). This rapid decline was accompanied by a significant decrease in wool growth even though there were only slight, non-significant decreases in food intake and liveweight gain during these first 2 weeks. In the subsequent 2 weeks, liveweight gain in the zinc-deficient lambs fell from 112 to 20 g/day and intake fell from 159 to 120 g/day. An intake of only 120 g/day [2·4 MJ metabolizable energy (M.E.)/day] is only slightly higher than the calculated maintenance energy requirement of the lambs (2·1 MJ M.E./day). Therefore the decline in growth rate caused by zinc deficiency was primarily caused by reduced food intake. Two 1 h periods of feeding may not have provided the best conditions for optimum intake by the zinc-deficient lambs.

Fig. 4. Autophagic vacuoles (→) in follicle bulbs of a lamb fed the zinc-deficient diet for 2 weeks. Haematoxylin and eosin staining. Bar line = 50 μm.

Fig. 5. Distortion and degradation of the wool fibres (*F*) within thickened outer root sheaths (*ORS*) of the distal parts of follicles in a lamb fed the zinc-deficient diet for 2 weeks. Some immature follicles (*I*) are present. Haematoxylin and eosin staining. Bar line = 100 μm.

Fig. 6. Masses of degraded fibres (*F*) and epithelial debris (*ED*) about to be expelled through enlarging follicle orifices (*O*). Haematoxylin and eosin staining. Bar line = 50 μm.

However, the reduction in food intake (41%) is similar to that reported by others for zinc-deficient sheep (Underwood and Somers 1969; Ott *et al.* 1964). Although zinc-deficient lambs continued to grow slowly, wool growth ceased and the wool was shed. This cessation in wool growth was then a specific effect of zinc deficiency on wool growth and was not due to depressed dry matter intake alone.

The shedding of the wool resulted from the degradation of the fibres in the distal part of the follicles within the thickened outer root sheaths. Similar distortion and degradation of fibres has been observed in adult sheep fed a diet of whole wheat grain and supplemented abomasally with methionine (Chapman and Reis 1978), in pre-ruminant lambs suspected of being deficient in biotin (Chapman and Black 1981), but subsequently found to be deficient in folic acid (Black, Colebrook and Chapman, unpublished observations), and in lambs on a lysine-deficient diet (Chapman *et al.* 1983). The condition also occurs in the BD (Pinkus 1965) and naked (*N*) (Raphael *et al.* 1982) strains of mice, and in humans with trichomalacia (Miescher and Schmuziger 1957). In each instance, the fibre distortion and degradation appear to be consequences of poor formation and impaired keratinization of the fibres lower in the follicles. Follis *et al.* (1941) claimed that hair follicles disappeared completely from the areas of skin from which the hair was shed in zinc-deficient rats. However, regressed follicles in an apparently inactive (telogen) stage were still present in their illustrative micrograph. In the present study with a shorter period of zinc deficiency regressed follicles were not observed, although numerous autophagic vacuoles developed in the follicle bulbs. Similar vacuoles develop during the phase of regression (catagen) of normal cyclic hair growth (Parakkal 1970). They also occurred in follicles in the lambs deficient in lysine and folic acid, mentioned above (Chapman *et al.* 1983; Black, Colebrook and Chapman, unpublished observations), and in sheep dosed with various depilatory agents (Chapman 1980; Hollis *et al.* 1983). Autophagic vacuoles develop in perfused rat liver within minutes of the hepatocytes being deprived of amino acids (Mortimer and Schworer 1977) and apoptotic bodies occur in a variety of biological phenomena involving changes in tissue architecture (Kerr *et al.* 1972; Wyllie *et al.* 1980).

Mills *et al.* (1967) reported that regrowth of fibres was visible on denuded areas of zinc-deficient lambs 24 h after the lambs were given a single drench of zinc sulfate. In the present study the regrowth of wool after the deficient lambs were given the control diet was slow. However, the amount of wool harvested by the clippers ($0 \cdot 19$ mg/cm^2. day) was an underestimate of the actual growth, because the skin was bare when the diet was changed and the clippers left behind a short pile ($1-1 \cdot 5$ mm) of the wool that had regrown. Also there is an apparent $3\frac{1}{2}$ week lag between feed intake and wool growth (Nagorcka 1977). The process of regrowth involved the exfoliation of fibre remnants and epithelial debris from the upper parts of the follicles and restoration of normal follicle structure. Because complete regression of the follicles did not occur the actual resurgence of growth was of fibres that had fractured in the distal parts of the follicles. So the fibres had fragmented tips in contrast to the tapered tips produced during cyclic hair growth.

The percentages of immature follicles appeared to be slightly higher in the deficient lambs. However, similar percentages of follicles matured in both the deficient and control lambs during the period of zinc deficiency. So it appears that the deficiency did not seriously affect follicle maturation.

During the 4 weeks of deficiency the wool-bearing skin on the midsides of the deficient lambs became slightly acanthotic and hyperkeratotic. However, it did not become

parakeratotic as did the skin in the flanks and hocks of older, ruminant lambs depleted of zinc for 10 weeks (Ott *et al.* 1964). The skin lesions around the mouths of our pre-ruminant lambs were similar to those reported on the faces of ruminant lambs (Ott *et al.* 1964; Mills *et al.* 1967; Underwood and Somers 1969), but they developed more quickly than in the ruminant lambs, even though the diet contained about twice as much zinc on a dry matter basis. Young pre-ruminant lambs may therefore have a higher zinc requirement and/or a smaller store of zinc than ruminant lambs, and be more susceptible than the latter to either marginal or short-term deficiency. The adverse effects reported here resulted from using a liquid diet which contained only 5 μg zinc per gram of dry matter, which is 20–30% of reported normal zinc content of ewes milk (assuming 20% of ewes milk is dry matter) (Underwood 1977). Further research is necessary to determine what dietary zinc concentrations first impair development of pre-ruminant lambs.

Acknowledgments

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