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Enterocyte ultrastructure and uptake of immunoglobulins in the small intestine of the neonatal lamb

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INTRODUCTION

During the perinatal period the newborn lamb, which is born agammaglobulinaemic, receives all its antibodies by means of transport across the intestinal epithelium, from ingested colostrum (McCarthy & McDougall, 1949). The digestive tract of the lamb (and the fetal sheep) therefore, provides an accessible animal model which, among many other possibilities (see Trahair & Harding, 1987), can be used to investigate the process of epithelial transport of proteins. However, there are no studies which have undertaken a comprehensive description of the ultrastructure of enterocytes within the perinatal period, an obvious prerequisite for transport studies.

The transfer of antibodies requires a highly efficient transferring mechanism to be present at birth. We have previously shown that the fetal lamb develops such a transferring system early *in utero* (Trahair & Robinson, 1986*a*) and we have studied the associated cellular kinetics which accompany the differentiation of enterocytes throughout the same period (Trahair, Perry, Silver & Robinson, 1986*a*, *b*). Because of the volatility of intestinal structure after birth (Trahair & Robinson, 1986*b*) it was thought appropriate to complete our earlier developmental study by extending our observations into the neonatal period. Particular note of the presence or absence of the structural basis for uptake and transfer will be made. To examine the uptake of immunoglobulins, rather than employing foreign substances or altered feeding regimens as most other studies have done, we will demonstrate the localisation of immunoreactive immunoglobulins, presumably of colostral/milk origin within the epithelium using immunohistochemistry.

MATERIALS AND METHODS

Experimental animals

Corriedale–Merino cross-bred lambs were used in this study. The animals were born in the laboratory and the day of birth was designated as Day 0. All lambs had free access to the ewe for suckling. The ages of the lambs when they were killed by barbiturate overdose was as follows: 4 hours (n = 2), 12 hours (1), 14 hours (1), 1 day (1), 2 days (4), 4 days (3), 5 days (2), 6 days (2), 7 days (1) and 8 days (1).

Tissue preparation

Tissue from each lamb from proximal (5 cm distal to ligament of Treitz), approximately mid, and distal (5 cm proximal to the ileo-caecal junction) portions of

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the small intestine were fixed in Bouin's fluid and wax-embedded for light microscopy and immunohistochemistry, or fixed in 2% paraformaldehyde/2·5% glutaraldehyde in phosphate buffer for electron microscopy.

Immunohistochemistry

Sections were dewaxed, hydrated and rinsed in phosphate buffer (0·1 M pH 7·4, PBS) and incubated in normal swine serum (1:10 in PBS, NSS; Dakopatts) for 20 minutes. Rabbit anti-sheep immunoglobulins (1:20–1:100 in 1:10 NSS; Cappel) were applied for 1 hour. After 3 PBS washes, endogenous peroxidase activity was blocked with 6% hydrogen peroxide in methanol for 10 minutes. After 3 further PBS washes the second antibody was applied (swine anti-rabbit immunoglobulins peroxidase conjugate, 1:25 in PBS; Dakopatts) for 30 minutes. After 3 further PBS washes DAB (3,3′-diaminobenzidine tetrachloride, 1·67 mM 0·05 M PBS, pH 7·6 with 0·01% hydrogen peroxide) was used to visualise the second antibody. After 5–7 minutes the slides were washed, stained with haematoxylin, dehydrated and mounted.

Absorption with sheep immunoglobulins (Sigma) and substitution of the first antibody with non-immune rabbit serum (1:20; Dakopatts) served as controls.

RESULTS

Enterocyte ultrastructure

Details of the fetal development of the intestine have already been described elsewhere (Trahair & Robinson, 1986*a*). At 2 days after birth, *proximal* and *mid portions* of the small intestine exhibited similar features of maturity which were already present *in utero* during the later weeks of fetal life (Trahair & Robinson, 1986*a*) (Fig. 1). However, some ultrastructural features clearly exhibited a greater degree of organisation: mid-villus enterocytes had a fully formed microvillous border (Figs. 1, 5, 7). The filamentous network of the terminal web was well-developed and the anchoring filaments (rootlets) of the microvilli penetrated through the terminal web to a considerable depth. The glycocalyx covering the microvilli formed an extensive complex radiating web at this time.

The intercellular spaces were swollen thereby forming a considerable extracellular compartment suggesting the substantial passage of material through the epithelium (Fig. 2). The lateral infoldings of adjacent cells were extended, remaining in contact by desmosomes at the tips of the slender processes. The junctional complex between cells in the terminal web region was often the only other point of contact between adjacent cells. Flocculent material was seen in the intercellular compartment, in the lamina propria tissue spaces and in the lacteals draining the villus. The Golgi region

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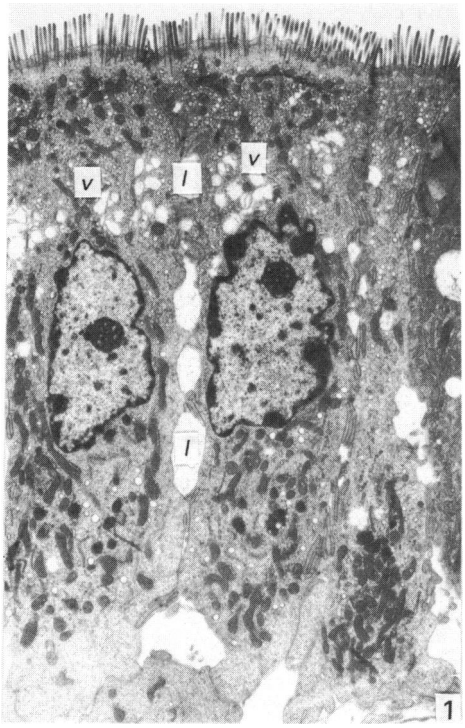
Fig. 1. Electron micrograph of the proximal small intestine of a 2 day old lamb. Columnar enterocytes have a well-developed brush border. At higher magnification it was noted that many vesicles (*v*) were filled with a flocculent material which was also seen in the swollen intercellular spaces (*I*). ×3700.

Fig. 2. A cross-section of enterocytes as shown in Figure 1. Cells remain in contact with each other by desmosomes at the tips of the extended lateral infoldings (arrows). The intercellular space was filled with a uniformly flocculent material. ×2650.

Fig. 3. The Golgi region (*G*) of enterocytes as shown in Figure 1. Numerous vesicles, some coated, were seen close by the swollen cisternae of the Golgi apparatus. Electron-dense bodies (*L*) are probably lysosomes. ×12000.

Fig. 4. Coated vesicles (arrows) at the basal and lateral cell membrane were extremely common. ×38000.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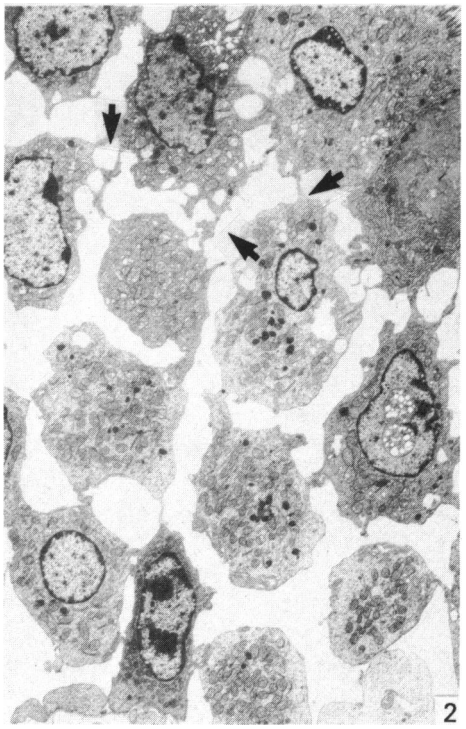
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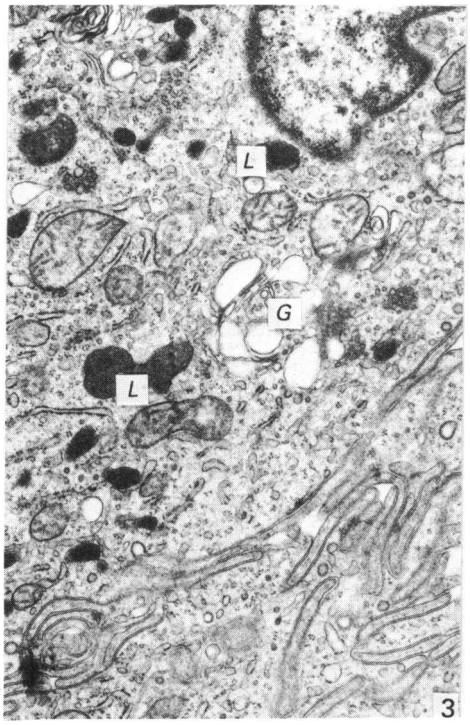
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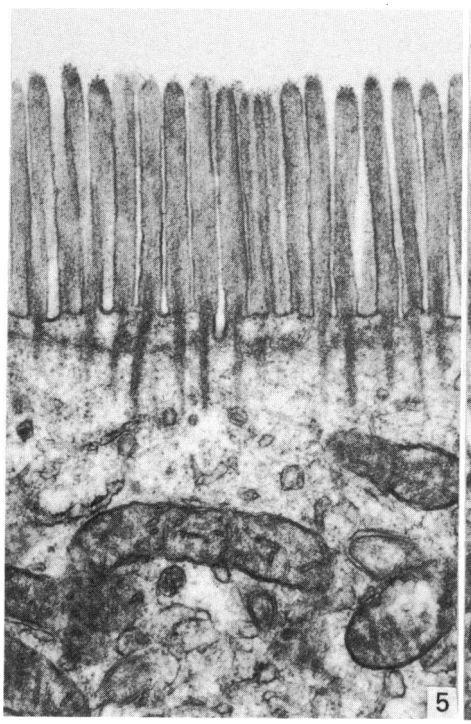
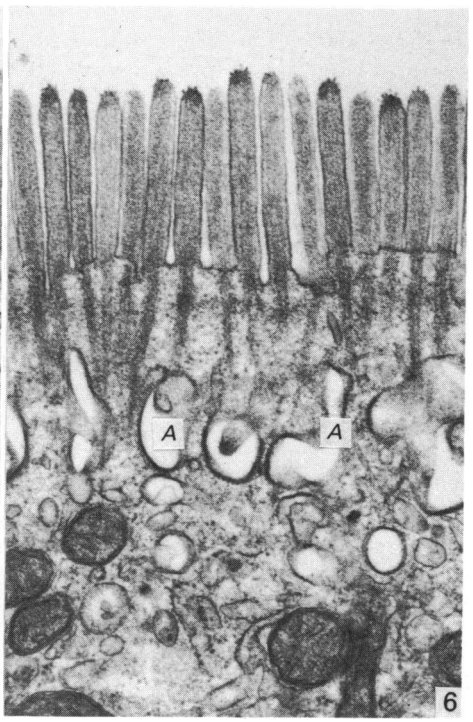




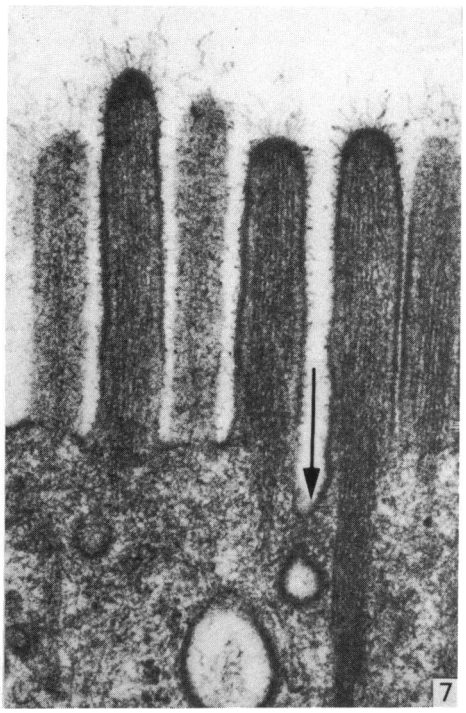


Fig. 5. The brush border of proximal regions of the intestine at 2 days of age was well-developed; usually there was no evidence of pinocytosis (see Figure 7). ×27000.

Fig. 6. The brush border of distal regions at 2 days of age still shows a well-developed apical endocytic network (*A*). ×29000.

Fig. 7. Only rarely was pinocytosis (arrow) seen in proximal regions at 2 days. ×60600.

of these cells occupied a supranuclear position and appeared swollen (Fig. 3). A considerable number of vesicles, some coated, with a range of densities and sizes was noted throughout the whole cell especially in the Golgi region. Numerous darkly staining bodies and multivesicular bodies were thought to be lysosomes.

Long strands of granular endoplasmic reticulum were abundant and mitochondria were distributed evenly. Only very occasionally was any evidence of pinocytotic activity on the luminal aspect of the cell observed (Fig. 7). The complex apical tubulo-vesicular network, which is a feature of the fetal intestine, was never seen in these cells. Coated vesicles were numerous, scattered throughout the cytoplasm, especially on its lateral and basal aspects (Fig. 4). Only rarely were they seen in the apical region. At the tips of the villi, cells appeared to become vesiculated and to die before being shed into the lumen.

In the *distal regions* of the small intestine, for at least 2 days after birth, vacuolated cells like those found throughout fetal development (Trahair & Robinson, 1986*b*) were still present on the upper third of the villi. The apical cytoplasm of these enterocytes possessed the characteristic well-developed apical endocytic network (Fig. 6). Numerous channels and vesicular profiles suggested that these cells were in the process of taking up material from the intestinal lumen. Although the vacuoles were smaller than those of earlier ages, a gradient of vacuolation could still be observed from non-vacuolated cells at the villus base to more extensively vacuolated cells at the villus tip.

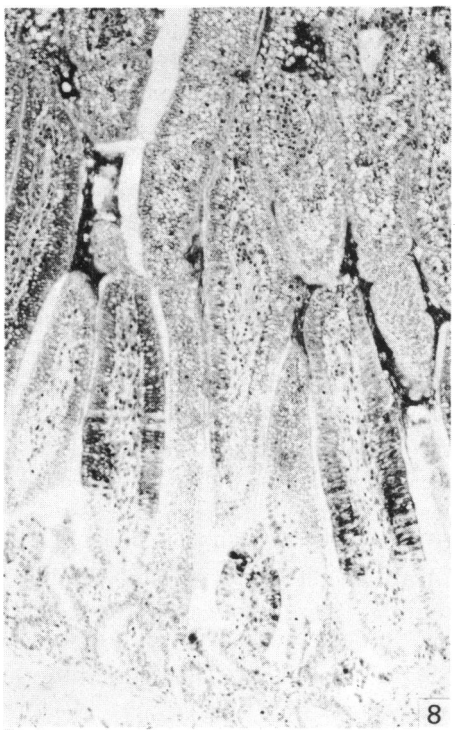
By 5 days after birth all regions of the small intestine had assumed a mature form. The brush border of distal enterocytes was devoid of any evidence of uptake. Vacuolated cells had entirely disappeared and distal enterocytes resembled the mature cells found in the proximal regions, described above.

Immunohistochemical localisation of immunoglobulins

Rabbit antibodies to sheep immunoglobulins detected immunopositive material in the mucosa and/or the lumen of both proximal and distal regions of the intestine at all ages examined (0–6 days). There was little qualitative difference in the staining patterns of proximal and distal regions (only proximal regions are shown in the Figures). All specific staining patterns were abolished by pre-absorption with sheep immunoglobulins. There was little or no background after incubation with non-immune serum.

Four hours after birth, immunostaining was found in the villus tip enterocytes in both regions. Not all villi stained to the same extent. There was no staining present in the lamina propria (Fig. 8). Fourteen hours after birth, staining of villus tips was still evident. Isolated groups of enterocytes at the base and along the sides of the villi were also conspicuously immunopositive in both regions (Fig. 9). Staining in the lamina propria in both regions indicated that transfer, and most likely circulation, of immunoglobulins had begun. Over the next few days overall staining of the villi became much reduced, though groups of cells were still strongly immunopositive (Fig. 10). An occasional crypt cell was also positive. Staining of all interstitial elements was very strong.

By 6 days there was little staining of the mucosal epithelium (Fig. 11), though (only rarely) there were still a few clusters of stained cells present.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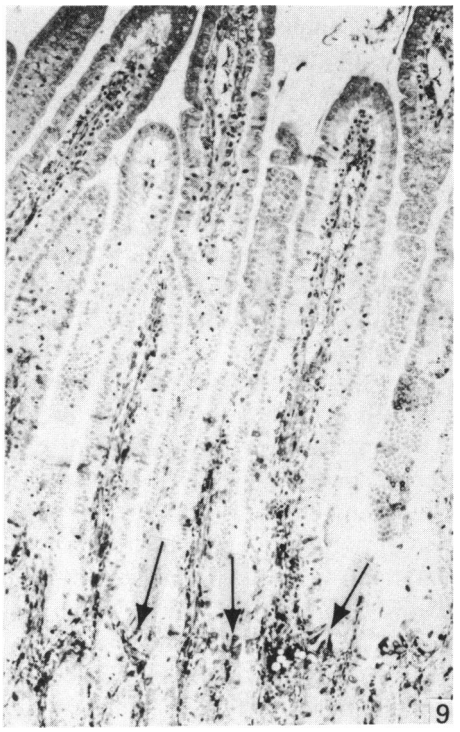
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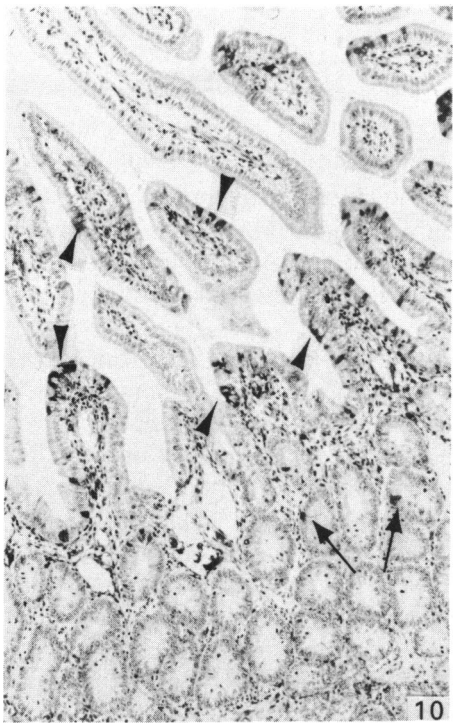
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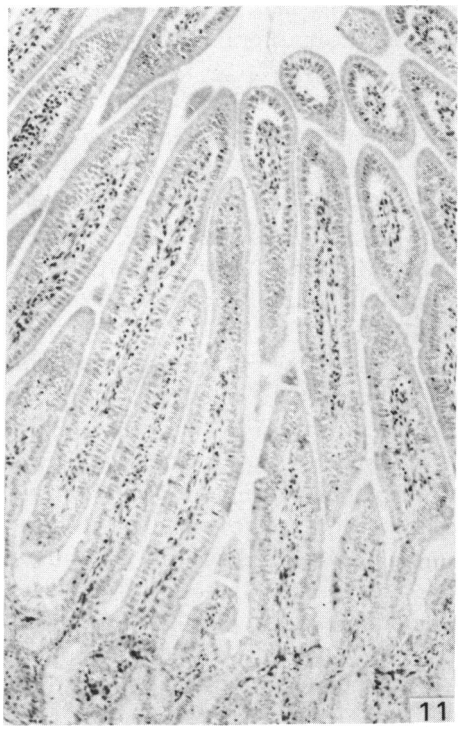
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DISCUSSION

Like the intestine of other species with a long gestation period, including man, the small intestine of the sheep at birth is relatively mature. Enterocytes in proximal regions reach significant morphological maturity at about 125 days of gestation (Trahair & Robinson, 1986*a*). Indeed, the levels of the major digestive enzymes achieved early in gestation suggest that biochemical maturity is likewise established precociously (P. M. Robinson, unpublished observations). The fetal or vacuolated enterocytes, which were present for a large portion of *in utero* development persist for a short period after birth in distal regions of the small intestine.

Recent studies have demonstrated that neonatal uptake and transfer of ferritin, when ingested with sheep serum, occurs in proximal regions (Dinsdale & Healy, 1982). The ultrastructural results of the present study confirm that, as in the suckling rat proximal small intestine (Rodewald, 1973), no vacuoles are present in enterocytes of this region in the lamb intestine, though many vesicles, some of which are coated, are present. By Day 2 these vesicles are most often found in basal and lateral regions of the cell. The mature intestine is also capable of taking up small amounts of whole protein by a process of pinocytosis (Cornell, Walker & Isselbacher, 1971). The present study only rarely showed any evidence of pinocytosis on the luminal aspect of mature enterocytes from proximal regions at 2 days. In distal regions, Dinsdale & Healy noted the presence of characteristic lysosomal enzymes, suggesting that when uptake occurs, presumably via the complex apical endocytic network as described in the present study, intracellular digestion would prevent any passage of ingested material across the epithelium. This view is consistent with the results of Rodewald (1973) who concluded that intracellular digestion was the fate of proteins taken up in the vacuolated enterocytes in distal regions.

However, more recent findings suggest that a dual system of uptake and transfer might be present in the suckling rat ileum (Siminoski *et al.*, 1986; Gonnella, Siminoski, Murphy & Neutra, 1987). In these experiments it was shown that biologically active peptides can be transferred transepithelially across the vacuolated cells. Careful ultrastructural examination revealed a range of vesicle morphology, both coated and uncoated types, being present. On the basis of these findings it was suggested that at least two separate pathways for endocytosed material might exist, possibly corresponding to the two types of vesicles. In our earlier published descriptions of vacuolated cells (Trahair & Robinson, 1986*b*), and in the present study, we found a similar diversity of vesicle morphology. The existence of more than one type of uptake (and possibly transferring) system may likewise be indicated by the immunohistochemical localisation of immunoglobulins in both regions of the intestine of the neonatal lamb, whether the cells are vacuolated or not, as revealed by the present study.

Fig. 8. Light micrograph of anti-sheep immunoglobulin immunoreactivity in the proximal small intestine of a suckled 4 hours old lamb. The upper two thirds of most villi were stained. × 240.

Fig. 9. By 14 hours after birth in proximal small intestine the villus tips were still stained. In addition the interstitium was stained. A smaller number of enterocytes at the villus base were strongly stained (arrows). × 220.

Fig. 10. Proximal small intestine. By 2 days after birth clusters of enterocytes were still stained (arrowheads). Some crypt cells were also stained (arrows). × 230.

Fig. 11. Proximal small intestine. By 6 days there was little epithelial staining present in either region of the intestine. × 220.

For some time 'closure' (the term used for the cessation of antibody transfer) has been thought to be related to the cellular replacement. If this was the case then it would be expected that the immunopositive cells we observed would have progressively moved up the villus to be replaced by cells incapable of immunoglobulin uptake. The staining patterns of immunoreactive immunoglobulins in the epithelium indicated that, with the exception of the youngest animal, the cells that were immunopositive did not form any clearly defined subpopulation. The appearance of strongly-stained cells at the base of the villi suggests that both newly-formed cells and the more fully differentiated cells further up the villus take up immunoglobulins. Another possibility is that the renewal patterns might be uneven, with newly-formed cells sliding past older cells which remain at the villus base. Such differential migration has been noted in the neonatal pig (Smith & Peacock, 1980). However, in our preliminary experiments, we examined the migration of tritiated thymidine pulse-labelled enterocytes in newborn lambs and noted that the labelling density profiles always suggested an orderly progressive replacement of the epithelium (unpublished observations). At the very least, the migration rates presented elsewhere (Moon & Joel, 1975) suggest in excess of 2–3 days for renewal; also the findings of Smeaton & Simpson-Morgan (1985) – 3 days; Attaix, Meslin & Combe (1984) – 4 days, would suggest that at the time of closure (2 days of age, Lecce & Morgan, 1962), the villi would still possess cells which had originated *in utero*. This may explain the persistence of vacuolated cells in the distal regions. More detailed investigation of the kinetics of the rapidly remodelling neonatal intestine (see Trahair & Robinson, 1986*a*) is needed before a clearer understanding of the process of enterocyte differentiation and closure can be obtained.

SUMMARY

Although the small intestine of the sheep is relatively mature at birth, there are still vacuolated enterocytes present for at least 2 days in distal regions. In the distal regions, vacuolated cells possess a range of vesicle morphology which might be indicative of at least 2 separate routes for enterocyte handling of proteins taken up from the lumen. The localisation of immunoreactive immunoglobulins within the enterocytes, presumably of colostral or milk origin, in both proximal (non-vacuolated) and distal (vacuolated) regions, does not follow patterns which suggest orderly renewal at closure. It is suggested that closure is not solely brought about by epithelial cell replacement.

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