

Perinatal development of the small intestine of the sheep

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Summary. Soon after birth, the villi in distal regions of the small intestine of the sheep rapidly decrease in size so that by day 6 the normal proximal-distal size gradient (tallest villi proximally) is established. This is the reverse of the gradient found throughout fetal life, where distal villi are taller than proximal villi. While crypt depth increases in both regions, the increase is more marked in proximal regions. The proportion of crypt cells labelled with a pulse of (³H) thymidine increases dramatically during day 1. Vacuolated cells are present in distal regions at day 2, but are absent by day 5. It is possible that the rapid loss of villus tip cells in distal regions is the means by which closure is achieved in this species. There is evidence to suggest that endogenous cortisol plays an important role in these changes.

Introduction.

We have previously reported changes in enterocyte morphology, intestinal structure and enterocyte kinetics throughout development in the fetal sheep (Trahair, Avila and Robinson, 1986 ; Trahair and Robinson, 1983, 1984, 1986a, b ; Trahair *et al.*, 1986a, b). As already noted in other species, many developmental changes are sensitive to the action of cortisol (see Moog, 1979 ; Klein and McKenzie, 1983a). In the fetal sheep, growth of intestinal structures is more rapid, and both the migration rate and proliferation of enterocytes increases later in gestation (Trahair, Avila and Robinson, 1986 ; Trahair and Robinson, 1984, 1986a, b ; Trahair *et al.*, 1986a, b). We have likewise observed that the development of the small intestine of the fetal sheep appears to be sensitive to cortisol (Trahair *et al.*, 1984, 1987a, b).

In most species a rise in plasma cortisol concentration is involved in the timing of the developmental changes of a number systems (see Ballard, 1979). In the rat this occurs towards the end of the suckling period, as weaning begins. In the sheep it occurs from 120 days gestation onwards where a dramatic rise in endogenous cortisol production is an important step in the initiation of parturition

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(see Wintour, 1984). The plasma cortisol concentration also remains relatively high in the early postnatal days (Nathanielsz, 1976).

The period from 120 days prior to birth until the early post natal days therefore provides an experimental model which might serve to illustrate the effects of endogenous cortisol *in vivo*, in addition to providing data which describes the neonatal small intestine in this species.

Materials and methods.

This study used Corriedale or Merino cross bred ewes of known mating dates and lambs of known neonatal age.

The age of the fetuses were 130 days gestation (n = 2) ; 132 (1) ; 139 (1) ; 141 (1) ; 145 (1). The lambs were aged 4 hours (2), 14 hours (1) ; 1 day (1) ; 2 days (1) ; 4 (1) ; 6 (1).

Birth of lambs occurred at either 147 or 148 days post conception. The lambs were kept with their mothers and allowed free access to suckling.

Pregnant ewes were anaesthetized by barbiturate infusion and their fetuses were catheterized, as described elsewhere (Trahair *et al.*, 1986a, b). The fetuses were either continuously infused with (³H) thymidine in saline (specific activity 925 GBq/mmol ; Amersham) at 125 μ Ci/hr, or injected intraperitoneally (18.5 kBq/g estimated body weight), 4 hrs prior to sacrifice. Elsewhere we (Trahair *et al.*, 1986b) and others (Fatemi *et al.*, 1985) have shown that the route of administration did not quantitatively affect the results obtained.

Lambs were injected intraperitoneally with (³H) thymidine (as for fetuses) 4 hrs prior to sacrifice. The ewes and lambs were killed by barbiturate overdose.

Tissue from proximal (within 5 cm from the ligament of Trietz) and distal (within 5 cm before the ileo-caecal junction) small intestine was removed and fixed in Bouin's fluid for 2 hrs. Paraffin sections (3 μ m) were cut and measurements of various intestinal parameters were made (see Trahair, Avila and Robinson 1986). Autoradiographs were prepared and the proportion of crypt cells labelled (PCL) was assessed (see Trahair *et al.*, 1986b).

Mean and standard deviations are given throughout the text. Comparison of means was by Student's t test for unpaired data.

Results.

Changes to intestinal wall components in late-gestation till 6 days after birth.

While there were no orderly age related changes in the values of most parameters, in some parameters systematic changes associated with the timing of birth were noted.

In proximal wall thickness (WT) (fig. 1a), there was a suggestion of an increase after birth, of which the mucosal thickness (MT) (fig. 1b) seemed to be

the chief determinant. Proximal MT increased after birth because both villus height (VH) (fig. 1c) and crypt depth (CD) (fig. 1d) increased.

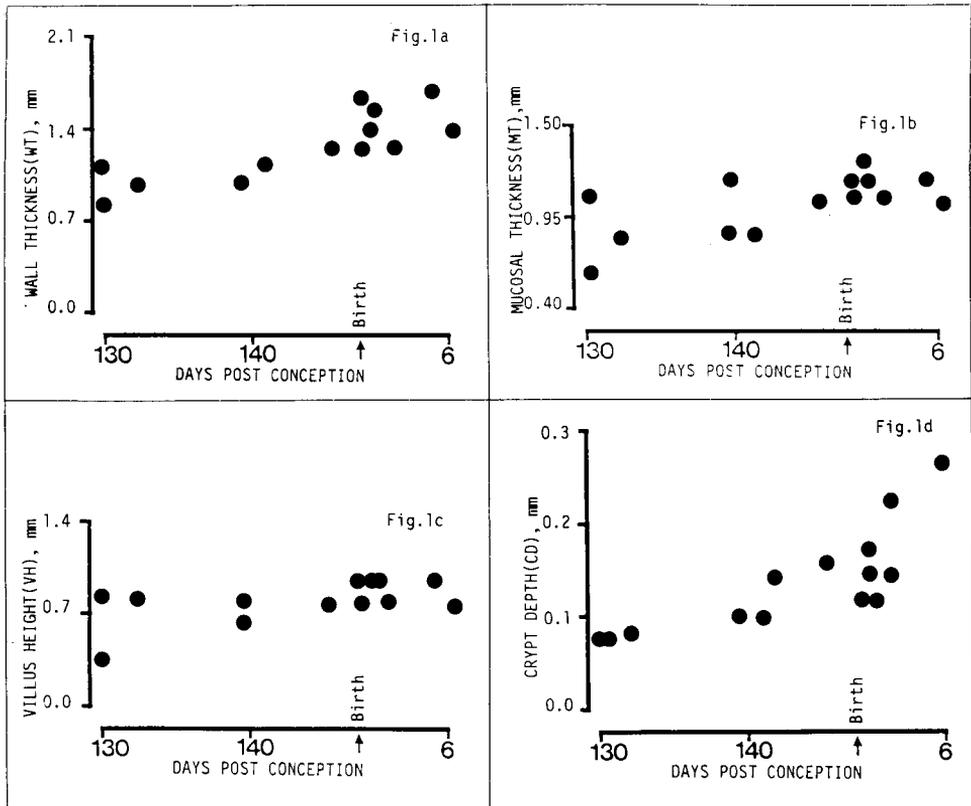


FIG. 1. — Changes in various intestinal parameters in the developing sheep small intestine from 130 days gestation to 6 days after birth (birth occurred at 147 or 148 days).

Proximal small intestine : a, Wall Thickness (WT) ; b, Mucosal Thickness (MT) ; c, Villus Height (VH) ; d, Crypt Depth (CD). (Note different scales).

In distal regions WT (fig. 2a) declined after birth. This could be explained by a marked decrease in MT (fig. 2b). While CD (fig. 2d) increased, there was a much greater decrease in VH (fig. 2c) throughout the same period. The decline in VH was therefore the main cause of the decline in MT and so in WT.

Enterocyte morphology of proximal regions soon after birth revealed no major changes from the mature appearance which has already been noted *in utero* since about 125 days (see Trahair and Robinson, 1983, 1986a). Fetal vacuolated-type enterocytes were present on the villus tips in distal regions at 2 days, but were absent by 6 days after birth. The ultrastructural features were comparable to those described for this cell type *in utero* (see Trahair and Robinson, 1986a).

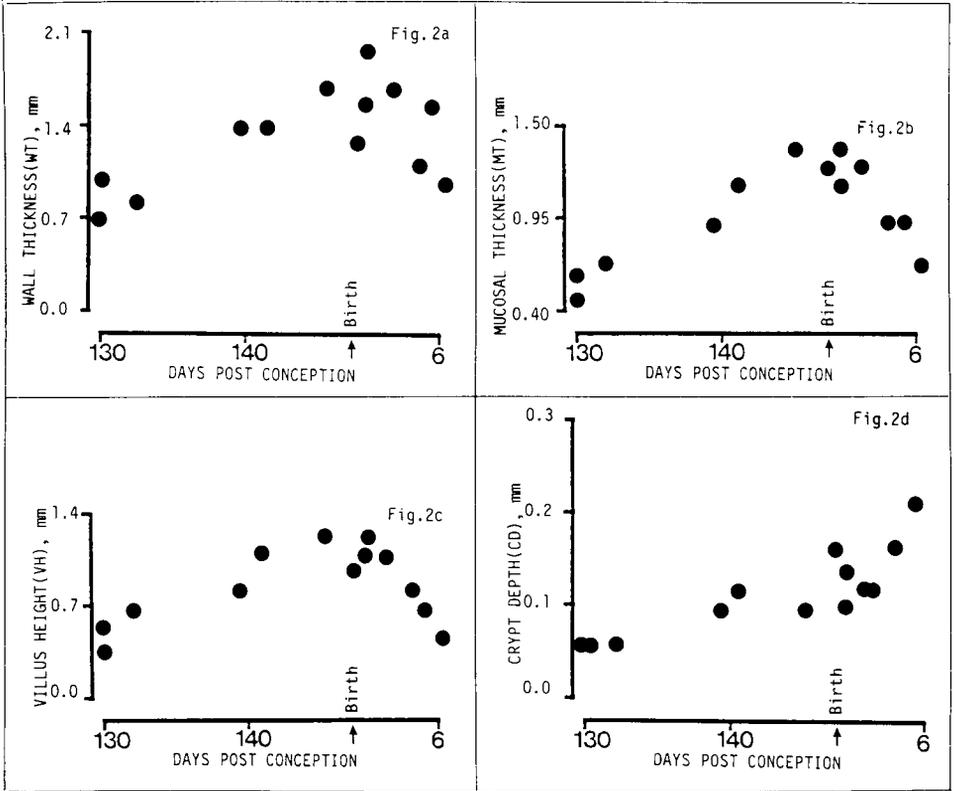


FIG. 2. — Changes in various intestinal parameters in the developing sheep small intestine from 30 days gestation to 6 days after birth (birth occurred at 147 or 148 days).

Distal small intestine : a, Wall Thickness (WT) ; b, Mucosal Thickness (MT) ; c, Villus Height (VH) ; d, Crypt Depth (CD). (Note different scales).

Changes in the proportion of cells labelled (PCL) at birth.

In proximal regions, for 4 hrs after birth the PCL remained at pre-birth levels, however, by 14 hours, the PCL had increased 70 %. Not until day 6 (153 days post-conception) did the PCL return to a lower level (fig. 3a).

In distal regions, by 14 hrs after birth, the PCL had increased by 92 %. After 6 days the PCL fell to levels comparable to late-term or at birth (fig. 3b).

The mean PCL for proximal regions during fetal development was 0.21 (± 0.01), while after birth it was 0.42 (± 0.11). Thus the average fetal proximal PCL was significantly lower than the average neonatal PCL ($p < 0.01$, Student's t-test for unpaired data, d.o.f. = 11).

The mean PCL for distal regions during fetal development was 0.23 (± 0.01) compared to 0.34 (± 0.09) after birth. Again, as for proximal regions, this increase was significant ($p < 0.05$).

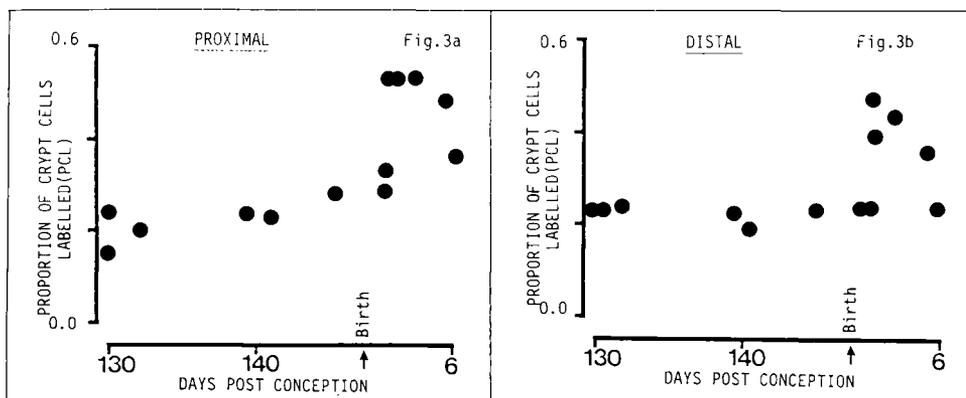


FIG. 3. — Change in the proportion of crypt cells labelled (PCL) with (^3H) thymidine from 130 days gestation to 6 days after birth (birth occurred at 147 or 148 days).
a, proximal small intestine ; b, distal small intestine.

Discussion.

The changes to structure associated with birth seen in this study were similar to those reported elsewhere (see below). Of particular note was the rapid change in some parameters. While throughout most of gestation villi in distal regions were longer than those in proximal regions (Trahair, Avila and Robinson 1986), which is the reverse of the pattern seen in the adult intestine of most species, in this study it appeared that after birth it was the rapid reduction of villus height in the distal regions rather than an increase in proximal regions which established the adult gradient.

In rats and mice villi and crypts increase in length after birth, but proximal villi are longer than distal villi (O'Connor, 1966 ; Herbst and Sunshine, 1969). In pigs, calves and sheep villi decrease in height after birth (Vodovar, 1964 ; Moon, 1971 ; Moon and Joel 1975 ; Attaix *et al.*, 1984).

In a report on renewal of the intestinal epithelium of 1 week old lambs, Attaix *et al.* (1984) noted the wide interanimal variability and rapid changes in intestinal structure which occur in the perinatal period. In support of this statement, they further cite work in other species which demonstrates the disparity which exists in similar measurements in other species made by different observers. Despite these reservations, the observations of a reduction of villus height after birth are in general accord with the results presented in the present paper. Furthermore, values for villus height and crypt depth agree reasonably well, although many of the lambs of the present study were much younger than the 10 day old group examined by those authors.

Moon and Joel (1975) also studied villus height and crypt depth in 1 day-old, 3 week-old and 1 year-old + lambs. The size of newborn proximal villi and crypts noted in the present study were also similar to the values cited by these authors. However, their values for distal villus height were smaller and for crypt depth

were larger. In addition, distal villi were shorter than proximal villi. Their findings demonstrated that the normal adult-like gradient of size was present by one day after birth.

The results of the present study demonstrated that in distal regions, villus height was greater and crypt depth was lesser than in proximal regions. The adult-like pattern was established by changes which began in the first days of life. Distal villi only became shorter than proximal villi by 6 days after birth. These findings are not in accord with the results of Moon and Joel, cited above. However, details of tissue handling or animal management procedures employed, especially with regard to the housing of the lambs and their access to suckling, both of which may have some bearing on the results obtained, were not given.

Soon after birth a dramatic increase in PCL was noted in both proximal and distal regions. Smeaton (1969) and Smeaton *et al.* (1985) also reported a significant increase in the mitotic index at birth. In both regions, the PCL fell rapidly over the first 6 days of life. Such a burst of cell proliferation might increase either villus height or crypt depth. It could also cause an increase migration rate and cell loss.

In proximal regions, villus height did not markedly change while crypt depth increased. In distal regions there was a rapid decrease in villus height and only a slight increase in crypt depth. In such a short time course (in the order of days), it is unlikely that significant changes to crypt and villus numbers would occur, hence the effects of the increase in PCL might be twofold. Firstly, an increase in migration rate which, on the basis of estimates made from the fetal renewal times (see Trahair *et al.*, 1986a), would bring about replacement within 8 days at the most. Secondly, a rapid removal of villus tip cells, in particular of vacuolated enterocytes in distal regions, as the villus size gradient typical of the adult is established. With reference to the first point, Attaix *et al.* (1984) have estimated renewal times in the 7 day old lamb to be between 2 and 4 days. However, it must be noted that because of rapidly changing size of the intestinal components, conventional techniques for assessing renewal times which assume a steady state over a number of days may be in considerable error. It was for this very reason that no such estimates were performed in the present study.

Results of some studies suggest that the rapid removal of specialized cells either by increased migration or increased cell loss, might be the means by which closure is achieved (see Patt, 1977). Whether this is the case in the newborn lamb and/or which regions of the intestine is responsible, cannot be established from this data alone. Either of the above possibilities would be consistent with much of the data relating to closure (see Dinsdale and Healy, 1982 ; Smeaton, 1969 ; Smeaton *et al.*, 1985).

We have suggested elsewhere that cortisol might be an important factor in controlling development of the sheep intestine (Trahair, Avila and Robinson, 1986 ; Trahair and Robinson, 1986a). We have furthermore demonstrated that growth of intestinal structures is reduced after fetal bilateral adrenalectomy (Trahair *et al.*, 1984, 1987a). Adrenalectomy also caused a reduction in enterocyte migration, while early cortisol infusion resulted in increases in both the PCL and migration rates (Trahair *et al.*, 1987b). In the sheep fetus the adrenal produces cortisol in response to hypophyseal ACTH from about 120 days onwards

(Wintour, 1984). Plasma cortisol concentrations reach their maximum levels prior to birth, and at birth are still much higher than those in the adult (see Nathanielsz, 1976). It is consistent therefore to suggest that the dramatic increases in PCL noted soon after birth may have been the result of the surge in cortisol production just prior to parturition. It should be remembered, however, that just as early cortisol infusion or adrenalectomy was unable to retard or advance development in all regards, it is unlikely that the regulation of the reorganization of the intestinal epithelium which we have noted to occur at birth would be simply the result of a single factor.

Other factors which have been cited to influence neonatal intestinal development (see Klein and McKenzie, 1983a, b for a review) include nutrient intake (Heyman *et al.*, 1984 ; Guiraldes and Hamilton 1981), thyroxine (see Yeh and Moog, 1977), insulin (Malo and Menard, 1983 ; Arsenault and Menard, 1984), cortisol (Herbst and Sunshine, 1969). Milk also contains a variety of factors including hormones (see Henning, 1982) which may affect development.

To what extent each of these, and possibly other, factors are involved in the neonatal development of the sheep small intestine remains to be elucidated.

Conclusions.

In the sheep, the transition to independent life results in rapid alterations to intestinal structure and function. Many of these changes are cortisol sensitive, indeed the early postnatal period is a time of markedly elevated plasma cortisol. The rate of proliferation of enterocytes rapidly increases, simultaneous with the establishment of adult size gradients of intestinal structures and the loss of fetal vacuolated cells from the distal small intestine. These events correspond to the timing of cessation of antibody transfer (closure). It is therefore possible that the rapid loss of villus tip cells might be the means by which closure is achieved in this species.

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Résumé. Développement périnatal de l'intestin grêle chez le mouton.

Peu de temps après la naissance, la longueur des villosités des régions distales de l'intestin grêle du mouton diminue de sorte qu'au sixième jour le gradient proximo-distal de longueur des villosités est établi (les villosités les plus longues se trouvant dans les régions proximales). Ceci est le contraire de ce qui est observé pendant toute la durée de la vie fœtale où les villosités des régions distales sont plus longues que celles des régions proximales. Alors que la profondeur des cryptes augmente dans ces deux régions, l'augmentation est plus marquée dans les régions proximales. La proportion de cellules marquées au niveau des cryptes par un pool de ^3H thymidine augmente d'une façon très importante pendant le premier jour. Les cellules vacuolées sont présentes dans les régions distales le deuxième jour, mais ont disparu le cinquième jour. Il est possible que la desquamation rapide des cellules à l'extrémité des villosités dans les régions distales représente le mécanisme par lequel la cessation de l'absorption des anticorps s'effectue chez cette espèce. Le rôle important joué par le cortisol endogène dans ces transformations est discuté.

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