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Altered intestinal development after jejunal ligation in fetal sheep

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Abstract. Experimental obstruction of the fetal small intestine resulted in massive hypertrophy of the segment proximal to the site of obstruction. Villus morphology was grossly abnormal. Enterocytes developed many irregular features, most notably cytoplasmic extensions (pseudopods, or blebs) from their apical surface. Distal to the site of obstruction, morphological anomalies which resembled those seen after experimental oesophageal ligation were found. These included delayed disappearance of the apical endocytic network, disrupted or absent microvilli, glycogen accumulation and inappropriate cell extrusion. Proximal to the obstruction, where stasis of swallowed fluid occurs, distension and abnormal intestinal development ensues. Distal to the obstruction where the intestine develops in the absence of swallowed fluid, development is also abnormal. The anomalies resemble those noted after oesophageal ligation in utero, and possibly are the results of reduced cellular nutrition. These results suggest that fetal ingestion provides the developing gastrointestinal tract with an important stimulus for normal growth.

Key words: Fetus – Small intestine – Ultrastructure – Intestinal atresia

Introduction

Enteral feeding is an important factor in maintaining normal gastrointestinal tract (GIT) structure and function. In the adult GIT, atrophy occurs in response to total parenteral nutrition, malnutrition, or as a response to surgical intervention, or in GIT disease where transit time is disturbed (Bragg et al. 1991). Likewise, in the developing GIT, enteral nutrition plays an important role in maintaining gut structure and function (Goldstein et al. 1985; Castillo et al. 1988; Trahair 1993). In addition, many of the ontogenic processes may be specifically influenced by growth factors present in either the amniotic fluid or milk which the fetus or neonate ingests (Castillo et al. 1992). Our experiments, where the fetal oesophagus has been ligated or fistulated, have demonstrated that GIT growth retardation occurs as a result of abolition of fetal ingestion (Trahair et al. 1986; Avila and Harding 1991). In addition, enterocyte differentiation is grossly aberrant in the absence of swallowed input (Trahair and Harding 1992). When Touloukian and Wright (1973) examined biopsies taken from the small intestine from 19 newborn infants with jejuno-ileal atresia, they noted a range of anomalies which included villus hypertrophy, and "tortuous, intertwined" villi. In a later study on fetal sheep, again, significant mucosal abnormalities were noted (Touloukian 1978). Similar features have been described in the chick embryo (Tovar et al. 1991).

Atresia of the oesophagus and intestine are alike in that they both deny the developing GIT exposure to enteral input. It seems highly likely therefore, that impaired GIT development could play a role in the failure to thrive observed in babies born with these relatively common anomalies (between 1:2000 and 1:300–1500 live births for eosophageal and intestinal atresia, respectively, see Flowers 1983), even after effective surgical repair (Cohen and Greecher 1979; Pierro et al. 1987; Puntis et al. 1990).

Our present study, therefore, was undertaken to examine carefully the changes in mucosal architecture as a response to intestinal atresia induced in utero, and to compare any changes to those we have already described in the absence of swallowing and to normal development.

Materials and methods

At 90–95 days of gestation, five date-mated ewes underwent surgery. Using sterile techniques and halothane anaesthesia, the fetus was exposed and its abdomen opened. Using cotton swabs and gentle manipulation, the duodenal-jejunal (DJ) flexure was visual-

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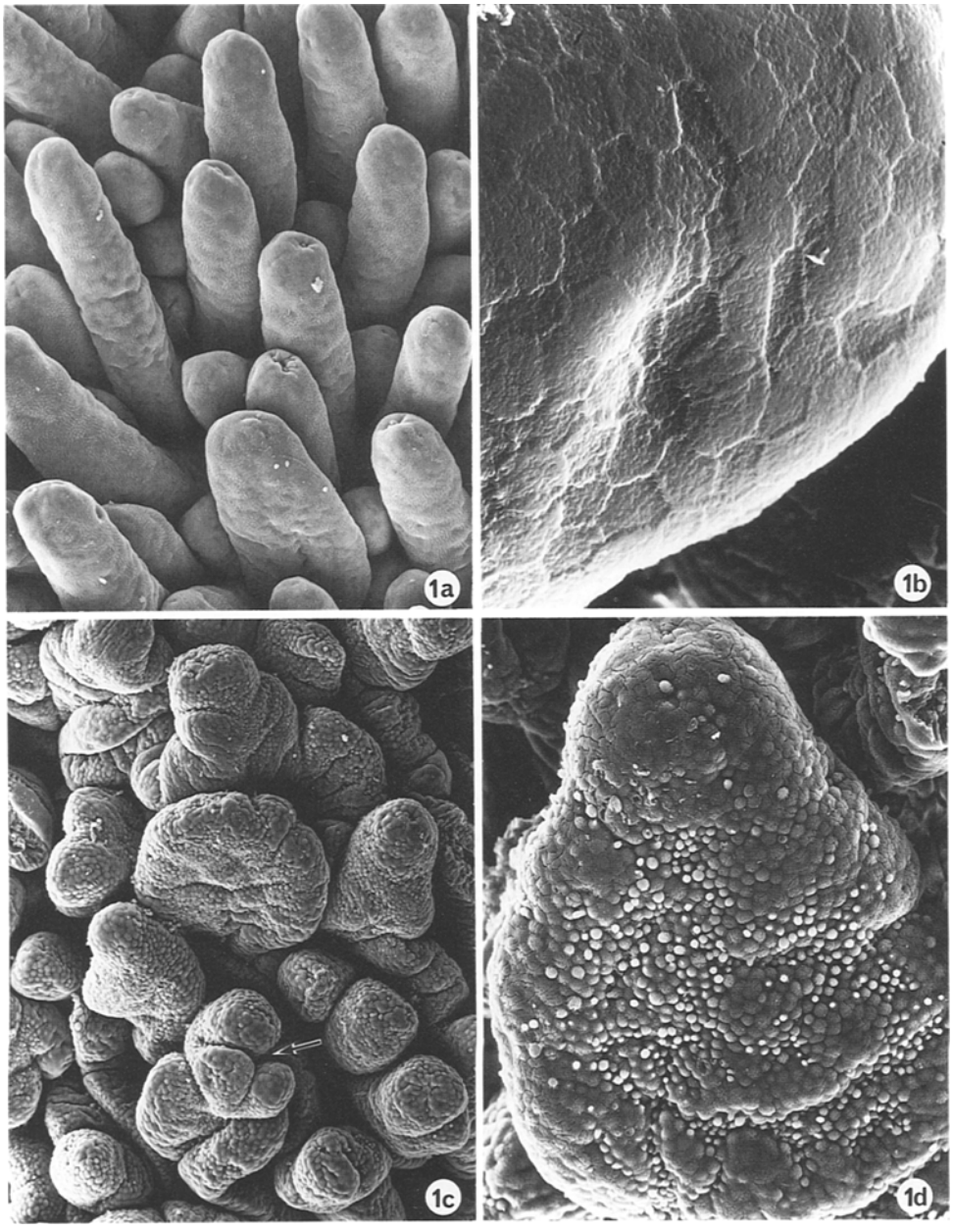

Fig. 1. a Scanning electron micrograph (SEM) of proximal small intestine from a normal fetus, 145 days of gestation. The villi are tall and cylindrical. ×160. **b** SEM detail **a**. Borders of the polygonal enterocytes are clearly visible. The microvillus coat is smooth and of even density. ×2700. **c** SEM of proximal small intestine, i.e. proximal to site of intestinal obstruction. Villus architecture is severely disrupted. Villi are broad, bluttened and branching (*arrow*). Epithelial cells bulge into the lumen, creating a very uneven mucosal surface. ×150. **d** SEM detail of Fig. 3. Enterocytes bulge into the lumen. ×400

ized. Distal 10 cm to this landmark the intestine was divided between two silk ligatures. The intestine was returned to the abdomen and all incisions closed. At 142–145 days gestation these ewes and a further three normal pregnant ewes were killed by barbiturate overdose and the fetus removed. Tissues were rapidly removed for electron microscopy from the following sites: duodenum, 5 cm proximal to the DJ flexure; jejunum, 5 cm proximal to the obstruction; jejunum, 5 cm distal to the obstruction; and ileum, 5 cm proximal to the ileo-caecal junction. The distance from the pylorus to the site of obstruction was measured in experimental fetuses (60 cm ± 5 cm) and the equivalent sites were sampled in the normal fetuses.

Tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and routinely processed into resin. Thin sections were viewed in a Hitachi 7000 transmission electron microscope (TEM). Tissues for scanning electron microscopy (SEM) were processed using the osmium thiocarbahydrazide method (two passes through osmium), dehydrated and dried using Peldri according to the manufacturers directions (Ted Pella Inc, Redding, CA, USA). The prepared specimens were mounted on stubs using conductive paint and lightly coated with carbon. Specimens were examined in a Phillips 505 SEM.

Results

Fifty-five days of intestinal obstruction produced massive hypertrophy of the segment proximal to the obstruction. All segments of the GIT proximal to the obstruction (including the duodenum, forestomachs and abomasum) were grossly distended. The distended portion of the GIT contained bile-stained swallowed fluid and gastric secretions. Distal to the obstruction the intestine was collapsed. The small amount of material present was yellow-orange, apparently accumulated cellular debris. Colon and caecum contained meconium. Peristalsis was observed in all segments.

In normal fetuses, flattened duodenal villi (leaf-like)

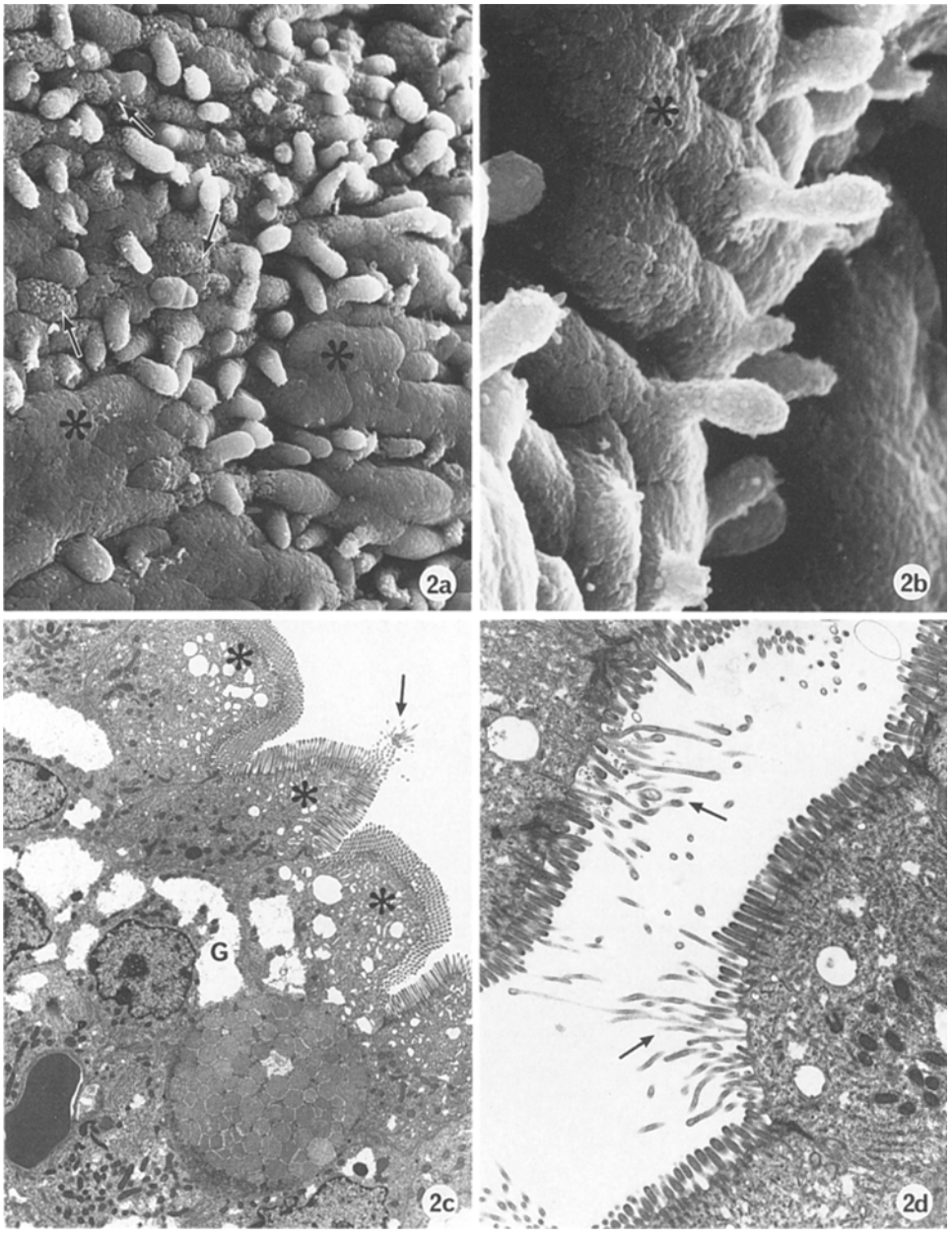

Fig. 2. Small intestine proximal to site of ligation. **a, b.** SEM detail of mucosal surface. Cytoplasmic blebs protrude into the lumen. Some cells have a smooth microvillus coat (*asterisks*), while on others the microvilli are coarse and clumped (*arrows*). **a** ×1500, **b** ×4700. **c** Bulging enterocytes result in a very irregular apical surface. An enterocyte with a cytoplasmic bleb is present (*arrow*). Numerous vesicles and tubules of the apical endocytic are conspicuous below the brush border (*asterisk*). Enterocytes accumulate cytoplasmic glycogen (*G*). Transmission electron micrograph (TEM) ×4000. **d** Towards the villus base and in the crypts, microvillus morphology is disrupted. Some cells develop patches of elongated microvilli (*arrows*). TEM ×8700

and tall jejunal villi (finger-like) were present (Fig. 1a). At higher magnification a smooth, even microvillus coat was visible (Fig. 1b). In fetuses with intestinal obstruction, the villi proximal to the site of obstruction were club-like, or segmented and branching in shape (Fig. 1c). Focally, either on individual or groups of villi, or on only one surface of particular villi, the apical surface of villus enterocytes bulged into the lumen, creating a bubbly appearance to the epithelial surface (Fig. 1d). At the cellular level, there were small, club-like processes (pseudopods) present, which bulged out of the apical surface of the enterocytes (Fig. 2a, b). These blebs were covered with microvilli. The microvilli of the rest of the same cell and of cells nearby were more clumped, compared to the normal smooth, velvet-like appearance (Fig. 2a).

TEM examination confirmed that the enterocytes were less columnar in shape, often with irregular apical borders (Fig. 2c). Sections through the pseudopods were noted along the apical surface (Fig. 2c). The granular endoplasmic reticulum of villus enterocytes was well-developed and dilated and the apical endocytic complex was still present. Many focal irregularities of the brush border were present including areas with unusually long microvilli, particularly in the crypts (Fig. 2d). Apical vesicles were present in crypt cells.

Distal to the site of obstruction, villi were cylindrical and finger-like in shape, i.e. similar to those in proximal sites of normal fetuses (see Fig. 1a, b). Enterocytes accumulated glycogen in the basal region of their cytoplasm and the nucleus was characteristically located in the upper third of the cell. The apical endocytic complex was

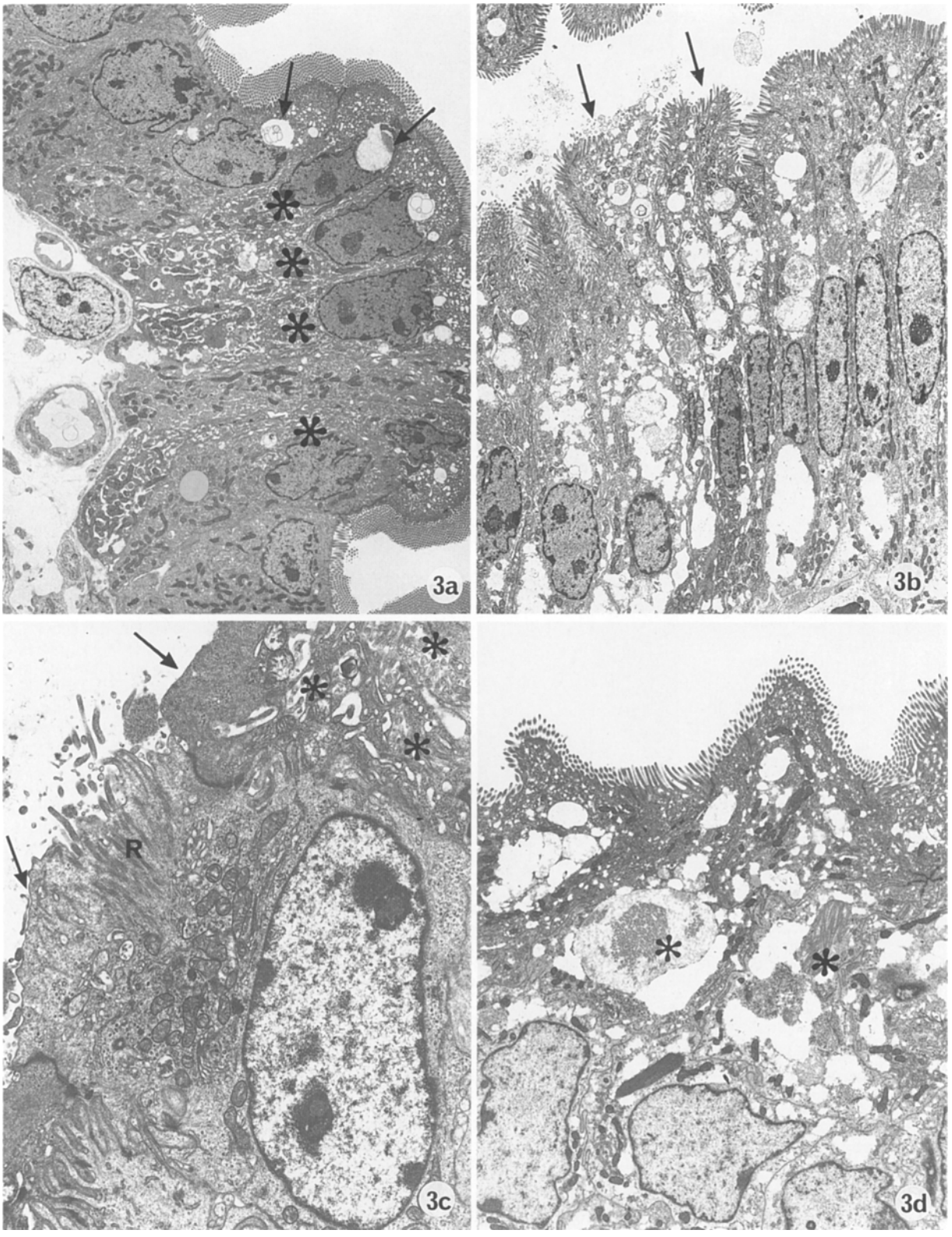

Fig. 3. Distall small intestine, proximal to the ileo-caecal junction, i.e. remotely distal to site of obstruction. **a** Areas of extensively vesiculated cells (*asterisks*) are present on some villi. Although the apical endocytic complex is present, vacuoles are small and poorly developed (*arrows*). TEM ×2800. **b** Cells towards the villus tip are crowded together. The apical surface is very distorted (*arrows*). Small vacuoles are present, some of which contain filamentous material (see **Fig. 4d**). TEM ×2800. **c** Focal areas of grossly disrupted brush border are present on many villi. Some cells have no brush border (*arrows*), others have aberrant microvilli, often with deep rootlets (*R*). Some cells are extensively vesiculated. Within this extensive intracytoplasmic membrane reticulum, there are many small finger-like profiles present (*asterisk*). TEM ×8200. **d** In many of the enterocytes, vacuoles contain a filamentous material (*asterisks*), shown here in both cross section and longitudinal section. TEM ×5000

still present in experimental fetuses, but sparse or absent in normal fetuses.

In experimental fetuses, in regions more distal (immediately proximal to the ileo-caecal junction), focal areas of vesiculated cells were found (Fig. 3a). Enterocytes bulged into the lumen, creating a very irregular luminal profile (Fig. 3b). There were focal areas with disrupted brush borders (including effacement and microvillus anomalies, Fig. 3c). Cells accumulated extensive pools of cytoplasmic glycogen and the granular endoplasmic reticulum was well-developed. Although the apical endocytic complex was present, the vacuoles were small, compared to control fetuses. Inclusion bodies containing filamentous material were present (Fig. 3d).

Discussion

Previous studies have established that abolition of fetal ingestion results in retarded development of the fetal GIT tissues in rabbits (Wesson et al. 1984; Mulvihill et al. 1985, 1986, 1989; Jacobs et al. 1989), rats (Morikawa et al. 1988) and sheep (Trahair et al. 1986; Avila and Harding 1991) (for a review see Trahair 1993). Our experiments in fetal sheep have demonstrated GIT specific growth retardation, most particularly of the epithelial components, in response to either fetal oesophageal ligation or fistulation. Consistent with results from experiments by other workers using a fetal rabbit model (Mulvihill et al. 1985), we have found that the growth retardation could be prevented if the oesophagus was reconstructed, and swallowing restored in utero (unpublished observations).

In addition to the significant changes to tissue architecture at the light microscope level (Trahair et al. 1986; Avila and Harding 1991), our recent study, using electron microscopy (Trahair and Harding 1992), demonstrated that enterocyte differentiation was adversely affected in the absence of swallowed fluid. We have proposed that swallowed fluid augments the substrate supply for GIT tissues, and so raises the possibility that enteral nutrition might be just as important for development and maintenance of the fetal GIT as it is in the neonate and adult organ (Trahair 1993). Such an effect would be in addition to the role swallowing might play in the delivery of putative GIT growth factors and hormones which are known to be present in amniotic fluid (Schindler 1982; Merimee et al. 1984; Scott et al. 1989), lung liquid (Spencer et al. 1983; Stahlman et al. 1989) which is swallowed (Harding et al. 1984), and any which may be present in GIT accessory gland secretions.

Clinical studies have noted that there are multiple biochemical and functional abnormalities in infants with intestinal obstruction: mucosal disaccharidases, absorption of glucose and vitamin A (Serrano and Zetterström 1987), and motor activity (Tepas et al. 1979) are all reduced in segments proximal to the obstruction. In addition, mucosal lactase is reduced distal to the obstruction (Serrano and Zetterström 1987). These data, together with our observations of vastly altered epithelial morphology might explain the deficiencies of intestinal function which produces failure to thrive persisting for months after correction (Cohen and Greecher 1979).

In the present study, while distal to the site of obstruction, development occurred in the absence of swallowed input, a manoeuvre we now consistently produces GIT growth retardation and abnormal enterocyte differentiation, in regions proximal to the atresia, the GIT was massively hypertrophied. Thus, in the one fetus the GIT can clearly exhibit widely variable tissue responses, especially at the cellular level (see below).

Maintenance of luminal volume is important for adult GIT structural and functional homeostasis (Harris and Kennedy 1988; Harris et al. 1988), it is possible therefore that dilation from the distending force of swallowed fluid could drive the hypertrophy we observed. In addition since pancreato-biliary secretions have been shown to be stimulators of growth (Altman 1974), excess of these secretions in proximal segments might provide an additional growth stimulus.

We did not observe filamentous inclusion bodies in enterocytes in our earlier studies, and their presence as a result of obstruction led us to re-examine archival sections. Though not as abundant, we can now report that these features were present after oesophageal ligation. Similar structures have been described in patients with microvillus atrophy (Phillips and Schmitz 1992). Cytoplasmic blebs, villus cell crowding and abnormal microvilli like those we have described have been noted in cases of childhood diarrhoea (Poley 1991; see also "tufting disease"; Reifen et al. 1992).

We have previously suggested that a common pathway for the altered mucosal growth and abnormal cellular differentiation could be local substrate deficiency, or tissue malnutrition. In chronic diarrhoea, reduced transit time and incomplete digestion markedly limit local substrate availability. Thus it is possible that local tissue malnutrition might be a common aetiology for the similarities seen in obstruction or diarrhoea. This is consistent with our proposition that fetal enteral nutrition is an important component of growth and maintenance of GIT tissues in utero (Trahair 1993).

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References

Altmann GG (1974) Demonstration of a morphological mechanism in the small intestine: role of pancreatic secretions and bile. In: Dowling RH, Riecken EO (eds) Intestinal adaptation. F.K. Schatteur, Stuttgart, pp 75–86

Avila GC, Harding R (1991) The development of the gastrointestinal system in fetal sheep in the absence of ingestion. J Pediatr Gastroenterol Nutr 12:96–104

Bragg LE, Thompson JS, Rikkers LF (1991) Influence of nutrient delivery on gut structure and function. Nutrition 7:237–243

Castillo RO, Pittler A, Costa F (1988) Intestinal maturation in the rat: the role of enteral nutrients. JPEN J Parenter Enteral Nutr 12:490–495

Castillo RO, Feng JJ, Stevenson DK, Kwong LK (1992) Maturation of jejunoileal gradients in rat intestine: the role of intraluminal nutrients. Biol Neonate 62:351–362

Cohen IT, Greecher CP (1979) Nutritional status following surgical correction of congenital gastrointestinal anomalies. J Pediatr Surg 14:386–389

Flowers WK (1983) Hydramnios and gastrointestinal atresia: a review. Obstet Gynecol Surv 38:685–688

Goldstein RM, Hebiguchi T, Luk GD, Taqi F, Guilarte TR, Franklin FA, Niemiec PW, Dudgeon DL (1985) The effects of total parenteral nutrition on gastrointestinal growth and development. J Pediatr Surg 20:785–791

Harding R, Bocking AD, Sigger JN, Wickham PJD (1984) Composition and volume of fluid swallowed by fetal sheep. Q J Exp Physiol 69:487–495

Harris MS, Kennedy JG (1988) Relationship between distension and absorption in rat intestine II. Effects of volume and flow rate on transport. Gastroenterology 94:1172–1179

Harris MS, Kennedy JG, Siegesmund KA, Yorde DE (1988) Relationship between distension and absorption in rat intestine I. Effect of luminal volume on the morphology of the absorbing surface. Gastroenterology 94:1164–1171

Jacobs DG, Wesson DE, Mago-Cao H, Muraji T, Konuma K, Mancer K, Kent G, Heim T (1989) Effect of esophageal ligation on the growth of fetal rabbits. J Pediatr Gastroenterol Nutr 8:245–251

Merimee TJ, Grant M, Tyson JE (1984) Insulin-like growth factors in amniotic fluid. J Clin Endocrinol Metab 59:752–755

Morikawa Y, Shimonaka H, Okada T (1988) Evidence for stimulative effect of amniotic fluid on the development of colonic goblet cells in fetal rats. Jpn J Vet Sci 50:1109–1111

Mulvihill SJ, Stone MM, Fonkalsrud EW (1985) The role of amniotic fluid in fetal nutrition. J Pediatr Surg 20:672–688

Mulvihill SJ, Stone MM, Fonkalsrud EW, Debas HT (1986) Trophic effect of amniotic fluid on fetal gastrointestinal development. J Surg Res 40:291–296

Mulvihill SJ, Halden G, Debas HT (1989) Trophic effect of amniotic fluid on cultured gastric mucosal cells. J Surg Res 46:329–337

Phillips AD, Schmitz J (1992) Familial microvillous atrophy: a clinicopathological survey of 23 cases. J Pediatr Gastroenterol Nutr 14:380–396

Pierro A, Cozzi F, Colarossi G, Pierce AM, Lister J (1987) Does fetal gut obstruction cause hydramnios and growth retardation? J Pediatr Surg 22:454–457

Poley JR (1991) The scanning electron microscope: how valuable in the evaluation of small bowel mucosal pathology in chronic childhood diarrhea? Scanning Microsc 5:1037–1063

Puntis JWL, Ritson DG, Holden CE, Buick RG (1990) Growth and feeding problems after repair of oesophageal atresia. Arch Dis Child 65:84–88

Reifen R, Cutz E, Griffiths A, Sherman P (1992) Tufting disease: a new entitiy causing prototracted diarrhea of infancy. Gastroenterology 102:A574

Schindler AE (1982) Hormones in amniotic fluid. Monogr Endocrinol 21:1–158

Scott SM, Buenaflor GG, Orth DN (1989) Immunoreactive human epidermal growth factor concentrations in amniotic fluid, umbilical artery and vein, serum, and placenta in full-term and preterm infants. Biol Neonate 56:246–251

Stahlman MT, Orth DN, Gray ME (1989) Immunocytochemical localization of epidermal growth factor in the developing human respiratory system and in acute and chronic lung disease in the neonate. Lab Invest 60:539–547

Serrano J, Zetterström R (1987) Disaccharidase activities and intestinal absorption in infants with congenital intestinal obstruction. J Pediatr Gastroenterol Nutr 6:238–243

Spencer GS, Hill DJ, Garssen GJ, MacDonald AA, Colenbrander B (1983) Somatomedin activity and growth hormone levels in body fluids of the fetal pig: effect of chronic hyperinsulinaemia. J Endocrinol 96:107–114

Tepas J, Wyllie RG, Shermeta DW, Inou AE, Pichard LR, Hader JA (1979) Comparison of histochemical studies of intestinal atresia in human newborn and fetal lamb. J Pediatr Surg 14:376–380

Touloukian RJ (1978) Antenatal intestinal adaptation with experimental jejunoileal atresia. J Pediatr Surg 13:468–474

Touloukian RJ, Wright HK (1973) Intrauterine villus hypertrophy with jejuno-ileal atresia. J Pediatr Surg 8:779–784

Tovar JA, Suñol M, Lopez de Torre B, Torrado J (1991) Mucosal morphology in experimental intestinal atresia: studies in the chick embryo. J Pediatr Surg 26:184–189

Trahair JF (1993) Is fetal enteral nutrition important for normal gastrointestinal growth?: a discussion. JPEN J Parenter Enteral Nutr 17:82–85

Trahair JF, Harding R (1992) Ultrastructural anomalies in the fetal small intestine indicate that fetal swallowing is important for normal development: an experimental study. Virchows Arch [A] 420:305–312

Trahair JF, Harding R, Bocking AD, Silver M, Robinson PM (1986) The role of ingestion in the development of the small intestine in fetal sheep. Q J Exp Physiol 71:99–104

Wesson DE, Muraji T, Kent G, Filler RM, Almachi T (1984) The effect of intrauterine esophageal ligation on growth of fetal rabbits. J Pediatr Surg 19:398–399