Acta vet. scand. 1979, *20*, 572—582.

From the Department of Pathology, Veterinary College of Norway, and the National Veterinary Institute, Oslo, Norway.

THE GASTROINTESTINAL MUCOSA IN YOUNG MILK-FED CALVES

A SCANNING ELECTRON AND LIGHT MICROSCOPIC INVESTIGATION *

By

Thor Landsverk

LANDSVERK, T.: *The gastrointestinal mucosa in young milk-fed calves. A scanning electron and light microscopic investigation.* Acta vet. scand. 1979, *20*, 572—582. — Gastrointestinal segments from 4 healthy, 17-, 21-, 22- and 23-day-old calves fed on whole cow's milk were examined. Scanning electron microscopy showed that the anterior duodenum had short villi varying in shape from leaf-shaped to nodular; the middle duodenum had broad, tongue-shaped villi and the anterior, middle, and parts of the posterior jejunum had slender, finger-shaped or leaf-shaped villi. The villi of the mucosa covering Peyer's patches in the posterior jejunum were short and either conical or tongue-shaped; there were also small "pseudovilli" caused by bulges in the lymphoid tissue. Morphometry showed that the villi were longer in the anterior jejunum than in the duodenum and the posterior parts of the jejunum ($P < 0.005$). Morphologically fat absorption was most heavy in the anterior third of the small intestine. Moderate amounts of fat were also found in the epithelium of the posterior jejunum and of the abomasum. Large fat droplets were seen in apical duodenal enterocytes, in contrast to the small epithelial droplets in other areas with fat absorption. Nile blue staining indicated that the fat in the large droplets was esterified.

scanning electron microscopy; intestinal villi; morphometry; fat absorption; milk diet; calves.

During studies of diarrhea in 2- to 4-week-old calves the need for additional information on the normal intestinal morphology became evident. Reports on this subject have been mostly restricted to calves in their first days of life. *Mebus et al.* (1975) studied the intestinal tract of a 2-day-old gnotobiotic calf using

* This study was supported by grants from the Agricultural Research Council of Norway.

techniques including light and scanning electron microscopy (SEM). With similar methods *Pearson et al.* (1978 a, b) studied the morphology of the small intestine in 2 colostrum-deprived, milk-fed, 4-day-old calves.

The purpose of the present investigation was to study the gastrointestinal mucosa in normal, about 3-week-old pre-ruminant calves, fed colostrum and whole cow's milk. Special emphasis was laid on the variation in shape and size of the villi throughout the small intestine and on signs of fat absorption.

MATERIALS AND METHODS

Animals and diet

Two male and 1 female Norwegian Red Cattle (NRF) calves and 1 male crossbred NRF × Charolais calf were born and raised at the Research Station, Heggedal. The calves were given colostrum after birth and then whole cow's milk at 38°C, 110 ml/kg body weight/day. They were bucket-fed 3 times a day during the first week of life and later twice a day at 7 a.m. and 4 p.m. No roughage was given and no bedding materials were used. The calves remained at the pre-ruminant stage as judged by the morphology of the forestomachs at necropsy, when the calves were 21, 17, 22 and 23 days old, for calves 1, 2, 3 and 4, respectively. Euthanasia was done by exsanguination during anesthesia.

Procedures of sampling specimens

Preliminary studies had emphasized the importance of fresh specimens, preferably taken under anesthesia. Within a few minutes of killing by shooting, the enterocytes had been observed to loosen from the lamina propria (*Landsverk* 1976, unpublished). Therefore, in the present investigation, the specimens were collected under anesthesia with pentobarbital sodium (20 mg/kg body weight) by a right laparatomy 3½, 4½, 6 and 7½ h after feeding, for calves 1, 2, 3 and 4, respectively. Specimens were taken from the fundic part of the abomasum, from various parts of the small intestine as listed in Table 1, from the cecum and from the middle of the spiral part of the colon. Sections for light microscopy (LM) were fixed in 10 % neutral buffered formalin or Carnoy's fixative, processed routinely and embedded in paraffin. Frozen sections were made using a modification of the method described by *Floch et al.* (1967): The

Table 1. Sites for samples from the small intestine and small intestinal lengths.

Sites	Duodenum			Anterior jejunum			Middle jejunum	Posterior jejunum			Ileum	Total length of the small intestine
	d1	d2	d3[1]	aj1	aj2	aj3	mj	pj1	pj2[2]	pj3		
Distance from pylorus in meters												
Calf No.												
1	0.05	0.2	0.35	0.55	1.4	2.7	—	12.7	14.0	15.0	16.3	16.5
2	0.05	0.2	—	—	1.0	3.0	8.5	—	—	14.8	16.1	16.3
3	0.05	0.2	0.35	0.55	2.2	7.2	10.2	15.2	16.2	17.5	18.8	19.0
4	0.05	0.2	0.35	0.55	1.2	2.5	10.7	13.3	13.8	14.8	16.1	16.3

[1] Duodenum by the iliac flexure.
[2] Point of transition to continuous thickening of jejunum by Peyer's patches.

sections were placed with the mucosal side down on specimens of liver tissue, attached to corrugated cardboard with pins, frozen in freon chilled with liquid nitrogen, wrapped in aluminium foil and stored at —70°C until tested. Specimens for scanning electron microscopy (SEM) were washed gently in phosphate-buffered isotonic saline, attached to dental wax and submerged and stored at 4°C in diluted Karnovsky's fixative (*Karnovsky* 1965) containing 0.14 M cacodylate buffer, 0.9 % glutaraldehyde and 0.7 % paraformaldehyde, for at least 1 month. Some sections were fixed in a solution containing 3 % glutaraldehyde and 3 % Macrodex® for 2 days at 4°C, and then stored in 70 % ethanol until further processing (*Landboe-Christensen & Parapat* 1972).

Histological methods

Formalin-fixed paraffin sections cut at about 5 µ were stained with hematoxylin and eosin (HE), van Gieson and the Alcian blue (pH 2.5) periodic acid-Schiff (AB-PAS) sequential stain according to *Mowry* (1963). Carnoy-fixed paraffin sections were stained with methyl green-pyronin (*Preece* 1972).

Unfixed frozen sections cut at 8 µ in a cryostat were used for Oil red 0 (*Segerra* 1970), Sudan black B (*Pearse* 1968) and Nile blue. The Nile blue stain was used largely according to *Chayen et al.* (1973). In order to prevent the sections from breaking up in the aqueous staining medium without the use of a fixative, 5 % polyvinyl alcohol was included in the staining medium (*Altman & Chayen* 1965).

Measurement of the mucosa

The lengths of the villi were measured with an ocular micrometer from the tip to the crypt-villus junction, and the crypt depths from the crypt-villus junction to the crypt base. Only longitudinally sectioned crypts and villi were measured. In each calf 10—15 measurements were made per site, at sites d1 and d2 (anterior and middle duodenum), aj2 (anterior jejunum 2), mj (middle jejunum), pj1 and pj3 (posterior jejunum 1 and 3); pj3 was measured over Peyer's patches.

Statistical evaluation

A sample mean with standard deviation was calculated for each site in each animal. The significance of the differences between intestinal sites was assessed by analysis of variance (2-way classification) and the chi-square test. The Neuman-Keul multiple test was also performed to reveal differences between all possible pairs of comparisons.

Scanning electron microscopy

Specimens fixed in Karnovsky's fixative were dehydrated in acetone and air-dried. The glutaraldehyde fixed specimens were air-dried after being dehydrated in ethanol and equilibrated in acetone. For control, some specimens were critical point dried with carbon dioxide as the transitional fluid. The dried specimens were attached to metal stubs with silver paste and coated with gold in a vacuum evaporator. Coated samples were examined in a Jeol 50 A scanning electron microscope with an accelerating voltage of 10—15 kV. Photographs were recorded on Polaroid Type 52 film.

Bacteriological examination

Routine bacteriological examinations of intestinal contents taken at necropsy including analysis for Salmonella spp. were made.

RESULTS

The calves seemed healthy. They were alert, had good appetite, and no diarrhea was seen.

At necropsy, gross examination showed that the serosal lymphatics in the anterior jejunum were filled with milk-white

lymph. The mucosal side of this part of the jejunum also appeared whitish. These areas of the small intestine constituted approx. 20 %, 45 %, 40 % and 30 % of the anterior jejunum of calves 1, 2, 3 and 4, respectively. No pathogenic bacteria were isolated from the intestinal contents.

With SEM the specimens gave identical results, independent of the somewhat varied methods of tissue processing: In the anterior duodenum the villi were short and showed great variation in shape. Within the same area leaf-, tongue- and finger-shaped villi could all be seen. In some areas, especially at the tips of the mucosal folds, the villi were nodular (Fig. 1). Some villi appeared fused or were interconnected with bridge-like structures (Fig. 2). The posterior part of the duodenum (d2 and d3) had relatively broad tongue-shaped villi (Fig. 3). The anterior, middle and the parts of the posterior jejunum, which were not occupied by Peyer's patches, had slender, finger- or leaf-shaped villi (Figs. 5, 6). The villi over Peyer's patches, which occupied about ¾ of the intestinal circumference and formed a continuous thickening of the posterior 15 % of the small intestine, were short, conical or tongue-shaped (Fig. 7). Between the "conventional" villi over Peyer's patches there were additional small "pseudovilli", with bulging of individual cells (Figs. 7, 9). Small pores were sometimes seen in enterocytes at the apex of the "pseudovilli" (Fig. 9). The colon had a relatively smooth mucosa with narrow clefts (Fig. 13).

The villi and crypt lengths in different parts of the small intestine are given in Fig. 15. The differences between villi lengths at the various sites of the small intestine were significant ($P < 0.005$). The ranging in villi lengths between the intestinal sites was as follows: d1 < pj3 < pj1 < d2 < mj < aj2. There was a significant difference between crypts at sites aj2 and pj1, and those at sites d1, d2, mj and pj3, so that aj2 and pj1 < d1, d2, mj and pj3 ($P < 0.05$). Within these 2 groups there were no significant differences ($P > 0.05$). The average villous to crypt ratios at intestinal sites d1, d2, aj2, mj, pj1 and pj3 were: 1.3, 3.4, 5.8, 4.3, 3.0 and 1.6, respectively.

With LM the villi were seen to have a columnar epithelium in which the cells were taller in the anterior parts of the small intestine than in the posterior parts. The nuclei of the enterocytes were situated near the cell base. The supranuclear cytoplasm of the villous enterocytes was only slightly pyroninophilic,

in contrast to the strongly pyroninophilic whole cytoplasm in the crypt cells. In apical duodenal enterocytes there were large unstained vacuoles occupying most of the cell cytoplasm (Fig. 11). They were located mainly in the supranuclear zone, but could also be seen at the base of the cell. The nucleus sometimes seemed to be pressed between the vacuoles or towards the base of the cell and was occasionally crescent-shaped.

With AB-PAS staining small intestinal goblet cells were most frequent in the anterior duodenum and in the posterior small intestine. Throughout the small intestine goblet cells were most frequent in the lower portions of the crypt-villus unit. Cecum and colon were especially rich in goblet cells (Fig. 14).

Intraepithelial lymphocytes were sometimes seen in the villi, and the lamina propria had a moderate cellular infiltration (Fig. 4) consisting of macrophages, plasma cells and lymphocytes. Cells with acidophilic granules were often seen. Most of them had an ovoid nucleus and were possibly globule leukocytes.

The "pseudovilli" resulted from bulges in the lymphoid tissue. This tissue was lined with a mostly columnar epithelium with small cytoplasmic vacuoles (Figs. 8, 10). There were few goblet cells, and the outer AB-PAS positive border was thinner than in ordinary villous enterocytes. There seemed to be a frequent migration of neutrophils. Within the epithelium, cells resembling globule leukocytes were frequently seen. Intraepithelial lymphocytes were sometimes observed.

Fat absorption, as seen in Oil red 0 and Sudan black B sections, was mostly found in areas corresponding to the whitish mucosal areas seen macroscopically. Fat absorption was most heavy in the apices of the villi, although it was present along the entire villous length. The apical duodenal enterocytes appeared to be completely filled with mostly large fat droplets which apparently corresponded to the vacuoles seen in the HE sections (Figs. 11, 12). In other areas with fat absorption the enterocytes contained mostly small fat droplets. With Nile blue the large as well as the small fat droplets stained pink or bright red. Moderate collections of small fat droplets were also seen in the epithelium of the posterior parts of the jejunum and of the abomasum. In areas where marked epithelial fat absorption was seen, the subepithelial villous stroma and central lacteals always contained large amounts of fat.

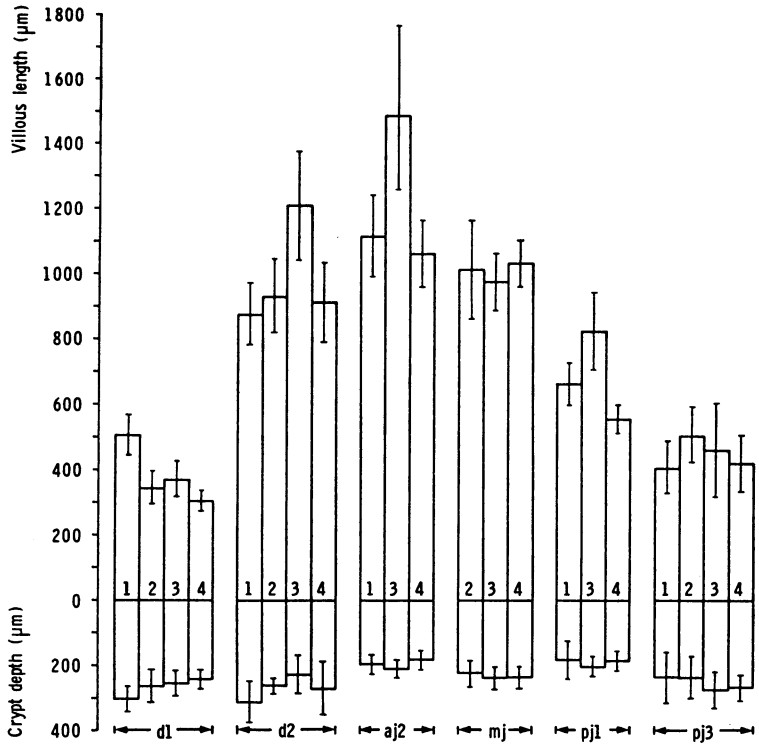

Figure 15. Mucosal measurements of the small intestine of the various calves (1—4) with the standard deviations indicated. d1 and d2 = anterior and middle duodenum, aj2 = anterior jejunum 2, mj = middle jejunum, pj1 and pj3 = posterior jejunum 1 and 3. pj3 was measured over Peyer's patches.

DISCUSSION

This study demonstrated more and less marked differences in the SEM appearance of the villi between different parts of the small intestine of the milk-fed calf. In the anterior, middle and parts of posterior jejunum the villi were relatively uniform in shape and generally resembled the villi reported in a 2-day-old gnotobiotic calf (*Mebus et al.* 1975) and in a 4-day-old calf (*Pearson et al.* 1978b). The short and sometimes nodular villi in anterior duodenum have not been described previously. In the adult rat *Baker et al.* (1963) reported low parallel ridges in the duodenal and upper jejunal mucosa in contrast to the leaf-shaped villi in the posterior parts of the jejunum.

The long, slender villi of the anterior jejunum found in this study create a large mucosal surface per square unit of intestine. The absorption of fat in this area was heavy, and according to *Mylrea* (1966) this applies to the intestinal absorption of other nutrients too. This intense absorptive activity may thus reflect the large mucosal surface.

The LM appearance of villi reported here is largely in accordance with the conventional textbook picture (*Stinson & Calhoun* 1976). The basal position of the nuclei in the villous enterocytes reported here is in contrast to the central or apical position of the nuclei described in the gnotobiotic calf studied by *Mebus et al.* This descrepancy may be related to the age difference since the calves in the present report were older and the intestinal epithelium was probably more mature than in the other one.

The occurrence of fat droplets in the abomasal epithelium coincides with similar observations in the stomach of puppies (*Weiss* 1912) and mice (*Turchini et al.* 1965). Calf pregastric esterase causes abomasal fat hydrolysis (*Otterby et al.* 1964), and the products of this triglyceride breakdown may permit fat absorption in the abomasum and in the anterior duodenum. According to *Mylrea,* however, any fat absorption that takes place in the abomasum seems to be limited.

Large fat droplets like those in the apical duodenal enterocytes have also been seen in neonatal rats (*Vacek et al.* 1962). In 4-day-old calves *Pearson et al.* (1978a) reported large vacuoles in apical enterocytes in the proximal small intestine which probably represented fat droplets. However, they did not use fat stains in their investigation. In the present study staining with Nile blue indicated that a substantial part of the fat in the large droplets was esterified. The accumulation of fat into large droplets in apical duodenal enterocytes may indicate inefficient fat transport, although the further transport of fat into the central lacteal seemed unimpaired. The mechanism causing this fat accumulation might possibly be insufficient intracellular chylomicron formation, somewhat similar to the fat accumulation seen in rats when intracellular protein synthesis is blocked with puromycin (*Friedman & Cardell* 1972). *Vacek et al.* reported that the size of fat droplets in apical enterocytes in the rats gradually decreased with age; the large fat droplets seen in this study might therefore be related to the young age of the calves.

The "pseudovilli" with their characteristic epithelium over-

lying lymphoid tissue probably represent specialized structures that play an important role in the intestinal immune response. Uptake of macromolecules in the epithelium overlying Peyer's patches has been demonstrated in mice (*Owen* 1977). It is possible that this epithelium may transport antigens from luminal contents to the immunocompetent cells of the lymphoid follicles. A separate report on the "pseudovilli" is in preparation.

ACKNOWLEDGEMENTS

I wish to thank the Department of Anatomy, Dental Faculty, University of Oslo, for allowing me to use its scanning electron microscope, and S. Stølen, engineer, for operation of the instrument. I also wish to express my gratitude to Dr. Ø. Lie, the National Veterinary Institute, for his help with the statistical calculations.

REFERENCES

Altman, F. P. & J. Chayen: Retention of nitrogenous material in unfixed sections during incubation for histochemical demonstration of enzymes. Nature (Lond.) 1965, *207*, 1205—1206.

Baker, S. J., V. I. Mathan & V. Cherian: The nature of the villi in the small intestine of the rat. Lancet 1963, I, 860.

Chayen, J., L. Bitensky & R. G. Butcher: Practical Histochemistry. John Wiley & Sons Ltd., London, New York, Sydney, Toronto 1973, 83—84.

Floch, M. H., S. Van Noorden & H. M. Spiro: Histochemical localization of gastric and small bowel mucosal enzymes of man, monkey and chimpanzee. Gastroenterology 1967, *52*, 230—238.

Friedman, H. I. & R. R. Cardell: Effects of puromycin on the structure of rat intestinal epithelial cells during fat absorption. J. Cell Biol. 1972, *52*, 15—40.

Karnovsky, M. J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 1965, *27*, 137A—138A.

Landboe-Christensen, E. & S. B. Parapat: The gastrointestinal mucosa in man, its surface architecture. Some observations by the scanning electron microscope. Jeol News 1972, *9* (4), 12—15.

Mebus, C. A., L. E. Newman & E. L. Stair: Scanning electron, light and transmission electron microscopy of intestine of gnotobiotic calf. Amer. J. vet. Res. 1975, *36*, 985—993.

Mowry, R. W.: The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of Alcian blue G8x and their combinations with the periodic acid-Schiff reaction. Ann. N.Y. Acad. Sci. 1963, *106*, 402—423.

Mylrea, P. J.: Digestion of milk in young calves. II. The absorption of nutrients from the small intestine. Res. vet. Sci. 1966, 7, 394—416.

Otterby, D. E., H. A. Ramsey & G. H. Wise: Lipolysis of milk fat by pregastric esterase in the abomasum of the calf. J. Dairy Sci. 1964, *47*, 993—996.

Owen, R. L.: Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: An ultrastructural study. Gastroenterology 1977, *72*, 440—451.

Pearse, A. G. E.: Histochemistry. Theoretical and Applied. J. & A. Churchill Ltd., London 1968, I, 691—692.

Pearson, G. R., M. S. McNulty & E. F. Logan: Pathological changes in the small intestine of neonatal calves with enteric colibacillosis. Vet. Path. 1978a, *15*, 92—101.

Pearson, G. R., E. F. Logan & G. P. Brennan: Scanning electron microscopy of the small intestine of a normal unsuckled calf and a calf with enteric colibacillosis. Vet. Path. 1978b, *15*, 400—406.

Preece, A.: A Manual for Histologic Technicians. Little, Brown and Company, Boston 1972, 359—361.

Segerra, J. M.: Histological and histochemical staining methods: A selection. *In* C. G. Tedeschi: Neuropathology, Methods & Diagnosis. Little Brown, Boston 1970, 233—269.

Stinson, A. W. & M. L. Calhoun: Digestive system. *In* H.-D. Dellmann & E. M. Brown: Textbook of Veterinary Histology. Lea & Febiger, Philadelphia 1976, 207—264.

Turchini, J. P., R. Pourhadi & P. Malet: Absorption de lipides et activités estérasiques dans la muqueuse gastrique fundique du nouveau-né (Souris). (Absorption of lipids and activity of esterases in the fundus gland region of the mucosa in the newborn (Souris). C. R. Soc. Biol. (Paris) 1965, *159*, 663—665.

Vacek, Z., P. Hahn & O. Koldovský: Histological study of fat distribution in the small intestine, liver and lungs following oral fat administration to rats of different postnatal ages. Čs. Morfol. 1962, *10*, 30—45.

Weiss, O.: Die Resorption des Fettes in Magen. (Fat absorption in the stomach). Pflügers Arch. ges. Physiol. 1912, *144*, 540—543.

SAMMENDRAG

Mage-tarmmukosa hos unge melkefórede kalver. En skanning elektron- og lysmikroskopisk undersøkelse.

Mage-tarmmukosa hos 4 friske kalver 17, 21, 22 og 23 dager gamle, fóret med helmelk, ble undersøkt. Ved skanning elektron-mikroskopi såes i fremre duodenum små villi som varierte fra blad- til knuteformede, i midtre duodenum brede, tungeformede villi og i fremre, midtre og deler av bakre jejunum slanke, finger-, eller bladformede

villi. Mukosa over Peyer-plettene i bakre jejunum hadde korte, koniske eller tungeformede villi, og mellom disse såes små "pseudovilli" som var epitelkledte fremhvelvinger av lymfoid vev.

Ved morfometriske undersøkelser viste det seg at villi i fremre jejunum var lengre enn i duodenum og i bakre avsnitt av jejunum ($P < 0{,}005$). Etter morfologien å dømme var fettabsorbsjonen størst i fremre tredjedel av tynntarmen. Moderate mengder fett ble også funnet i epitelet i bakre tynntarm og i løpe. I epitelcellene apikalt i duodenalvilli såes store fettdråper, mens det i andre områder med fettabsorbsjon var små dråper i epitelcellene. Nilblått farging indikerte at fettet i de store dråpene var forestret.

(Received March 22, 1979).

Reprints may be requested from: T. Landsverk, the Department of Pathology, Veterinary College of Norway, P.O.Box 8146, Dep., Oslo 1, Norway.