The Ultrastructure of Neonatal Calf Intestine and Absorption of Heterologous Proteins

THEODORE E. STALEY,1 LANE D. CORLEY, LINVILLE J. BUSH AND E. WYNN JONES
College of Veterinary Medicine and College of Agriculture, Oklahoma State University, Stillwater, Oklahoma

ABSTRACT The ultrastructural morphology of the jejunal and ileal cells of newborn calves was similar to the intestinal absorptive cells of other newborn ungulates. Microvilli were well developed, tubules or invaginations in the apical cytoplasm were extensive. Large supranuclear vacuoles were limited to the ileal cells. After injection of ferritin-IgG or ferritin into ligated intestinal loops, the ferritin particles were found around the microvilli and within the tubular system. After 2–6 hours ileal vacuoles containing ferritin were found near the basal membrane. In the jejunal cell ferritin was found only in the tubules. No ferritin could be detected in calf sera after injection into the intestinal loops. To establish that heterologous proteins were absorbed, calves were given human serum via stomach tube and their sera subsequently was found to contain circulating levels of human albumin and gamma globulin. Also newborn pigs and suckling rats also were given ferritin; but it could not be detected in their sera. The results of these experiments suggest that while the neonatal intestine is permeable to some heterologous proteins, ferritin is not transported across the absorptive cell into the circulation.

The permeability of the small intestine in the calf to large molecular weight proteins has been reviewed by Brambell ('58). This permeability in other neonatal animals i.e. pigs (Mattisson and Karlsson, '64; Staley et al., '68a, '69a) pups (Anderson, '65; Rubin, '66; Staley et al., '69b) and rats (Clark, '59; Graney, '68; Rodewald, '70) has been shown to be due to the capability of the apical surface membrane of the intestinal absorptive cells to invaginate and form tubules that subsequently ended in vacuoles. The vacuoles then contacted the lateral intercellular space or passed the length of the cell and discharged their contents through the basal cell membrane into the lamina propria, depending on the species involved.

While considerable physiological information is available on absorption of colostrum (Bangham et al., '58) and immunoglobulins (Klaus et al., '69) in the calf, little information has been presented on the ultrastructural events of absorption.

The objects of this investigation were to characterize the ultrastructural morphology of the jejunal and ileal mucosa of the newborn calf and to demonstrate the pathway for absorption of large molecular weight proteins such as ferritin and the immunoglobulin IgG. Further, evidence will be presented to demonstrate that heterologous proteins such as human serum proteins will pass the intestinal absorptive cells into the circulation.

MATERIALS AND METHODS

1. Morphological studies of normal intestine

In experiment 1, seven Holstein and Ayshire calves obtained at birth were not allowed to nurse, and were sacrificed within four-six hours for morphological studies.

2. Morphological and immunological studies of ferritin absorption in calves

Experiment 2 was designed to study the absorption of ferritin suspended in two dif-
ferent carrier solutions, as well as ferritin conjugated to IgG.

In five newborn unfed calves, three ligated intestinal segments were prepared in both the jejunum and ileum. Anesthesia was induced with intravenous sodium pentobarbital, a laparotomy was performed and intestinal segments three inches in length were isolated by ligation. Care was taken not to obstruct the intestinal vessels and lymphatics. Areas of the intestine were selected as follows: jejunum—midportion of the small bowel, and ileum—five inches from the attachment to the large intestine. Each of the three ligated loops of each area of the intestine was injected with 2 ml of one of the three test absorptive markers. Beginning with the most distal loop and proceeding proximally: (1) rabbit antihuman IgG conjugated to ferritin (12–16 mg/100 ml protein) (Cappel Laboratories), (2) horse spleen ferritin (1 mg/ml) (Pentex Biochemicals) which had been dialyzed cadmium free with 0.7 M EDTA, then redialyzed with 0.1 M sodium phosphate, (3) cadmium free ferritin suspended in boiled colostral whey. Boiled colostral whey was prepared according to the methods of Balfour and Comline ('62). The loops were then returned to the abdominal cavity. After two-six hours the intestinal loops were surgically removed from the living anesthesized calves, and jugular vein blood samples were taken for radial immunodiffusion analysis of ferritin.

Tissue processing

Sections of the jejunum and ileum were placed in (4°C) normal saline, opened along their mesenteric border, and immersed in cold (4°C) 3% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 7.4. After initial fixation overnight, the segments were washed in 0.1 M sodium phosphate and cut into pie-shaped segments. Secondary fixation was by (4°C) 1% osmium tetroxide in 0.1 M sodium phosphate for one and one-half hours. The tissues were subsequently washed in the same buffer, dehydrated in graded series of cold ethanol-water, and embedded in Epon (812) (Luft, '61). Gold to silver sections were cut on a Porter-Blum M-2 microtome, were mounted on unsupported 120 mesh copper grids, and were stained in lead citrate and uranyl acetate. A Philips EM-200 was used for examination and photography of the sections. Tissues from all calves were fixed in glutaraldehyde, embedded in Epon, sectioned at 1 μ, mounted on glass slides, and stained with alkaline 1.0% toluidine blue or Periodic Acid Schiff reagent.

3. Absorption of human serum proteins

The third experiment was to determine the amount of absorption of three human serum proteins by the newborn and day old calf.

Human serum was obtained from the blood bank of a local hospital and mixed to form a 20 l pool. The serum was packaged in quart containers and frozen until use.

Newborn calves

Calves were obtained within one-two hours after birth. A peripheral blood sample was taken, the calves were weighed and given human serum via stomach tube into the underdeveloped rumen at the rate of 2 ml/100 gm of body weight. Peripheral blood samples were taken at 2, 6, and 24 hours later, allowed to clot, and the serum was separated and frozen until analysis. Following the initial dose of serum, calves were fed bovine colostrum at the rate of 8% of body weight in two feedings during the 24 hour period.

Twenty-four hour old colostrum fed calves

The time of birth, and body weight was recorded. Calves were fed colostrum on the basis of 8% of body weight for 24 hours at which time they were given human serum (2 ml/100 gm body weight). Peripheral blood samples were obtained as in the previous group.

Composition of human serum

The total protein of human serum was 5.31 gm/100 ml. The albumin (16.93 mg/ml) and gamma globulin (26.17 mg/ml) content was determined by radial immunodiffusion (Fahey and McKelvey, '65).

*Ethylenediamine tetraacetate.
Reference proteins

The reference for determining human serum protein absorbed and present in calf serum was the human serum pool (total protein 5.31 gm/100 ml) given to all calves. The two other reference proteins were human albumin (16.0 mg/ml) (American Hospital Supply Corporation) and human gamma globulin (31.0 mg/ml) (Pentex Biochemicals).

Antisera

Antisera prepared in rabbits to whole human serum protein, human albumin, and human gamma globulin was obtained from Pentex.

Serum analysis

The sera of calves was analyzed by separate radial immunodiffusion assays (Fahey and McKelvey, '65) for whole human serum protein, human albumin, and human gamma globulin. One milliliter of one of the respective antisera, 7 ml of phosphate buffer, and 8 ml of 2.5% ionagar (Colab Laboratories) was poured onto a three and one-quarter × four inch glass plate and allowed to solidify. A plastic template with 35, 2 mm diameter holes was used to cut wells in the agar plate. The sera to be tested was added to the wells with a capillary tube and allowed to diffuse into the agar containing the antisera. Triplicate determinations were made on all sera. Plates were refrigerated at 4° in a humid, air tight container for 24 hours, was washed in cold normal saline for 48 hours, dried over-night, and stained with amido Black B. The precipitate ring diameters were measured with a magnified millimeter scale.

4. Immunological studies of ferritin absorption in pigs and rats

The fourth experiment was to determine whether ferritin per se could be absorbed into the circulation. Small neonatal animals were chosen to dose via stomach tube with ferritin. Unsuckled newborn pigs and two-day old suckled rats were used for this experiment because it was not practical to dose 50 kg calves with ferritin solutions.

Five pigs and five rats were given 2 ml/100 gm body weight of 100 mg/100 ml ferritin solution. After six hours suborbital blood samples were taken for analysis of serum by radial immunodiffusion with an antiferritin serum (Cappel). The sensitivity of the radial immunodiffusion technique to ferritin was 12 µg/ml as determined by serial dilution of a standard ferritin solution.

A summary of the experimental procedures is presented in table 1.

RESULTS

Normal ultrastructure: Jejunal absorptive cells

Jejunal villous cells (fig. 1) were typically columnar with well developed microvillous borders. The nuclei of the jejunal cells were generally oval, were located in

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number and kind of animals</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>1</td>
<td>7 Newborn calves</td>
<td>Sacrifice for normal morphology of intestine</td>
</tr>
<tr>
<td>2</td>
<td>5 Newborn calves</td>
<td>Inject separate ligated loops of jejunum and ileum with ferritin-phosphate, or ferritin-IgG conjugate, or ferritin in boiled colostral whey. Sacrifice at two to 6 hours. Remove intestinal mucosa for electron microscopy, and obtained peripheral blood samples for analysis of ferritin.</td>
</tr>
<tr>
<td>3</td>
<td>5 Newborn calves</td>
<td>Intragastric administration of human serum, obtain peripheral blood samples prior to dose (0 hour), then 2, 6, and 24 hours after dose.</td>
</tr>
<tr>
<td>4</td>
<td>5 Newborn pigs</td>
<td>Intragastric administration of ferritin, obtain peripheral blood samples prior to dose (0 hour) and 6 hours later.</td>
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the apical cytoplasm, and contained dense chromatin aggregations around the periphery. Nucleoli were present in the nuclei of most sections. The Golgi apparatus tended to be subnuclear or arranged around the lower one-third of the nucleus. Most mitochondria were in the base of the cell, with a small number beneath the microvillus border, and scattered through the cytoplasm. The lateral cell membranes were extremely interdigitated with adjacent epithelial cells. Migrating lymphocytes were common between the epithelial cells. Few strands of endoplasmic reticulum were visible in the base of the cells. Small vesicles were located adjacent to the lateral and basal cell membranes. These vesicles were found contacting and fusing with cell membranes. Multi-laminated bodies were generally found throughout the cytoplasm. The basal lamina was present as a distinct fuzzy filamentous structure beneath the epithelial cells.

The fuzzy coat or glycocalyx was present on the microvillus tips and decreased in amount on the lateral microvillous membranes. The filamentous core of the microvilli was very prominent and extended through the terminal web for approximately one-half the length of the microvilli.

Immediately beneath and at places extending through the terminal web, was the apical tubular complex (fig. 10). This tubular complex was demonstrated to best advantage by oblique sections through the apical cytoplasm of the cell (fig. 3). Tubules arose by invaginations between the base of the microvilli, but connections with the lumen of the intestine were not often apparent. The tubular system occurred in two major forms or profiles. The most readily apparent form which was visible microscopically at magnifications of 2000 times, was the dilated tubule. Dilated forms attained a diameter of 0.2 μ and assumed a variety of rounded to spherical shapes. The non-dilated tubules, the other forms were most apparent as a longitudinal convoluted profile which appeared to lie at right angles to the microvillus border of terminal web. Structurally both tubular forms were composed of a trilaminar membrane. The lamina lining the tubules contained spines or leaflets which projected into the tubular lumen. The next most apparent structures were the apical cytoplasmic vacuoles which were 0.3 μ in diameter. These vacuoles were found in direct connection with the tubules, as in the ileal cells (fig. 12). In addition the trilaminar membrane forming the vacuole wall was similar and leaflets were found on the inner most lamina.

Normal ultrastructure: Ileal absorptive cells

The ileal cells (fig. 2, 4) had many similarities to the jejunal cells. The microvilli on the cells of the villus tip were short, no glycocalyx was evident, and the intermicrovillous membrane was irregular. Tubules and vacuoles were prominent below and within the terminal web. The cells on the lower portion of the villi appeared more like the conventional mature cell, with orderly microvilli and no tubules or vacuoles (fig. 5). The most apparent difference between the ileal cell and the jejunal cell was the large supranuclear vacuole which occupied the apical two-thirds of the ileal cell, extending almost to the terminal web (fig. 4). Many ileal cells appeared to be composed of slender peripheral portions of cytoplasm surrounding a large vacuole (fig. 2). The supranuclear ileal vacuole contained an electron dense flocculent material that tended to aggregate adjacent to the enclosing vacuole membrane. This vacular material was Periodic Acid Schiff positive on 1 μ light microscopic preparations. There was a progression in vacuolar size from small at the mid-portion of the villus, and larger at the villous tips. In the ileal cell the Golgi apparatus was supranuclear and in direct contact with the supranuclear vacuoles. The enclosing vacuole membrane of the supranuclear vacuole was smooth on the inner most lamina. In addition, dilations of the Golgi cisternae were present between the supranuclear vacuoles and the Golgi center (fig. 6). Fixation of vacuole membranes was generally fair to poor and many discontinuities of ileal vacuoles were evident. The nuclei and mitochondria were restricted primarily to the basal portions of the cell. Nuclei had irregular outlines, especially in the cells of the villous tips, with many cyto-
plasmic channels passing between the folds of the nuclear membrane.

Small coated vesicles were abundant adjacent to the lateral and basal cell membranes. Oblique sections through the apical cytoplasm provided, as in the jejunal cell, the best profile of tubules. Projections from the luminal lamina of the tubules were evident in dilated or non-dilated tubules (fig. 7). Continuities of the tubules and apical vacuoles were occasionally observed (fig. 12).

**Lamina propria of the jejunum**

The subepithelial space was composed of a basal lamina beneath which was scattered electron opaque material, presumably serum protein, reticular fibers, and a variety of connective cellular elements. Fenestrated blood capillaries occurred at intervals in close association with the absorptive epithelium (fig. 8). Within the lamina propria were phagocytes, lymphocytes, fibroblasts, and few plasma cells and eosinophils (fig. 1). The cytoplasm of the capillary endothelium was quite electron dense, contained many vesicles, free ribosomes, and an irregular luminal border. The lymphatic endothelium, characterized by endothelial cells with homogenous electron dense granules. Some granules had a definite crystalline arrangement of their contents (fig. 8). The lymphatic endothelial cell was quite complex with a well developed Golgi apparatus and many vesicles, abundant ribosomes, endoplasmic reticulum, and mitochondria. The cell membrane which formed the lining of the lymphatic lumen was irregular with deep infoldings and cytoplasmic projections. The intervening space between these vessels contained a faint basement membrane around the capillary endothelium, but none was apparent around the lymphatic. Reticular fibers filled the remaining space with occasional fibroblasts.

**Absorption of ferritin-IgG or ferritin**

The summation of the morphological picture of the uptake of ferritin-IgG conjugate or ferritin suspending in 0.1 M sodium phosphate or ferritin in boiled colostral whey by absorptive cells of the jejunum and ileum is shown in table 2.

In this morphological description the term ferritin tetrad or ferritin is used to describe all forms of ferritin since no distinction can be made in tissue sections. The jejunal cells did not take up ferritin but ferritin-IgG was observed in the apical tubular complex (fig. 10). The cells of the ileum were the most active in the uptake of ferritin-IgG or ferritin.

In the ileum the ferritin tetrad was found between the microvilli and within the apical tubules. The tubules did not become filled to capacity but contained randomly scattered particles of ferritin. The ferritin tetrad tended to lie adjacent to the outer lamina of the trilaminar membrane of the tubules and contact with the membrane was frequently observed. Both tubular profiles (dilated and non-dilated) contained ferritin, however, photographic estimates of amounts was not reliable and considerable variation occurred between sections (fig. 11). At the level of the 0.2 \( \mu \) vacuoles, large aggregates of ferritin were dispersed within the vacuoles. Clear areas or areas filled with a slightly flocculent material were interspersed between the aggregations. Portions of apical tubules were
Ferritin absorption. Summary of: (1) Calf intestinal cell uptake of ferritin, (2) radial immunodiffusion assays for ferritin of peripheral blood serum from (a) calves which were given ferritin intraluminal into ligated intestinal loops, and (b) pigs and (c) rats which were given ferritin by intragastric tube

<table>
<thead>
<tr>
<th>Ferritin solution</th>
<th>Calf intestinal cell</th>
<th>Radial immunodiffusion</th>
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<tr>
<td></td>
<td>Jejunal</td>
<td>Ileal</td>
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<tr>
<td>Ferritin-IgG</td>
<td>Apical tubules</td>
<td>Vacuoles near BCM</td>
</tr>
<tr>
<td>Ferritin-phosphate</td>
<td>NU</td>
<td>Vacuoles near BCM</td>
</tr>
<tr>
<td>Ferritin-BCW</td>
<td>NU</td>
<td>Vacuoles near BCM</td>
</tr>
</tbody>
</table>

NU, no uptake; BCM, basal cell membrane; BCW, boiled colostral whey; (—), negative reaction of sera with antiferritin sera; NT, not tested.

Peripheral sera from calves which received all ferritin solutions simultaneously into separate ligated loops was tested immunologically for ferritin only, irrespective of suspending solution or conjugation with IgG.

Table 2

<table>
<thead>
<tr>
<th>Ferritin solution</th>
<th>Average serum levels of human serum protein (HS), human gamma globulin (HGG) and human albumin (HA) in newborn and day old calves (mg/ml)</th>
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<tr>
<td></td>
<td>Time in hours after intragastric instillation of human sera</td>
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<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>NB</td>
</tr>
<tr>
<td>HS</td>
<td>(5)</td>
</tr>
<tr>
<td>HGG</td>
<td>(5)</td>
</tr>
<tr>
<td>HA</td>
<td>(5)</td>
</tr>
</tbody>
</table>

¹ Mean of NB./mean of DO expressed as a per cent.
( ), Number in parentheses is number of animals used in establishing the mean.
NB, Newborn.
DO, Day old.
±, Standard error.

Ferritin was not detected by radial immunodiffusion assays of peripheral blood sera from calves which received ferritin-IgG, ferritin in phosphate buffer or ferritin in boiled colostral whey in ligated intestinal loops, or from neonatal pigs or two-day old sucking rats which were given ferritin intragastrically table 2.

Absorption of human serum proteins

Human serum proteins were absorbed by the intestine of both newborn and day old calves, table 3. The total amount of all human serum components absorbed and measured by radial immunodiffusion analysis of human serum protein (HS), human gamma globulin (HGG), and human albumin (HA) showed a substantial difference between day old and newborn calves. Analysis of HS component showed a maximum amount in the calves sera of both groups six hours after intraluminal instillation of 2 ml human serum/100 gm of body weight. The day old calves mean serum level of HS at six hours was 46% of that of the newborn calves, a substantial decrease in absorption. HGG in the sera of
both groups of calves was the largest component absorbed. Maximum levels in both groups occurred by six hours. Comparison of the mean serum level of HGG between the two groups indicated a 56% lower level in day old calves. Although HA was absorbed least of all the human serum components measured, maximum levels also occurred by six hours. Comparison of the mean serum levels of HA between the two groups, showed as for HGG, a 56% lower level in day old calves.

**DISCUSSION**

Histological changes in the intestinal epithelium of the calf following colostral protein absorption have been described at the light microscopic level (Smith, '24; Comline et al., '51). In addition electron microscopic studies of the intestinal epithelial cells of nonfed and colostrum fed goats have shown cytological changes during protein absorption (Veress and Baintner, '69). The ultrastructure of the intestinal absorptive cells of the newborn pig has received the most attention of the domestic animals, and undergoes a cytological transition during the first few hours after birth (Mattisson and Karlsson, '66; Sibalín and Bjorkman, '66; Vodovar and Flechon, '66; Staley et al., '68a,b; '69a; Hardy et al., '71). This transition in cytology is apparent in the duodenum of the pig during the first 36 hours of life (Staley et al., '68a; '69, '70). The jejunal tubular system is a feature common to all epithelial cells which take up undigested proteins, i.e., yolk sac (King and Enders, '70), ductuli efferentes (Montorzi and Burgos, '67), in the intestine of fish lacking stomachs (Yamamoto, '66) as well as the proximal convoluted tubule of the kidney (Neustein, '67). The presence of this structure does not necessarily imply that protein is transported across the epithelium but merely that the cell is capable of engulfing protein much in the manner of a macrophage.

The terminal ileum of the calf intestine was initially considered to be the site of protein absorption, due to the presence of periodic acid Schiff positive vacuoles in the epithelial cells (Smith, '24). Balfour and Comline ('62) suggested that the mucopolysaccharide of the ileal cell combines in some manner with the engulfed protein and may be a necessary prerequisite for absorption. The large lamina proprial cells with PAS positive vacuoles found in the apical one-fourth of the ileal villi of the calf have also been reported in the neonatal guinea pig (Clarke and Hardy, '70). The significants of these cells is unknown.

The structure of intestinal lymphatics has been described for the cat (Papp et al., '62) and for man (Dobbins, '66), however, endothelial lymphatic granules have not been reported. It is of importance to recognize and differentiate these granules from
experimental proteins, i.e., ferritin, or peroxidase, used in absorptive studies.

El-Nageh ('67) has presented histological evidence that absorption of gamma globulin occurs mainly in the jejunum and not in the duodenum or ileum of the calf. Fluorescent globulin was shown entering the lymphatics of the jejunum. In the present study ferritin-IgG, but not ferritin suspended in phosphate buffer or in boiled colostral whey, was taken up by the jejunal cell. However, ferritin-IgG was not transported past the apical end of the nucleus. In the ileal cell all ferritin solutions were observed to be transported into the base of the cell. However, in neither area of the intestine was ferritin observed in transit out of the cell. The two hours that ferritin solutions were in contact with the intestinal mucosa would seem to be an adequate time for absorption to occur into the circulation, since human serum proteins were absorbed and present in the circulation within this period. The morphological picture of ferritin absorption had not changed by six hours, at which time human serum proteins were at a maximum concentration. Ferritin did not appear to come into contact with the polysaccharide containing vacuoles. This feature may explain the reason for failure of ferritin to be released from the cell. However, Graney ('68) reported ferritin in the supranuclear vacuoles, but not the release of ferritin from the intestinal cell of the suckling rat.

In the calf accelerated absorption of I125-globulin and polyvinyl pyrrolidone occurred when suspended in bovine colostral whey or boiled bovine colostral whey (Balfour and Comline, '62). This work has been substantiated, and other compounds such as lactate, pyruvate, and potassium isobutyrate will also accelerate absorption (Hardy, '69a). In this study boiled colostral whey did not appear to enhance uptake or release of ferritin from the intestinal cell of the calf.

The inability of ungulates: pigs and calves, to absorb ferritin into the circulation is an enigma since polyvinyl pyrrolidone in calves (Hardy, '69a) and pigs (Hardy, '69b); heterologous and homologous globulins and albumins (Leece et al., '61; Pierce and Smith, '67) and large particles, i.e., Escherichia coli in pigs (Staley et al., '69c) appear to be readily transported. The molecular weight of ferritin (450,000 M.W.) or ferritin-IgG conjugate (450,000 plus 163,000 M.W.) might at first appear to be the determining factor except that in this study human albumin (68,000 M.W.) and human gamma globulin (163,000 M.W.) were absorbed equally well in relation to total dose. Also in the work of Klaus et al. ('69) in calves, IgG (163,000 M.W.) and IgM (10* M.W.) were absorbed with equal facility. From this evidence it appears that the neonatal calf intestinal epithelium has a degree of selectivity and absorbs on the basis of protein rather than molecular weight. The decrease in absorption of human serum proteins in day old calves confirms the process of closure described by Deutsch and Smith, ('57). However, this quantitative analysis shows a decrease in serum levels of human serum protein, human gamma globulin, and human albumin of approximately 44% in day old calves as compared to newborn calves. The reason for this decline in absorption has been related to the cessation of release of proteins from the intestinal absorptive cell rather than cessation of uptake (Clarke and Hardy, '71). Apparently proteolytic activity within the intestinal lumen is not of significance during the initial 24 hours of life in the calf (Hardy, '69c).

Closure to ferritin absorption, in the sense that ferritin is not released into the circulation, had already occurred in the neonatal animal tested. This fact should be borne in mind in future work utilizing electron microscopic markers in absorptive studies.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service Research grants FR-05567 and AI-06461 and Oklahoma Agricultural Experiment Station Project 1450.

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testinal epithelium from young ruminants with

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study of the columnar epithelial cell in the in-
testine of fresh water teleosts: Goldfish (Caras-
sius auratus) and rainbow trout (Salmo irideus).

Abbreviations

E, absorptive epithelium
at, apical tubules
av, apical vacuoles
bm, basement membrane
cap, capillaries
dt, dilated tubules
eos, eosinophil
fb, fibroblast
gc, glycocalyx
G, Golgi
lym, lymphatic
M, microvilli
mv, supranuclear polysaccharide
containing vacuole
nt, nondilated tubule
pv, PAS positive vacuole
sm, smooth muscle
TW, terminal web

PLATE 1

EXPLANATION OF FIGURES

All tissues for electron microscopy were fixed initially in 3% glutaralde-
hyde, postfixed in 1% osmium tetroxide and stained with lead citrate and
uranyl acetate.

1 Columnar intestinal absorptive cells from the jejunum of a newborn
calf. Well developed microvilli (M), tubules (at), and vacuoles (av)
are visible in the apical end of the cell. Nuclei are also located in
the apical end of the cell. The area of the Golgi (G) apparatus and
multilaminated bodies are subnuclear. Capillaries (cap) are in close
apposition to the intestinal epithelial cells, and lamina proprial cells
with bizarre granules are interspersed between the capillaries. × 2,600.

2 Columnar intestinal absorptive cells from the ileum of a newborn
calf. The most apparent feature is the highly vacuolated nuclei. A
flocculent polysaccharide material is dispersed throughout most of the
supranuclear vacuoles (mv). The Golgi apparatus, not readily appar-
ent in this low magnification micrograph, is in a supranuclear posi-
tion. Large cells with PAS positive vacuoles (pv) fill the lamina pro-
pra. Eosinophils are also prominent. × 1,900.
PLATE 2

EXPLANATION OF FIGURE

3 An oblique section through the apical end of two absorptive cells from the jejunum of a newborn calf. Microvilli (M) appear in cross section above the terminal web (TW), with a glycocalyx (gc) extending into the intestinal lumen. Two forms of tubules are apparent, a dilated tubule (dt) and a nondilated tubule (nt). This view exemplifies the degree of surface membrane which has invaginated into the apical cytoplasm. Note tubules (arrows) within the terminal web. The leaflet projections of the lamina lining the tubules are similar to those found in the ileum and are demonstrated in figure 7. × 20,700.
PLATE 3
EXPLANATION OF FIGURES

4 Apical surface of an ileal absorptive cell from the upper portion of an intestinal villous of a newborn calf. Microvilli appear irregular and no glycocalyx is evident. Tubules appear like smooth membranes (arrows) at this magnification. The supranuclear vacuole occupies the apical portion of the cell. Polysaccharide (mv) is present as flocculent electron dense aggregates within the vacuole. × 13,200.

5 Apical surface of an ileal absorptive cell from the lower portion of the intestinal villous of a newborn calf. Microvilli are orderly and no tubules or vacuoles are present. × 14,200.

6 The Golgi apparatus (G) in the ileal cells from the midportion of an intestinal villous are complex and diffuse. The Golgi membranes and vacuole membranes appear similar. Budding portions (arrows) of the Golgi cisternae are suggestive that large supranuclear vacuoles containing polysaccharide (mv) are formed from these membranes. The polysaccharide appears as a flocculent material within the vacuoles and some portions of the Golgi. × 13,400.
PLATE 4

EXPLANATION OF FIGURES

7 An oblique section through the apical end of an ileal absorptive cell of a newborn calf. Dilated tubules (dt) and nondilated tubules (nt), both have leaflets which project into the lumens of the tubules. × 69,000.

8 Lamina propria of the jejunum of a newborn calf with a portion of a capillary (cap) and the adjacent lymphatic (lym). The cytoplasms of the capillary and lymphatic endothelia are very irregular and vesicular. Fenestrations (arrows) in the capillary cytoplasm are abundant beneath the intestinal absorptive epithelium. Insert A, × 16,000. The lymphatic endothelium is identifiable by electron dense granules in the cytoplasm. × 5,600. A crystalline pattern is present in some granules. Insert B, × 13,000.
PLATE 5
EXPLANATION OF FIGURES

9 A large vacuolated cell in the lamina propria of the ileum of a newborn calf. These cells contain PAS positive vacuoles (pv) of various sizes filled with a flocculent polysaccharide similar in appearance to the contents of the ileal absorptive cell vacuoles. This vacuolated cell is in intimate association with the absorptive epithelium (E). A capillary (cap) with a vesicular cytoplasm is present in the lower right corner. An eosinophil (eos), fibroblast (fb), and a portion of a smooth muscle (sm) cell make up the remainder of the electron micrograph. × 6,000.

10 Apical end of a jejunal cell from a ligated intestinal loop of a calf exposed to ferritin-IgG. Ferritin particles (arrows) are seen between the microvilli (M) and within the apical tubular complex. × 43,700.
PLATE 6
EXPLANATION OF FIGURES

All electron micrographs illustrated here were taken from ligated ileal loops of newborn calves after exposure to ferritin-IgG or ferritin for six hours.

11 Oblique section through the apical end of an ileal cell. Ferritin particles (arrows) are seen within the dilated and nondilated tubules. × 65,000.

12 Section through the apical end of an ileal cell. Ferritin particles partially fill an apical vacuole. Tubules (arrows) are seen extending from opposite sides of the apical vacuole. × 16,600.

13 Cytoplasmic vacuole in the midportion of an ileal cell is partially filled with ferritin particles. These vacuoles did not become confluent with the supranuclear vacuole (mv) but were contained in membrane derived from the apical tubules or plasmalemma. × 58,800.

14 Basal portion of an ileal cell overlying the basement membrane (bm) and capillary (cap) endothelium. A small vacuole (arrow) partially filled with ferritin is seen near the basal cell membrane. × 25,200.