Functional organization of the bovine rumen epithelium

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Graham, C., and N. L. Simmons. Functional organization of the bovine rumen epithelium. Am J Physiol Regul Integr Comp Physiol 288: R173–R181, 2005. First published August 19, 2004; doi: 10.1152/ajpregu.00425.2004.—The functional organization of the bovine rumen epithelium has been examined by electron and light microscopy combined with immunocytochemistry to define a transport model for this epithelium. Expression of connexin 43, an integral component of gap junctions, the tight-junction molecules claudin-1 and zonula occludens 1 (ZO-1), and the catalytic α-subunit of Na⁺-K⁺-ATPase was demonstrated by SDS-PAGE and Western blotting. From the lumen surface, four cell layers can be distinguished: the stratum corneum, the stratum granulosum, the stratum spinosum, and the stratum basale. Both claudin-1 and ZO-1 immunostaining showed plasma membrane staining, which was present at the stratum granulosum with decreasing intensity through the stratum spinosum to the stratum basale. The stratum corneum was negative for claudin-1 immunostaining. Transmission electron microscopy confirmed that occluding tight junctions were present at the stratum granulosum. Plasma membrane connexin 43 immunostaining was most intense at the stratum granulosum and decreased in intensity through stratum spinosum and stratum basale. There was intense immunostaining of the stratum basale for Na⁺-K⁺-ATPase with weak staining of the stratum spinosum. Both the stratum granulosum and the stratum corneum were essentially negative. Stratum basale cells also displayed a high mitochondrial density relative to more apical cell layers. We conclude that epithelial barrier function may be attributed to the stratum granulosum and that cell-cell gap junctions allow diffusion to interconnect the barrier cell layer with the stratum basale where Na⁺-K⁺-ATPase is concentrated.

IT IS NOW WELL RECOGNIZED that rumina of sheep and cattle do not act solely to prepare food for subsequent digestion in the abomasum and later intestines but also allow selective uptake of nutrients in the form of large quantities of short-chain fatty acids such as acetate, propionate, and butyrate generated by intraruminal microbial fermentation (8, 19, 20, 23, 24, 27). The rumen is covered by a stratified epithelium that consists of leaflike papillae (10–15 mm in length in the cow), which greatly increase the absorptive surface area (26). That these papillae serve as absorptive structures is suggested by observations that the extent of the papillary proliferation and size respond to dietary-dependent alterations. The intake of high levels of protein and carbohydrate appears to increase papillary size and density via butyrate and propionate regulation of IGF-I production (25).

The transport of NaCl and nutrients across rumen epithelium is not considered to be purely passive. Sheep and cattle rumina may develop spontaneous electrical potential differences and short-circuit currents when isolated and clamped in Ussing chambers bathed in identical medium (17); thus absorption is likely to be mediated by active and secondary active transport processes (23). Interpretation of such rumen transport events has relied on the Koefoed-Ussing model (14), which comprises three compartments: a cellular compartment, an apical (outside rumen)-facing barrier, and a basal (blood)-facing inner membrane (17, 26). In addition, this model implies the existence of a permeability barrier composed of tight junctions delimiting the outside-facing lumen compartment, whereas the basal membrane must sit on a basal lamina (14). To generate a transepithelial short-circuit current in Na⁺-rich medium, an inherent polarity must exist with leak and pump pathways asymmetrically distributed to apical and plasma membrane domains, respectively (5, 14).

Morphologically, from the lumen surface, four distinct cell layers can be distinguished: the stratum corneum, the stratum granulosum, the stratum spinosum, and the stratum basale (26). Electron microscopy of these cell layers suggests that occluding junctions exist at the outer surface of the stratum granulosum (12). There is at present a paucity of information regarding the cellular and subcellular distribution of key transport elements required for the rumen epithelium to generate vectorial substrate flow. Hansen (10) has investigated the amount and the isoforms of the catalytic subunits of Na⁺-K⁺-ATPase expressed in rumen papillae using direct measurements of 86Rb uptake, enzyme activity in homogenates, [³H]ouabain binding, and gel electrophoresis-Western blotting. He found that the α₁-isofrom was expressed and that the Na⁺-K⁺ pump density was 2.6 nmol/g wet wt of tissue, which is two- to threefold higher in density than that found in skeletal muscle. Earlier histochemical localizations of total ATPase activity within the rumen showed that the outer nonkeratinized cell layers were responsible for Na⁺ pumping (11). However, these histochemical techniques to identify Na⁺- and K⁺-stimulated ATPase activity in bovine rumen epithelium were hampered by the presence of a large nonspecific Mg²⁺-stimulated activity (9). Therefore, there is no information on the relative cellular and subcellular distribution of the Na⁺-K⁺-ATPase within the constituent cells of the bovine rumen epithelium.

The nature of the permeability barrier in rumen epithelium has also not been fully defined. Specialized cell-cell junctions in rumen epithelium have been described, but no attempt has been made to link this to absorptive epithelial function. Indeed, as noted in recent papers from Franke’s group (4, 16), the presence of extended tight-junction structures and their spatial arrangements in mammalian-stratified epithelium have been the subject of controversy. A key concept of the arrangement

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for a multicellular-stratified epithelium capable of active Na⁺
transport (such as the frog skin) is that the epithelial barrier
layer communicates with lower basal cell layers via low-
resistance intercellular gap-junctional pathways (18).

The purpose of the present paper was to investigate a
transport model for the bovine rumen epithelium in which the
epithelial barrier function has been investigated by studying the
distribution of the integral membrane tight-junction protein
claudin-1 (7, 13), cell-cell gap-junction cellular connectivity by

METHODS

Tissue collection and preparation. Bovine rumen samples were
obtained postmortem from commercial slaughterhouses after humane
slaughter in accordance with U.K. legislation. Samples were excised
from the forestomachs of cattle within 15–40 min of slaughter, and
~100 cm² of rumen from the ventral sac and ~10 cm from the left
longitudinal groove were immediately washed and then transported to
the University of Newcastle upon Tyne. Camel S240 scanning electron microscope. All sample process-
with 10-nm gold with a Polatron sputter coater, and viewed with a
microscope with an accelerating voltage of 100 kV. For scanning

60°C. Survey sections (1
duration) steps to 100% resin for 3 h before a curing step for 24 h at

impregnation with epoxy resin via 25, 50, 75% acetone-resin (1-h
min steps), and 100% acetone (2 changes of 1-h duration) before
sequential dehydration was performed via 25, 50, 75% acetone (30-
min steps) followed by 80% acetone, and 2 ml of water, rinsed two times
for 15 min with Sorenson’s buffer, and then postfixed with 1% OsO₄.
After we rinsed two times for 15 min with Sorenson’s buffer, sequential dehydration was performed via 25, 50, 75% acetone (30-
min steps), and 100% acetone (2 changes of 1-h duration) before
impregnation with epoxy resin via 25, 50, 75% acetone-resin (1-h
duration) steps to 100% resin for 3 h before a curing step for 24 h at
60°C. Survey sections (1 µm) were cut and stained with 1% toluidine
blue in 1% borax. Ultrathin sections were cut with a Diatome diamond
knife on an RMC MT-XL ultramicrotome and then stained with
uranyl acetate (90 min at 25°C) followed by lead citrate (40 min at
20°C) and finally viewed with a Phillips CM100 transmission electron
microscope with an accelerating voltage of 100 kV. For scanning
electron microscopy, postfixed tissues (as above) were dehydrated
through graded ethanol concentrations, critical point dried, coated
with 10-nm gold with a Polaron sputter coater, and viewed with a
Cambridge S240 scanning electron microscope. All sample process-
ing was carried out by the Biomedical Electron Microscopy Unit,
University of Newcastle upon Tyne.

Immunocytochemistry. Sections (5 µm) were washed three times
with PBS, permeabilized with 0.1% saponin in PBS for 20 min, and
then blocked with 10% serum (species dependent on host animal used
for secondary antibody production) in PBS. Sections were then
incubated overnight at 4°C with primary antibody (typically diluted
1:50 in 10% serum-PBS), washed three times, and then incubated for
1 h at room temperature with an appropriate Alexa fluor-conjugated
secondary antibody (Molecular Probes, Eugene, OR). Appropriate
controls were run where the primary antibody was omitted (to test for
specificity); in all cases, sections were negative for fluorescence.
Typically, sections were counterstained with ethidium homodimer 1
(Molecular Probes) to highlight the nuclei. Immunofluorescence was
detected by confocal laser scanning microscopy.

All immunocytochemistry was repeated for material obtained from
at least three animals.

Western blots. Protein samples were loaded at 10–20 µg protein/well
on 4–20% polyacrylamide Criterion gels (Bio-Rad). After elec-
trophoresis, proteins were transferred to 0.45-µm nitrocellulose mem-
branes and blocked with 5% milk powder in PBS with 0.1% Tween 20
(Blotto) for 1 h at room temperature. Membranes were probed with
primary antibodies overnight at 4°C, washed three times with PBS-
0.1% Tween 20, and then incubated for 1 h at room temperature with an
appropriate horseradish peroxidase-labeled secondary antibody.
After a further three washes, signals were detected by enhanced
chemiluminescence. Western analysis was representative of data from
at least three animals.

Antibodies. The monoclonal mouse anti-sheep Na⁺-K⁺-ATPase antibody MA3-928 (1, 3) (Affinity Bioreagents, Golden, CO) recog-
nizes an epitope between residues 646 and 652 of sheep Na⁺-K⁺-
ATPase (NIPVSQ). BLAST searching (2) of the database showed that this epitope is common to mouse, human, and sheep α-Na⁺-K⁺-
ATPase subunits but does not discriminate between α-isofoms.

The rabbit polyclonal antibodies raised against claudin-1 (human-
mouse COOH-terminal sequence) and connexins 26, 32 (both mouse
COOH-terminal sequence), and 43 (rat COOH-terminal sequence)
were obtained from Zymed Laboratories (San Francisco, CA). The
anti-zonula occludens 1 (ZO-1) antibody (Zymed) was raised against
an epitope mapping to amino acids 334–634 of the human sequence.
All antibodies were chosen for their cross-reactivity for several
mammalian species.

The monoclonal mouse anti-bovine oxidative complex V (ATP
synthase, α-subunit ATP5A1) 7H10 was obtained from Molecular
Probes (A-21350) and was used to map mitochondrial density in
rumen sections.

For immunocytochemistry, Alexa fluor (Molecular Probes)-conju-
gated secondary antibodies were used at a concentration of 1:20 to
1:50. For Western blotting, horseradish peroxidase-conjugated anti-
bodies were used at a concentration of 1:100,000 (Sigma goat anti-
rabbit IgG) or 1:250,000 (Sigma goat anti-mouse IgG and Santa Cruz
donkey anti-goat IgG).

RESULTS

Overall structure of the rumen epithelium. The rumen epi-
thelium consists of a stratified epithelium. Luminal extensions
consist of leaflike papillae up to 15 mm in length that greatly
extend the total surface area. Figure 1A shows the gross
structure of the rumen papillae. Note the characteristic ridges
and hollows on the surface. A higher magnification view of the
ridges and hollows reveals the highly keratinized squamous
cells on the surface of the epithelium (Fig. 1B). The extensive
cell sloughing seen on papillary ridges is evidence that the
outermost keratinized cell layer is unlikely to act as an epithe-
lial permeability barrier. The trough between papillary ridges
contains a heterogeneous microflora (not shown).

Light microscopy (Fig. 1C) reveals that the microflora at the
lumen surface are loosely associated with the outermost layers
of the stratum corneum. The four cell layers (labeled from the
rumen lumen) are the intensely stained keratinized outer layer
of the stratum corneum, then the underlying stratum granulosum, the stratum spinosum, and finally the stratum basale (26). Both the limits of the stratum corneum and the stratum basale are clearly defined even at low power. Note the characteristic columnar cells of the stratum basale, whose long axes lie perpendicular to the basement membrane. Electron microscopy defines the desmosome-rich stratum spinosum and the stratum granulosum containing developing cytoplasmic keratin aggregates (not shown). The presumptive absorptive function of the papillae is emphasized by the full thickness of the papilla (twice that shown in Fig. 1A of 150–300 μm) relative to its length (Fig. 1A).

Cellular localization of the epithelial permeability barrier in rumen epithelial tissue. Simple mucosal epithelia possess tight junctions at the apical pole of the cells, which serves to seal the epithelial surface and to delimit the specialized apical and basal membrane domains (5, 6). The multicellular structure of the rumen epithelium renders such a model simplistic. To determine the localization of the permeability barrier in the rumen-stratified epithelium, we investigated localization of claudin-1 (7, 13) and ZO-1, an integral membrane protein and the MAGUK-plaque protein of tight junctions (6), respectively (Figs. 2 and 3), and sought morphological correlates of occluding junctions by electron microscopy (Fig. 4).

Western analysis with the use of a claudin-1-specific polyclonal antibody revealed a specific signal (not seen when primary or secondary antibodies were omitted) of the predicted apparent molecular size of ~23 kDa in rumen homogenates (Fig. 2A), but weaker signals were evident in bovine kidney cortical or medullary samples (not shown). In rumen sections, claudin-1 immunofluorescence was localized to the membrane of the stratum granulosum cells (Fig. 2B). At this outer cell layer bordering the stratum corneum (compare transmitted light image to claudin-1 immunofluorescence), there was evidence of lateralized claudin-1 staining at the cell borders. Immunostaining intensity decreased from the stratum granulosum toward the stratum spinosum and was weak at the stratum basale. Note that the stratum corneum is negative for claudin-1.

The ZO-1 antibody chosen (see METHODS) was unsuccessful in Western blotting of the bovine protein. However, a weak specific immunofluorescence similar to the pattern seen with claudin-1 was observed. Discrete membrane immunofluorescence was observed in the stratum granulosum with decreasing intensity across the rumen epithelium toward the stratum basale. (Fig. 3). Dispersed cytoplasmic immunofluorescence was observed in some cells of the stratum corneum.

The similar pattern of expression of the occluding junction proteins claudin-1 and ZO-1 is consistent with the presence of an occluding barrier at the stratum granulosum of bovine rumen epithelium (7, 29). It is apparent that neither the stratum corneum nor the stratum basale shows appropriate membrane expression of junctional proteins to allow formation of a functional occluding junction. Transmission electron microscopy was therefore employed to examine the nature of cell-cell junctions between adjacent cells in each of the four cell layers. Figure 4 shows cell-cell junctions in the stratum granulosum (Fig. 4A) and stratum corneum (Fig. 4B). Note the electron-dense occluding junction at the outer-facing side between adjacent stratum granulosum cells, which is completely absent in the stratum corneum. Desmosomes, assessed by morpholog-
ical criteria, were prominent between cells of the stratum basale and the stratum spinosum, between cells of the stratum spinosum, and at the level of the stratum granulosum. In addition, frequent junctions of the ribbon (fascia), lamellated, or sandwich type of junction (16) were observed between the cell layers of the stratum granulosum, spinosum, and basale, often being present in the interdesmosomal regions (not shown).

The observed claudin-1 and ZO-1 distribution and the presence of occluding junctions between cells of the stratum granulosum indicate that a permeability barrier is most likely to be present at the level of the stratum granulosum in bovine rumen epithelium.

Localization of the Na\(^+\)/K\(^+\)-ATPase in the rumen epithelium. The Na\(^+\)/K\(^+\)-ATPase is the primary molecule by which cellular energy is utilized to generate the asymmetric ion gradients that energize polarized secondary active solute transport by epithelial tissue (5, 15, 18). It has previously been demonstrated by ouabain binding assays and Western blotting that rumen homogenates contain the \(\alpha_1\) subunit of the Na\(^+\)/K\(^+\)-ATPase (9); however, the precise cellular location of the Na\(^+\)/K\(^+\)-ATPase has not yet been determined. The present study uses an \(\alpha\)-specific anti-Na\(^+\)/K\(^+\)-ATPase antibody (see METHODS). Figure 5A shows by Western blotting that a major band at \(\sim 110-120\) kDa was present in bovine rumen tissue, together with a faint additional band at \(\sim 97\) kDa; these bands most probably represent the \(\alpha\)-subunits of the bovine protein(s). Similar bands with similar relative intensities were observed for renal tissue (\(\alpha_1\)-subunit of the Na\(^+\)/K\(^+\)-ATPase predominates). Parallel determinations of the relative abundance of the \(\sim 110\) to \(120\)-kDa band in renal or rumen tissue homogenates at identical protein loading show that this band intensity is low in rumen compared with renal cortical and medullary homogenates (Fig. 5A and by densitometry 1 vs. 8.3-fold for rumen vs. renal cortical homogenate).

Figure 5B shows that Na\(^+\)/K\(^+\)-ATPase density is highly heterogeneous between cells of the rumen epithelium, predominately being expressed toward the basal plasma membranes of the stratum basale cells. The plasma membrane distribution of
the α-subunit is not however restricted to the basolateral membrane, as cells of the stratum basale show circumferential staining at the apical pole in connection with the stratum spinosum cells. Thus occluding junctions, which are not present (see above), cannot delimit plasma membrane expression as would be the case in a single cell-layered epithelium between apical and basolateral plasma membrane domains (5, 15). Equivalent immunostaining of bovine renal sections shows basolateral staining of tubule profiles only (not shown). Figure 6 shows that there is extensive infolding of the basal membrane at the point of contact with the basal lamina (Fig. 6A) and that there are extensive interdigitations of the lateral membranes between cells of the stratum basale (Fig. 6B). Thus membrane amplification may underlie the apparent polarization of Na⁺-K⁺-ATPase expression at the stratum basale. In any case, the decreases in intensity of immunostaining in the cells of the stratum spinosum and the stratum granulosum indicate that these cells would be largely incapable of generating polarized transruminal Na⁺ transport (see DISCUSSION).

Mitochondrial density and distribution in the rumen epithelium. The monoclonal mouse anti-bovine oxidative complex V (see METHODS) was used to map mitochondrial density in rumen sections in conjunction with Na⁺-K⁺ pump density. Figure 7 shows that the most intense staining for complex V is associated with the apical pole of the cells of the stratum basale. This was directly shown by electron microscopy, which confirmed a high cytoplasmic mitochondrial density. Cell mitochondrial density decreases toward the rumen lumen, with cells of the stratum spinosum and the stratum granulosum showing progressively lower staining. The stratum corneum was essentially

Fig. 4. Electron micrograph of occluding junctions between cells at the stratum corneum (SC) where no occluding junction is present (A) and between neighboring cells of the stratum granulosum (SG) at the interface with the stratum corneum (B). Note occluding junction and desmosome. Scale bars = 1.0 μm (A) and 0.5 μm (B).

Fig. 5. Na⁺-K⁺-ATPase α-subunit expression in rumen epithelium. A: Western blot of Na⁺-K⁺-ATPase α-subunit expression in bovine rumen. B: Immunolocalization of Na⁺-K⁺-ATPase α-subunit expression (green). Cells were costained with ethidium homodimer 1 (red) to label nucleic acids. Note prominent membrane staining of Na⁺-K⁺-ATPase α-subunit at the stratum basale at the basal lamina with decreasing intensity across the stratum spinosum to the stratum granulosum and stratum corneum. Scale bar = 20 μm.
negative. The mitochondrial density within cells of the stratum basale does not directly parallel the subcellular distribution of the Na\(^+-\)K\(^+\) pump; thus, although ATP usage via the Na\(^+-\)K\(^+\)-ATPase will be maximal within this cell type, additional metabolic functions are indicated.

**Connexin 43 expression in the rumen epithelium.** Western analysis (Fig. 8A) confirms expression of connexin 43 in bovine rumen with a single band of ~43 kDa being evident. Immunolocalization of connexin 43 expression (Fig. 8B) shows membrane staining at the level of the cells of stratum granulosum and stratum spinosum. More punctate staining is seen at the level of the stratum basale. The stratum corneum is negative. This pattern of staining is consistent with intercellular communication within and between the cells of the stratum granulosum, spinosum, and basale, forming a syncytium (21, 30). No specific protein by immunoblotting or immunofluorescence was evident in bovine rumen epithelium with the rabbit anti-mouse connexin 32 polyclonal antibody. Immunoblotting with the rabbit anti-mouse connexin 26 antibody failed to detect a single band at ~26 kDa; however, a band at ~90 kDa was evident, perhaps representing a multimeric (or oligomeric) complex (not shown). Connexin 26 immunofluorescence was present in cells of the stratum granulosum and stratum spinosum, whereas the stratum corneum and stratum basale were negative (not shown).

**DISCUSSION**

It is well established that there is active transport of Na\(^+\) alone and stimulated by the presence of short-chain fatty acids across isolated sheets of rumen epithelium (8, 17, 19, 20, 23, 24, 27). The fundamental model of how such polarized active transport might be established was developed by Koefoed-Johnson and Ussing (14) in their seminal study on frog-skin epithelium. A two-membrane model was proposed in which the outer membrane was permeable to Na\(^+\) by electrodiffusion, whereas the inner membrane expressed the Na\(^+-\)K\(^+\)-ATPase and was highly selective for K\(^+\). Thus metabolic energy is transduced by the Na\(^+-\)K\(^+\) pump to form a low internal Na\(^+\) concentration and elevated intracellular concentration. Na\(^+\) flow from the outside solution down its electrochemical gradient is then matched by Na\(^+\) extrusion across the inner membrane to the blood side, with uptake being matched by electrodiffusive loss (and hence recycling) across the inner-facing membrane (14). The polarized location of the Na\(^+-\)K\(^+\)-ATPase is therefore a crucial characteristic of all epithelia capable of generating transepithelial Na\(^+\) transport. In simple, single-cell-layered epithelial layers (renal tubule epithelium, intestinal mucosal cells, and model cultured epithelia such as Madin-Darby canine kidney cells), the Na\(^+-\)K\(^+\)-ATPase is restricted to the lateral and basal cell membranes (3, 15).

The cellular structure of the rumen epithelium is complex, consisting of a number of distinct cell layers (see Figs. 1 and 2). In a similar fashion, the frog skin structure is also complex. Mills et al. (18) investigated the localization of Na\(^+-\)K\(^+\) pump sites in frog skin using \(^{3}H\)ouabain autoradiography. By analysis of grain density, they showed that the Na\(^+-\)K\(^+\) pump was predominately localized to the innermost living cell layers of the stratum spinosum and the stratum germinativum. These
authors proposed a model for frog skin in which all living cell layers functioned as a syncytium with respect to active trans-epithelial Na\(^{+}\)/H\(^{+}\) transport (18).

Studies of rumen epithelia have focused on the magnitude of Na\(^{+}\)/H\(^{+}\)-K\(^{+}\)/H\(^{+}\)-ATPase activity compared with other tissues such as kidney and also on the molecular nature of the catalytic subunit expressed in rumen [the \(\alpha_1\)-subunit only is expressed (10)]. We demonstrate here for the first time the cellular distribution of the Na\(^{+}\)/H\(^{+}\)-K\(^{+}\)/H\(^{+}\) pump within rumen epithelium. Previous work with heavy metal capture techniques (9, 11) lacked specificity in measurement of ATPase activity; in addition, work based on the likely metabolic activity of the tissue was indirect (26). Indeed, Henrikson (11) concluded that the Na\(^{+}\)/H\(^{+}\) pump is concentrated in the midepithelial cell layers of sheep rumen. The present results show that, for bovine rumen, Na\(^{+}\)/H\(^{+}\)-K\(^{+}\)/H\(^{+}\) pump density is mostly concentrated in the cells of the stratum basale, with decreasing density toward the lumen (stratum spinosum, stratum granulosum). The stratum corneum was not immunostained. The distribution of immunostaining in the stratum basale was consistent with plasma membrane staining but was concentrated in the basal areas of the lateral membranes and the basal membranes abutting the basal lamina. These areas showed the greatest membrane amplification with interdigitations and infoldings, respectively. The presence of immunostaining at the “apical” pole of the stratum basale is consistent with the absence of tight junctions between the cells of the stratum basale. No separate membrane domains would thus be established. The cells of the stratum basale also contain a high density of mitochondria relative to cells of the stratum spinosum or stratum granulosum, consistent with the energy requirements of primary active Na\(^{+}\)/K\(^{+}\) pumps.

Establishment of an epithelial barrier requires the formation of morphologically distinct tight junctions. Tight junctions determine the extent and maintain distinct apical and basolateral membrane domains in mucosal epithelial cells as well as being the location of the permeability barrier (5, 6, 13). The present data have investigated the distribution of the integral membrane tight-junctional protein claudin-1, together with the tight-junctional plaque protein ZO-1. Both ZO-1 and claudin-1 are present in the stratum granulosum and the stratum spinosum, but the density of these proteins declines toward the stratum basale. Recently, Franke’s group (4, 16) investigated the distribution and spatial arrangements of tight-junctional proteins in diverse stratified epithelia (epidermis, heel pad, snout, gingival, esophagus, vagina, uroepithelium, etc.) in several mammalian species, including bovine epithelia. Tight-junction proteins ZO-1, claudin-1, and claudin-4 are commonly abundant in the upper third of suprabasal cell layers. The present distribution reported here for bovine rumen is therefore consistent with that seen for other stratified mammalian epithelia, including bovine gingiva (16). Electron microscopy showing the cellular localization of a tight-junctional barrier indicates that true tight junctions (“kissing”), where there is close approach of the adjacent plasma membranes, occur in the outer (nonkeratinized) cell layer of stratified epithelia, the stratum granulosum in bovine rumen. Heterogeneous junction structures in which occludin is also expressed include “laminated tight junctions” (coniunctioes laminosae) and “sandwich junctions” (iuncturae structae), which are also observed in suprabasal areas (16). Consistent with the occurrence of a true tight-junctional permeability barrier in bovine rumen epithelium, we observed kissing tight junctions at the outermost layer of stratum granulosum at the interface with the stratum corneum.

Confirmation of the importance of claudin-1 in the formation of the permeability barrier in the stratified epithelium of mam-
mals has been derived from recent studies of claudin-1 knockout mice (7). These mice die in the early neonatal period from failure of the epidermal barrier to prevent water loss. Claudin-1 is localized to the outermost layer of the stratum granulosum of mouse epidermis where continuous tight junctions are found (7). Thus it seems that the present data in bovine rumen are formally similar to those studies of mouse epidermis. It had been thought that the primary permeation barrier may reside in the stratum corneum. Early studies of the permeation of electron-dense tracers (horseradish peroxidase, lanthanum, and ferritin) from the lumen side showed only limited and incomplete permeation of the stratum corneum (12). However, it should be noted that perfusion of these tracers from the arteriolar side of rumen allows permeation up to but not including the stratum corneum (12), consistent with diffusion limitation at the electron microscopic level alone do not allow functional classification of such junctional complexes (16). Further work with immunoelectron microscopy is required to colocalize connexin 43 with tight-junctional proteins such as occludin and claudin-1.

In this model of bovine rumen epithelium in which multiple cell layers form a functional syncytium, the suprabasal cell layers would not of necessity have to display high metabolic capacity or Na\(^+\)-K\(^+\) pump density; rather, the presence of gap junctions allowing passage of small molecules, e.g., Na\(^+\), would allow diffusional influx at the stratum granulosum to be transmitted to stratum basale for ATP-dependent extrusion.

The rumen is known to mediate the vectorial transport of a number of metabolic substrates such as volatile fatty acids (acetate, butyrate, and propionate) (8, 17, 19, 20, 23, 24, 27). The present data emphasize the need for such transport processes to be interpreted in terms of a distributed model in which polarity is achieved via cell-specific membrane transport protein expression.

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REFERENCES


