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Ultrastructure of the Rumen Epithelium of the Goat
(Capra hircus)

by
Nobuko O. Kuhn
John H. Thompson

May 1965

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ULTRASTRUCTURE OF THE RUMEN EPITHELIUM
OF THE GOAT (Capra hircus)

by

Nobuko O. Kuhn
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Biophysics Division
Directorate of Medical Research

May 1965

US Army Edgewood Arsenal
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Edgewood Arsenal, Maryland 21010

FOREWORD

The work described in this report was authorized under Project 1M012501A27, Wound Ballistics (U). This work was started in January 1964 and completed in February 1965. The experimental data are contained in notebook MN-1797.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Acknowledgments

We are indebted to Dr. Frederick W. Light, Jr., and Capt. Mart R. Krigman, MC, USAR, for their continuous interest and support in this study, to Dr. Robert Schwebel, formerly Capt., VC, USAR, for doing most of the autopsies, to all members of the Wound Assessment Branch for technical support, and to Joseph B. Scott for making all the prints.

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DIGEST

The electron microscopy of the rumen of the goat was correlated with the physiology of that organ. The rumens of five normal goats, which had been used as subjects in experiments involving wounding by high-velocity missiles, were sampled and prepared for examination in the electron microscope by routine methods utilizing osmic acid fixation and Maraglas embedding.

The ruminant forestomach (the rumen) is lined by parakeratotic, stratified squamous epithelium and is an active site of absorption of nutrients, including fatty acids, water, and electrolytes. The fine structure correlates with this absorptive capacity in the following ways: (1) keratinization is partial; (2) the lesser degree of keratinization is associated with a corresponding decrease in the number and size of tonofibrils as compared to skin; (3) mitochondria are numerous in all cell layers, except in the corneum, and many of them contain inclusion granules; (4) cytoplasmic processes are prominent, thus increasing surface area; (5) much intercellular space occurs between the processes; and (6) no mucous or sebaceous glands are present.

Some interesting morphologic findings are noted, including the presence of clear cells in the rumen epithelium, a possible receptor organ, three different types of cell connections, and cross striations in desmosomes and half-desmosomes.

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ULTRASTRUCTURE OF THE RUMEN EPITHELIUM OF THE GOAT (Capra hircus)

I. INTRODUCTION.

The ruminant stomach is divided into four compartments in this order: rumen, reticulum, omasum, and abomasum. The first three are nonglandular and lined by keratinizing, stratified squamous epithelium, whereas the fourth has glandular epithelium and corresponds to the human stomach in histology and function. In the adult animal, the rumen is the largest of all the compartments. The mucosa in the rumen of the goat forms grossly visible papillae measuring 1 to 5 mm in height and approximately 1 mm in diameter at the base. In the reticulum, a muscle lamella forms ridges like a honeycomb, whereas in the omasum it forms large overlapping flaps, and both surfaces are then dotted with stubby papillae.¹

Until recently, the rumen was considered as no more than a large fermentation vat, and study of its physiology was centered about its motility. Since 1940, however, increasing attention has been paid to the microbiology of the rumen and to the considerable nutritional absorption that takes place across the epithelium.² The short-chain fatty acids and acetic, propionic, and butyric acids, which are synthesized from ingesta by cellulose-digesting bacteria in the rumen, are absorbed and partially oxidized in the epithelium itself.²⁻⁶ Most of the soluble carbohydrates ingested by goats disappear in the rumen, presumably all fermented to fatty acids and absorbed. Some protein absorption also takes place in the rumen, but most of it is removed in the small intestine.^{7, 8} There is also active transport of ions and water across the epithelium. Potassium, sodium, and chloride ions have been studied in this regard.⁹⁻¹¹ The order of magnitude of sodium uptake and electrical energies involved is comparable to that of frog skin.⁹

In view of the active nature of this epithelium, an attempt will be made to correlate the fine structure of the goat rumen epithelium with some of its known physiologic functions and to examine its similarities to other stratified squamous epithelia from man and laboratory animals.

II. METHODS AND MATERIALS.

Five clinically healthy, castrated, male, white, Texas Angora goats were sampled in all. One goat was sacrificed by electrocution, which

resulted in instantaneous death of the animal, and specimens were taken within 4 min following death. Specimens were removed from two other goats under anesthesia (intravenous Nembutal-Veterinary). The remaining two goats died while under anesthesia. Specimens in these cases were placed in the fixative within 6.5 and 7.5 min after death.

Although no particular selection was planned, the specimens were usually taken from the midventral area of the rumen, close to the reticulo-rumen border, because this was the most accessible site. The gross rumen contents adhering to the piece of tissue were gently flicked off with dry surgical sponge. For electron microscopy, the papillae were removed at their bases by using a new, grease-free razor blade and were minced further before being placed in 1% veronal-buffered osmium tetroxide. Tissues were fixed for 2 hr at 0°C, dehydrated through an alcohol series, and embedded in Maraglas. After curing, ultrathin sections were cut either on a Porter-Blum MT-1 or an LKB ultramicrotome, stained with 3% uranyl acetate, lead citrate, or 1.5% phosphotungstic acid (PTA), and examined in the RCA EMU-3G electron microscope.

For light microscopy, tissue was obtained from all the above animals, fixed in 10% formalin, and stained with hematoxylin and eosin (H and E). In addition, specimens for special stains were obtained at different times from routine goat autopsies done in the laboratory. Special stains included oil red O for neutral fat, periodic acid-Schiff (PAS) with and without diastase for glycogen and mucopolysaccharides, and the β -(3,4-dihydroxyphenyl)- α -alanine (DOPA) reaction for melanocytes.

III. RESULTS.

A. Light Microscopy.

By H and E staining, individual papillae were seen to consist of a loose, connective-tissue core, lined by a parakeratotic type of stratified squamous epithelium of varying thickness, the dermal portion of which rested

on a basement membrane and formed rete pegs (figure 1). Many capillaries and lymphatics were seen in close proximity to the basal cells. The basal layer consisted of cuboidal to columnar cells that were overlaid by a spinous layer composed of round cells with intercellular bridges. A stratum granulosum was present but discontinuous, and a stratum lucidum was absent. Near the surface, cells became flattened and densely eosinophilic or swollen and light-staining. Frequently, swollen cells formed the deep layer of the stratum corneum, and the more superficial cells were flat. The deep layer of "primary swelling" and the granular layer below it have together been called the stratum transitionale.¹² In the stratum corneum, nuclei were still present but were flattened, shrunken, and densely basophilic.

There was PAS-positive material in the corneum, basement membrane, and around capillaries, with no change following digestion with diastase, thus agreeing with the findings of others^{13, 14} on the absence of glycogen from the epithelium. Oil red O showed lipid droplets in the corneum, as was described by Habel.¹⁵ However, Dobson used Nile blue sulphate and found no fats or fatty acids.¹³

Although the many clear cells that can be seen by H and E in the basal cell layer arouse the suspicion that they are melanocytes, melanin granules were not seen in the routine sections. Moreover, the DOPA reaction for identification of melanocytes was repeatedly negative in the present studies.

B. Electron Microscopy.

1. Lamina Propria.

Only that area immediately contiguous to the basement membrane was investigated. This area contained young collagen^{16, 17} in widths varying from 100Å to 600Å, in intimate relation with fine, 20Å fibrils extending from the basement membrane. In addition to fibroblasts and lymphatics, capillaries with the "fenestrated" type of endothelium¹⁸ seen in capillaries of the intestine, kidney, and some endocrine organs were also present. This morphology was not affected by anesthesia. Actual openings were not seen; "pores" were always spanned by diaphragms consisting of fused cell membranes (figure 2).¹⁹



FIGURE 1

ONE RUMEN PAPILLA

[Epithelial fingers dip into the loose connective tissue core, and many small vessels can be seen among them (arrows). The granular layer appears discontinuous. Swollen cells are typically seen on the sides and bases of papillae as shown here, whereas cells at the tip are flat. H and E, X90.]



FIGURE 2

A SOMEWHAT LARGE CAPILLARY SHOWING ITS RELATIONSHIP TO THE
BASAL CELLS AND ITS ATTENUATED ENDOTHELIUM

(Fine fibrils can be seen extending from the two basement membranes into
the connective tissue space, as well as making contact with the cells they
border. Uranyl acetate, X13,500.)

2. Basement Membrane.

A continuous, finely fibrillar basement membrane consisting of 20Å fibrils separated the lamina propria from the basal cells (figures 2 to 6). The fibrillar composition made the boundaries indistinct and the measurements inaccurate. If low-magnification micrographs were measured, however, the basement membrane was 500Å to 650Å in diameter and was separated from the basal cells by a low-density space measuring 650Å to 800Å. At high magnifications, this space was seen to contain a low-density amorphous substance and was frequently traversed by fibrils from the basement membrane. At frequent but irregular intervals, this light space contained lamellar densities that resembled half-desmosomes and attached thickened areas of basement membrane to thickened basal-cell membrane. At the latter sites, increased cytoplasmic density and convergence of tonofibrils were observed in the epithelial cell, in the manner of a typical desmosome (figure 4). This arrangement was noted in mouse epidermis and called the "basement attachment device" by Setälä et al.²⁰ Since then it has been reported by others in human oral mucosa, cornea, amphibian skin, human and rat epidermis, mouse esophagus, and rat tongue²¹⁻²⁵ and referred to as a 'half-desmosome.' Frequently, 20Å fibrils were seen crossing the light space from the cytoplasm to merge with the basement membrane (figure 3). Other fibrils extended from the basement membrane and mingled with the collagen fibers (figures 2 to 5).

3. Stratum Basale.

The border between epithelium and lamina propria was highly convoluted. On the deep side of the basal layer, slender, tortuous, interlacing, cytoplasmic processes of epithelial cells were numerous and frequently formed the sites of attachment to the basement membrane. There was much intercellular space among these processes (figure 6).

There were two types of cells noted in this layer. The first type, the epithelial cell, which was by far the more numerous, had a cell border with modest processes (except on its basal side, as noted) and was attached to its neighbors by means of desmosomes. The dominant feature of these cells was the numerous mitochondria that often contained large osmiophilic granules measuring 600Å to 900Å and sometimes exhibited cristae that were arranged linearly along the axis of the organelle (figure 7).

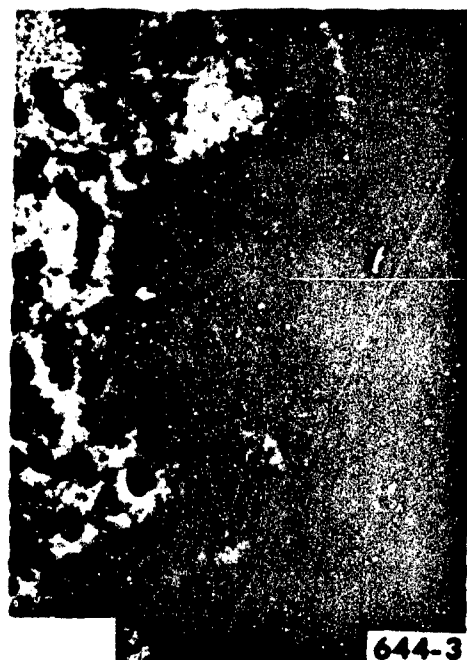


FIGURE 3

A HALF-DESMOSOME

(The typical lamination is not seen. Apparent fibrillar connections are seen extending between basement membrane and epithelial cytoplasm. Note two pinocytotic vesicles just below it. The finely fibrillar feltwork of the basement membrane is evident. Uranyl acetate, $\times 86,000$.)

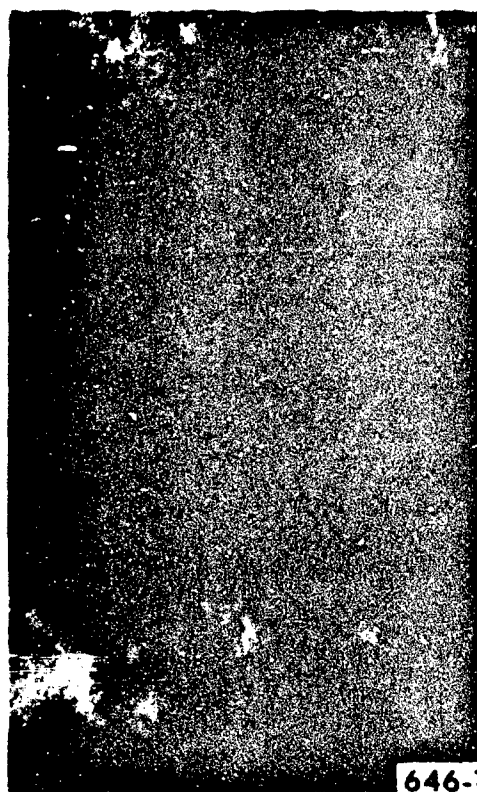


FIGURE 4

TYPICAL HALF-DESMOSOME, WITH THREE DARK LAYERS
AND TWO LIGHT LAYERS

(Cytoplasmic thickening and some tonofibrils
are seen. Uranyl acetate, 36,000.)

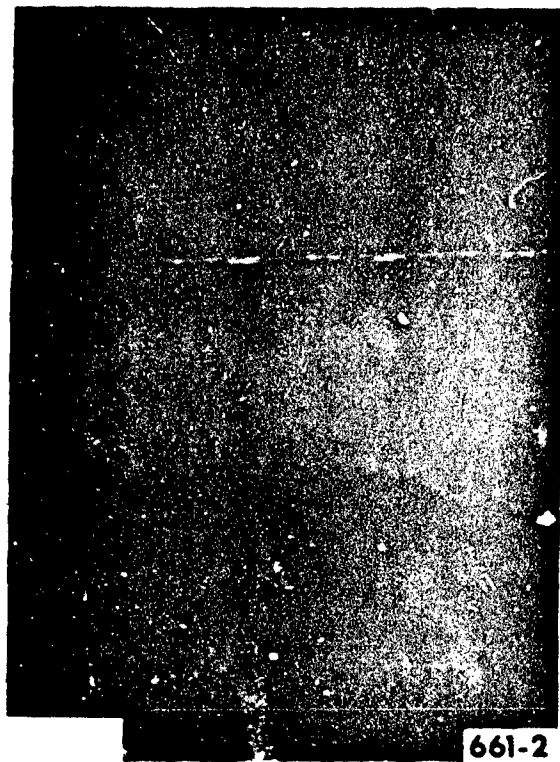


FIGURE 5

SEPTATE DESMOSOME IN BASAL LAYER

[Where septa do not cross the intercellular space, the cell membranes appear beady (arrow). Uranyl acetate, X86,000.]

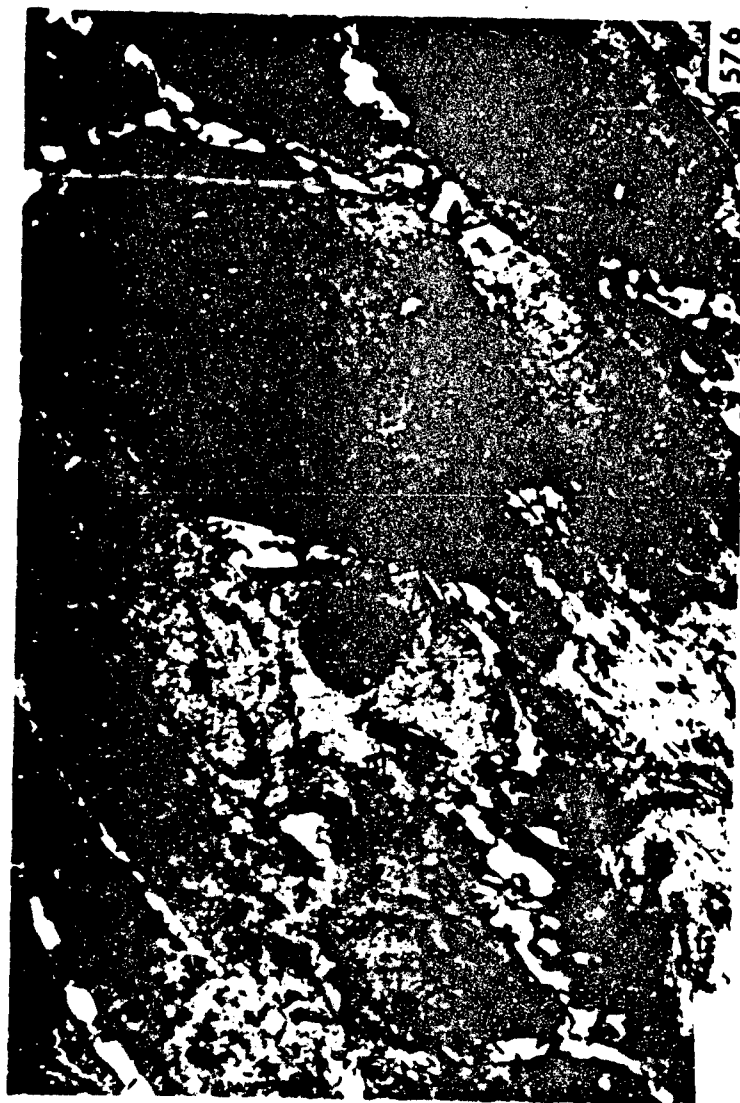


FIGURE 6

BASAL LAYER

[Three clear cells are seen, one of which contains a few round osmiophilic bodies. The cross section of a small dendrite is visible just to the left of the two contiguous clear cells (arrow). The complex border along the basement membrane and the intercellular spaces are evident. Uranyl acetate, X4,800.]

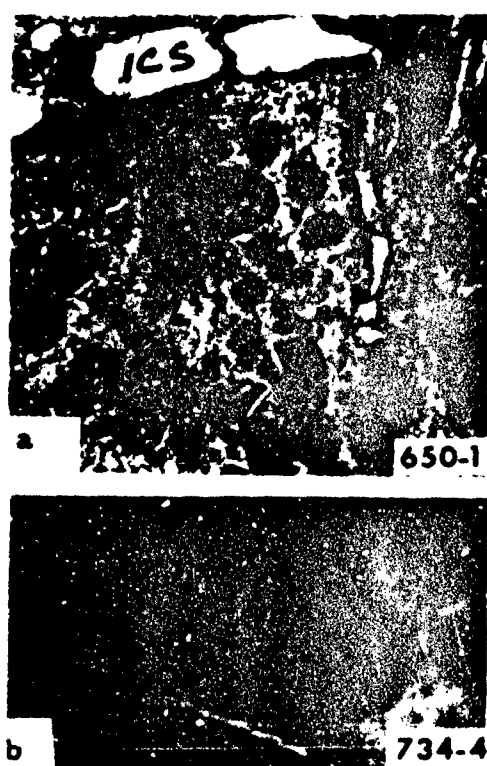


FIGURE 7

CELL MITOCHONDRIA

[(a) Mitochondria containing linear and semicircular cristae. Stratum spinosum. ICS, intercellular space. Uranyl acetate, X15,500.
 (b) Two large and one small mitochondrial granules, showing internal structure. Lead citrate, X127,500.]

The Golgi apparatus and ergastoplasmic reticulum were inconspicuous. Many small vesicles were seen in the cytoplasm and in pinocytotic arrangement along the cell membrane, with openings into the intercellular space or the light space bordering the basement membrane. Small, narrow bundles of short osmiophilic filaments were seen, generally around the periphery of the cell, although there were also such bundles among the centrally located mitochondria. The cytoplasm was coarsely granular and contained fine filaments. The nucleus was round to oval, with one or two nucleoli (figures 6 and 8).

The second cell type was dendritic in character, with a smooth cell membrane, no desmosomes, and no tonofilaments. When compared with the epithelial cell, it possessed a much lighter staining cytoplasm, fewer mitochondria, more ergastoplasm, and a more prominent Golgi zone. In one such cell, several round, osmiophilic inclusions measuring $320\text{\AA}$ in diameter were seen in the Golgi area. The cell resembled the dendritic "clear cell" described in squamous epithelium by others²⁵⁻³⁰ and demonstrated to be a melanocyte.

Two types of cell attachments were seen in the basal layer. The first was the usual lamellated desmosome,³⁰⁻³² and the second was a septate desmosome similar to that described by Wood³³ in *Hydra* epithelium, and to which he attributed a water-barrier function (figure 5). His dimensions are larger than those of the present report, however. This septate desmosome was usually seen between cell processes and consisted of thickened apposing cell membranes $30\text{\AA}$ in diameter and $80\text{\AA}$ apart, connected by osmiophilic septa $20\text{\AA}$ thick and spaced at $60\text{\AA}$ intervals.

4. Stratum Spinosum.

The cells in the stratum spinosum were generally rounded and attached to their neighbors by desmosomes on cytoplasmic processes. Deep in this layer, the processes were usually stubby, with much intercellular space between them (figures 7 and 8). Sometimes the desmosomes were bounded on one or both sides by "tight junctions" in which the outer cell membranes of apposing cells fused into one middle line (figure 9).^{31, 34} The septate type of desmosome was also seen in this layer. Also, fine, $20\text{\AA}$ cross striations within desmosomes seemed to occur frequently. This has also been reported for corneal epithelium in the rabbit.²⁴ The striations could not be followed from the cytoplasm of one cell to another, but certainly crossed from one attachment plaque to the other. When this occurred, the central disc was usually missing (figure 10). There is believed to be a fibrillar component to the desmosome, although it may be confined to a certain stage of maturation as suggested by Teng,²⁴ or its presence may only be detected in a certain plane of section.



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FIGURE 8

A LOW-MAGNIFICATION SURVEY OF THE RUMEN EPITHELIUM

[B, basal layer; S, spinous layer; G, granular layer. The corneum is not seen. One clear cell is present in the lower left. Note the numerous mitochondria up to the granular layer. Small cytoplasmic granules can be seen

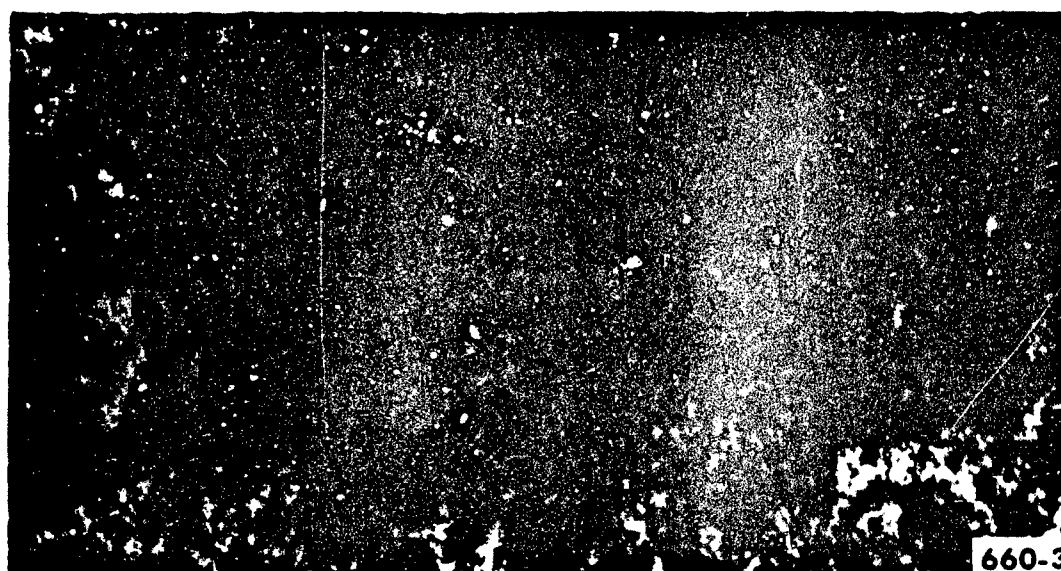


FIGURE 9

INTERDIGITATING CELL PROCESSES, SHOWING THE RELATIONSHIP
BETWEEN TIGHT JUNCTIONS (ARROWS) AND DESMOSOMES

(ICS, intercellular space. Uranyl acetate, X86,000.)

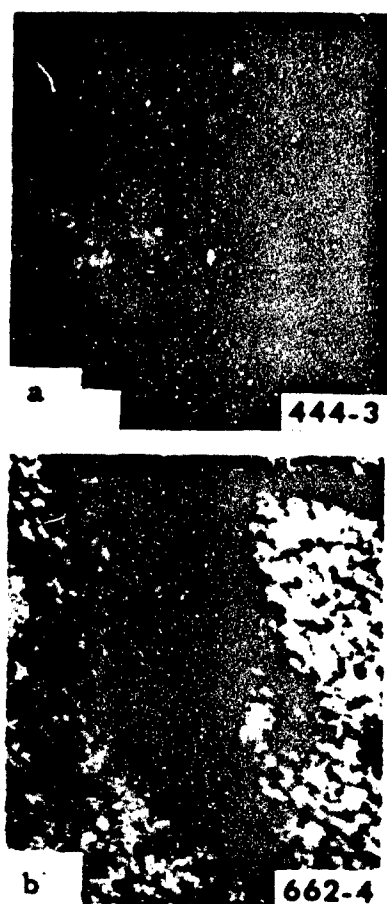


FIGURE 10

DESMOSOME WITH ALL COMPONENTS VISIBLE
EXCEPT THE MIDDLE DENSITY

[Cross striations are seen. (a) Uranyl
acetate, $\times 89,000$; (b) uranyl acetate,
 $\times 86,000$.]

In the more superficial layer, the processes became elongated and interdigitated, with the result that intercellular space decreased. Tonofibrils became longer and thicker, while mitochondria, though still plentiful, occupied a diminishing area in the center of the cell. More and more of the periphery was taken up by isolated bundles of tonofilaments and small, dark, cytoplasmic granules (figure 8). These granules were also seen in the granular layer and ranged greatly in size, but were generally less than 0.15μ . Such granules have been described in rat oral epithelium, mouse-embryo epidermis, adult-mouse epidermis, and human epidermis.^{16, 35-38} Their origin is unknown. Moderate-sized (e. g., $0.8\mu \times 1.6\mu$) keratohyalin granules sometimes appeared in an otherwise typical spinous cell.

In the deeper layer of the stratum spinosum, a two-cell arrangement was seen in which one cell with cytoplasmic processes encircled the other in a manner analogous to the Schwann cell-nerve axon relationship (figure 11). Both cells usually appeared similar, with clear cytoplasm and similar organelles. Both contained nuclei in some micrographs. Therefore, whenever a nucleus was not seen, it was assumed to be caused by the plane of sectioning. The cells resembled melanocytes in some sections and unmyelinated peripheral nerve endings in others. It was not possible to determine the extent of this relationship.

Solitary clear cells were also seen. Some of these were melanocytelike cells, and their dendrites cut in cross section. If a cell body was not in the vicinity, however, dendrites were impossible to distinguish from unmyelinated peripheral nerve endings, which are known to occur in the rumen epithelium³⁹ as in the skin.⁴⁰

5. Stratum Granulosum.

The change to a fully developed granulosum cell could be abrupt even though keratohyalin granules appeared below this layer (figure 8). Also, in electron micrographs, keratohyalin granules, albeit small, were consistently seen at this level, and for this reason the discontinuity of the granular layer as noted by light microscopy was considered more apparent than real.



FIGURE 11

EXAMPLE OF THE TWO-CLEAR-CELL ARRANGEMENT
IN THE STRATUM SPINOSUM

(A nucleus is seen in the lower cell.
Lead citrate, X16,000.)

The nucleus became flattened and its margins frequently appeared scalloped, but the nuclear membrane remained intact. Very large, densely osmiophilic keratohyalin granules appeared in close proximity to the nucleus, and smaller granules were scattered throughout the now-rarefied cytoplasm. The low-density cytoplasm in the central portion of these cells was composed of a finely filamentous meshwork in which diminished numbers of normal-appearing mitochondria devoid of inclusion granules were present. Lysosome-like round bodies were frequently seen. Along the periphery, short tonofibrils occurred, oriented roughly parallel to the cell membrane. These bundles were shorter and narrower than those in the stratum spinosum.

The keratohyalin granule itself showed a structure combining fibrillar and amorphous components identical to those discussed by Brody and others.^{22, 23, 34, 35, 37, 41}

6. Stratum Corneum.

In the deeper portion of the corneum, the cells were flattened and osmiophilic or distended and of low density. The swollen areas contained a fine, 80Å, filamentous network that could not be identified either by special staining or electron microscopy (figure 12). The cell borders were thickened and prominent, and the cell contents showed the 'keratin pattern'⁴² admixed with a more definitely fibrillar component, with the latter frequently predominating. A three-layered stratum corneum, such as was described by Brody,⁴² was not present.

Blunted cytoplasmic processes containing remnants of desmosomes were present in all layers of the corneum (figures 13 and 14). Separation of desmosomal attachments began in places between the granular layer and the corneum, with more and more separation occurring in succeeding layers until the cell finally sloughed off, still bearing debris of cytoplasmic organelles, nuclear remnants, and desmosomal components. Lipid droplets were occasionally seen in all the cell layers of the corneum but hardly seemed enough to account for the results seen with oil red O. Rumen micro-organisms were invariably seen on the surface.

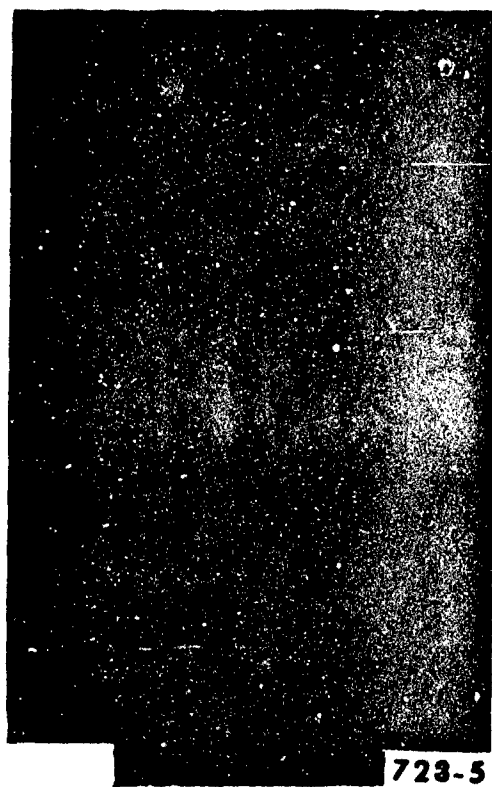


FIGURE 12

A SWOLLEN SURFACE CELL IN THE CORNEUM

(The swollen area occupies the center of the field and contains a fine filamentous network. Lead citrate, X48,000.)



FIGURE 13

THREE CELL LAYERS IN THE STRATUM CORNEUM

(The cytoplasm of the cells shows the keratin pattern. The cell membranes are thickened and lamination reminiscent of desmosomes can be seen between apposing cell membranes. Lead citrate, $\times 48,000$.)



735-2

FIGURE 14

A HIGHLY FIBRILLAR SURFACE CELL

(Cytoplasmic processes with tonofibrillar remnants of disrupted desmosomes can be seen projecting into the rumen cavity. Uranyl acetate, X69,400.)

IV. DISCUSSION.

A. Absorption.

When the fine structure of the rumen is compared with that of other stratified squamous epithelia, the morphologic basis for its absorptive capacity is better understood. The most extensively studied stratified squamous epithelium is epidermis, which is known to have limited absorptive capacities, most likely for three reasons: (1) there is a highly efficient barrier at the level of the deep, keratinized layer (the stratum corneum conjunctum); (2) there is in the corneum a lipid substance, the source of which may be sebaceous glands; (3) there is no demonstrable, active transport system in these cells.^{22, 43, 44} Removing the corneum or extracting it with combined lipid solvents renders it freely permeable to water. Furthermore, partially keratinized or parakeratotic mucous membranes are 13 to 15 times more permeable than goatskin, and frogskin is 10 times more permeable.²² In frogskin, the basement membrane layer seems to be the chief barrier and the one at which the bioelectric skin potential originates.⁴⁵ In human skin, this polarizing current is thought to originate at the boundary of the corneum and noncornified layer, although the dermal-epidermal junction may provide a second barrier.²²

By electron microscopy, of the three layers of the corneum, the cells of the deepest layer contain the most keratin pattern, whereas the intermediate and superficial layers are more fibrillar. Brody⁴² considers this deepest layer the main part of the stratum conjunctum, which is the water barrier.

Observations on goatskin in the present study show that tonofibrils in epidermal cells are strikingly more abundant, coarser, and more prominent in deep cell layers, and all organelles are less abundant than in rumen.* Such a definite conclusion, however, could not be drawn regarding contrasts between other skin (as reported in the literature^{16, 17, 20, 22, 30, 34, 35, 38}) and rumen.

Frogskin, which also is active in electrolyte transport, may possess cells with many mitochondria, many of which contain inclusion granules. At the same time, it performs a water-barrier function, the epithelial cells are strikingly fibrillar, and mucous cells are present.^{3, 46, 47} Voute could not find a consistent correlation between fine structure and electrolyte uptake.⁴⁷

* Kuhn, N. O. Unpublished observations.

In its ultrastructure, rumen resembles mucous membrane more than skin, since the former does not always undergo keratinization.^{25, 41, 48, 49} Since the surface of rumen epithelium is constantly bathed in large volumes of liquid and subjected to friction from ingesta, it is not surprising that a horny layer, such as is found in the skin, is not present. This alone probably allows for appreciable diffusion to occur, but there is another factor; namely, the many intercellular spaces between cytoplasmic processes. A pathway through such intercellular spaces was proposed as a "shunt pathway" in frog skin by Ussing and Windhager.⁵⁰

Another requisite for an absorptive surface is the presence of active transport systems. In the rumen, the numerous mitochondria can fulfill this function, since these organelles are known to be involved in active water and electrolyte transport⁵¹⁻⁵⁵ and probably also in storage in granule form.⁵⁶⁻⁵⁸ In considering the fatty acids absorbed and metabolized by rumen epithelium, the mitochondria are probably responsible for this function also, since they are known to contain the enzymes and cofactors necessary for the oxidation of fatty acids.⁵⁴

In addition, the tortuous cell processes of the epithelial cells increase the surface area available for absorption to take place.^{17, 30} The lack of secretions from mucous or sebaceous glands must also contribute to enhanced uptake.

B. Morphology.

The clear cells in the basal layer were at first assumed to be the "clear cells" of Masson, which give a positive DOPA reaction and are regularly found in other stratified squamous epithelia.²⁵⁻³⁰ Therefore, the authors' negative result with the DOPA reagent is disturbing, but perhaps can be explained on the basis of unfamiliarity with this special stain. It is also possible that the specific enzyme was absent from the clear cells.

The explanation for the two-clear-cell arrangement in the stratum spinosum is not immediately obvious, especially since their identities remain obscure. They may form a simple receptor organ, although the authors are not aware of any receptor organ that has a similar structure. Receptors would not be unexpected in the rumen, which is an organ possessing varying patterns of motility under different circumstances.⁵⁹

V. CONCLUSIONS.

The ruminant forestomach (the rumen) is lined by parakeratotic, stratified squamous epithelium and is an active site of absorption of nutriments, including fatty acids, water, and electrolytes. The fine structure correlates with this absorptive capacity in the following ways: (1) keratinization is partial; (2) the lesser degree of keratinization is associated with a corresponding decrease in the number and size of tonofibrils as compared to skin; (3) mitochondria are numerous in all cell layers, except in the corneum, and many of them contain inclusion granules; (4) cytoplasmic processes are prominent, thus increasing surface area; (5) much intercellular space occurs between the processes; and (6) no mucous or sebaceous glands are present.

Some interesting morphologic findings are noted, including the presence of clear cells in the rumen epithelium, a possible receptor organ, three different types of cell connections, and cross striations in desmosomes and half-desmosomes.

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