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Successive Changes in the Epimural Bacterial Community of Young Lambs as Revealed by Scanning Electron Microscopy†

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Scanning electron microscopy was used to determine the time of initial colonization of the rumen epithelium of young lambs and successive changes with time in the morphological composition of the epimural community. Tissue samples were obtained from two groups of lambs at 1, 2, 4, 6, 8, and 10 weeks of age. Comparisons were made with the epimural communities observed at 12 well-distributed sites in the rumen of a mature wether. Epimural bacteria were already present on the epithelium at 1 week of age. The morphological composition of the epimural community changed with age, with the pattern of succession being similar in both groups of lambs. A total of 24 morphotypes were distinguished by scanning electron microscopy; 17 were rod shaped, 4 were cocci, 2 were spiral, and 1 was filamentous. These morphotypes were further subdivided into: (i) those persisting after their initial colonization in young lambs and present in the adult (7 morphotypes), (ii) those seen only in the adult (2 morphotypes), and (iii) those present only in young lambs (15 morphotypes). The seven morphotypes present in both the lamb and the adult could be considered indigenous members of the epimural community. Several morphotypes appeared restricted in their colonization to certain regions of the papillae, suggesting the presence of microhabitats within the epithelial habitat. Two rod-shaped bacteria were repeatedly seen specifically attached to one another, suggesting an interspecific association.

Three interacting bacterial communities are now recognized in the rumen ecosystem: (i) bacteria floating free in the rumen fluid, (ii) bacteria which adhere to feed particles in the fluid, and (iii) bacteria which adhere to the rumen epithelium (6). The first two groups have been studied quite extensively, whereas the third group has been studied only recently. The term "epimural" (upon the wall) has been proposed for bacteria which adhere to the epithelium to distinguish them from bacteria which adhere to feed particles in the lumen (17). The presence of an epimural bacterial community on the bovine rumen epithelium was first confirmed incidentally by Tamate et al. (22) in a scanning electron microscopy study of rumen mucosal surface structure. The first specific studies of the epimural community in sheep and cattle (2, 14) revealed a morphologically heterogeneous bacterial community, including rods, cocci, and spiral morphotypes. Bacterial density on the tissue varied between sampling sites within the rumen of individual animals, but was similar at the same site in different animals. Bacteria were seen adhering to both the lumenal and nonlumenal surfaces of desquamating epithelial cells, suggesting a role in tissue recycling. Cheng et al. (6) discussed the role of epimural bacteria in the rumen and proposed three functions: (i) tissue recycling, (ii) oxygen scavenging, and (iii) urea metabolism. Various amounts of evidence now exist for each of these proposed functions (7, 9, 23); however, additional definitive studies are needed to further clarify the role of the epimural bacteria. The physical nature of the diet has been shown to directly affect the distribution of the epimural bacteria due to mechanical removal of adherent bacteria from the tissue surface by abrasive action (16). It is unclear at the present time whether the epimural bacterial community is taxonomically distinct from the other two bacterial communities in the rumen. Mead and Jones (17) tentatively identified 161 strains of epimural bacteria from mature sheep and were able to place 95% of isolates into previously described genera of functionally significant rumen bacteria. Dehority and Grubb (8) similarly characterized 95 strains of epimural bacteria from mature sheep and were able to place them into four genera of common anaerobic rumen bacteria. Preliminary reports on the isolation of epimural bacteria from cattle (5, 6), however, suggested that the bovine epimural community was taxonomically distinct from that of rumen contents. The isolated strains were identified as species of *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Lactobacillus*, *Fusobacterium*, and *Propionibacterium*, along with additional strains of unidentified anaerobes.

To determine the taxonomic uniqueness and more clearly define the role of the epimural community, indigenous members need to be identified and distinguished from transient bacteria. If transient members of the epimural community are not differentiated from indigenous members, invalid conclusions may be drawn with regard to the taxonomic and functional uniqueness of this community. One of the distinguishing features of indigenous bacteria is that they colonize their habitat during succession (20). The present scanning electron microscopy study of epimural bacterial succession was undertaken to determine the time of establishment in young lambs and to identify the indigenous morphotypes within the epimural community.

MATERIALS AND METHODS

Tissue sources. In a preliminary study the epimural community of an adult ruminant was examined to allow comparisons with the developing epimural community in lambs. The source of tissues representative of the mature ruminant was a 43-kg cross-bred wether receiving a corn-cottonseed hull-based diet containing 13% crude protein. Tissue disks (1.2 cm in diameter) were excised from 12 well-distributed sites in the ruminoreticulum while the animal was anesthetized

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with Nembutal (sodium pentobarbital). These tissue samples were washed once by rapid agitation in 0.85% saline to remove digesta and unattached microorganisms as described by Bauchop et al. (2) and then immediately fixed in 0.5% glutaraldehyde as described below.

Twelve lambs, two groups of six lambs each, were used as the source of tissue samples to study successive changes in the epimural bacterial community. Both sexes were used; males were not castrated. A time interval of 6 months existed between the sampling of the two groups. Lambs were allowed to nurse the ewe until 8 weeks of age. They also had access to alfalfa hay and a creep diet consisting of 50% cracked corn, 20% soybean meal, 15% rolled oats, 10% wheat bran, and 5% dehydrated alfalfa from birth through 10 weeks of age. Tissue samples were obtained from anesthetized (sodium pentobarbital) lambs in both groups at 1, 2, 4, 6, 8, and 10 weeks of age. Within each animal, two tissue disks (1.2 cm diameter) were excised from each of the four sites shown in Fig. 1. Both tissue disks were washed three times in succession 100-ml amounts of sterile anaerobic dilution solution (4) according to the procedure of Cheng et al. (6). One disk was immediately fixed and prepared for scanning electron microscopy as described below; the second disk was used for bacterial colony counts and isolation studies (18). Wet tissue weights of the reticulorumen, abomasum, and omasum were also obtained at the time of biopsy to enable determination of the stage of rumen development. The entire stomach was removed and slit open to remove contents. The reticulorumen, abomasum, and omasum were then separated and washed in isotonic saline to remove any remaining contents. Stomach parts were blotted slightly to remove excess saline and then weighed. The weight of tissue disks removed from the rumen was determined and added to the reticulorumen weight.

Scanning electron microscopy. After washing, tissue samples were prefixed in cold 0.5% glutaraldehyde in 0.067 M cacodylate buffer for approximately 2 h, followed by fixation in 5.0% glutaraldehyde in cacodylate buffer overnight. They were then washed five times in 0.067 M cacodylate buffer and carried through the modified thiocarbohydrazide (O-T-O-T-O) procedure of Malick and Wilson (13). This was followed by dehydration through a graded series of ethanol and critical point drying in liquid CO_2. Dry tissues were mounted on aluminum stubs with copper printer glue, sputter coated with gold-palladium, and then viewed in either a JEOL JSM-S1 or a JEOL JSM-35C scanning electron microscope at an accelerating voltage of 10 kV. Each sample was scanned for 2 to 3 h, examining as many different papillae

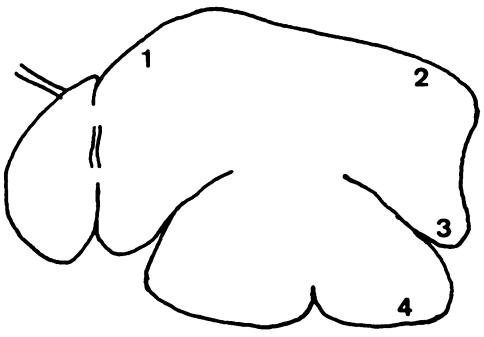

FIG. 1. Diagram of reticulorumen from left side showing tissue sampling sites. All sites were on the midline: 1, roof of cranial rumen; 2, roof of dorsal rumen; 3, floor of caudodorsal blind sac; 4, floor of caudoventral blind sac.

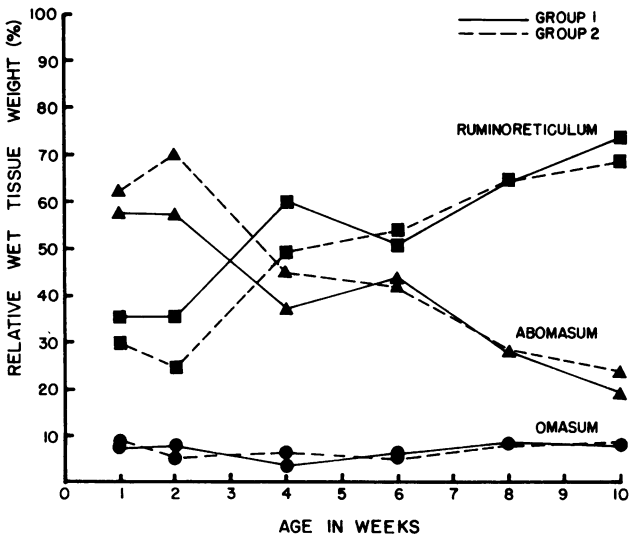

FIG. 2. Relative wet tissue weights of the ruminoreticulum, abomasum, and omasum of lambs in groups 1 and 2. Relative wet tissue weight (%) = (wet tissue weight of stomach part/wet tissue weight of whole stomach) × 100.

within the sample as possible. Low-magnification photomicrographs (×30 to ×100) were taken of each sample for measurement of papillae length.

RESULTS

Rumen content and anatomy. Relative wet tissue weights of the ruminoreticulum, omasum and abomasum were determined to establish the stage of rumen development during sampling times. These findings (Fig. 2) closely paralleled those of Oh et al. (19), and based upon these relative weights, samples from the 1- and 2-week-old lambs represented the preruminant stage (abomasum weight > ruminoreticulum weight), the 4-, 6-, and 8-week samples represented the transition stage (relative ruminoreticulum weight still increasing and greater than abomasum weight), and the 10-week samples represented the ruminant stage of development (ruminoreticulum at constant relative weight [70 to 75%] > abomasum weight).

A number of physical changes were observed in the rumen epithelial habitat over the 10-week sampling period which influenced the epimural bacterial community. Rumen content changed markedly in lambs over the 10-week period. At 1 week the rumen contained a small amount of clear fluids. At 2 weeks a few particles of undigested straw bedding were present along with the clear fluid. By 4 to 6 weeks the rumen was filled with the creep diet. By 8 to 10 weeks a mixture of the creep diet and alfalfa hay was present.

Papillae changed dramatically in length and surface characteristics over the 10-week period. Mean papillae length increased each week, from a mean of 0.32 mm at 1 week of age to 1.42 mm at 10 weeks of age, a 4.4-fold increase (Table 1). Papillae in group II lambs developed more rapidly than those in lambs from group I and were longer at all sampling times. In both groups of lambs, papillae at site 1 (roof of cranial rumen) were significantly longer then those at the other three sites. During the early transition stage (weeks 4 and 6), papillae were occasionally cone shaped rather than the more typical tongue shape. The tips of these cone-shaped papillae were nearly devoid of bacteria, due presumably to the rapid sloughing of cells around the tips.

Additional changes in tissue topography occurred with the

TABLE 1. Length of papillae

Sample	Mean papilla length (mm)		
	Group I	Group II	Overall
Age (wk)			
1	0.31[d]	0.33[d]	0.32[d]
2	0.29[d]	0.38[d]	0.34[d]
4	0.52[c]	0.76[c]	0.64[c]
6	0.61[b,c]	1.02[b,c]	0.81[b,c]
8	0.74[b]	1.16[b]	0.95[b]
10	1.07[a]	1.78[a]	1.42[a]
Site in rumen			
1	0.84[a]	1.15[a]	1.00[a]
2	0.43[b]	0.80[b]	0.62[b]
3	0.52[b]	0.93[a,b]	0.72[b]
4	0.56[b]	0.75[b]	0.65[b]

[a–d] Values in the same column with the same superscript and from the same type of sample (age or rumen site) are not significantly different ($p < 0.05$).

[e] For site descriptions, see Fig. 1.

development of longitudinal "grooves" (Fig. 3A) and large circular depressions on the surface of some papillae (Fig. 3B). These latter changes were first evident in samples from 6-week-old lambs and persisted thereafter. Figure 3C illustrates an ultrastructural difference that was observed between epithelial cells. Both cells were completely devoid of bacteria; one cell was covered with small granular projections, whereas the immediately adjacent cell was smooth. No clear pattern was observed that would explain these differences in cell surface characteristics.

In early samples, the papilla surface was relatively smooth, and the sloughing epithelial cells were relatively thin and flat (Fig. 3D). By week 6 the tissue topography was typically rough, especially on the sides and base of the papillae (Fig. 4A). Epithelial cells in this rough topography appeared swollen, presumably due to the deposition of keratin. This swelling created a topography consisting of more highly exposed, raised areas corresponding roughly to the centers of the cells and "micro-protected," depressed areas corresponding to the tapered, joined edges of the cells

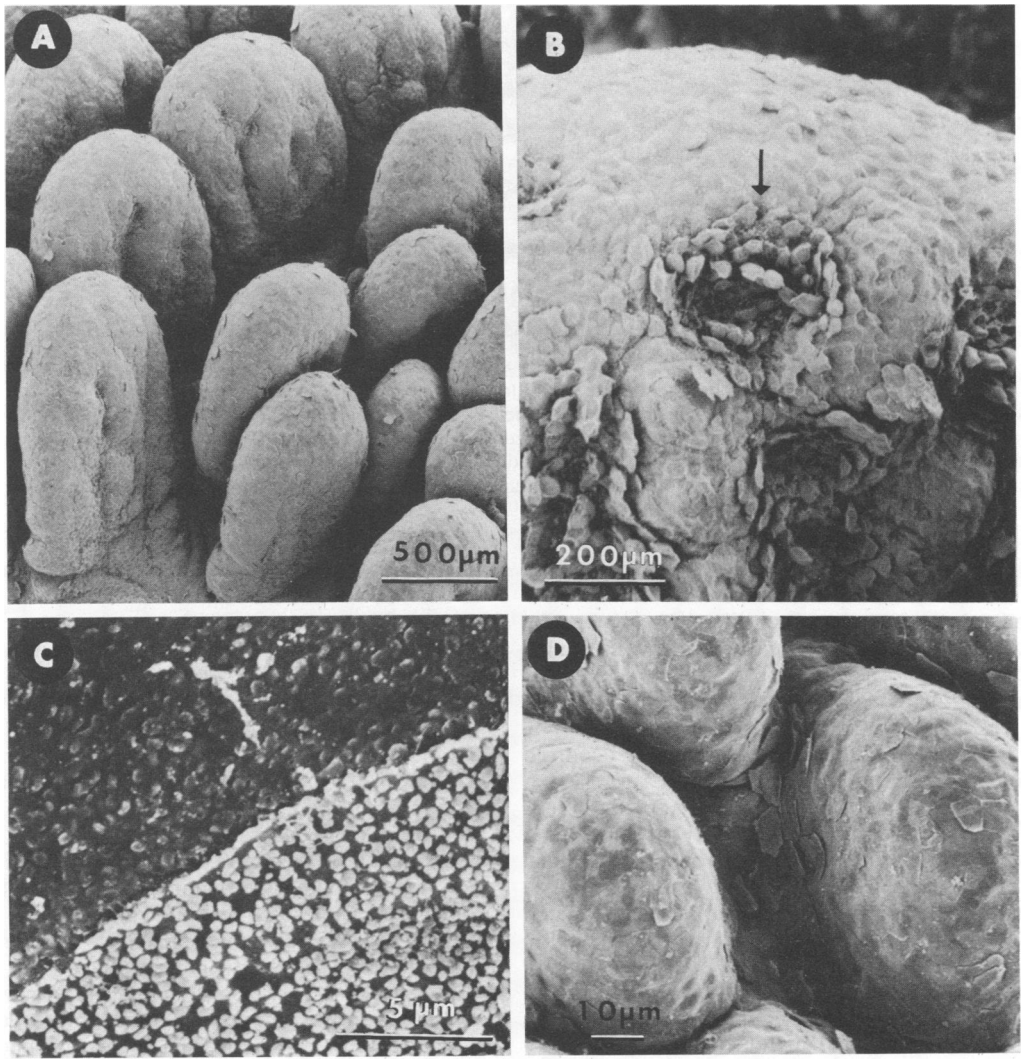

FIG. 3. Structural changes in papillae during the period from 1 to 10 weeks. (A) Longitudinal grooves became evident on the sides of papillae by 6 weeks of age. (B) Circular depressions with raised epithelial cells were also seen on some papillae at 6 weeks. These circular depressions in some cases appeared to be the upper limits of the lateral grooves. (C) Surface texture also differed from cell to cell in some cases. Some cells were covered by small granular projections, whereas other adjacent cell surfaces were relatively smooth. (D) In early samples (1 to 2 weeks) the tissue topography on the papillae surface was relatively smooth, with the sloughing epithelial cells being typically thin and flat.

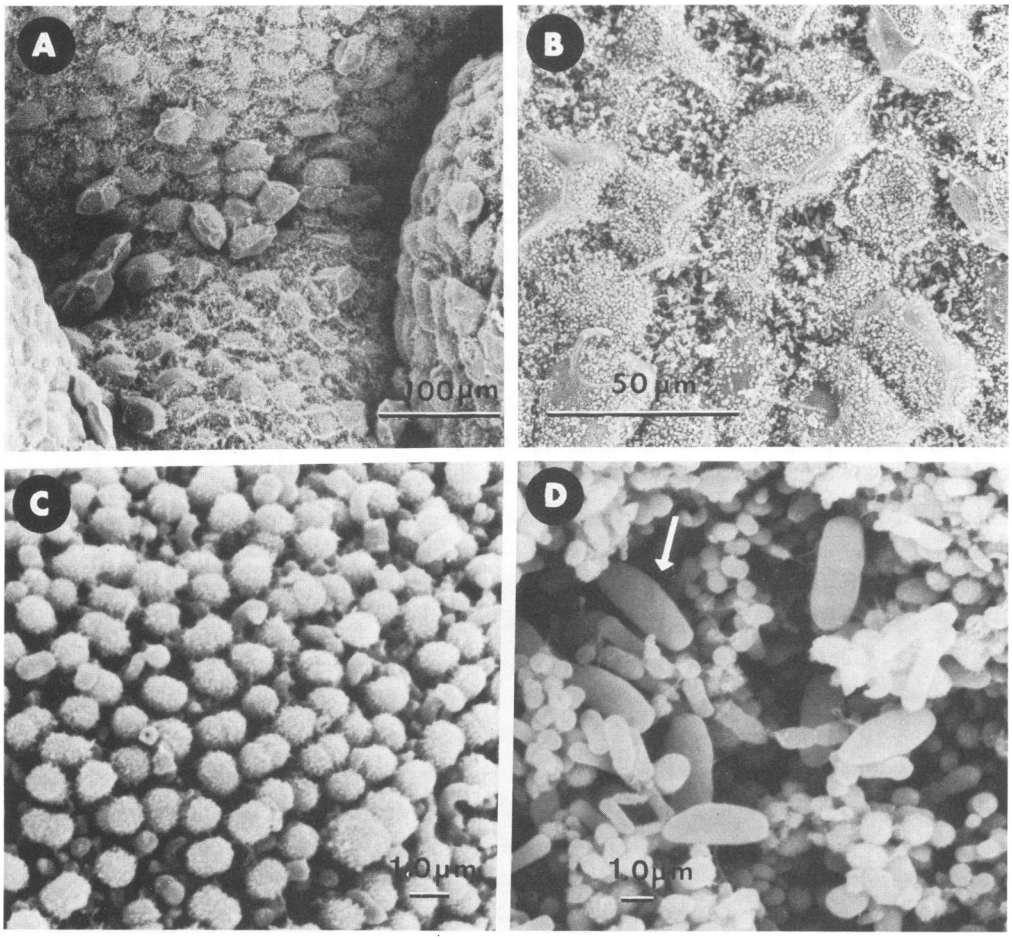

FIG. 4. (A) In later samples (6 weeks through adult) the tissue topography became typically rough, especially at the base and sides of papillae. Epithelial cells in these areas were swollen, presumably due to keratin deposition. (B) This rough topography was characterized by raised areas with intermittent depressions. Raised areas corresponded roughly to the centers of the cells, and depressions corresponded to the tapered cell edges. (C) Raised areas in this topography were colonized by a nearly pure culture of a coccus. (D) Depressions were colonized by a mixture of morphotypes including cocci and rods, with occasional spiral organisms. The large rod with tapered ends (arrow) is referred to in the text.

(Fig. 4B). Differences were seen in the types of bacteria colonizing these two microhabitats. The raised areas were colonized almost exclusively by cocci (Fig. 4C), whereas the depressions were colonized by a mixture of bacterial morphotypes (Fig. 4D).

Successive changes in morphotypes. An epimural community had already established itself on the rumen epithelium by 1 week of age, indicating that bacterial colonization occurred shortly after birth. The epimural community at 1 week was, however, considerably less complex than that of the adult. There were a few clearly dominant morphotypes, and bacteria were typically only one layer deep on the epithelial surface. On the tips of papillae, bacteria were present as a scattered monolayer, with much of the surface uncolonized and isolated microcolonies of a single morphotype quite common.

The dominant morphotype at 1 week was a coccus which colonized the sides and base of the papillae (Fig. 5A). This coccus was 0.6 to 0.8 μm in diameter and occurred singly and in pairs. The cell surface was characteristically granular in appearance, presumably due to capsular material which collapsed during dehydration. Small cavities or "pits," seemingly suggestive of enzymatic degradation, were occasionally observed in epithelial cells underlying these cocci (Fig. 5A). This morphotype was also present in the 2-week-old lamb, but at much lower levels and was not seen in later samples.

Another morphotype present at 1 week was a curved rod with tapered ends (0.5 by 1.9 μm; Fig. 5B). This morphotype appeared restricted in its colonization to the base of the papillae and was present in all samples from 1 week through adult, being most dense at 2 weeks. The microcolony of a straight rod (1.3 to 2.5 by 0.5 μm) shown in Fig. 5C was a common morphotype on the papillae tip at 1 week. A short rod or coccobacillus (Fig. 5D) which was typically seen in long chains was also present at low population levels on the tips, sides, and base of papillae in the 1-week-old lamb. This morphotype was also seen at slightly higher density in the 2-week-old lamb; it was not present in samples after 2 weeks.

A morphotype seen only at site 3 (floor of caudodorsal blind sac) in the 1-week-old lamb is shown in Fig. 6A and B. Because of its unique structure, this organism could be identified strictly on the basis of its morphology. The cells occurred in pairs and adhered on one side only, thus forming a palisade-like chain. This morphotype was identified as *Alysiella filiformis*, an aerobic, gram-negative bacterium which has been isolated from sheep saliva (21) and recently was reported to colonize cattle tongue (15). To our knowl-

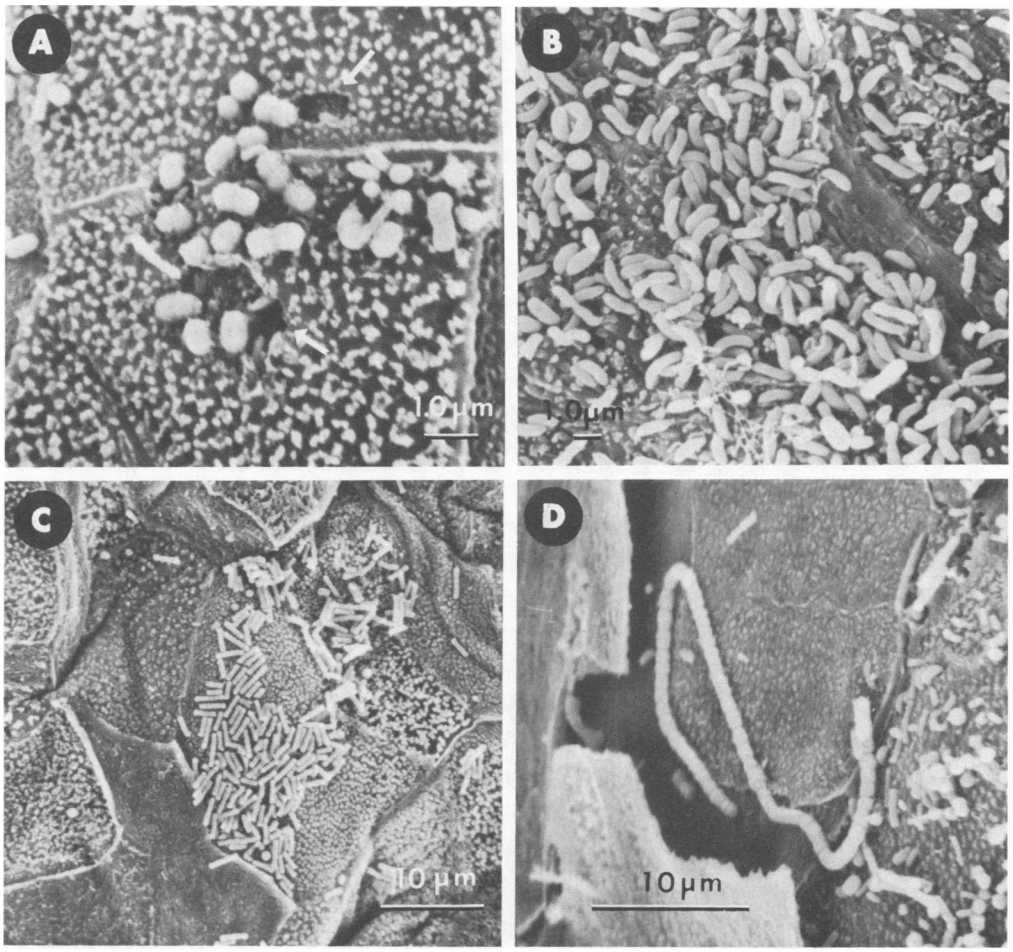

FIG. 5. Four of the bacterial morphotypes seen on the epithelium in the 1- and 2-week-old lamb. (A) A coccus which was prevalent in the 1-week-old lamb often occurred as a diplococcus. Note the "pits" underlying these cells (arrow) suggestive of enzymatic degradation. (B) The curved rods in this micrograph were present from 1 week through adult, being most dense in the 2-week-old lamb. (C) This microcolony of a straight rod was present on the tips of papillae in the 1-week-old lamb. (D) This short rod or coccobacillus was typically seen in long chains at low population levels in the 1- and 2-week-old lamb; it was not present in later samples.

edge, this is the first report of its colonization of the rumen epithelium.

In the 2-week-old lamb, the dominant morphotype was the curved rod first seen at lower density in the 1-week-old lamb (Fig. 5B). At 2 weeks it colonized the base and lower sides of papillae to the near exclusion of other morphotypes. At 4 to 10 weeks and in the adult it was still a prominent morphotype but was present at a level intermediate to those seen at weeks 1 and 2. This pattern of colonization, i.e., initially low, then high, then stabilizing at intermediate population levels, was also typical of other persistent morphotypes. Nonpersistent morphotypes which were present at more than one sampling time tended to follow a similar pattern, except that they disappeared after reaching high population levels instead of stabilizing.

The coccus shown in Fig. 6C formed a dense mat on some areas of the papillae tip in the 2-week-old lamb. Extracellular fibers, seemingly collapsed capsular material, were evident, extending from cell to cell and to the underlying epithelium. This organism did not persist in later samples.

At 4 weeks the epimural community became notably more complex in that no single morphotype was obviously dominant and the number of different morphotypes increased. The typical form of colonization also changed from a scattered monolayer to a dense monolayer or bilayer. This increase in complexity at 4 weeks was in conjunction with the presence of the creep diet in the rumen and thus the ingestion of solid food by the lamb. Since the morphological compositions of the 4- and 6-week-old lambs were not markedly different, they will be presented together. The curved rod which was dominant at 2 weeks was still a prominent morphotype at the base of the papillae at 4 and 6 weeks; however, it was in some cases interspersed with other morphotypes. A rod-shaped bacterium (2.4 to 4.0 by 0.7 μm) that attached end-on was a dominant morphotype in the 6-week-old lamb (Fig. 6D). A very tightly coiled spiral organism (2.9 to 3.5 by 0.3 μm) that attached end-on was also seen frequently on the tips and sides of papillae in the 4- and 6-week-old lamb (Fig. 7A). This morphotype was present at very low population levels in the 1- and 2-week-old lamb, at slightly higher levels in the 4-week-old lamb, and was most dense in the 6-week-old lamb. It was not seen at 8 and 10 weeks or in the adult. Another morphotype seen occasionally at 4 weeks and most dense at 6 weeks was a large cocci (1.6- to 2.5-μm diameter; Fig. 7B). The surface of this coccus was in some cases colonized by another rod-shaped organism in the 6-week-old lamb. This coccus did not persist beyond 6 weeks.

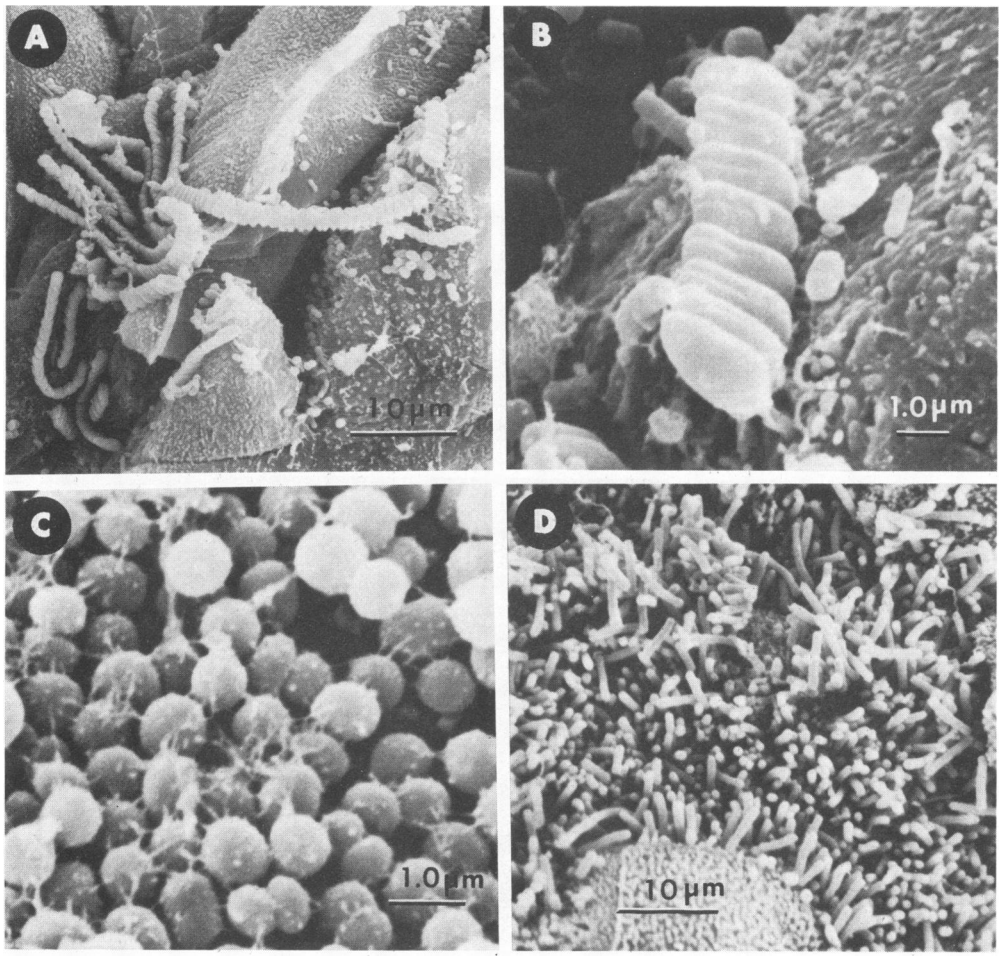

FIG. 6. (A and B) Floor of caudoventral blind sac at 1 week showing colonization by *A. filiformis*, a reportedly aerobic, gram-negative gliding bacterium. Cells occurred in pairs in chain form, with extracellular material produced only on one side of the cell. (C) The cocci in this micrograph formed a dense monolayer on some areas of the epithelium in the 2-week-old lamb. Extracellular fibers are evident extending from cell to cell. (D) This rod-shaped bacterium which attached end-on was a dominant morphotype in the 6-week-old lamb.

The morphological compositions of the 8- and 10-week-old lambs were also quite similar and will be presented together. One morphotype was again dominant in the 8- and 10-week-old lambs. The dominant morphotype was a coccus (1.0-μm diameter) which colonized the tips of papillae and raised areas in rough-type topography on the sides of papillae (Fig. 7C). In the adult animal, cocci of similar morphology were found to be dominant on the tips of papillae in 8 of the 12 sites examined, often colonizing these areas to the near exclusion of other morphotypes. A short rod (0.6 by 0.4 μm) with blunt ends (Fig. 7C) appeared along with the coccus on the tips of papillae at 8 weeks and colonized the same areas, but at much lower population levels. A spiral organism (8.0 by 0.3 μm; Fig. 7D) was usually present at low population levels mixed with other morphotypes. It first appeared at 4 to 6 weeks at low population levels, was most dense at 8 weeks, and was present at a lower density in 10-week and adult samples. Another morphotype commonly seen in 8- and 10-week-old lambs as well as the adult was the large rod with tapered ends shown in Fig. 7D. This rod colonized the depressed areas in rough topography.

The two morphotypes shown attached to one another in Fig. 7E were present at 8 weeks of age unassociated and they were repeatedly seen specifically attached to one another in 10-week samples and in the adult. All of the bacterial cells in the epimural community were in close proximity to one another; however, these two rod-shaped bacteria were more intimately associated than the general community. Cell dimensions were 2.5 to 4.5 by 0.4 μm and 3.4 to 8.0 μm by 2.0 to 2.5 μm for the thin rod (epibiont) and the large rod, respectively. The two rods were typically aligned with their long axes parallel; however, occasionally the epibiont was seen attached end-on (Fig. 7F), and even less frequently it was seen partially aligned. From these observations it appeared that the association was initiated by end-on attachment followed by alignment along the longitudinal axis. This attachment did not interfere with cellular division of either morphotype.

Indigenous morphotypes. Bacteria on the epithelial surface were differentiated on the basis of cell shape, size and surface characteristics into 24 distinguishable morphotypes. Of these 24 morphotypes, 17 were rod shaped, 4 were cocci, 2 were spiral shaped, and 1 was filamentous. These 24 morphotypes were further divided into the following three groups: those which persisted after their initial colonization and were present in the climax or adult community (7 morphotypes, group I), those seen only in the adult (2 morphotypes, group II), and those present only in young

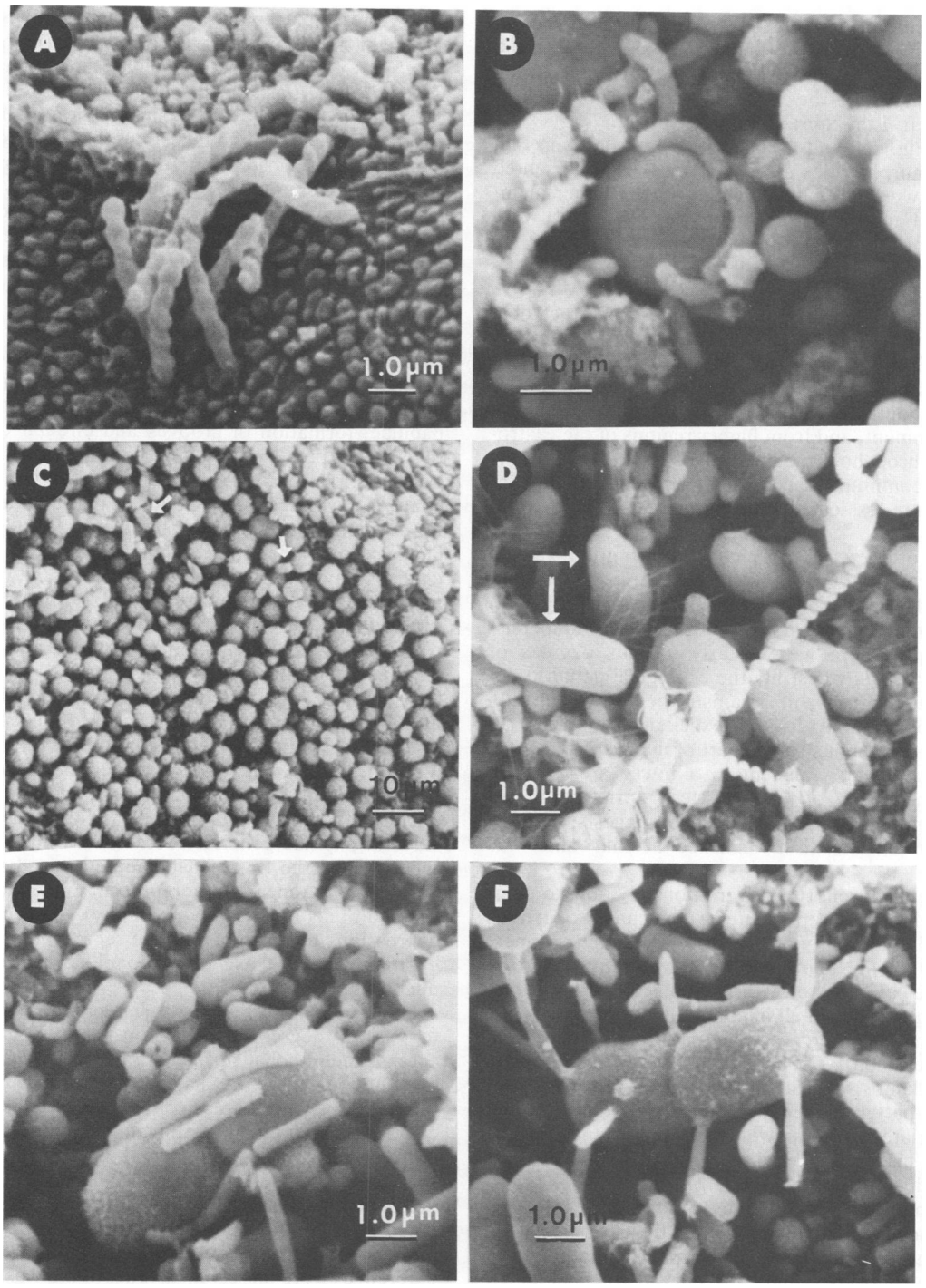

FIG. 7. (A) A very tightly coiled spiral organism that attached end-on was a prominent morphotype in the 6-week-old lamb. It was present at low levels at weeks 1, 2, and 4 and was absent from samples after 6 weeks. (B) The large coccus shown in this micrograph with a rod attached to it was present at low levels at 4 weeks and at higher population levels at 6 weeks. It was not seen in later samples. (C) This coccus was clearly the dominant morphotype at 8 and 10 weeks and in the adult. It colonized the tips of papillae and raised areas in rough topography on the sides and base. Note also the short rod (arrow) which colonized the same areas at much lower population levels. (D) This spiral organism was present at low population levels at 4 and 6 weeks and was most dense at 8 weeks; it persisted at lower density in the 10-week and adult samples. The large rod with tapered ends (arrow) was a prominent morphotype in depressed areas of rough topography. (E) Two rod-shaped bacteria were repeatedly seen intimately associated with one another in an interspecific relationship, with the thin rod attached to the surface of the larger rod. This bacterial association did not interfere with cellular division of either morphotype. (F) This association appeared to be initiated by end-on attachment of the epibiont followed by alignment along the longitudinal axes. Globules of extracellular material mediating attachment can be clearly seen at the attached end of the thin rods.

lambs (15 morphotypes, group III). Those morphotypes in group I would most likely be indigenous members of the epimural community. These seven morphotypes were (i) the curved rod in Fig. 5B, (ii) the coccus in Fig. 7C, (iii) the short rod in Fig. 7C, (iv) the spiral organism in Fig. 7D, (v) the tapered rod in Fig. 7D, and (vi and vii) the two rods in Fig. 7E. The other morphotypes discussed above and presented in micrographs were all placed into group III. Other members of group III and the two morphotypes in group II were not presented for the sake of brevity.

DISCUSSION

Pristine habitats, such as the gastrointestinal tract of a newborn lamb, are colonized in characteristic successions (1, 20). The first microbes to colonize the habitat are primarily contaminants from passage through the birth canal. Microbes continue to enter with the diet, from the external environment, and from higher regions of the gastrointestinal tract throughout the life of the animal; however, as the indigenous community becomes established, these transient microbes are less capable of competing in the ecosystem.

The division of bacterial morphotypes into groups based upon persistence in colonization was an attempt to differentiate between the indigenous and transient morphotypes in the epimural community. Among the transient or nonpersistent morphotypes there were clearly various degrees of transiency. *A. filiformis* best fits the ecological definition of an allochthonous or transient organism, being present only at one site in one animal. It is an excellent example of an organism being indigenous to one part of the gastrointestinal tract (oral cavity) and transient in a lower area (rumen epithelium). The ability of this reportedly strict aerobe to colonize the epithelium at 1 week also suggests that the oxygen tension on the tissue surface at that time was relatively high.

Other transient morphotypes were not only able to colonize the epithelium but represented significant proportions of the epimural community at one or more sampling times. Examples include the spiral morphotype in Fig. 7A, the cocci in Fig. 6C, and the rod which attached end-on in Fig. 6D. These morphotypes in all likelihood induced changes in the environment that affected the pattern of succession (autogenic succession). The fact that they were present in both groups of lambs suggests that they are a normal part of the succession pattern. Their transiency may therefore not be an appropriate indication of their importance to the animal, as they may be essential to the subsequent establishment of the indigenous community.

The seven morphotypes included in group I exhibited evidence of being indigenous based upon their persistence in colonization and their presence in the adult community. Of these seven morphotypes, only the curved rod in Fig. 5B was established on the tissue during the preruminant stage of development. The other six morphotypes were established during the later part of the transition stage (6 to 8 weeks). The curved rod in Fig. 5B, spiral morphotype in Fig. 7D, coccus in Fig. 7C, and tapered rod in Fig. 7D appear similar to morphotypes present in electron micrographs taken of epimural bacteria in mature ruminants by previous workers (2, 14, 16, 17). The fact that they are commonly found in the mature animal is further evidence that they are indigenous members of the epimural community.

The succession of epimural bacteria in lambs closely followed the stages of rumen development as determined by relative wet tissue weights. Preruminant lambs possessed a relatively simple epimural community consisting of a few dominant morphotypes, with other morphotypes present at very low density. Bacterial cells during this stage were present on the tissue primarily as a scattered monolayer or isolated microcolonies. The beginning of the transition stage was associated with the ingestion of solid food. At this time there was an apparent increase in the complexity of the epimural community. Layering of bacteria was more common, and a number of morphotypes were codominant. The complexity of the epimural community increased in the ruminant stage, with certain bacteria establishing interspecific associations. Bacteria were several layers deep in some areas, with bacteria in outer layers attached to other bacteria rather than directly to the epithelium.

These successive changes observed in the epimural community were apparently related to a combination of influencing factors. These included changes in length and shape of papillae, formation of grooves and circular depressions on the sides of papillae, swelling of epithelial cells after keratin deposition, and changes in dietary content. Additionally, changes in volatile fatty acid levels in the rumen and changes in oxygen tension would be expected to contribute to changes in the epimural community. These environmental changes divided the epithelial habitat into a number of microhabitats as evident by the different morphotypes colonizing them. For example, the curved rod in Fig. 5B was restricted in its colonization to the base and lower sides of papillae. Also, in areas of rough topography, one morphotype colonized the raised areas (the cocci in Fig. 7C), and a mixture of different morphotypes colonized adjacent depressed areas. Previous investigators have also noted these differences between raised and depressed areas in mature sheep (2). The tips of papillae were also colonized almost exclusively by a coccus after 8 weeks of age, whereas the sides of papillae generally contained a mixture of morphotypes. Thus, the tips of papillae and raised areas (in rough topography) appeared to represent one microhabitat; depressed areas (in rough topography), grooves, and circular depressions on sides of papillae represented another microhabitat; and the base of the papillae represented a third microhabitat.

One would expect these three microhabitats to be exposed to various degrees of abrasion by movement of digesta across the epithelial surface during rumen contractions. The level of fiber in the diet has been reported to affect distribution patterns within areas of the rumen (16). These abrasive effects of fiber would logically affect bacterial distribution on both a macroscopic (regions within the rumen) and a microscopic (regions on the papilla) level. These differences between raised and depressed areas in rough topography could also be explained by variation in the degree of washing in these areas during sample preparation. However, if these differences were merely an artifact of the washing technique, the cocci would logically have formed an underlying layer in depressed areas, and this was not observed to be the case.

Bacterial species residing in close proximity or regularly occurring together make up an interspecific association (1). Interdependency and interactions would be expected in the epimural community since so many diverse morphotypes are found in a limited area. Layering of bacteria and intimate associations between bacterial cells in the epimural community of the rumen can be seen in electron micrographs of previous investigators (9, 17). Although these investigators mentioned this layering and cell-to-cell attachment, they did not report seeing the same morphotypes repeatedly and specifically associated to one another as was the case for the

two rods in Fig. 7E. This type of specific attachment has been reported in other ecosystems, however, such as the termite gut (3) and the human oral cavity (10).

The role of bacterial attachment has been discussed as it relates to the digestion of plant particles and epithelial cells within the rumen (5); however, the role (if any) of attachment in bacterial interactions in the rumen, such as those reviewed by Wolin (24), has not been discussed. The attachment of some bacteria to plant particles in the rumen is known to be necessary to juxtapose membrane-associated cellulase enzymes with substrates, thus enabling digestion of the constituent plant polymers (11, 12). This association of cellulases, and perhaps amylases, with the bacterial glycocalyx is thought to conserve these enzymes and prevent their loss by diffusion. It seems entirely feasible that bacterial-bacterial adhesion would similarly facilitate interspecific transfer of nutrients by limiting their loss due to diffusion.

We have seen a similar type of specific adherence between *Methanosarcina* species, a methanogenic bacterium, and other unidentified bacteria from a mesophilic, swine manure digester (unpublished data). Zhilina and Zavarin (25) have reported two types of trophic relationships in which *Methanosarcina* cells are involved. One group of trophic organisms (primarily *Desulfovibrio vulgaris*) is in regular, close association with *Methanosarcina* cells due to the fact that they obtain hydrogen from the *Methanosarcina* cells. The second trophic bacteria are commensals or putrefactive organisms that use disintegrated *Methanosarcina* cells as a substrate. It is impossible to determine exactly which type of trophic relationship is occurring between the two rod-shaped bacteria seen in the epimural community. It is tempting to speculate, however, that this physical bacterial-bacterial attachment is a necessary prerequisite to some type of nutrient transfer.

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