Scanning Electron Microscope Study of Bacteria Associated with the Rumen Epithelium of Sheep

T. BAUCHOP,* R. T. J. CLARKE, AND J. C. NEWHOOK

Applied Biochemistry Division, Department of Scientific and Industrial Research,* and Department of Physiology and Anatomy, Massey University, Palmerston North, New Zealand

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Examination of the rumen epithelium of sheep by scanning electron microscopy revealed bacteria associated with the epithelial surface. Comparison of epithelial surfaces from 10 sheep revealed areas that were consistently densely covered with bacteria and other areas where the cover was consistently light. The bacterial populations were frequently of mixed morphological types, but areas populated with a single type were also observed. This finding, together with the discovery of bacterial forms not previously described in rumen contents, suggests that a specific flora may exist on the rumen epithelial surface. The functional significance of such a population is discussed.

Bacteria associated with the gut mucosa have been described in a number of mammals (2, 5, 6) but the phenomenon has received only brief mention in ruminants (7). In a study of the rumen epithelium of sheep with the scanning electron microscope, objects resembling bacteria were observed on the epithelial surfaces. The persistence of these bacteria through extensive and thorough washing of the tissue suggested that they might be firmly attached to the epithelium. The present study was undertaken to investigate the extent and nature of the epithelial bacterial population.

MATERIALS AND METHODS

Romney wether sheep, aged from 1 to 4 yr and fed on mixed white clover (Trifolium repens L.)-perennial ryegrass (Lolium perenne L.; "Grasslands Ruanui") pasture, were slaughtered by severing the jugular vein. The rumen and reticulum were rapidly exteriorized and pieces of wall about 2 cm square were removed from specific sites. Each tissue sample was washed by shaking vigorously in 500 ml of 0.85% (wt/vol) sodium chloride at room temperature, and when free of adhering rumen contents (approximately 10 s) was immediately fixed in 10% (wt/vol) formalin. To minimize postmortem changes in the tissue, the manipulations were performed as rapidly as possible and all samples were fixed within 3 min of sacrificing the animal.

The pieces of fixed tissue were washed further with jets of distilled water from a squeeze-bottle, attention being paid to the areas between the papillae, and were freeze-dried at -30 C. Dried tissue pieces were mounted, suitably orientated, on aluminum stubs with rubber cement. The preparations were coated with 20 nm of carbon, followed by 20 nm of gold-palladium, while being rotated in a vacuum chamber. The coated preparations were examined and photographed with a Cambridge Stereoscan scanning electron microscope, Mk 2a.

Each epithelial sample was assessed for degree of bacterial cover by examination of a number of sites on two or more papillae. The degree of cover was scored on a scale from 1 (a few scattered bacteria) to 4 (dense mat of bacteria). The types of bacteria present were also recorded. Magnifications were mainly in the range ×2,000 to 5,000.

RESULTS

The distribution and degree of bacterial cover on the epithelial surfaces were investigated by examining pieces of tissue from 14 sampling sites in the ruminoreticulum of a single sheep. The sites examined are shown in Fig. 1. With each sample a search was made of surfaces of suitably orientated papillae. An example of the papillae in a typical preparation from the roof of the dorsal rumen is shown in Fig. 2.

Bacteria were found on all of the surfaces examined but the numbers varied greatly in different areas of the ruminoreticulum. Most of the bacteria seen were on the dorsal, caudal, and lateral surfaces of the rumen, with the most dense populations on the roof of the dorsal rumen and on the floor of the caudodorsal blind sac (positions 4 and 5, Fig. 1). The rest of the rumen surfaces had only light or moderately heavy cover of bacteria. With the exception of one sample (see Fig. 5b) only scattered bacteria were seen on the reticulum samples. Areas on some of the papillae were covered with populations composed almost entirely of a single morphological type.

To substantiate the observations that bacterial populations varied in both density and type
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on surfaces from various sampling sites, samples were obtained from nine other sheep. In each of these sheep pieces of tissue were taken from four areas in the rumen (positions 3, 4, 5, and 7 as described in Fig. 1). These areas had been found to vary in density and type of bacterial cover in the first sheep examined. A summary of the results for all 10 sheep is shown in Table 1. The differences found in the original animal were confirmed; similar bacterial cover and types were found in the same epithelial sampling sites in all animals.

Examples of the degrees of cover observed are illustrated in Fig. 3a and b. In Fig. 3a, only a few bacteria are present on the epithelial surface and the granular projections covering the surface of the epithelial cell can be clearly seen. In Fig. 3b, the surface of the sample from the floor of the caudodorsal blind sac is densely covered with a mixed population. Minute local areas with a "pure" population were frequently found and Fig. 4a shows such an area on a papilla from the roof of the dorsal rumen. A coccus can be seen colonizing the raised portion of the epithelial surface while the lower surfaces are covered by a population composed almost entirely of a long rod. Figure 4b illustrates the discrete spatial separation of the two forms. Details of structure of the two morphological types are shown in Fig. 4c and 4d. The coccus measured 0.7 to 1.0 \( \mu m \) in diameter and ap-

Fig. 1. Diagram of reticulorumen from left side showing tissue sampling sites. All sites on midline except (13) and (14): (1) pole of reticulum, (2) cranial reticulum, (3) roof of cranial rumen, (4) roof of dorsal rumen, (5) floor of caudodorsal blind sac, (6) roof of caudoventral blind sac, (7) floor of caudoventral blind sac, (8) floor of ventral rumen, (9) caudal surface of cranial pillar, (10) rim of cranial pillar, (11) cranial surface of cranial pillar, (12) caudal surface of ruminoreticular fold, (13) left lateral surface of caudoventral blind sac, and (14) left lateral surface of caudodorsal blind sac.

Fig. 2. Washed papillae from floor of the caudodorsal blind sac of ovine rumen. x31.
TABLE 1. Degree of bacterial cover and the major bacterial forms present on the epithelial samples from four areas in the rumens of 10 sheep

| Sites in rumen | Bacterial cover | Total score for bacterial cover |
|---------------|-----------------|---------------------------------|
|               | A'   | B  | C  | D  | E  | F  | G  | H  | I  | J'  |
| Roof of cranial rumen (3) | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 15 |
| Roof of dorsal rumen (4) | 4 | 4 | 4 | 4 | 4 | 1 | 3 | 2 | 3 | 4 | 33 |
| Floor of caudodorsal blind sac (5) | 3 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 4 | 28 |
| Floor of caudoventral blind sac (7) | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 3 | 17 |

* Bacterial cover scored 1 (a few scattered bacteria) to 4 (heavy mat of bacteria). R = rods, C = cocci, and M = mixed morphological types.
* Site numbers refer to Fig. 1.
* Sheep no.
* This sheep was sampled in 14 sites in the ruminoreticulum. Bacterial scores for the positions (in parentheses) not given above were as follows: (1) 1; (2) 1; (6) 3; (8) 2; (9) 2; (10) 1; (11) 2; (12) 2; (13) 3; and (14) 3.

peared to have a rough, pimpled surface. The rods were 0.3 to 0.5 \( \mu m \) by 3 to 7 \( \mu m \), usually curved and thickened in the polar region.

Figure 6a represents a degree of cover intermediate between those in Fig. 3a and b, and shows a relatively pure population of a curved rod on a sample from the floor of the caudoventral blind sac. Figure 5b shows a localized high concentration of a short rod on a sample from the mid-reticulum. This was the only dense population of bacteria seen in any reticulum sample.

In a number of sites throughout the ruminoreticulum, pockets of a spiral organism were found. Figure 5c is typical of the heaviest population seen. Figure 5d gives more detail of its morphology. Although this spiral is associated with mixed populations on the epithelial surfaces, it does not correspond with any of the spiral organisms previously reported from rumen contents (1). It has been isolated and cultured and is being characterized. It differs in its small size (0.3 by 4 \( \mu m \)) and in the extremely tight coiling of the spirals, the coils of which are just visible under critical phase-contrast illumination.

At high magnifications, thin thread-like structures were frequently seen between bacterial cells (Fig. 6a and b).

**DISCUSSION**

The bacteria found on the epithelial surfaces of the ruminoreticulum are occupying a specific ecological niche or are merely contaminants from the bacteria-rich rumen contents. A number of observations indicate that the bacteria may be true inhabitants of the epithelial surface. First, there was variation in bacterial cover in different areas of the ruminoreticulum. Second, when four areas in each of the nine animals were examined in detail, two areas consistently had heavy cover and two were consistently lightly covered. Third, epithelial areas were found with a single morphological type predominating, and these areas were consistent in location in all sheep. Fourth, at least one of the bacteria, the spiral (Fig. 5c and d), has not been reported previously from rumen contents. However, failure to demonstrate its presence might be explained by its small size.

The main types of bacteria found on the epithelium are rod shaped, as is the case in normal rumen contents. It is not possible to identify them on the basis of morphology so comparison with known rumen bacteria cannot be made. Isolation and characterization of some of these bacteria are in progress.

Tamat et al. (7), in their study of bovine rumen epithelium, described colonies of a coc- cus with thread-like structures between the cells. The similar structures observed by us were associated with both coccal and rod-shaped bacteria (Fig. 6a and b). The threads appear similar to mucus. However, mucus is
Fig. 3. Epithelial surface of the ovine rumen. (a) The floor of the caudoventral blind sac with few attached bacteria. x2,500. (b) The floor of the caudodorsal blind sac showing a dense cover of mixed types of bacteria. x2,500.
Fig. 4. Bacteria on the epithelial surface of the ovine rumen. (a) Two relatively pure bacterial populations on the mid-dorsal surface of the rumen. \( \times 1,350 \). (b) Higher magnification showing the spatial separation of the two types. \( \times 2,400 \). (c) Details of the cocci in (b). \( \times 11,400 \). (d) Details of the rods in (b). \( \times 6,600 \).
FIG. 5. Bacteria on the epithelial surface of the ovine ruminoreticulum. (a) Relatively homogeneous population of a curved rod on the floor of the caudoventral blind sac. ×7,000. (b) Pure population from the pole of the reticulum. ×9,200. (c) Spiral bacteria on the caudal surface of the ruminoreticular fold. ×5,500. (d) Spiral bacteria showing the closed nature of the coils. ×10,750.
Fig. 6. Bacteria on the epithelial surface of the ovine rumen. Network of threads connecting some of the bacteria. (a) On the floor of the caudodorsal blind sac. ×12,750. (b) On the roof of the cranial rumen. ×12,500.
not secreted by the epithelium of the ruminoreticulum and it seems unlikely that the material could be derived from salivary mucus. The close association of the threads with individual bacterial cells suggests that they may be an extracellular product of bacterial metabolism.

Nothing is known of the functional significance of these bacteria on the rumen epithelium. Indigenous populations of bacteria have been observed, by conventional histological methods and by transmission electron microscopy, on the mucosal epithelia of various regions of the gut of many animals. These animals include man, monkeys, swine, hamsters, rats, mice, and birds (2, 5, 6). The bacteria involved are believed to interact physiologically with the epithelium (5, 6) but the mechanisms are not fully understood. Some of these indigenous populations are firmly attached to the epithelium and some are merely embedded in mucus; most are in a position to receive nutrients from the adjacent epithelial cells. Interference with alkaline phosphatase has been postulated for bacteria in the duodenal epithelium of mice (8).

The epithelium of the ruminoreticulum is an important absorptive surface and passage of some metabolites into the rumen also occurs (3, 4). Attached bacteria would thus be in a favorable position to utilize nutrients passing through the wall.

Other benefits of attachment to the epithelium can be postulated. An advantage would be conferred on organisms whose growth rate was insufficient to avoid being washed out of the gut. Also, in the rumen, ingestion by ciliate protozoa might be avoided.

Further studies of these bacteria, including their isolation and growth in vitro, and determination of their substrate affinities may provide evidence for their role in this niche.

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