ELECTRON-MICROSCOPIC INVESTIGATION OF THE FLORA OF SHEEP ALIMENTARY TRACT

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Summary

Digesta from different regions of the sheep alimentary tract have been examined using electron microscopy. Direct examination of digesta from the rumen has revealed the presence of a number of phages and of a large variety of bacteria differing considerably in their morphology. An atlas of electron micrographs of these bacteria has been presented. The presence of large numbers of bacterial cell walls in the rumen indicates that the breakdown of bacteria commences in this organ. The major sites of digestion of bacteria are in the abomasum and small intestine where there is a substantial removal and modification of bacteria.

I. Introduction

Bacteria appear to undergo lysis in the rumen of sheep as indicated by the presence of large numbers of cell walls in any sample examined (Hoogenraad *et al.* 1967). Some of the factors responsible for such lysis have been indicated by Jarvis (1968). In addition it has been shown that rumen bacteria, and their cell walls, are susceptible to degradation under conditions similar to those existing in the alimentary tract of sheep (Hoogenraad and Hird 1970) and are digested during their passage through it (Hoogenraad *et al.* 1970).

The present paper arose out of an attempt to follow such digestive processes by examining morphological changes in rumen bacteria as they pass along the alimentary tract. As an initial step, a detailed study of bacteria from rumen contents of sheep was undertaken using the electron microscope.

In the studies of Moir and Masson (1952) by visual light microscopy, and of Smiles and Dobson (1955) by ultraviolet microscopy, many of the larger organisms found in the rumen were described. Several electron micrographs of rumen bacteria have also been published by Hungate (1966). However, most of the smaller organisms which form the bulk of the rumen population have not been described. Accordingly, we present electron micrographs of bacteria taken directly from rumen contents.

Smiles and Dobson (1955) examined samples from rumen, omasum, and abomasum of sheep using ultraviolet microscopy and found that degenerative changes of various bacteria occur in the omasum and particularly the abomasum. To extend this study, samples from the rumen, omasum, abomasum, and different regions of the small intestine were inspected with the electron microscope to see whether degenerative changes also occur with the smaller bacteria. Parallel to this study, total bacterial counts were made by light microscopy.

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II. Materials and Methods

(a) *Materials*

Rumen digesta were collected from sheep fitted with permanent rumen fistulae and fed on a diet of lucerne chaff, and from freshly killed sheep from the slaughter house. For the experiments comparing the different regions of the alimentary tract, material was obtained from sheep which had access to a mixed pasture of perennial rye grass and white clover until immediately before slaughter. Due to the degenerative changes which occur on storage, examination was limited to fresh material.

(b) *Electron Microscopy of Rumen Contents*

Material was filtered through gauze and centrifuged at 250 *g* to remove plant material, protozoa, and very large bacteria (greater than 10 μm in diameter), the latter not being suitable for examination by electron microscopy using negative staining. Samples were examined in the electron microscope (Hitachi HS-11A and Hitachi HS-8) after staining with potassium phosphotungstate at pH 6·5. To obtain an even distribution of the microorganisms present in the samples, the agar stripping technique described by Mayor *et al.* (1965) was also used.

(c) *Electron Microscopy of Different Regions of the Alimentary Tract*

After slaughter of the sheep, the alimentary tract was ligated so that samples could be removed from the rumen, omasum, and abomasum. The small intestine was ligated into sections of 3 ft so that samples could be taken from three segments nearest the abomasum and two segments nearest the caecum. The samples were filtered through gauze and mounted on grids using the agar plating technique, and stained with 1% potassium phosphotungstate. Five sheep were inspected in this fashion.

The total number of bacteria present in each sample was determined using the nigrosine slide technique as described by Gall, Stark, and Loosli (1947).

III. Results

(a) *Examination of Rumen Contents*

Inspection of samples from the rumen of sheep by visual light microscopy showed a wide variety of both Gram-positive and Gram-negative bacteria to be present. *Lampropedia* and *Oscillospira* were conspicuous (Fig. 1). Also present were Gram-positive spiral forms and large Gram-negative cocci. Inspection of rumen contents with the electron microscope shows an even greater variety of bacteria than can be seen by light microscopy. Some of the species have morphological features enabling them to be named from a description given of the bacteria grown in pure culture. Accordingly, tentative names have been assigned to these; the remainder are simply numbered and presented as such.

Irrespective of the source of material inspected, samples from the rumen always contained bacteria which showed some degenerative changes and a large number of bacterial cell walls (Fig. 2).

Another constant feature of the samples inspected was the presence of a number of bacteriophages (Hoogenraad *et al.* 1967). Some additional phages found in various rumens since this publication are included in the present paper. A particularly large phage with long appendages arising from either the shoulder region or a contracted sheath (Fig. 3) was found free in rumen liquor in large numbers. It was also found associated with a small coccus as in Figure 4 where the phage particles are shown present inside a cell and dispersing from it. A different phage with a long tail (Fig. 5)

is also frequently found in rumen liquor, and a phage with an even longer tail has also been found (Fig. 6). It appears that the latter has a partly collapsed head and a fine, lacy material can be seen at the tip of the long tail which may be newly discharged nucleic acid.

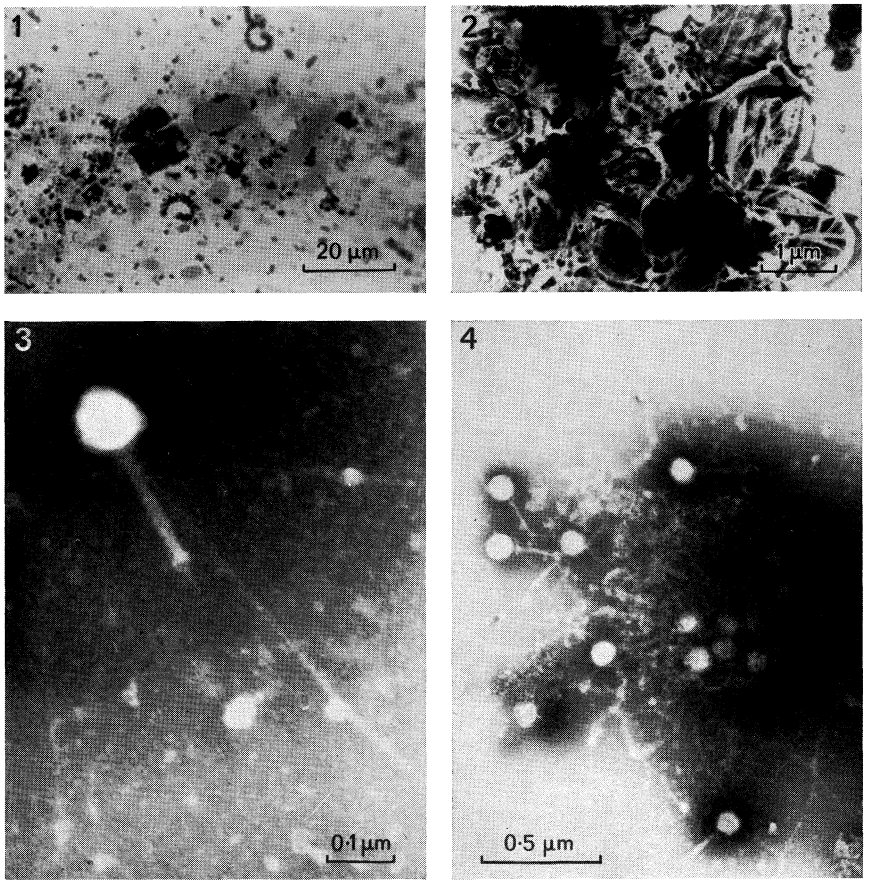


Fig. 1.—A smear of rumen contents, Gram-stained and photographed by light microscopy using an oil-immersion lens. The preparation shows Gram-positive "window-pane sarcina", spiral forms, and rods and cocci, and Gram-negative *Oscillospira* and a very large number of other organisms. ×600.

Fig. 2.—Large numbers of cell walls and pieces of cell wall are present, as well as a small coccus and rods. ×12,500. [In Figures 2–50, samples were negatively stained with 1–2% potassium phosphotungstate, at pH 6·5, before being photographed.]

Fig. 3.—The largest phage seen in the rumen contents. Long appendages arise from the shoulder region of the phage, but may be connected to the base of a contracted sheath.

Fig. 4.—A small coccus heavily infected by a large phage. The coccus appears to have been lysed by the large phage seen in Figure 3.

Rumen bacteria were usually found in mixed clusters as shown in Figure 7. Attempts to separate bacteria in order to obtain photographs of individual bacteria

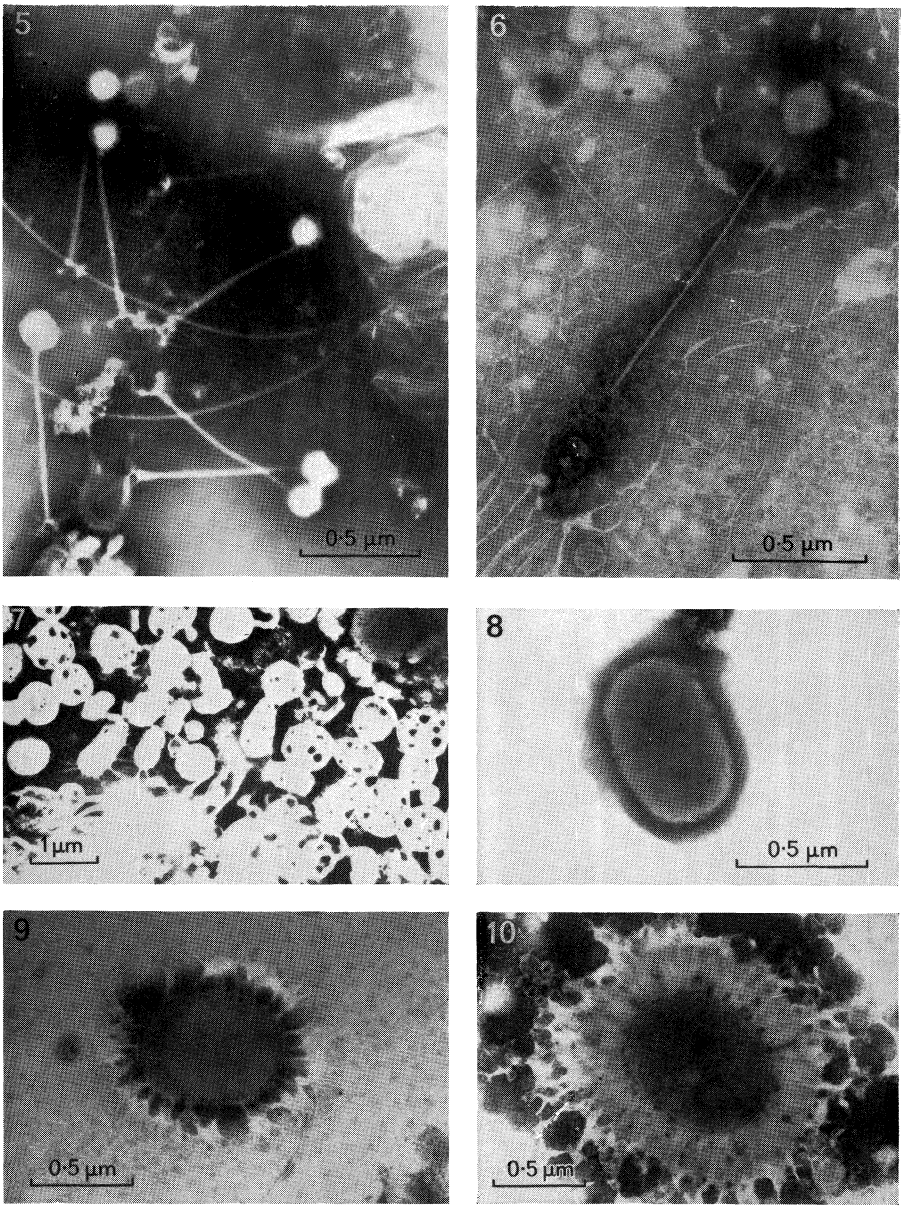


Fig. 5.—Phage with a long tail found free in rumen liquor.

Fig. 6.—Phage with a very long tail around the base of which is a fine, lacy material which may be nucleic acid discharged from the phage.

Fig. 7.—A mixed cluster of small rumen microbes. The predominant type present is a small coccus (organism 1) and also present are small rods and some cell debris. ×9,000.

Fig. 8.—Organism 2, diplococcus or small coccus undergoing cell division. ×27,000.

Fig. 9.—Organism 3, diplococcus or small coccus with mucoid capsule, undergoing cell division. ×27,000.

Fig. 10.—Organism 4, coccus with a very dense capsule. The outer layer of the cell wall is folded in places forming vesicles. ×25,000.

were unsuccessful as treatment which was successful in separating bacteria unfortunately damaged them. Some of the bacteria described hereafter could therefore not be photographed alone.

(*b*) *Cocci*

The most common bacteria found in the rumen of sheep are small cocci, usually less than 1 μm in diameter.

Organism 1 (Fig. 7) shows many small cocci ranging in size from 0·5 to 0·8 μm in diameter. Electron-dense "granules" are present.

Organism 2 (Fig. 8) is a diplococcus or a small coccus which may be in the process of cell division.

Organism 3 (Fig. 9) is a diplococcus or a small coccus which may also be undergoing cell division; it possesses a capsule. This bacterium ranges in size from 0·5 to 0·7 μm and has a thicker cell wall than organism 2.

Organism 4 (Fig. 10) is a slightly larger coccus (0·7–0·8 μm) with a very thick capsule. The outer layer of the cell wall is folded in places forming vesicles which in one place extend to the outside of the capsule.

Organism 5 (Fig. 11) is a coccus with a capsule similar to that in Figure 9. It is, however, somewhat larger (1·0 μm) and many electron-dense granules of varying sizes are to be seen. It may be a diplococcus or in a state of cell division.

Organism 6 (Fig. 12) shows a large coccus (1·5–2·0 μm in diameter) with many electron-dense granules. A disrupted spirochaete in contact with a small diplococcus may be seen. Other organisms and cell wall material are also present.

Organism 7 (Fig. 13) is one of the most distinctive bacterial types present in the rumen of sheep. It is a fimbriated, small coccus, 0·8–1·2 μm in diameter. This bacterium is often seen in the "apparent" process of cell division. The fimbriae appear to be flat ribbons which have evenly spaced, minute processes on them. The small laminated bodies seemingly caught up in the fimbriae are of interest. Figure 14 shows a variation with finer and more numerous fimbriae; it was taken from another animal. This organism is particularly abundant in the rumen of pasture-fed animals and is less frequently found in animals housed in metabolism cages and fed a diet of lucerne chaff.

Organism 8 (Fig. 15) is a coccus similar in size to organism 5 (1 μm in diameter) but is without a capsule. Two other rod-shaped organisms are present.

Organism 9 (Fig. 16) is a streptococcal type. The chains consist of cocci 0·6–0·8 μm in diameter. A thick cell wall is visible in a number of the cells and one of them appears to be empty.

Organism 10 (Fig. 17) is 3·0–4·0 μm in diameter and has many electron-dense granules in it. Smaller organisms and cell wall material may be seen in close proximity. Flagella, which appear to arise from the coccus, can be seen on the right-hand side of the organism. Figure 18 shows an organism similar in size, but without electron-dense granules. The flagella may be seen more clearly in this electron micrograph.

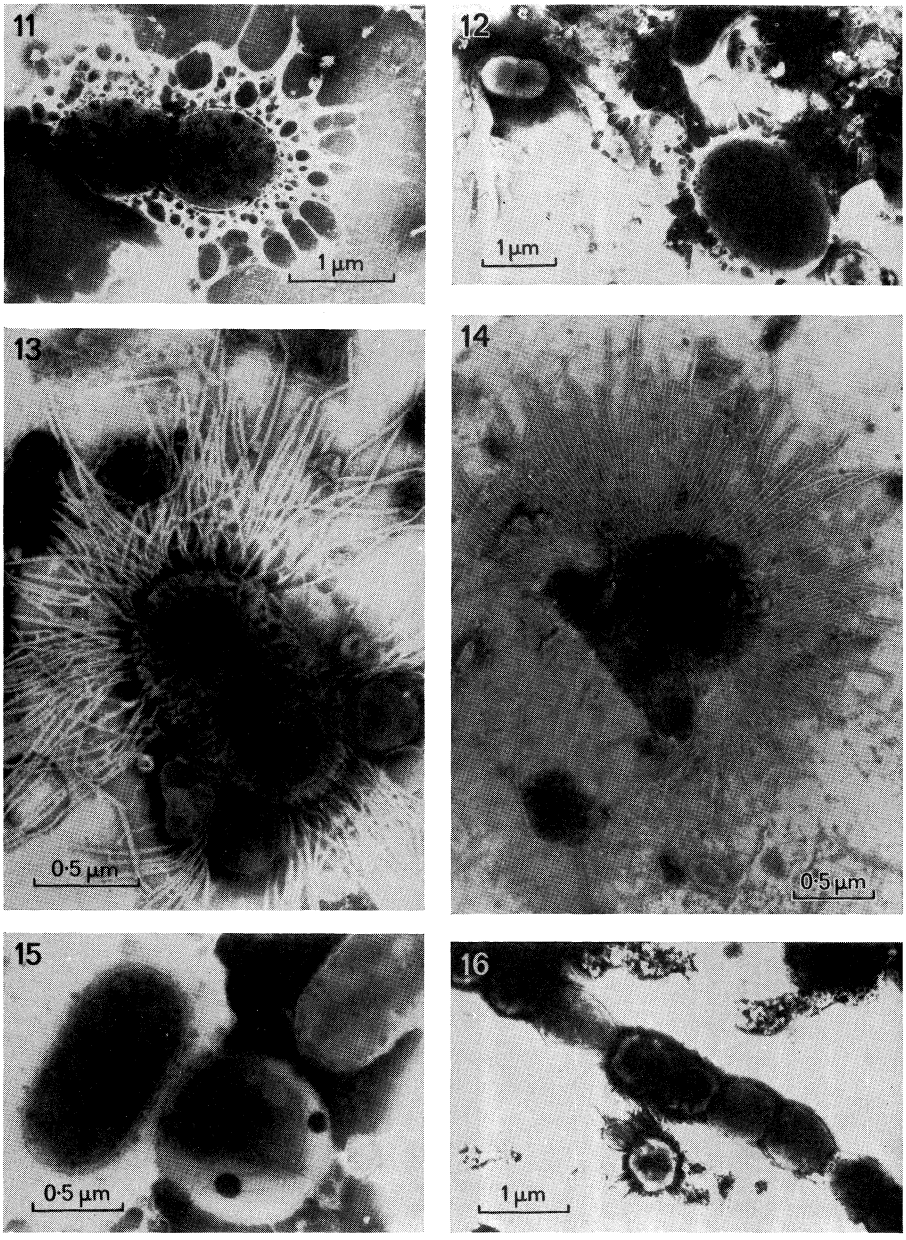


Fig. 11.—Organism 5, coccus or diplococcus with a thick cell wall and mucoid capsule. The cell contains irregular-shaped electron-dense granules. ×14,000.

Fig. 12.—Organism 6, large coccus with electron-dense granules. Also present are two curved rods similar to organism 23 (Fig. 33), a small diplococcus, a disrupted spirochaete, cell walls, and other debris. ×10,000.

Fig. 13.—Organism 7, coccus with fimbriae which have evenly spaced minute processes on them. ×28,000.

Fig. 14.—Organism 7, coccus with more abundant fimbriae than the organism in Figure 13. The evenly spaced processes present in Figure 13 do not appear to be present in this organism. ×21,500.

Fig. 15.—Organism 8, a coccus similar in size to organism 5 (Fig. 11) but without a capsule, and organism 16, a rod-shaped bacterium with a dense capsule. ×25,000.

Fig. 16.— Organism 9, streptococcal type of organism. The cells are not all alike and one appears to be empty. ×12,000.

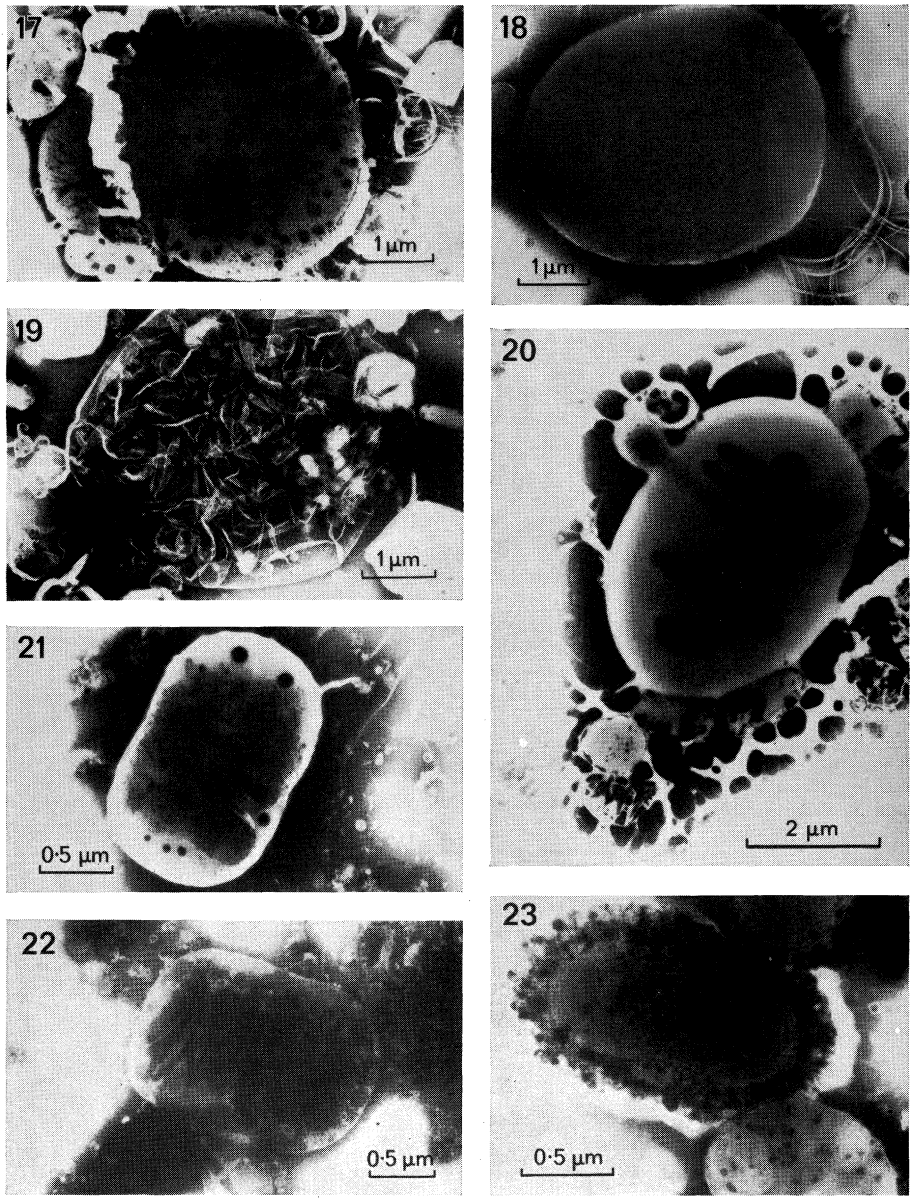


Fig. 17.—Organism 10, large coccus containing electron-dense granules. Flagella appear to arise from the right-hand side of the organism. ×10,000.

Fig. 18.—Organism 10, similar-sized coccus as in Figure 17, but lacking the electron-dense granules. The organism has flagella and a sculptured surface. ×9,500.

Fig. 19.—Cell wall, which was found free in rumen fluid, derived from a large coccus similar in size to organism 10. ×10,000.

Fig. 20.—Organism 11, a large oval form with flagella. The irregular-shaped, electron-dense regions are a constant feature of this organism. ×9000.

Fig. 21.—Organism 12, a short rod-shaped organism which appears to be in the process of cell division. ×20,000.

Fig. 22.—Cell wall probably derived from a short rod as seen in Figure 21. ×20,000.

Fig. 23.—Organism 13, a rod-shaped organism with a spiked capsule surrounding a thick, multi-layered cell wall. ×25,000.

Figure 19 shows a large cell wall, approximately 4 μm in diameter, which may be derived from a large coccus of this type.

Organism 11 (Fig. 20) is a large oval form (approx. 4 μm in diameter) which is also flagellated. The bands of electron-dense regions are a constant feature in all specimens seen. This organism is similar to the oval form described by Woodcock and Lapage (1913).

(*c*) *Rods*

Organism 12 (Fig. 21) is a short rod measuring 1·0 by 1·5 μm, which may be undergoing cell division. Figure 22 shows a cell wall found in rumen liquor which may be derived from a similar organism.

Organism 13 (Fig. 23) is a rod measuring 0·5–0·7 by 1·5 μm with a spiked capsule surrounding the thick, multilayered cell wall. The fine material adhering to the spikes is of interest. A similar organism is shown in the top left of Figure 24 in which are also present many small pieces of cell wall and cell debris and a selenomonad-like organism. Organism 13 is most commonly found in sheep which have been fasted for a period of time and was rarely found in samples from a fistulated sheep fed a diet of lucerne chaff.

Organism 14 (Fig. 25) is a slightly constricted bacillus measuring 0·5 by 1·8 μm which has a thick cell wall. The cell wall is surrounded by a capsule.

Organism 15 (Fig. 26) is a larger bacillus than organism 14, measuring 0·7 by 2·6 μm; it does not have a capsule. It appears to be filled with spheres which in places appear to be joined by disks.

Organism 16 (Fig. 15) is a small rod-shaped organism (0·6 by 1·2 μm). It has similarities to organism 4 (Fig. 10), having a very thick capsule, and the outer layer of the cell wall is folded in places forming vesicles.

Organism 17 (Fig. 27) is a long slender rod (0·3 by 2·5 μm). A mucoid-like capsule is present.

Organism 18 (Fig. 28) is a slightly larger rod than organism 17, measuring 0·5 by 3·0 μm. The cell wall appears to be thin.

Organism 19 (Fig. 29) is a long, very thin rod (0·15 by 3·5 μm). Other micrographs show this organism undergoing cell division in the central region. The organism in this photograph shows a constriction in this position.

Organism 20 (Fig. 30) is a long curved rod with a constricted end (0·3 by 2·4 μm). It is commonly found in the rumen of sheep. It is lacking in a capsule which distinguishes it from the following organism.

Organism 21 (Fig. 31) is similar in size to organism 20, measuring 0·4 by 2·7 μm. However, it has a thick cell wall and is surrounded by a mucoid-like substance attached in places to the bacterial body.

Fig. 28.—Organism 18, a long, slender rod with a very thin cell wall. ×17,000.

Fig. 29.—Organism 19, a long, very thin rod with a constriction in the middle of the cell which may be related to cell division. ×17,000.

Fig. 30.—Organism 20, a long, curved rod which is constricted at one end. ×27,000.

Fig. 31.—Organism 21, a long, curved rod with mucoid capsule. ×25,000.

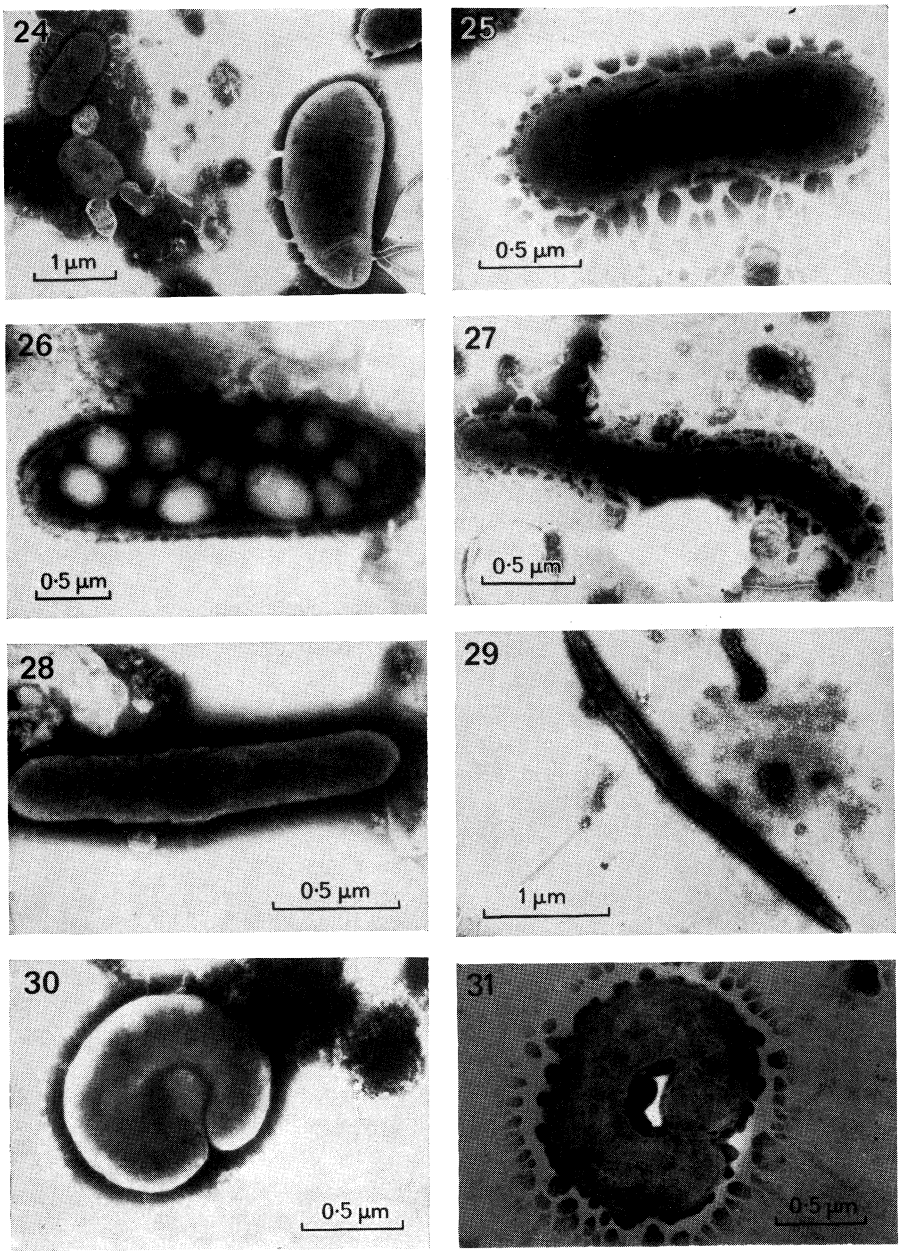


Fig. 24.—Organism 13, a rod-shaped organism with a spiked capsule (top left-hand corner of micrograph). Also present is a selenomonad-like organism. ×11,000.

Fig. 25.—Organism 14, a slightly constricted bacillus surrounded by a thick, multi-layered cell wall. A mucous capsule surrounds the cell. ×27,000.

Fig. 26.—Organism 15, a bacillus larger than organism 14, but without a capsule. The cell is filled with low-density spheres. ×20,000.

Fig. 27.—Organism 17, a long, slender rod with a mucoid capsule. ×25,000.

Organism 22 (Fig. 32) is a shorter curved rod (0·3 by 1·6 μm) which is possibly undergoing cell division.

Organism 23 (Fig. 33) is a curved rod (0·5 by 1·5 μm) which is usually present in the rumen arranged in pairs forming a "doughnut". They contain a number of electron-dense granules.

Organism 24 (Fig. 34) is a helically coiled organism which consists of a number of coiled rods measuring 0·3 by 2·5 μm. A similar organism was isolated from the caecum of rats by Fitzgerald *et al.* (1965) and was found to resemble most closely the genus *Eubacterium* (Prévot).

(*d*) *Selenomonads*

There are a number of different *Selenomonas* species and bacteria similar in appearance commonly found in rumen digesta.

Organism 25 (Fig. 35) is a crescent-shaped bacterium with a tuft of long flagella. It may be *Selenomonas ruminantum*. Its dimensions are 2 by 6 μm.

Organism 26 (Fig. 36) is also a *Selenomonas* species (1·0 by 4 μm) the ends of which are more pointed than organism 25. It may be the same as that described by Moir and Masson (1952) (in their list, organism 5).

Organism 27 (Fig. 37) is a larger selenomonad measuring 1·9 by 4·7 μm with rounded ends and a tuft of lateral flagella. This organism may correspond to organism 4 described by Moir and Masson (1952). The rounded ends and dense tuft of flagella make this species of *Selenomonas* look similar to organism 26, although this cell is only slightly curved compared with organisms 25 and 26.

(*e*) *Spirochaetes*

Organism 28 (Fig. 38) is most likely a member of *Borrelia* sp. The organism is wrapped around a number of small cocci. The protoplasmic core of the bacterium is surrounded by a sheath, the structure of which is clearly visible. Also surrounded by this sheath are the axial filaments. These are inserted into the body of the organism at both ends as shown in the micrograph. These structures are also shown in the thin sections prepared by Bladen and Hampp (1964).

In a number of places along the bacterial body, protruding patch-like structures are repeated. These structures are thought to be sporing bodies (Ovčinnikov and Delektorskij 1966). The spirochaete measures 0·5 by 8 μm.

Organism 29 (Fig. 39). The spirochaete in this figure is similar to organism 28, but has a smaller number of axial fibres. Also, there are fewer fibres in the middle section than at the end of the organism and this may be associated with cell division (Listgarten and Socransky 1964). Disrupted bacterium may be seen nearby and also a species similar to organism 13.

(*f*) *Changes in Bacterial Composition along the Alimentary Tract*

As digesta passed from the rumen, through the omasum, abomasum, and the small intestine there was a decrease in the number of bacteria per unit wet weight until the vicinity of the caecum was reached, where there was a gradual increase in bacterial numbers. In Figure 51 the total number of bacteria per gram wet weight in the different regions of the alimentary tract is given. The histogram represents the average of five sheep and also shows the range of values obtained in the five experiments.

Although there was a marked difference in numbers of bacteria between the rumen and omasum, careful inspection with the electron microscope showed that the bacterial composition of the omasum and rumen contents were similar. There were

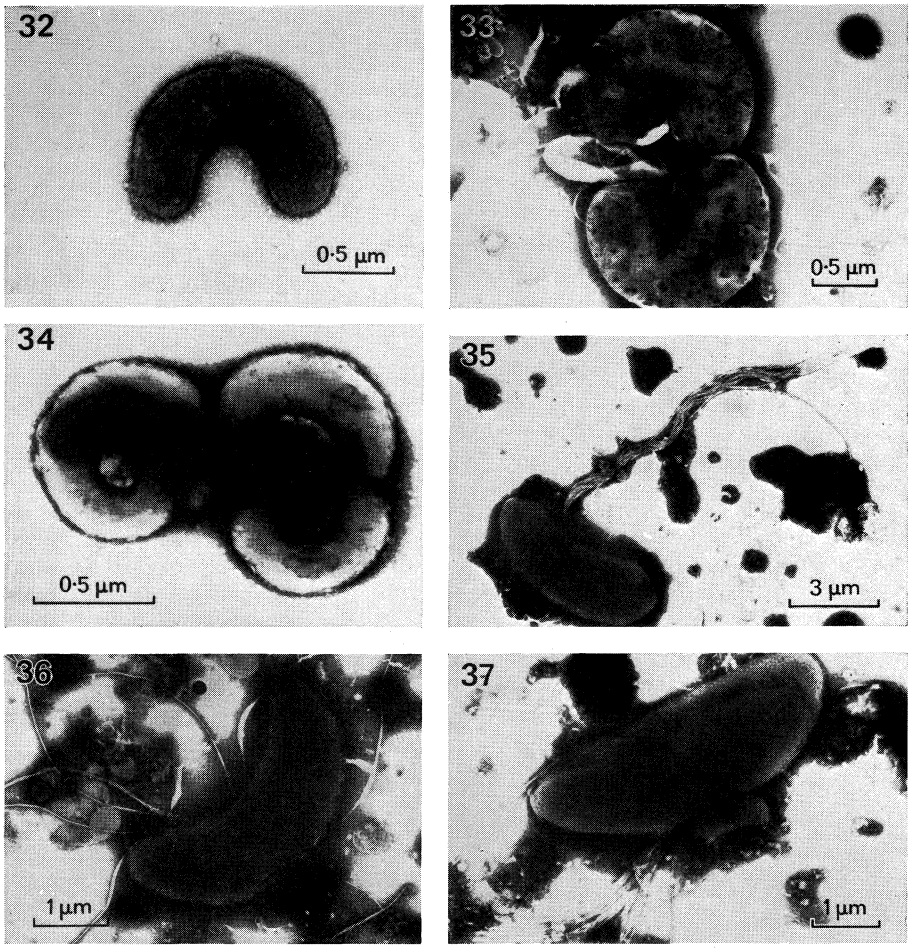


Fig. 32.—Organism 22, a short, curved rod undergoing cell division. ×25,000.

Fig. 33.—Organism 23, a pair of squat, curved rods. This organism usually contains electron-dense granules as shown. ×17,500.

Fig. 34.—Organism 24, a helically coiled organism. The curved rods are arranged to form a helix, and it is similar to an organism found in the caecum of rats (Fitzgerald *et al.* 1965). ×32,000.

Fig. 35.—Organism 25, selenomonad with a tuft of many flagella. ×4,000.

Fig. 36.—Organism 26, a crescent-shaped cell with more pointed ends than organism 25. There are also fewer flagella. ×10,000.

Fig. 37.—Organism 27, selenomonad with a less curved bacterial body than organism 25, but with a large tuft of lateral flagella. ×9,000.

also similar proportions of entire bacteria to cell walls and phages were present in both. Spirochaetes and organism 7 (Figs. 13 and 14), which show greater structural detail than most other organisms found in the rumen, were found completely

undamaged as often in the omasum as in the rumen. An important point is that there were many cells, cell walls, and broken cells present in both of these regions of the alimentary tract, and it can be concluded that breakdown of bacteria occurs in the rumen at least. Figure 40 shows a spirochaete from the rumen, which has lost its axial filaments. Figure 12 also shows a disrupted spirochaete from the rumen. Figure 41 shows another spirochaete from the omasum which, although it still has its axial filaments and sheath, has begun to disintegrate in the middle. Another way in which bacteria have been seen to deteriorate is shown in Figure 42. This micrograph shows three selenomonads found in the omasum, two of which still have their characteristic lateral flagella. But all three have become etched, and have sculptured surfaces. This feature was shown more clearly in the small intestine, where nearly all of the whole cells had this appearance (Fig. 47).

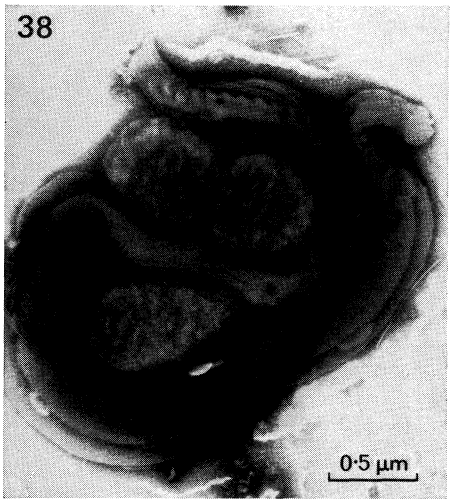
A marked transition occurred from the omasum to the abomasum where only occasionally whole cells were seen. The cocci shown in Figure 43 were not seen in the rumen, although similar organisms were seen in the small intestine (Fig. 46). Most of the whole cells either had etched surfaces as described previously, or showed other signs of disintegration such as may be seen in Figure 44 where the bacterial cell appears to form regular-sized granules. These granules may be seen free in abomasal contents as shown in Figure 45 (top left-hand corner). In the abomasum, cells in an advanced stage of breakdown and pieces of cell wall and other cell debris are most common (Fig. 45).

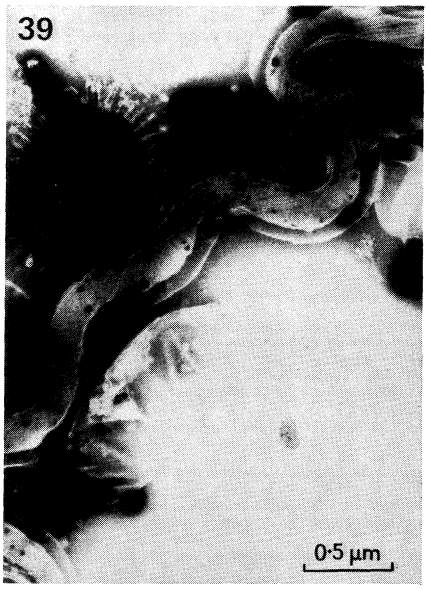
The difference between the abomasum and the duodenum was not as distinct as that between the omasum and abomasum. The duodenal contents contained much mucoid material, and the material seen under the electron microscope was therefore embedded in this mucus. No morphological differences could be detected in bacteria between the different regions of the duodenum–jejunum. Intact bacteria were occasionally observed as in Figure 46, but mostly the few cells present were distinctly etched as in Figure 47. What was most commonly seen in this region is shown in Figure 48, i.e. some large pieces of unrecognizable material (cf. Fig. 45), together with many other very fine pieces of debris. The two circular patches on the micrograph are holes in the mucoid background on which the cell debris are found.

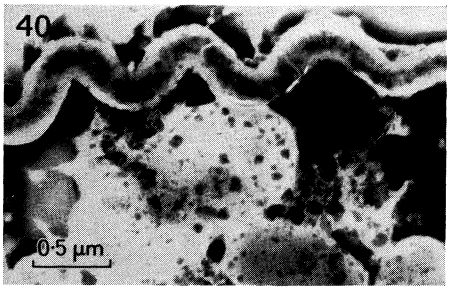
As the caecum was approached, the numbers of bacteria in the ileum increased. It was found that there was a greater proportion of cell debris to entire cells in the vicinity of the caecum (Fig. 49) as compared with the rumen and omasum. An organism similar to the fimbriated organism 7 of the rumen was also found in large numbers in the region of the alimentary tract close to the caecum. The sample shown in Figure 50 shows that the fimbriae are usually matted. Fewer bacterial types were found in the caecum or in the adjacent ileum than were found in the rumen.

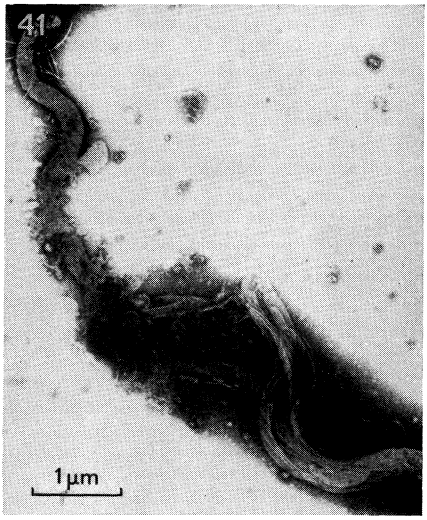
IV. Discussion

Most of the very large bacteria found in the rumen are distinguishable on the basis of their morphology. One of the most distinctive types observed on inspection of rumen contents under the light microscope is *Oscillospira guillermondii* (Moir 1951), one of the largest types of bacteria present in the rumen. Likewise, the "window-pane sarcina", which is probably *Lampropedia merismopedioides* (Hungate









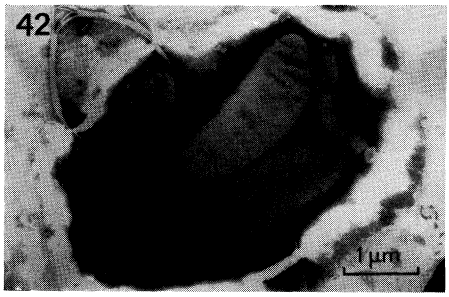


Fig. 38.—Organism 28, a spirochaete wrapped around three small cocci. The points of attachment of the fibrils are clearly shown and the fine structure of the sheath may also be seen. ×24,000.

Fig. 39.—Organism 29, a spirochaete with a smaller number of axial filaments than found in organism 28. Also present in the top left-hand corner is organism 13 (see also Figs. 23 and 24). ×24,000.

Fig. 40.—A spirochaete from the rumen which has lost its axial filaments. Pieces of cell debris and damaged bacteria may also be seen. ×21,000.

Fig. 41.—A disrupted spirochaete from the omasum. The middle of the organism has disintegrated, but the axial filaments are still attached to the end of the spirochaete and the fine structure of the sheath has been unaffected. ×12,000.

Fig. 42.—Three selenomonads from the rumen which have become etched and have sculptured surfaces. ×10,000.

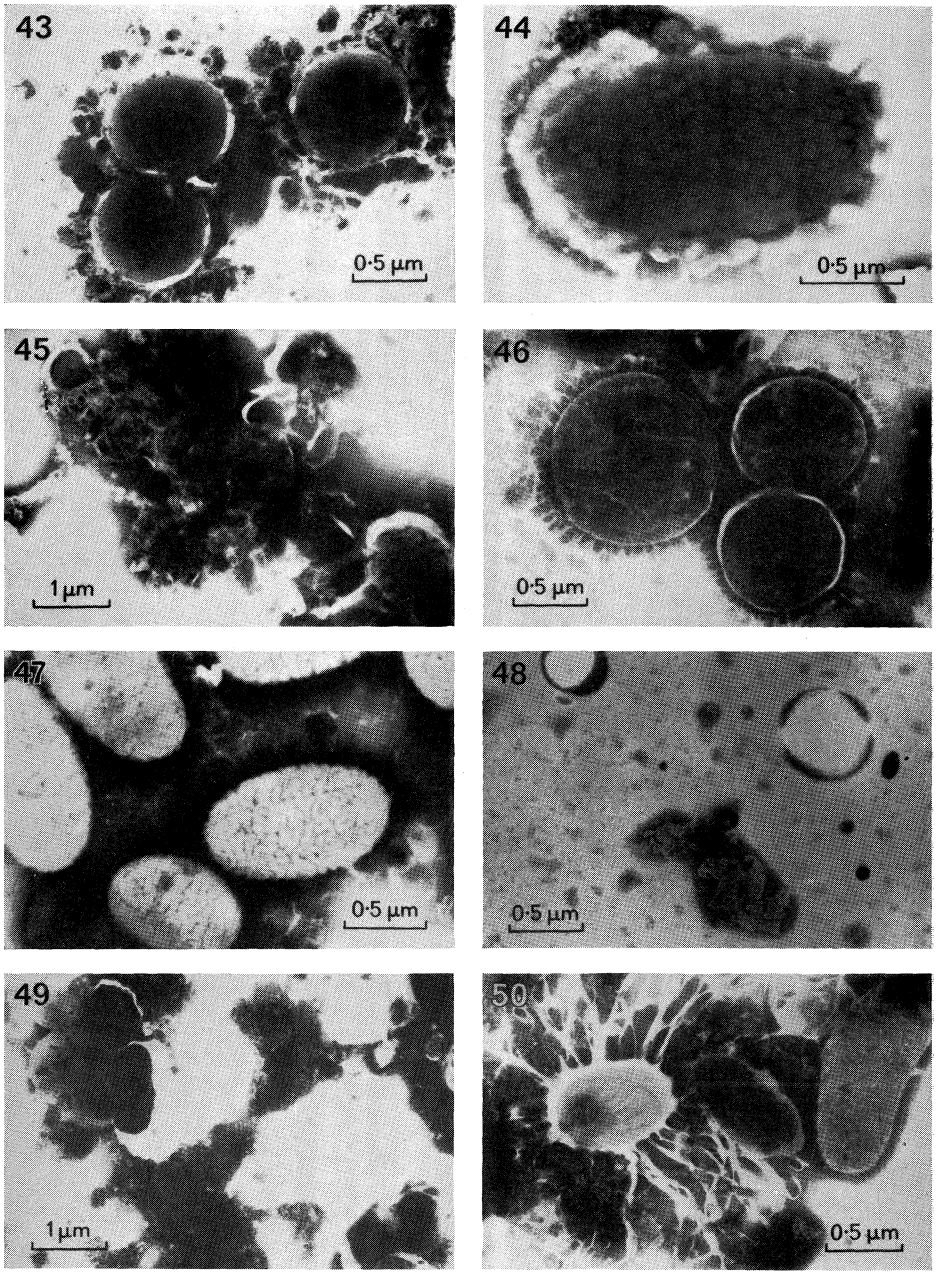


Fig. 43.—Three cocci found in the abomasum which appear to be quite intact and are similar to cocci seen in the small intestine (Fig. 46). This organism has not been seen in the rumen. ×20,000.

Fig. 44.—An oval-shaped cell found in the abomasum, showing signs of disintegration. The cell contains regular-shaped granules which are similar to granules found free in abomasal contents (Fig. 45). ×20,000.

Fig. 45.—A sample from the abomasum which gives a representative picture of what is found in this organ. A number of cells are in an advanced state of breakdown, and also present are pieces of cell wall and debris. ×10,000.

1966), is also readily identified on the basis of morphology. Very few of the small bacteria are really distinctive, and most of them simply appear as Gram-positive and Gram-negative cocci and short rods, usually 0·4–1·0 μm in diameter and 1–3 μm long.

Moir and Masson (1952) classified 33 organisms on the basis of morphology, histochemical staining, ecology, and function. However, because of the limitations of visual light microscopy, little detailed description could be made of many of the organisms, particularly the abundant small bacteria of less than 1 μm in diameter. The electron micrographs presented here show considerable morphological detail of the rumen bacteria and suggest that electron microscopy is a useful technique for identification and for following the more gross changes in numbers of certain bacterial species with changes in diet. Indeed, morphological matching of bacteria in the rumen with bacteria isolated in pure culture would seem to be a desirable object before identification of rumen species could be said to be complete.

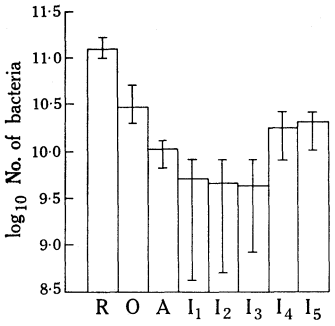


Fig. 51.—Numbers of bacteria found in different regions of the sheep alimentary tract. The histograms give the $\log_{10}$ total number of bacteria per gram wet weight, found in the following regions of the alimentary tract:

R, rumen and reticulum;
O, omasum;
A, abomasum;
I_1, 0–3 ft along small intestine from abomasum;
I_2, 3–6 ft from abomasum;
I_3, 6–9 ft from abomasum;
I_4, 3–6 ft from caecum;
I_5, 0–3 ft from caecum.

The total number of bacteria was counted using the nigrosine slide technique described by Gall, Stark, and Loosli (1947), and represents the average of five different sheep. The range of counts obtained is indicated.

Smith (1965) found that the abomasum is the site where the lowest number of live bacteria are found and that numbers gradually increase along the alimentary tract as conditions become more favourable for bacterial growth. The present study has confirmed that the abomasum is the major site of breakdown of rumen bacteria, but that this process begins in the rumen where many bacteriophages were found,

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Fig. 46.—Three fimbriated cocci found in the duodenum–jejunum. Intact bacteria such as these were rarely found in the duodenum–jejunum. ×20,000.

Fig. 47.—A number of etched cells found in the duodenum–jejunum. Most cells in this region of the alimentary tract were distinctly etched in a similar way. ×22,000.

Fig. 48.—Representative sample of duodenal–jejunum contents. Some large pieces of debris (similar to that seen in Fig. 45) and many other very fine pieces of debris are seen on a mucoid background. ×20,000.

Fig. 49.—A sample taken from small intestine, 0–3 ft from the caecum. Present are a number of small bacteria, cell walls, and other cell debris. ×10,000.

Fig. 50.—A fimbriated coccus, similar to organism 7, found in the small intestine near the caecum. The fimbriae are matted and this organism was rarely found as complete in this region as in the rumen and omasum. Also present are two other bacteria, which appear to be partially disintegrated, and many pieces of cell debris. ×20,000.

both free in rumen fluid and associated with a number of different bacterial types (Hoogenraad *et al.* 1967). It has also been shown that not only are bacteria killed in the abomasum but there is a marked digestion and removal of bacteria so that the distinctive morphological types found in the rumen and omasum are no longer identifiable in the abomasum and small intestine.

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