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Attachment of the Ciliate *Epidinium* Crawley to Plant Fragments in the Sheep Rumen

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High concentrations of the ciliate *Epidinium* Crawley are associated with damaged regions of fresh plant material undergoing digestion in the sheep rumen. This finding supports the postulate that sequestration in the rumen explains the low rate of passage of protozoa despite the high flow rate of liquid from the rumen. The maintenance of *Epidinium* in the rumen, despite their slow growth rate, is also explained.

There has been considerable interest in the passage of rumen protozoa to the lower gut in relation to the contribution of protozoal nitrogen to total microbial nitrogen available to the host (7, 10). The actual rates at which this passage occurs have been the subject of considerable discussion.

It was long assumed that liquid and particles of microbial dimensions passed from the rumen at the same rate. However, using this assumption the division rates of protozoa (12 to 36 h), measured in vitro, were inadequate to account for their maintenance in the rumen (3, 4, 6, 7, 9). Turnover times for rumen liquid vary with the diet, but range up to 22 h for cattle and sheep (7). For an organism to maintain itself in the rumen of a pasture-fed animal, its average division time must be approximately 69% of the liquid turnover time (7).

It is now known that the passage rate of liquid from the rumen greatly exceeds that of solids (12) and that protozoa may be preferentially retained (1, 11). Information on the mode of retention has been lacking. This paper contains direct observations with the scanning electron microscope (SEM) of the ciliate *Epidinium* Crawley (5) attached to plant fragments in the rumen.

MATERIALS AND METHODS

Animals. Two Romney wether sheep fitted with a rumen cannula were stall fed once daily. One animal was fed pelleted lucerne (*Medicago sativa* L.), and the other was fed freshly cut white clover (*Trifolium repens* L.).

Plant fragments. Pieces of lucerne stem (10 mm long) were cut from near the growing tip of a freshly harvested plant, and some were sliced longitudinally. Several pieces, both whole and sliced, were put in a polyethylene centrifuge tube (65 by 15 mm) that contained numerous 1.6-mm holes. The tube was sealed with a rubber stopper and enclosed in a mesh nylon bag (ca. 275 by 200 μm). At the commencement of feeding the weighted bag was suspended in the rumen. At timed intervals, pieces of stem were removed, rinsed in normal saline, and fixed in 4% (wt/vol) unbuffered formaldehyde.

When natural plant fragments were required, samples of rumen contents were obtained through the fistula at timed intervals after the commencement of feeding. The samples were strained through cheesecloth, and the solids were fixed with an equal volume of 4% (wt/vol) formaldehyde. Pieces of physically damaged tissue were selected by examination with a dissecting microscope.

SEM. Fixed stem samples were prepared by several methods, all of which gave similar results. (i) Samples were rinsed with distilled water, vacuum dried, and mounted on aluminum stubs with conductive specimen cement. (ii) Samples were rinsed with distilled water, blotted, mounted, and freeze dried. (iii) Samples were vacuum dried and mounted. Vacuum drying was over P_2O_5 at room temperature. Specimens for freeze drying were rapidly frozen in Freon 12 cooled with liquid N_2. Mounted specimens were sputtered with gold (Minicoater; Film-Vac, Inc.) and examined and photographed with a Cwikscan 100 field emission SEM.

RESULTS

During an investigation of plant digestion in sheep rumen, pieces of whole and longitudinally cut lucerne stem in nylon bags were suspended in the rumen, and samples were removed at intervals for examination with the light microscope and the SEM.

Microorganisms resembling entodiniomorph ciliate protozoa were found attached in large numbers to stem samples suspended in the rumen for periods from 1 to 40 h. Ciliates attached to a piece of cut lucerne stem are shown in Fig. 1. The distribution of the protozoa on the stem was highly specific, with large numbers

concentrated beneath the epidermis on the cut surface. Even after long incubations (40 h) in the rumen, high numbers of protozoa were still present on whole stem pieces (Fig. 2).

Although the nylon bag technique is used commonly in digestibility studies, it was considered that under these conditions the use of cut stems might produce artificial results. Therefore, SEM examinations were made on natural plant digesta by removing pieces of plant tissue at different times after feeding from the rumen of a sheep fed fresh white clo-

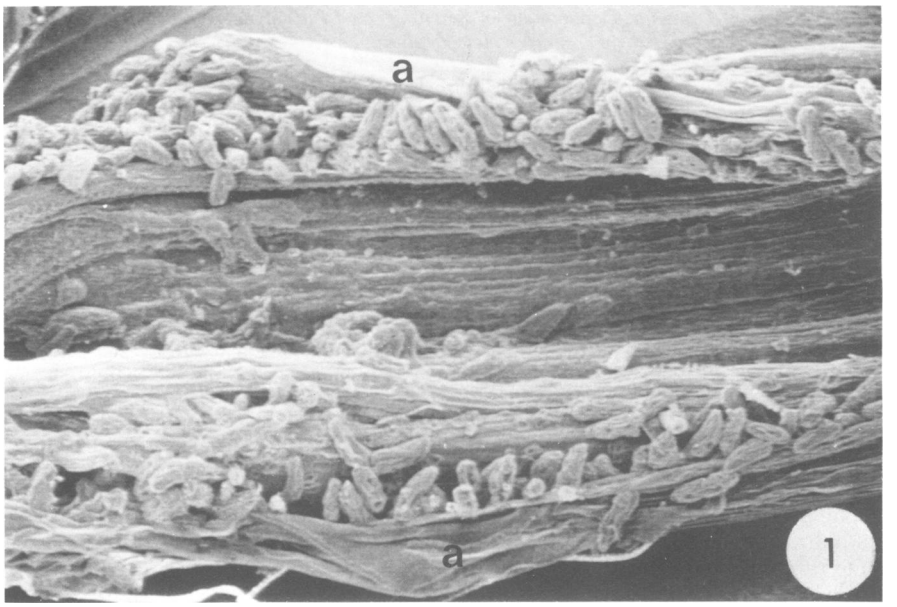

FIG. 1. *Protozoa on cut surface of lucerne stem after suspension in a nylon bag in sheep rumen for 6 h. ×70. a, Epidermal layer.*

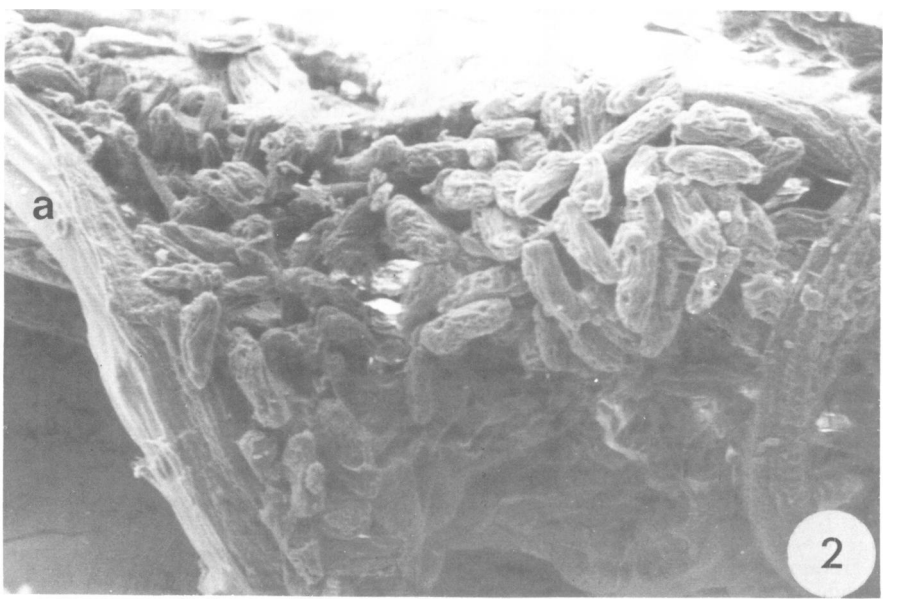

FIG. 2. *Protozoa on cut lucerne stem after suspension in a nylon bag in sheep rumen for 40 h. ×140. a, Epidermal layer.*

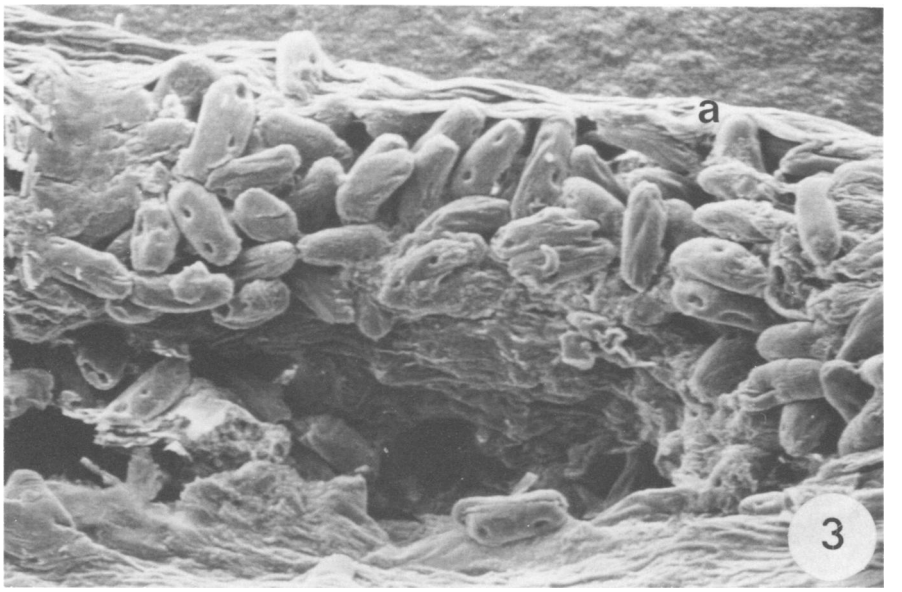

FIG. 3. *Protozoa on white clover fragment from sheep rumen contents 3 h after commencement of feeding. ×170. a, Epidermal layer.*

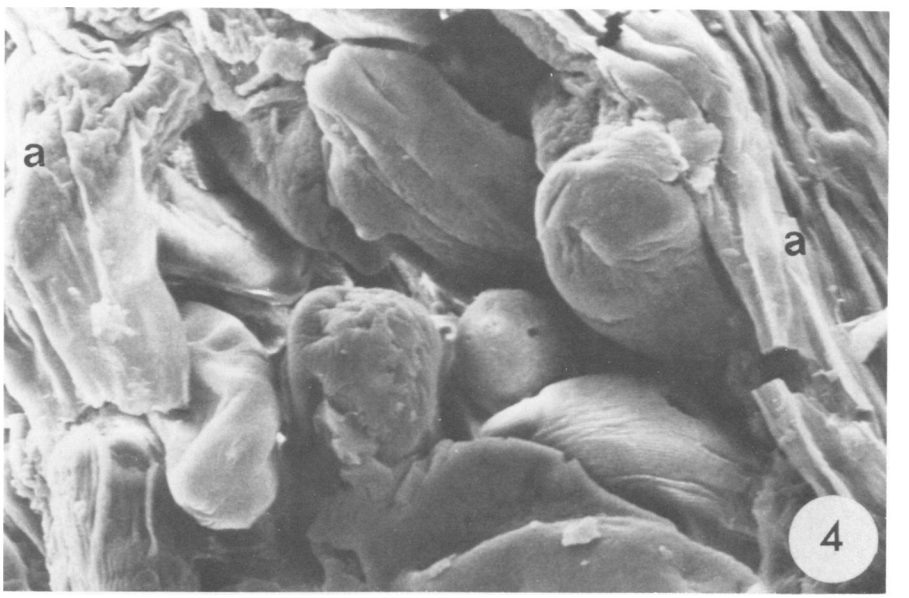

FIG. 4. *Protozoa wedged in white clover fragment from sheep rumen contents 3 h after commencement of feeding. ×700. a, Epidermal layer.*

ver. Figure 3 shows that identical results were obtained, with high concentrations of protozoa at a subepidermal level on damaged stem tissue. These high numbers were associated only with areas of the plant fragments where physical damage was obvious and were found as early as 1 h after the commencement of feeding.

Protozoa were also found associated with damaged areas of clover leaf but, as this type of tissue is very fragile when damaged, it was not possible to obtain satisfactory preparations for SEM examination. However, with light microscopy ciliates were easily demonstrated on leaf pieces.

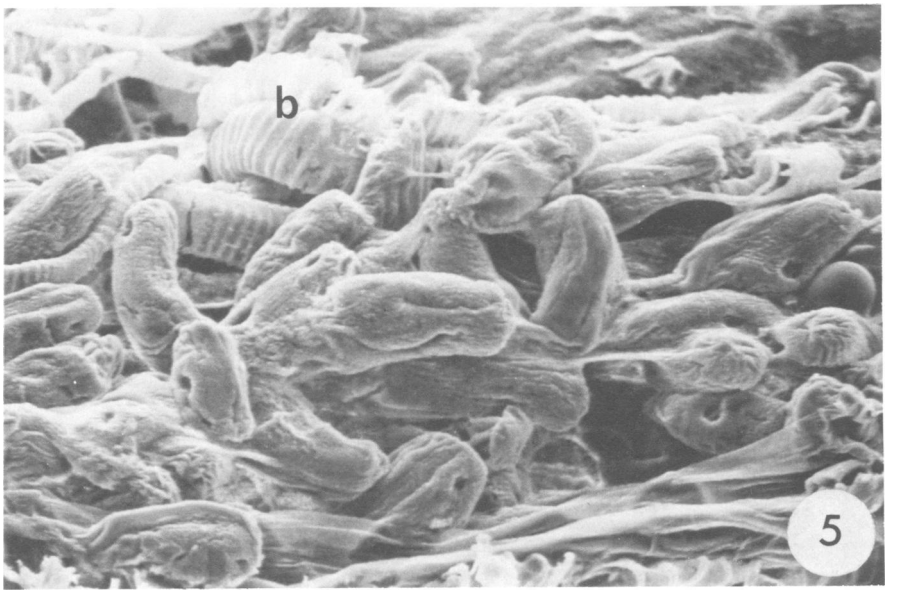

FIG. 5. *Protozoa on vessel elements of white clover from sheep rumen contents 1 h after commencement of feeding. ×320. b, Vessel elements.*

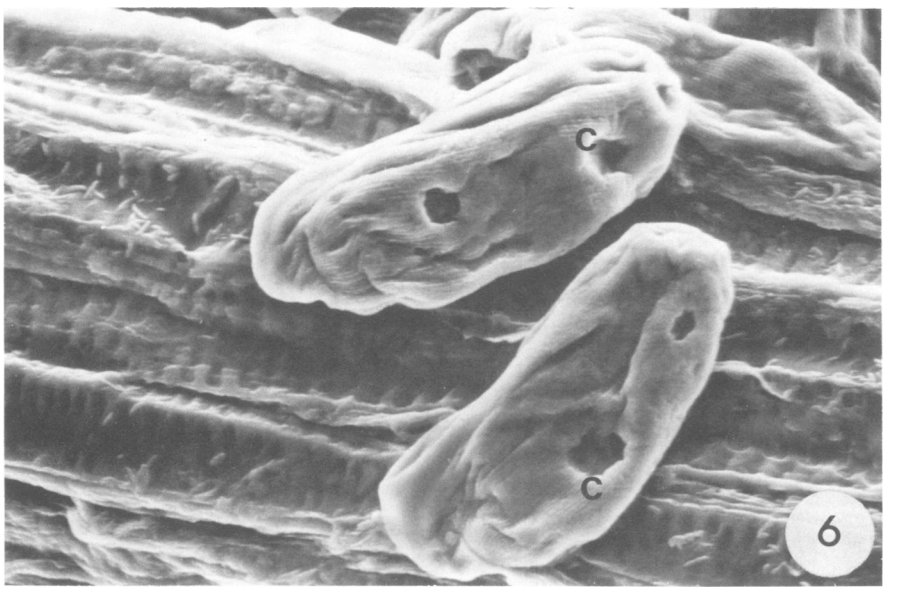

FIG. 6. *Cells of Epidinium ecaudatum on stem. ×700. c, Vacuole.*

Although most of the protozoa were apparently lying on the damaged surfaces of the plant fragments, closer examination revealed that many were wedged between layers of plant cells (Fig. 4) and among the vessel elements (Fig. 5).

With the exception of a few individual organisms, the ciliates were identified as *Epidinium ecaudatum*. Identification, which was facilitated by higher magnifications (Fig. 6 and 7), was based on size, number and position of vacuoles, number and position of membranelle zones, tortional displacement of the body, and spination. The other ciliates, seen occasionally

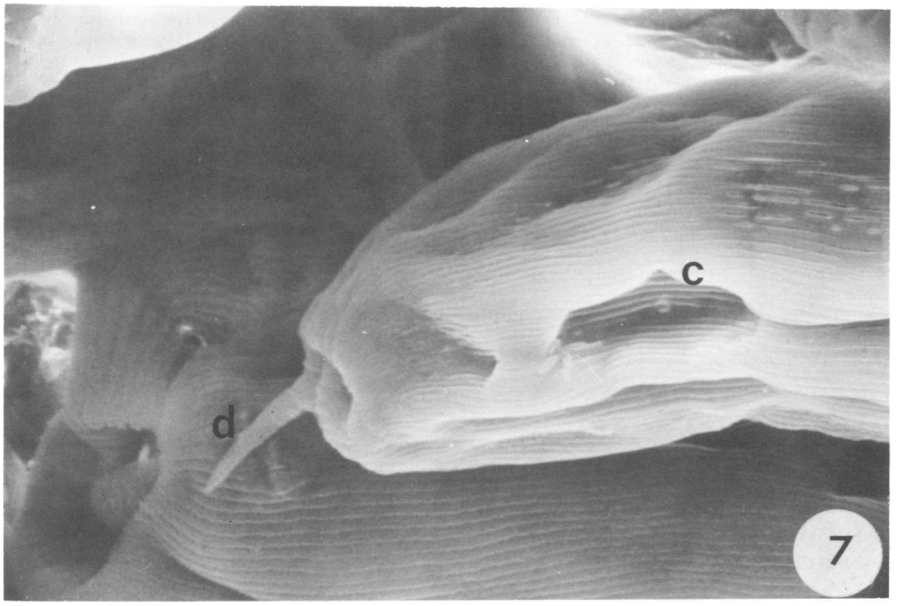

FIG. 7. *Detail of Epidinium ecaudatum f. caudatum.* ×3,100. *c, Vacuole; d, caudal spine.*

(<1%), were probably the entodiniomorphs *Eudiplodinium* and *Diplodinium* and the holotrich *Dasytricha*.

DISCUSSION

The composition of the rumen protozoal fauna may vary with the host species and the geographic distribution of the host and is affected by diet (7). On diets of fresh pasture in New Zealand, *Epidinium* is a major component of the protozoal population in sheep and cattle (2, 9). Thus the present observations leave no doubt that, at least on diets of fresh pasture, sequestration of important members of the rumen protozoal fauna by food particles does occur.

Although shown here only with legume stems, the phenomenon occurs with other plant tissues in the rumen. As well as with stems, examination of partly digested clover and grass with the dissecting microscope revealed the presence of many protozoa concentrated at sites of physical damage. However, due to the difficulty of working with leaf samples some limitations were imposed on the investigations by the techniques needed to prepare specimens for SEM examination. The main advantage of stem samples in this respect is their physical rigidity, which permits them to retain their structure through the preparative steps. Chewed and partly digested leaf samples are too fragile to withstand the required manipulations.

The distribution of *Epidinium* on the damaged stem and leaf surfaces is in agreement with its dietary habits. *Epidinium* is known to ingest whole or damaged chloroplasts and starch grains, and it is in the regions that contain chloroplasts that the ciliates concentrate (9).

The present findings may explain the observation of Weller and Pilgrim (11) that the contribution of protozoal protein to total microbial protein leaving the rumen is lower than expected on a relative population basis. It has generally been accepted that rumen protozoa benefit the host by synthesis of protein. If this concept is no longer tenable because of a low passage rate from the rumen, then the fundamental questions of a protozoal contribution to the host requires reconsideration also. In addition, the close association of protozoa with plant particles could result in underestimation of rumen ciliate populations by the normal methods of enumeration, which depend upon protozoa being free in the liquid fraction of rumen contents.

ACKNOWLEDGMENTS

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