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The Rumen Ciliate *Epidinium* in Primary Degradation of Plant Tissues

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Pieces of lucerne stem suspended in a sheep rumen in nylon bags were removed after different time intervals and examined by scanning electron microscopy. By 15 min large numbers of the ciliate protozoan *Epidinium* Crawley were attached to damaged areas of the stem, although a complex protozoal fauna was present in the rumen contents. Highest concentrations were on cortex and phloem tissues, with densely packed protozoa forming a complete ring around the transversely cut end of the stem between the epidermis and the vascular cylinder. Within 2 h there was extensive degradation of thin-walled tissues, as indicated by the amount of exposed vascular cylinder. Epidermis was not degraded but slid down the side of the stem as the underlying tissues were removed. This rapid degradation of plant tissue is explained by direct ingestion of tissues by the protozoa on the plant fragments. Many of the epidinia attached to the stem pieces were ingesting phloem elements and chlorophyllous tissue. The rumen protozoa have not previously been shown to participate on this scale in the physical degradation of plant material.

The rumen ciliate *Epidinium* Crawley was shown previously to attach to damaged regions of fresh plant materials undergoing digestion in the sheep rumen (2). With lucerne and clover stems highest populations were found concentrated in tissues adjacent to the epidermis. The known dietary habit of chloroplast ingestion by *Epidinium* was proposed as an explanation for the specificity of attachment to this particular region of stem (2). Recently, studies (1) with bovine rumen contents in vitro demonstrated an association of *Epidinium* with mesophyll tissue of grass leaves. It was suggested that the location of starch grains in different grasses might influence the attack by these protozoa.

Further details of this protozoan-plant tissue interaction have been revealed by scanning electron microscope (SEM) examination of lucerne stems suspended in a rumen for short periods of time. Rumen epidinia have been found to be involved in the primary degradation of large plant fragments by direct ingestion of specific tissues.

MATERIALS AND METHODS

Animal. A Romney wether sheep fitted with a rumen cannula was stall fed once daily with pelleted lucerne (*Medicago sativa* L.).

Plant fragments. Pieces of fresh lucerne stem, which had been suspended in a nylon bag in the rumen as the sheep commenced eating, were removed at timed intervals and fixed in 4% (wt/vol) unbuffered formaldehyde.

Plant fragments from rumen natural digesta were obtained from sheep fed fresh lucerne or chaffed lucerne, from cattle (*Bos taurus*) fed meadow hay, as well as from feral red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus*). Rumen samples from sheep and cattle were obtained through fistulae; samples from red deer and reindeer were obtained at sacrifice. All samples were fixed with an equal volume of 4% (wt/vol) unbuffered formaldehyde and stored in sealed containers until required for further processing.

SEM. Stem samples were rinsed three times with distilled water, blotted dry, and freeze-dried (2). Other details of sample preparation for SEM examination were as described previously (2).

Light microscopy. Epidinia attached to lucerne stem pieces from the rumen were dislodged by scraping the cut ends of the stem with the back of a scalpel blade. The scrapings were suspended in a drop of normal saline on a microscope slide and examined by phase-contrast illumination.

RESULTS

Examination of pieces of lucerne stem suspended in the rumen and removed at intervals showed an extensive population of *Epidinium* on longitudinally sliced stem pieces, even after incubation periods of 30 min (Fig. 1). A study of stem pieces cut transversely revealed more details of this rapid protozoal colonization. Large numbers of *Epidinium* were found attached to the cut ends of the stem after incubation for 15

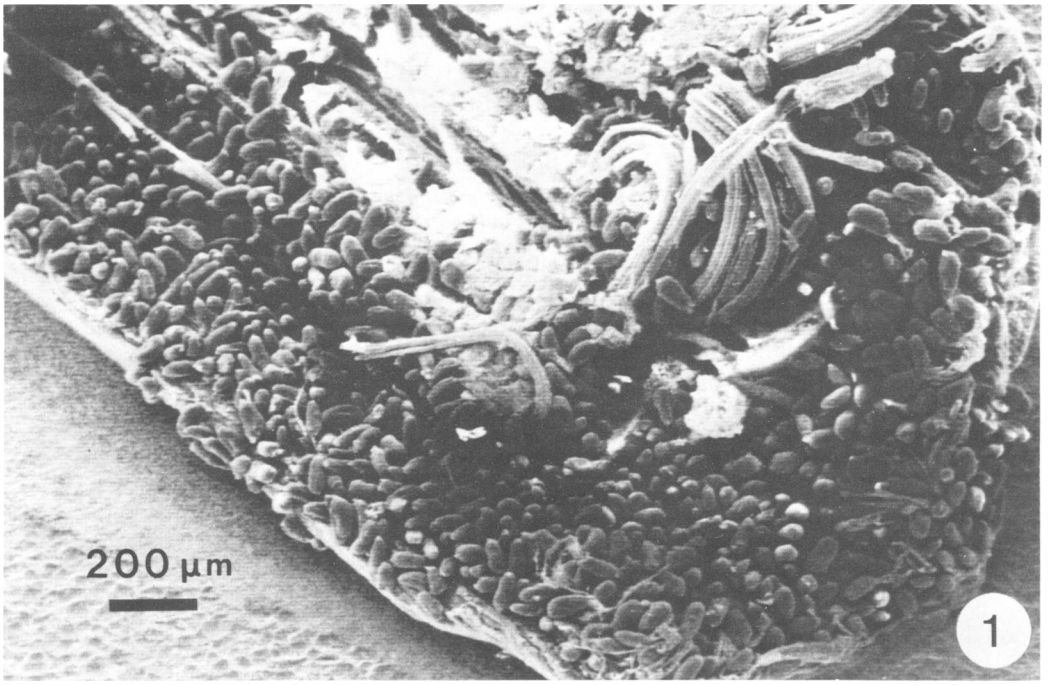


FIG. 1. *Epidinium on a longitudinally cut surface of lucerne stem suspended in a sheep rumen for 30 min.*

min (Fig. 2a). Most of the protozoa were densely packed in the region of the cortex and phloem tissues and formed a ring around the cut end of the stem between the epidermis and the vascular cylinder. Greater details are shown in Fig. 2b. Considerable degradation of cortex and phloem tissues with resultant exposure of the vascular cylinder was evident in these preparations. The epidermis did not appear degraded.

Degradation of the central pith-containing region also occurred, and *Epidinium* colonized this area too, although a lower population was present (Fig. 2c).

Degradation of tissues at the cut ends of the stem progressed in a similar pattern for the 2-h duration of the experiment (Fig. 3a and b). Epidinia were still present at this time and were found colonizing plant fragments even after 40 h in the rumen (2). By 2 h considerable amounts of thin-walled tissues had been removed, with large expanses of vascular cylinder exposed, and undegraded epidermis corrugated down the outside of the stem. The degradation of tissues at the stem cut ends was apparent even to the unaided eye after incubation periods of 1 h.

Effects similar to these were not obtained when control pieces of stem (not incubated in the rumen) were similarly processed. Thus, these effects were not explained by collapse or retraction of thin-walled tissues during preparation for SEM.

More detailed examination of the attached protozoa disclosed additional information on the rapid degradation of the thin-walled stem tissues. The epidinia ingested plant tissues directly on the large stem pieces. Epidinia are shown ingesting phloem tissue in Fig. 4a and chlorophyllous and phloem tissues in Fig. 4b. In Fig. 5a a single *Epidinium* is shown ingesting pith tissue. In all cases the mouth parts were greatly distended around the tissues.

During the short incubation times studied in these experiments many bacteria also attached to specific stem tissues. During ingestion of plant tissues by *Epidinium*, some of these bacteria were also consumed. (Fig. 5b).

The extent and degree of plant tissue ingestion by epidinia on stem pieces was examined next. Epidinia attached directly to lucerne stem pieces from the rumen were dislodged from the cut end of the stems and examined by light microscopy. The majority of the dislodged protozoa were found to contain large plant fragments extending from the mouth and penetrating deep inside (Fig. 6).

The association of *Epidinium* with large plant fragments in the rumen appears to be a general one and was noted also in cattle (*Bos taurus*), red deer (*Cervus elaphus*), and reindeer (*Rangifer tarandus*). Similar findings showing direct ingestion of plant tissues by *Epidinium* attached to large plant fragments from natural digesta

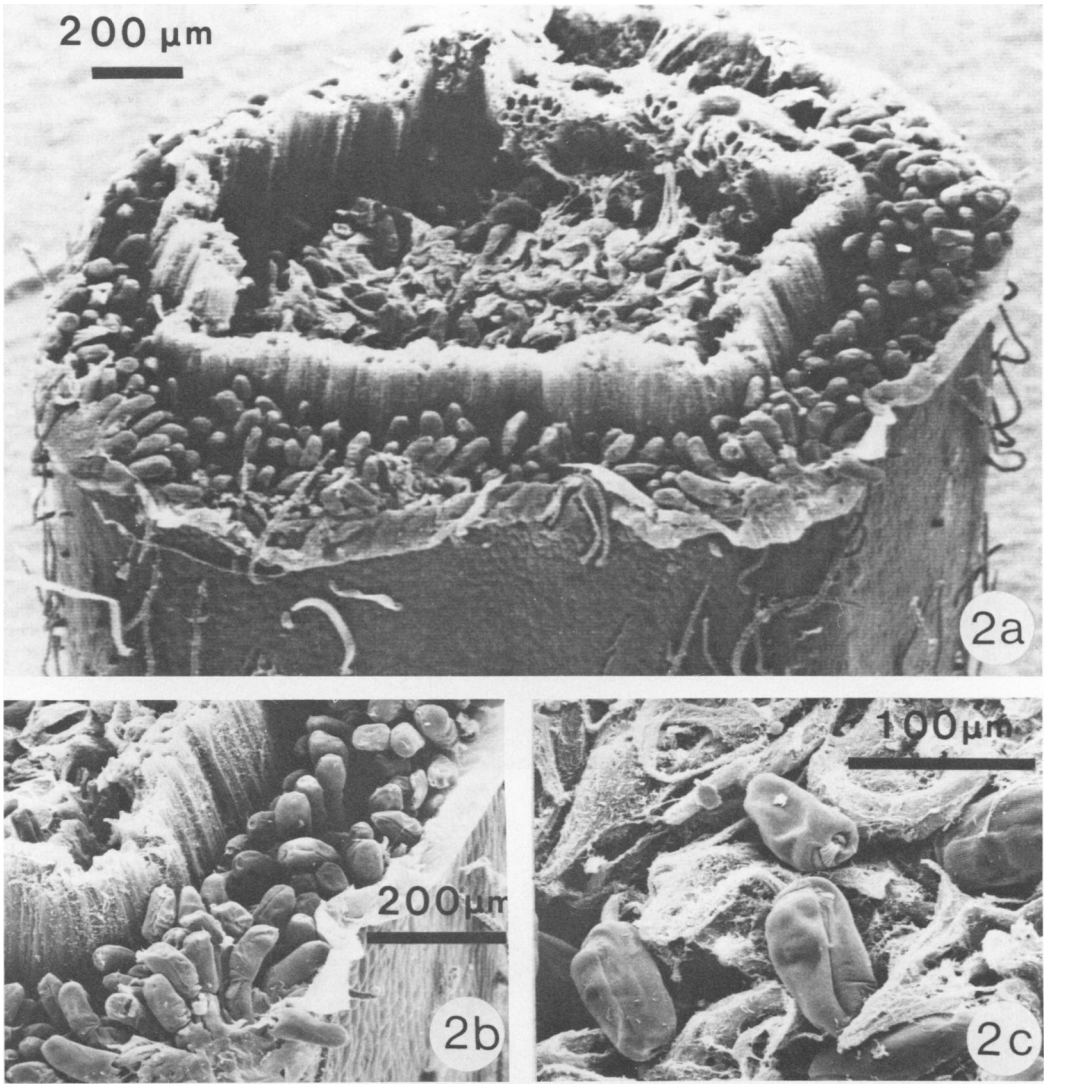


FIG. 2. *Epidinium on the cut end of a piece of lucerne stem suspended in a sheep rumen for 15 min. (a) Epidinium forming a ring around the cut end between the epidermis and the vascular cylinder; cortex, phloem, and pith tissues are degraded, with xylem remaining. (b) Epidinium densely packed between the epidermis and the vascular cylinder. (c) Epidinium on pith.*

have been obtained with sheep fed fresh or chaffed alfalfa and with cattle fed meadow hay.

DISCUSSION

The results reported here on protozoal attachment to large plant fragments in the rumen are an extension of those described earlier (2). The use of shorter incubation periods has revealed that attachment is even more extensive than shown previously, although again only damaged plant tissues were colonized. The high specificity of this protozoan-plant tissue interaction is expressed in two forms. First, only a single protozoan genus, *Epidinium*, was found attached to the tissues, despite the fact that a complex protozoal fauna was present in the rumen. Second, the attraction and attachment to the cortex and phloem tissues of the stem, although previously noted, was more clearly demonstrated by using shorter incubation periods. Both of these aspects of the plant tissue-protozoan relationship suggest that a chemotactic response is operative, particularly in view of the brief incubation period (15 min) required to obtain this effect. Presumably, water-soluble compounds must be involved to produce such rapid attraction to

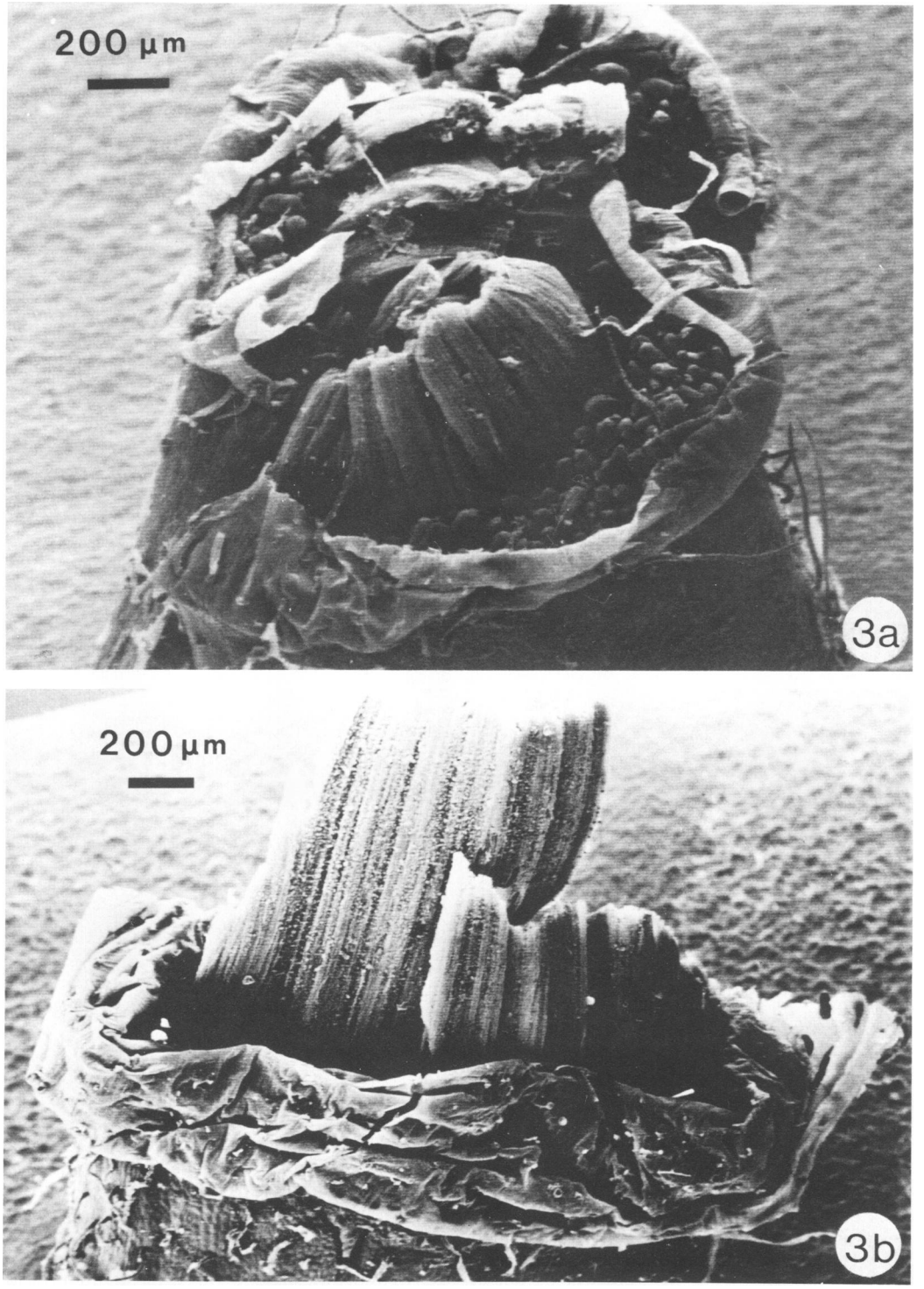


FIG. 3. *(a) Epidinium colonizing the tissues between the epidermis and the vascular cylinder at the cut end of lucerne stem. The amount of the vascular cylinder exposed shows the extent to which cortex and phloem tissues were degraded. Suspended in sheep rumen for 60 min. (b) At 120 min, with more extensive degradation of cortex and phloem tissues and additional vascular cylinder exposed.*

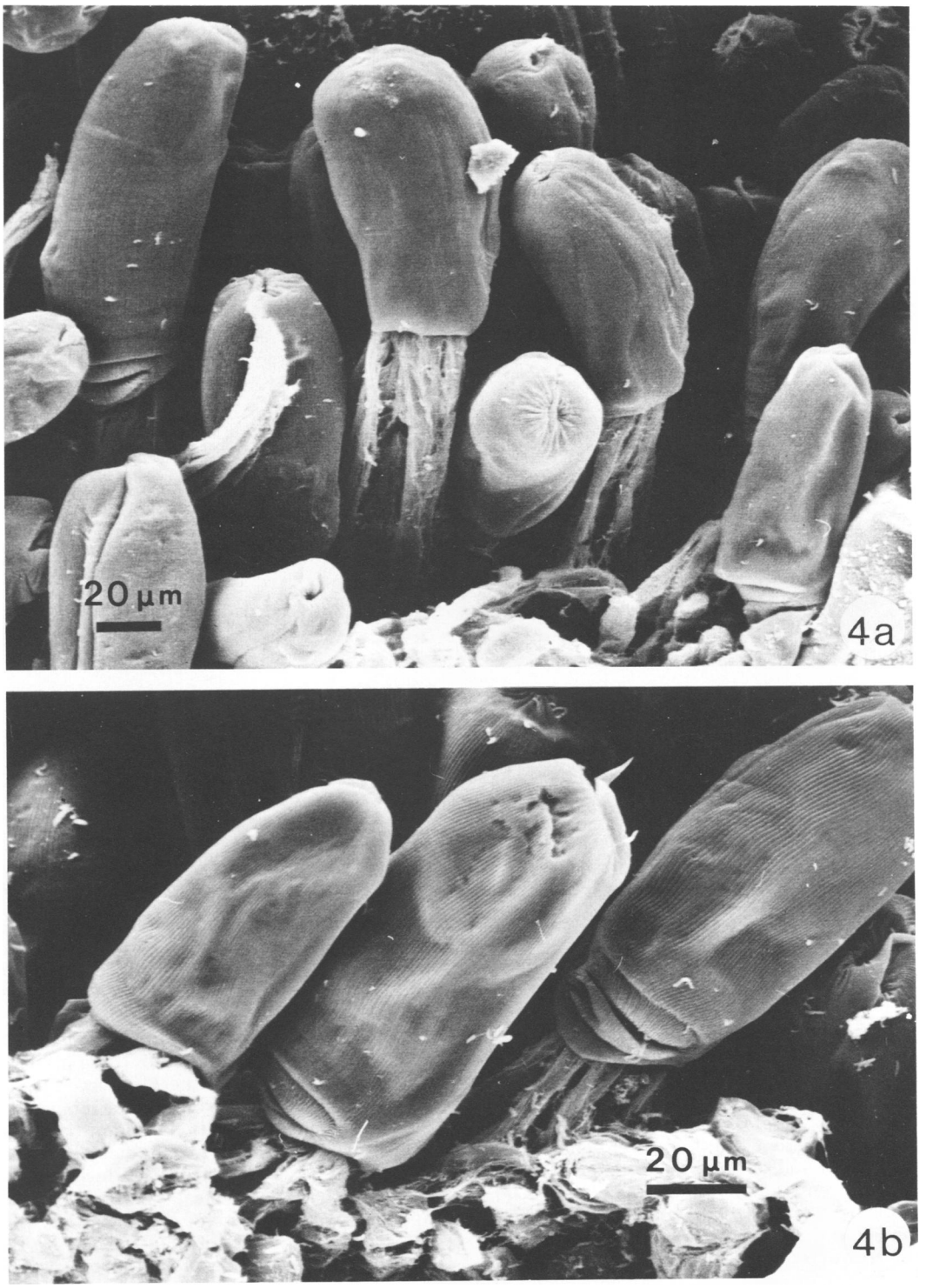
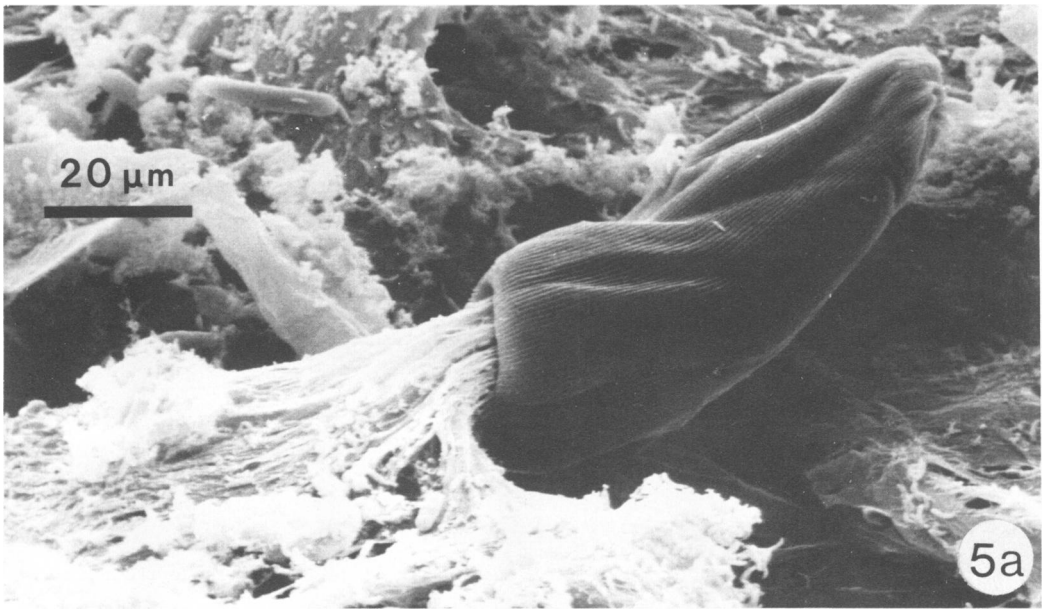
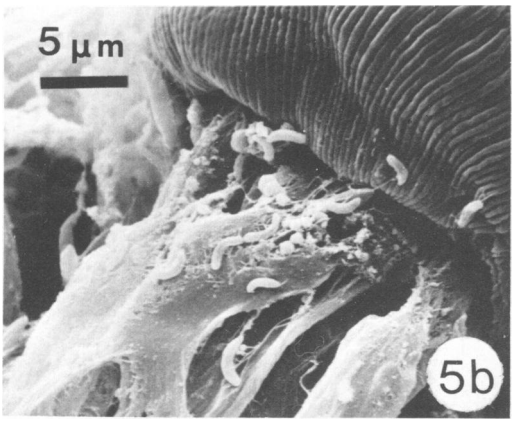


FIG. 4. *Epidinium ingesting phloem elements (a) and chlorophyllous tissue and phloem elements (b) of lucerne stem suspended in a sheep rumen for 15 min. Note the fully distended position of the mouth.*





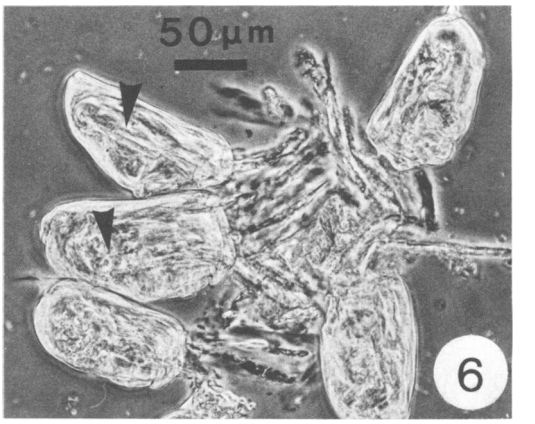


FIG. 5. *Epidinium ingesting pith tissue (a) and bacteria on phloem tissue (b) of lucerne stem suspended in a sheep rumen for 15 min.*

FIG. 6. *Epidinium dislodged from a lucerne stem suspended in a sheep rumen for 15 min. Thin-walled plant fragments extend from the mouths and penetrate deep inside the protozoa. Phase-contrast illumination.*

phloem cells, and they may relate to the sugars and other nutrients transported by phloem elements.

The plant pieces studied in these experiments underwent several washings during transfer through different solutions after removal from the rumen and during preparation for SEM. It appears remarkable, therefore, that such large numbers of *Epidinium* remained firmly attached to the stem pieces. A possible reason for this firm attachment may be the protozoal ingestion of the exposed ends of phloem elements on the large plant pieces and the deep engulfment of these tissues by the protozoa. The fully distended position of the mouth found during feeding on the stem was common in these preparations, although it has not been observed previously in the liquid phase of rumen contents.

The extensive degradation of plant tissues at the stem cut ends, visible to the unaided eye at 1 h, appeared too rapid to be produced by the action of digestive enzymes. It can, however, be explained by attachment of *Epidinium* followed by the rapid ingestion of plant tissues by the protozoa. In order to explain the progressive degradation of thin-walled tissues in these experiments, either rapid digestion of ingested tissue must occur, with epidinia remaining in po-

sition on the stem, or, more likely, epidinia break off tissue pieces and leave their sites of colonization. If the latter is correct, digestion of the engulfed tissues could then proceed over a more prolonged period. Successive colonizations by protozoa with continued removal of plant tissues could then account for the degree of degradation observed. However, regardless of the mechanism involved, these findings reveal a new role for epidinia in the rumen; epidinia participate in the physical degradation of forage, formerly attributed entirely to the action of chewing and rumination. The degradation by *Epidinium* also results in the exposure of additional internal plant tissues for further microbial colonization and digestion.

The extent of plant tissue ingestion by *Epidinium* has not been appreciated previously (5). Although chloroplast ingestion by *Epidinium* has been noted often (6), ingestion of large plant fragments has been observed much less frequently and is seldom seen with *Epidinium* free in the liquid phase of rumen contents. The demonstration of ingestion of such large quantities of cellulosic plant material raises the question of the digestive capabilities of *Epidinium*. Evidence for intrinsic cellulolytic digestion by rumen protozoa remains inconclusive (3), and, although cellulolytic bacteria may function within the protozoa, the results of this study suggest a need for a re-examination of this problem. It is possible, of course, that *Epidinium* utilizes mainly the soluble components in the ingested tissues and that indigestible fractions are simply ejected from the mouth later.

Although *Epidinium* has also been shown to ingest bacteria, this appears merely adventitious under these circumstances. Our observations by SEM suggest that the protozoa are attracted specifically to the plant tissues and that under these conditions the bacteria or their fermentation products or both probably do not attract *Epidinium*. The bacterial cover on the cortex and phloem tissues was relatively low compared with the dense cover on adjacent vascular cylinder tissues (Bauchop, unpublished data) which did not attract *Epidinium*, although the bacteria appeared identical in both cases. Results of Coleman (4) also indicate that bacteria are not the major component in the diet of *Epidinium*.

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