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CONTRIBUTION OF PROTOZOA TO THE RUMEN CELLULOLYTIC ACTIVITY

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Symbiotic microorganisms, bacteria and ciliates enable ruminants to utilize plant material, which is not available to man. It is well known that bacteria degrade cellulose in the rumen, but the participation of ciliates in this process is still somewhat controversial. Electron microscopical study of ciliates collected from a sheep under controlled feeding gave an unequivocal answer to this question. Ciliates degrade plant cell walls. The origin of the enzymes responsible for this digestion remains unclear but the hypothesis that there is a closer symbiosis between ciliates and bacteria than was previously believed, has been studied.

Material and Methods

Ciliate preparation

Samples of ciliates were collected at seven different time intervals from half an hour before until 6-5 h after the morning feeding, from a fistulated Texel sheep receiving a standard hay diet, containing 25% cellulose. Rumen fluid was strained through surgical gauze. Filtrate aliquots were maintained at 37°C under N2 in test tubes. Grass waste particles accumulated at the top of the test tubes, ciliates at the bottom. Ciliate samples were centrifuged at 300 g for 10 min and the ciliate pellets washed with physiological solution to free them from bacteria.

Electron microscopy

Ciliates were fixed for 2 hours in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and postfixed for 1 hour in 2% OsO4 in cacodylate buffer. The samples were dehydrated and embedded in Epon. Ultrathin sections were stained in uranyl acetate and poststained in lead nitrate. They were examined with the aid of a Philips EM 301 electron microscope.

Results

The observation of ciliates at different times after ingestion of hay by the sheep shows that the endoplasm contains numerous vegetal fibres and that these plant cell wall fragments are degraded, while other structure modifications can also be observed.

(1) Figures related to these results will be given on the poster which will be displayed during the Symposium. The authors can produce them on request.
This sequence of events can be simplified into five steps.

1. Vegetal fibres of various sizes and shapes already attacked by bacteria in the rumen appear enclosed in big pockets surrounded by a membrane and most likely contain rumen fluid (fig. 1). In the vicinity of the cell walls in degradation are small and big vacuoles containing one or many sorts of bacteria, of which most of them are intact and cellulolytic (fig. 1 and 2). Golgi saccules and vesicles are present in the vicinity of the fibres. Up to 8 groups of 10 cisternae near one cell wall have been observed.

Fig. 2 shows that the endoplasm is filled with small tubules between the fibres and the vacuoles. Some of these tubules appear rigid. Others are connected to bacteria containing vacuoles. The arrowhead on fig. 2 points to such a tubule, linked at one end to the vacuole, and at the other extremity to a more or less circular grey vesicle, similar to the smaller structure near it.

2. Cell wall fragments become very different (fig. 3). They can be indented in many places, and their edges become very dark. They are still surrounded by a membrane but it adheres very closely to the fibre so that it is not always visible. A great number of dark vesicles (0.1 - 0.6 µm) burst (fig. 3 and 4). Their shape varies from circular and ovoid to elongated and curved. Some are linked together. Others are fixed to the fibre pockets, sometimes by a small peduncle. A black dot is visible in some of them. The Golgi apparatus is further differentiated; the smooth and coated vesicles appear more numerous. The cisternae of rough endoplasmic reticulum develop along the fibres.

3. The ground substance of the fibres is reduced and darker. The edges become puffed up (fig. 5) by a material similar to that in the fibres. The dark vesicles of stage 2 have disappeared. Small and rigid, straight or curved tubules appear. They look clear or granular. Vesicles containing a bacterium occur, showing one or more tubular expansions (fig. 8).

5. Narrow canaliculi go through the endoplasm in network (fig. 9). They are connected to each other and divide up the cytoplasm. In some places there are pockets containing intact bacteria (fig. 10) where 5 to 6 canaliculi end.

Discussion

Degradation of the vegetal fibres.

Electron micrographs show that the vegetal fibres are ingested by the ciliates after a process of endocytosis similar to that observed for bacteria. They are all enclosed in a vacuole surrounded by a membrane.

The cell walls are degraded progressively. They are first reduced to smaller pieces (stage 2). Cellulase spreads between the sheets of the fibre (stage 3). Cell walls are totally transformed and no constituent is recognizable anymore. Only indigestible residues (stage 4) such as lignin remain.

Origin of the digestive enzymes.

The main feature at stage 2 is the outburst of the black vesicles. Their relation to the fibre digestion is evident because of their localization near or on the fibre pockets and also because they are particularly abundant at that moment but have disappeared at stage 4. Moreover, they are acid phosphate positive (Absil, personal communication). So, according to the definition, they are lysosomes.

The cellulase that they carry together with other enzymes could originate in the ciliate itself. The presence of numerous intact cellulolytic bacteria in vacuoles in the neighbourhood of the fibres rather suggest that the bacteria might be at the origin of the cellulase utilized by the ciliate.

The tubules free in the cytoplasm or fused with the vacuoles containing the bacteria might be of Golgi origin. They would concentrate the bacterial cellulase and become the dark granules or lysosomes. Lysosomes fuse further with the phagosomes containing the vegetal fibres after the classical schema of de Duve and Wattiaux (1966).
The involvement of Golgi apparatus in the degradation process could concern the processing and/or the differentiation of the lysosomal membranes but also of some other acid hydrolases. Absil (personal communication) showed that Golgi cisternae were indeed acid phosphate positive as were some free smooth tubes.

It can be concluded that celluloses degradation by ciliates probably results from the mutualism between them and the bacteria. Degradation products are stored in the ciliates which are of higher nutritive value for the host than the bacteria.

Bacteria also are probably at the origin of the digestion of the ciliates. Observations at stage 5 show a relation between bacteria and the compartmentalization of the ciliates. This correlates with the hypothesis of Baker (1943) that ciliates are only degraded after polysaccharide storage.

References
