Re-establishment of *Entodinium caudatum*, Cultured *in vitro*, in the Rumen of a Defaunated Sheep

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Rumen Entodiniomorphid protozoa have been grown *in vitro* (e.g. Hungate, 1942, 1943; Coleman, 1958, 1969), but certain morphological structures were sometimes lost after a period of growth under these conditions. It was therefore of interest to determine if such protozoa were still capable of growth in the rumen.

The protozoon chosen was *Entodinium caudatum* that had lost the caudal spine during *in vitro* culture for $9\frac{3}{4}$ years (Coleman, 1960). A Clun Forest ewe with a rumen cannula was defaunated with dioctyl sodium sulphosuccinate (Abou Akkada *et al.* 1968). It was fed daily on 800 g. hay and 200 g. crushed oats, kept in a building that contained no other ruminants and tended by persons having no other contact with ruminants. Under these conditions the animal was maintained without rumen ciliate protozoa for over 2 months until deliberately reinoculated. However, the microscopic appearance of the rumen contents changed on defaunation and remained abnormal even after reinoculation in that they contained large numbers of *Oscillospira guillermoidii* and other 'large bacteria'. The inoculum was 5 ml. of a washed suspension of *E. caudatum* containing 10^7 protozoa. A week after inoculation protozoa were visible in a drop of rumen contents and after 4 weeks 1.3×10^6 protozoa/ml. were present compared with only 3×10^5 /ml. in the culture *in vitro*. The protozoa appeared to grow directly after inoculation. *E. caudatum* was the only ciliate protozoon in the sheep for 3 months until the animal was returned to housing where it had contact with other sheep and where it became infected with small numbers of *E. simplex*. Seven months after reinoculation (at the time of writing) these were still the only ciliate protozoa present and there was no evidence of the reappearance of the caudal spine in *E. caudatum*. This observation was not unexpected, as Eadie (1967) showed that when *E. caudatum* was grown as the major ciliate species in an isolated sheep it gradually lost its caudal spine.

White (1969) found that each *Entodinium caudatum* organism grown *in vitro* (Coleman, 1960) contained 21.8 ± 8.2 bacteria. These were principally *Klebsiella aerogenes* and *Proteus mirabilis*, which grew equally well aerobically or anaerobically. It was therefore of interest to investigate the change, if any, in the number and nature of the intracellular bacteria on transfer to the rumen. Rumen samples were taken from the sheep at weekly intervals for 10 weeks, beginning 6 weeks after reinoculation and continuing until the appearance of *Entodinium simplex*. Fifty ml. rumen contents strained through muslin were diluted with 50 ml. of salts medium (Coleman, 1958) containing 0.25% (w/v) L-cysteine hydrochloride. This diluted material was stood for 1 min. and then the top 90 ml. was centrifuged for 1 min. from starting the centrifuge

(maximum R.C.F. 500g). The pelleted protozoa were washed 4 to 5 times on the centrifuge in the salts-cysteine medium and finally resuspended at a population density of approximately 5×10^5 /ml. Thereafter the procedures for ultrasonic breakage of the protozoa and bacteriological examination of the material were as described by White (1969). The average bacterial count per protozoon in a suspension of the washed protozoa was 1.66 ± 0.71 before breakage and 22.29 ± 12.55 after breakage. As each protozoon disintegrated in the agar medium after sonic treatment, liberating its intracellular bacteria, the minimal count obtainable with intact protozoa was 1. The washed protozoa thus contained very few extracellular bacteria. Although the number of intracellular bacteria had not changed markedly the nature of the flora in the protozoa had altered as no bacteria grew aerobically on plates of Oxoid nutrient agar containing 0.5% (w/v) glucose (Oxoid Division of Oxo Limited, Thomas House, Queen Street Place, London, E.C. 4). Examination of liquid cultures in the medium of Bryant & Robinson (1961) grown from colonies taken from the isolation medium showed the existence of three main morphological types of bacteria: (a) small Gram-negative cocci resembling *Veillonella* spp.; (b) small curved or curled Gram-negative bacteria resembling *Butyrivibrio* spp.; and (c) Gram-positive cocci resembling *Streptococcus bovis*. Occasionally other morphological types were found, and for the purposes of determining the numbers of each type present these were included in group (c). In 76 separate cultures obtained from disrupted protozoa, bacteria of type (a) were seen 49 times, those of type (b) 25 times and those of type (c) 12 times.

These results show that when *Entodinium caudatum* was resettled in the rumen there was a change in the intracellular bacteria to ones morphologically similar to those found in the rumen.

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