— Research Note —

Aggregation and dispersion of holotrich protozoa *in vitro*

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Remen ciliate protozoa are classified into two main groups, oligotrichs (entodiniomorphs) and holotrichs. As for the diurnal cycles of population changes of different ciliates in the rumen, most of oligotrichs including *Entodinia* decrease once after feeding and increase gradually to the original levels^{3,4}. On the other hand, holotrich populations increase abruptly with feeding and reach maximum shortly after feeding, and then diminish gradually^{1,2}. It seems likely that the initial drop in the oligotrich cycle is mainly due to the physical dilution of eating, drinking and/or salivation of the host animal⁴. This, however, would not be the case for holotrichs, and up to date no reasonable explication has been given why such a sudden increase in the holotrich cycle should occur. Recently in the process of our studies on the *in vitro* simulation of rumen fermentation with a special artificial rumen, which was developed by attaching a continuous dialysis system to the continuous culture apparatus of modified Slyter type³, we have found a key phenomenon to answer the question of the holotrich cycle.

In the continuous dialysis system, the perfusion liquid (1.08% NaHCO_3) was circulated between 20 l tank and eight dialysis tubes (each of which was 3.2 cm diam. and 25 cm long; "Naturin" Sausage Casing, No. 30/32, Becker & Co., Germany) at a rate of 1.2-1.3 l/min. The perfusion liquid was renewed two times per day at the "feeding" time of the morning and the evening. When the dialysis tubes were covered with 100 mesh silk sac, fine sludge was formed rapidly between the sak and the semipermeable membrane. The microscopic examination did show the fact that the sludge was mainly composed of holotrich protozoa. The holotrichs (*Isotricha* and *Dasytricha*) were observed to adhere serriedly to the surface of the membrane (Fig. 1). Thereafter, we found the facts that more than 60% of holotrichs in the

Fig. 1. Microscopic picture of the aggregation of holotrich protozoa ($\times 70$)

fermentor gathered on the surface of the dialysis membrane before the morning "feeding" and that the mass of them dispersed just after the "feeding." Consequently, the pattern of changes of holotrich concentrations in the fermentor was just accorded with *in vivo*. Namely, the holotrich cycle *in vivo* is explainable with this alternate repetition of aggregation and dispersion of holotrichs.

Judging from the results of some experiments on the factors which bring about the aggregation in holotrich protozoa, the dialysis seems to be essential for holotrichs to aggregate at least in the *in vitro* condition. This suggests that a provable aggregative place might be offered *in vivo* by the rumen wall which is known to be the place where the absorption of fermentation products is accomplished in the rumen. Furthermore, it is also important to know the factor(s) which cause the holotrichs to disperse, and the ecological meaning of the cycles of aggregation and dispersion in the life of holotrich protozoa. The studies are still in progress, and full details will be published later.

References

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