Encystment of *Bodo caudatus*

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With 17 Figures

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Summary

Before formation of the cyst wall, the food vacuoles are lost, the cell rounds up and the flagella lie close against the body in a flagellar groove. At this early stage, the contractile vacuole is very active, the Golgi apparatus is prominent and the basophilic cytoplasm is composed of closely packed ribosomes. As the cyst wall is secreted, layer by layer, the large Golgi apparatus is replaced by several smaller membrane stacks and mitochondrial changes occur involving local loss and modification of the cristae. Some parts of the mitochondrion undergo degenerative changes and may become surrounded by bacilliform bodies. These same bodies are also associated with small particles of sequestered cytoplasm which are present throughout the encystment process and are believed to be autophagic vacuoles. As the cyst wall thickens, cell shrinkage is manifest as a number of membrane invaginations. The final cyst wall is of uneven thickness and possesses a single operculum which is visible only by electron microscopy. Probable cyst wall precursor is found in small vesicles scattered throughout the cytoplasm.

1. Introduction

Although encystment is an event important for the survival of many free living and parasitic protozoa, detailed physiological and fine structural studies of this process and the resulting cyst have been largely confined to amoebae (Vickerman 1962 a, Band 1963, Griffiths, Lloyd, Roach, and Hughes 1967, Bowers and Korn 1969). This paper describes the formation and structure of the cyst in a flagellate, *Bodo caudatus*, the trophozoite of which has been described in detail elsewhere (Brooker 1971 a). *B. caudatus* is a free living kinetoplastid flagellate and as such possesses a conspicuous mass of DNA, the kinetoplast, located in a capsular expansion of the single mitochondrion. In at least some of the closely related trypanosomatids, this structure has been implicated in the morphogenetic and presumed adaptive metabolic changes which occur in response to environmental changes when these parasites pass between their vertebrate and insect hosts (Vickerman...
1962 b, 1971, Rudzinska, D'Alesandro, and Trager 1964). In those parasitic bodonids which are transmitted by leeches, a comparable role in the transformations which take place on passing from one host to the other can be visualized, but in the free living species such as B. caudatus, the only known environmentally induced changes in which the kinetoplast may play some part occur at encystment and excystment. The changes accompanying encystment are described here although the possible effect of dyskinetoplasty on these events has yet to be investigated.

2. Materials and Methods

The strain of Bodo caudatus used in this study was isolated from a sample of pig faeces obtained from Winches Farm near St. Albans, England. The trophozoites, which were grown with the original population of bacteria in a medium containing 0.4% yeast extract (Oxoid) and 0.2% liver infusion (Oxoid), were allowed to encyst and the cysts collected at varying intervals one week after the initiation of the culture. For electron microscopy, the mixture of cysts and trophozoites was gently centrifuged and the resulting pellet fixed for two minutes at room temperature in 0.5% osmium tetroxide buffered to pH 7.4 with 0.05 M sodium cacodylate. After treatment with 1% uranyl acetate in 25% ethanol for 30 minutes, the pellet was dehydrated using ethanol-water mixtures and absolute alcohol. Brief treatment with propylene oxide was followed by embedding in Epon. Thin sections were cut using a Porter-Blum MT2 ultramicrotome and stained in lead citrate prior to examination in an EM 6B electron microscope. Thick sections of embedded material were examined by phase contrast microscopy and after toluidine blue staining by bright field microscopy. Whole cysts were either fixed in Schaudinn's fluid and stained using Heidenhain's iron haematoxylin or examined alive by phase contrast microscopy.

3. Results

3.1. Light Microscopy

Shortly before encystment begins, the trophozoites round up and the flagella become progressively less active until they are almost immotile. Throughout cyst wall formation, which is marked by the appearance of numerous peripheral granules, occasional slight movements of the flagella persist and cause

Abbreviations used in figures: bb bacilliform body, cw cyst wall, cyt cytopharynx, f flagellum, fg flagellar groove, G Golgi apparatus, k kinetoplast, m mitochondrion, n nucleus, nm nuclear membrane, op operculum, res reservoir.

Fig. 1. Transverse section of the flagellar groove. A particle of segregated cytoplasm is surrounded by four bacilliform bodies. ×31,500

Fig. 2. An immature cyst in which the position of the cytopharynx relative to the flagellar groove is clearly seen. Cyst wall precursor (granular material) is still being added to the cyst wall and is discharged from small vacuoles (arrows). Cell shrinkage has begun. ×26,400

Fig. 3. Periphery of an immature cyst showing a dense layer of ribosomes below the cell membrane. ×82,700