Hibernatory Behaviour of the Heterotrichidan Protozoan *Spirostomum* Ehrenberg

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Introduction

THE ABILITY of ciliated protozoa to form cysts is a remarkable phenomenon and it is one which, as Gravé (1985) reminded us, is amenable to observation by optical microscopy. The occurrence of ciliates in infusions of boiled hay and straw has been known for many years, and gave rise to the term INFUSORIA by which the group was known in an earlier century (Dallinger, 1901). This phenomenon allows us to infer that encystment must be a widespread ability among such forms, yet it remains true that some well-known forms have not been shown to form cysts (Ford, 1976). Observations of autumnal free-living *Spirostomum* Ehrenberg have shown a stage that may indicate an early state of cyst formation. The behaviour of the organisms in connection with this observed phenomenon reveals a form of association which shows something of the gregarious nature of these ciliates under conditions of environmental stress, and may indicate a further means by which they can protect themselves during the adverse conditions of winter-time.

The Organism

Spirostomum is one of the largest of protozoa and has been well characterised by Corliss (1982) and Curds *et al* (1983). The genus is well known to devotees of freshwater microscopy. Looking most like fragments of india rubber left on the page after the erasure of a mistake whilst drawing, the organisms are pale grey in colour, sometimes yellow, lilac or brown, and move through water with a rotating movement that seems to convey a 'nodding' manner to the cell. The cells may range from 150 μm to 3 mm in length, and are cigar-shaped with conical or truncated terminal poles. Characteristic of the genus, and visible under low magnification, is the system of spiral sub-pellicular myonemes associated with the ciliary pattern with which the cell is marked. The cell surface is covered throughout with cilia, apparently less well marked in the peristomial area, and these are so profuse as to confer an almost bushy appearance to the cell seen under higher magnifications.

There is a large macronucleus, which may be elongated and lobed, and a contractile vacuole which often shows a long collecting canal. *Spirostomum* grazes on swimming bacteria and other small unicellular organisms. The cell is easily disrupted, and the cytoplasm will stream from the cell membrane, leaving isolated an area of ciliated pellicle in which the cilia can be seen to remain beating for some time afterwards. The genus is frequently found in water systems in which beech leaves occur. There are blooms during the warmer months, but when the ambient temperature is $\leq 5°C$ the cells cease to be evident. It is the phenomena associated with this disappearance which have been investigated, and which are the subject of this report.

Procedure

Microscopical observation of pond-water samples in Autumn when the temperature was measured at <5°C confirmed that *Spirostomum* was absent from areas where the organisms were known to abound in the warmer seasons. Samples of beech leaf detritus from the bottom of the pond were collected and removed to the laboratory. In each case, substantial amounts approximating to 250 ml of material were scooped up, care being taken to avoid undue disturbance. The samples were allowed to stabilise in a cold cabinet at 4°C for two days and then examination was carried out.

Observations

A binocular ×12·5 dissecting microscope was utilised to search for organisms in the leaf material. Clumps of an ivory white were seen to occur, in which large numbers of feebly moving ciliates were congregated together (Fig. 1). Each group had the appearance of a small tuft of fibres when seen by the unaided eye, not unlike a wooly aphid on a branch, each measuring approximately 5-8 mm across. Clustering closely together were the individual *Spirostomum* organisms, each ≅ 800 μm in length. Several thousand organisms appear to occur in each group.

Under higher magnifications the organisms can be seen to be tightly packed (Fig. 2). In this example, the warmth of the microscope lamp has initiated more active motility and individual organisms can seen to be separating from the cluster.

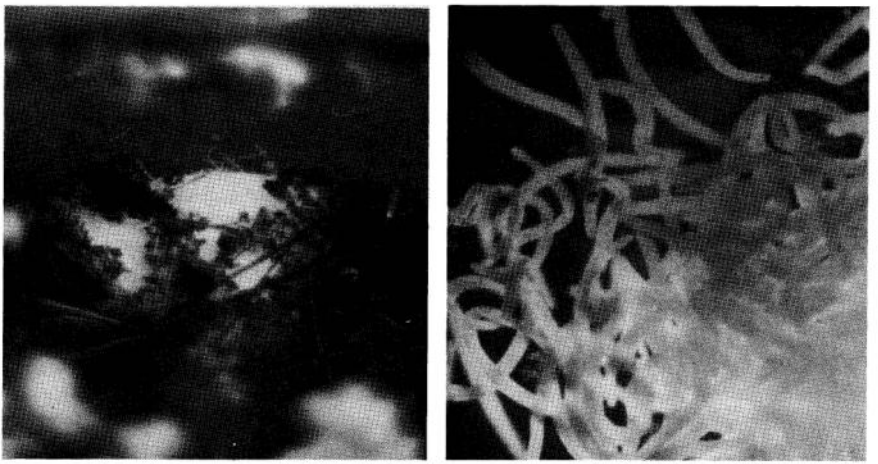
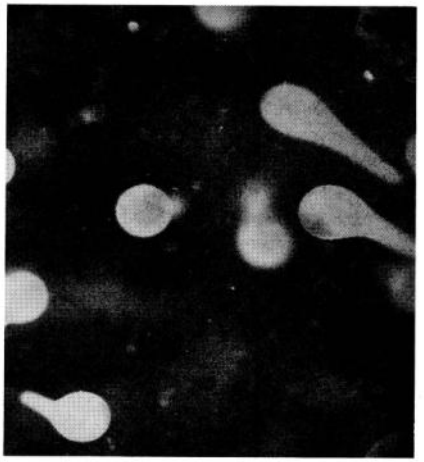


FIG. 1. Macrograph showing cluster of resting *Spirostomum* organisms. Each cluster is visible to the unaided eye, and may contain several thousand individuals. ×12.5

FIG. 2. The periphery of a cluster shows individuals ready to separate from the group under the influence of warmth from the microscope lamp. The intimacy of association is clearly apparent. ×75.

Samples taken in a similar fashion five weeks later show organisms which have adopted a rounded configuration. Individual *Spirostomum* were observed to have formed spherical bodies each ≅ 180 μm in diameter. Dark-ground microscopy showed organisms in stages of revival, as before (Fig. 3). However, on first observation the ciliates were observed in the form of perfect spherules. It would be premature to associate this phenomenon with encystment, though clearly we are here witnessing a resting stage in the *Spirostomum* cycle.



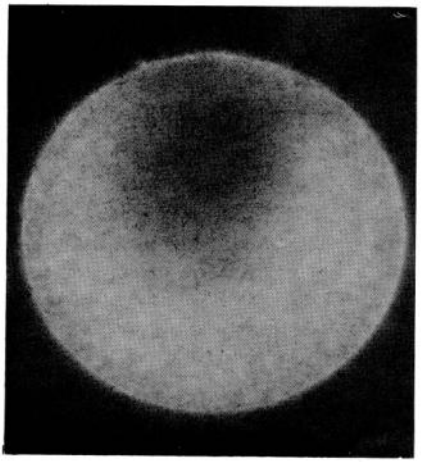


FIG. 3. A later stage of reaction to winter conditions shows a tendency to form rounded bodies, not unlike an early form of cyst formation. Motility can be restored by warming from the lamp. ×75.

FIG. 4. *Spirostomum* in mid-winter conditions becomes rounded. Each of these resting stages is ≅ 180 μm diameter. These 'hibernatory' bodies may be related to cyst formation. ×450.

The most fascinating phenomena here, however, are those associated with the clustering of ciliates in groups, apparently in readiness for the onslaught of winter conditions. The intimacy with which the organisms become associated is remarkable. Group action of this kind suggests that *Spirostomum* possesses refined trophic and recognition mechanisms. The realisation that protozoa, in a manner analogous to the behaviour of some insects and mammals, recognise the value of 'safety in numbers', poses intriguing insights into microbial sensibility.

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