

# The influence of an eucaryotic intranuclear cell parasite on the production of gemmules in *Ephydatia fluviatilis* (Porifera, Spongillidae)

Norbert Weissenfels

Abteilung für Entwicklungsbiologie, Zoologisches Institut, Universität Bonn, Poppelsdorfer Schloß,  
W-5300 Bonn 1, Federal Republic of Germany

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**Summary.** A prerequisite for the production of gemmules is the presence of intact archaeocytes and trophocytes, which give rise to the thesocytes with which the gemmule is eventually filled. The coat enclosing the gemmule requires spongioblasts for its formation and incorporates amphidisk spicules, which develop in amphidiskoblasts. The cell parasite, the development of which is described here, infects mainly archaeocytes but also spongioblasts and amphidiskoblasts. Even a moderate infection results in significant malformation of the gemmule covering. In the thesocyte nucleus, the parasite can survive the resting phase of the gemmule. After the gemmule has hatched, the parasite, again in the virulent form, is present in the young, developing sponge. The parasite may be a microsporidian of the primitive type, close to the genus *Metchnikovella*, which typically occurs in gregarines.

in a refrigerator at 4°C. Groups of five gemmules were hatched in polypropylene dishes and cultured in a well-aerated aquarium at 16°C (Langenbruch and Weissenfels 1987). Gemmule anlagen at various stages of development were obtained for examination in the light and electron microscopes by treating many sponges from cultures started at different times (age of the sponges 78, 119, and 216 days) with theophylline (Rasmont 1974). Doses of theophylline ( $10^{-4}$  M) were administered three times, at three-day intervals. All the sponges initiated gemmulation one day after the final dose of theophylline. Hence the age of the gemmule anlagen at the time of examination was known accurately.

The sponges were fixed in 1% OsO<sub>4</sub> + 1% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 0.02 M cacodylate buffer. Semi- and ultrathin sections were made with an LKB Ultrotome III and observed with a Zeiss EM 9 S-2. Phase-contrast microscopy was carried out with a Leitz-Dialux microscope.

*Abbreviations for the microscopic procedures:* PhM, phase-contrast microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy

## C. Results

Infective stages of a eucaryotic intranuclear parasite were found in all specimens of *E. fluviatilis* that had been growing for several months in our laboratory aquaria. The main cells affected are the archaeocytes of the mesenchyme. The parasites are most abundant in the vicinity of gemmule anlagen, into which archaeocytes, trophocytes, spongioblasts, and amphidiskoblasts are known to migrate. In Fig. 1, the circles mark ten of these infective organisms, at different stages of development, near a tangentially cut gemmule anlage (GA).

The parasitic infection impairs the development of the sponge. The damage is clearly apparent in the process of gemmule formation, particularly that of the thick coat enclosing the gemmule. The normal structure of this shell is shown in Fig. 2a (GS); in an infected sponge, the shell is incompletely formed (GS in Fig. 2b). It is only about half as thick as normal, so that the amphidisks (AD) project outward and are not covered by the outer layer of the shell (OS). Closer examination provides an explanation of this phenomenon.

## A. Introduction

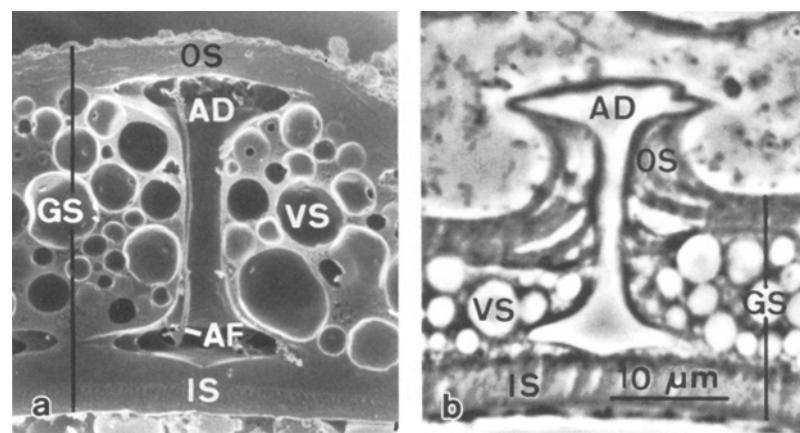
The process by which gemmules (resistant asexual reproductive bodies) arise is well known for *Ephydatia fluviatilis* and for other spongillids (Langenbruch 1981, 1984; Weissenfels 1989). Study of gemmulation is facilitated by the fact that the process can be induced by theophylline (Rasmont 1974a, b). The eucaryotic intranuclear parasite with which this paper is concerned has also been documented previously, and the basic features of its developmental and reproductive cycle are known (Weissenfels et al. 1989). Here these observations are extended and new information is provided about the way in which the parasite interferes with gemmule production and is transmitted to subsequent sponge generations.

## B. Materials and methods

Resistant stages (gemmales) of the freshwater sponge *Ephydatia fluviatilis* (Linné, 1758) were collected in the River Sieg (tributary of the Rhine) near Au and Operzau in 1989 and 1990 and stored



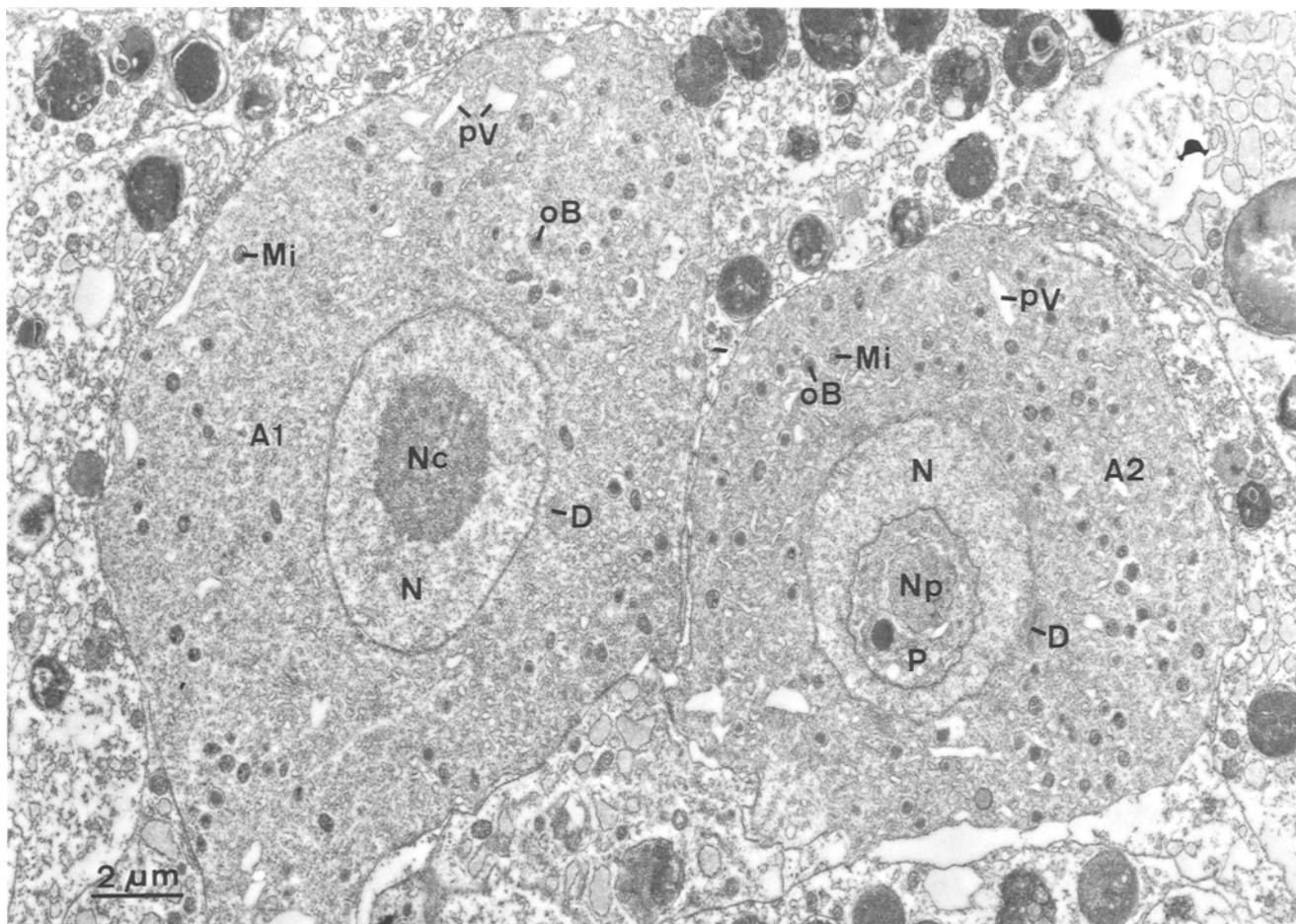
**Fig. 1.** Tangentially sectioned gemmule anlage (GA) of *Ephydatia fluviatilis*, in the vicinity of which are ten examples of parasitic infection of archaeocytes (○) at various stages of development. 119-day-old culture. PhM



**Fig. 2a, b.** Region of the gemmule shell (GS) of *E. fluviatilis*, sectioned perpendicular to the surface. The amphidisks (AD) were removed by treatment with hydrofluoric acid. AF axial filaments; IS inner layer; VS vacuolar layer; OS outer layer of the gemmule shell. **a** Section through the shell of a mature gemmule collected in the field (Langenbruch 1984), SEM. **b** Section through a gemmule shell incompletely developed following parasite infection. 216-day-old culture. PhM

The ultrathin section of Fig. 3 passes through two adjacent archaeocytes, the matching, dense fine structure of which indicates that they are young daughter cells. The left archaeocyte (A1) contains in its nucleus (N) a voluminous nucleolus (Nc). In the right archaeocyte (A2) the section does not pass through a nucleolus, but it does show an intranuclear parasite (P) with its nucleus (NP). Within the cytoplasm of the parasite is a relatively

large osmiophilic body of unknown origin and significance. Both archaeocytes exhibit a large number of spherical to oval mitochondria (Mi) and similarly shaped vacuoles with highly contrasting content (oB). The relative abundance of these two different, approximately equal-sized structures is Mi:oB=1.4:1 for A1 and 1:5.5 for A2. The difference is largely due to a considerable reduction in the absolute number of mitochondria (Mi)



**Fig. 3.** Ultrathin section of two young daughter archaeocytes lying side by side: archaeocyte *A*1 with nucleus (*N*) and nucleolus (*Nc*), and archaeocyte *A*2 with intranuclear parasite (*P*), showing the parasite nucleus (*NP*). *A*1 and *A*2 contain many mitochondria (*Mi*)

and equally large osmiophilic bodies (*oB*), in the numerical ratios 1.4:1 in *A*1 and 1:5.5 in *A*2. The pulsatile vacuole system (*PV*) is visible in both daughter cells. *D* dictyosome. 119-day-old culture of *E. fluviatilis*. TEM

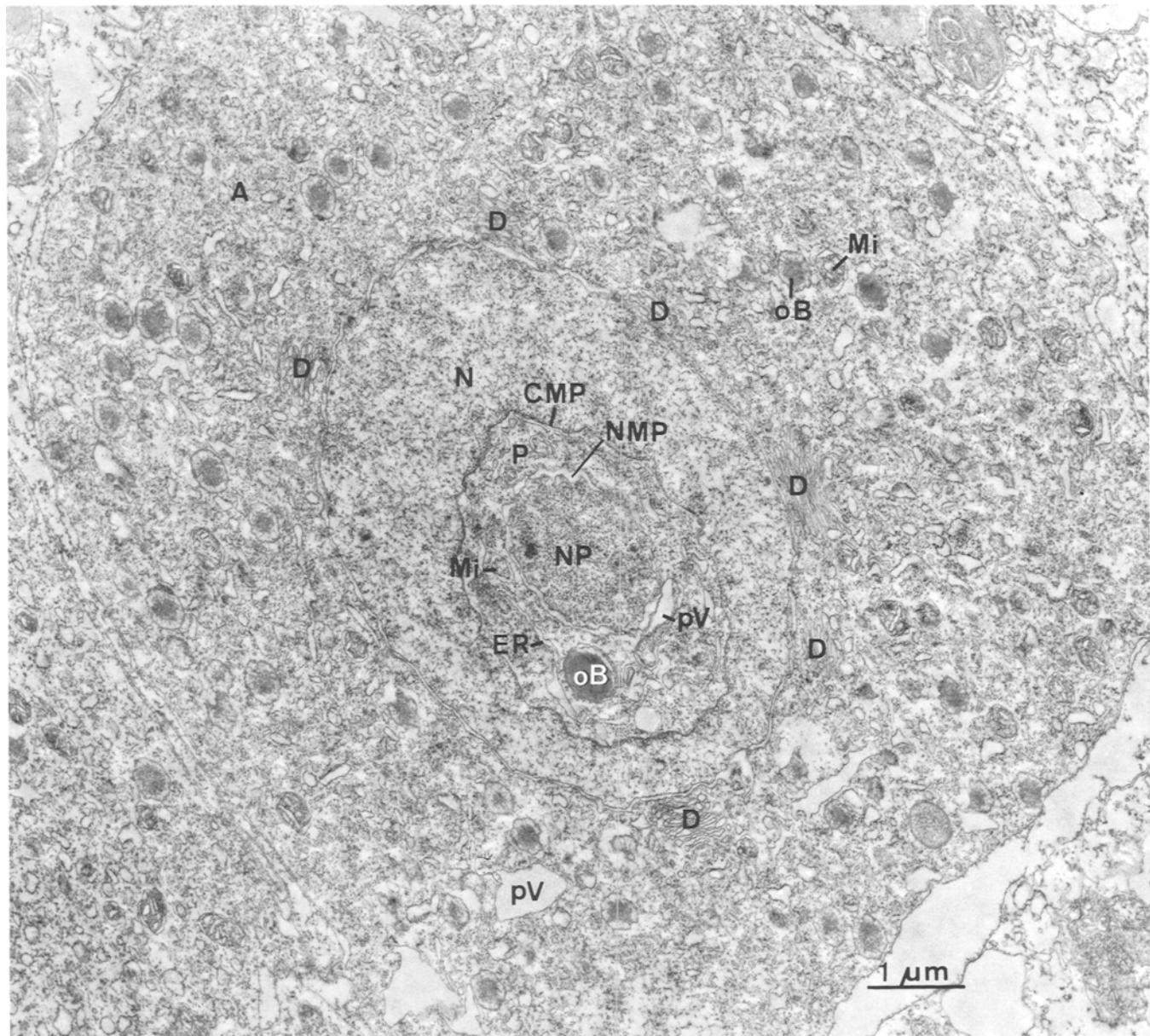
in *A*2 as compared with *A*1. In the cytoplasm of both archaeocytes (*A*1 and *A*2) profiles of the pulsatile vacuole system (*PV*) can be seen. Similar profiles are also found in the parasite (*P*) of archaeocyte *A*2. Fine-structural details are more clearly shown in the enlarged view of *A*2 in the following figure.

The nucleus (*NP*) of the parasite (*P*) in Fig. 4 is enclosed in the double membrane (*NMP*) typical of eucaryotes. The cytoplasm of the parasite contains ribosomes and endoplasmic reticulum (*ER*). Small structures with double membranes are reminiscent of mitochondria (*Mi*). The round and elongated vacuoles (*PV*) in the parasite are comparable to the pulsatile vacuoles of the infected cell. The cell membrane (*CMP*) of the parasite is unusually osmiophilic and hence quite distinct in appearance from all the other membranes present. The oval, osmiophilic inclusion body (*oB*) is surrounded by a double membrane. Structures of this kind are routinely found in the parasites.

Apposed to the outer membrane of the nucleus (*N*) of the host cell are dictyosomes (*D*), six of which can be seen in the ultrathin section of Fig. 4. Such an increase in the number of dictyosomes is characteristic

of parasitized sponge cells. Figure 4 also clearly reveals the difference in structure between the mitochondria (*Mi*) and the osmiophilic bodies (*oB*). There are indications that the latter are a product of the dictyosomes.

The development of the parasite in the nucleus of the host cell is illustrated by photomicrographs in Fig. 5 and electron micrographs in Fig. 6. The parasite (*P*) is clearly visible in the nucleus (*N*) of the archaeocyte (*A*) (Fig. 5a). It is enclosed in a well-defined cell membrane (*CMP*) and contains one nucleus (*NP*). The section passes through the nucleolus (*Nc*) of the archaeocyte nucleus (*N*). The number of nuclei in the parasite (*P*) gradually increases by caryokinesis, from two (Fig. 5b) to several (Fig. 5c) to many (Fig. 5d). As it becomes multinucleate the parasite enlarges, crowding the nuclear material of the host cell into a narrow peripheral band and ultimately filling the host nucleus (*N*) completely. The nuclei of the plasmodial parasite (*NP*) in Fig. 5b-d are capped by darker spots. The plasmodium then separates into spore-like individual cells (Fig. 5). All that remains of the host cell nucleus is a highly contrasting peripheral zone that looks like a capsule wall. When the daughter cells of the parasite are fully mature they



**Fig. 4.** A parasite (*P*) with nucleus (*NP*) and nuclear membrane (*NMP*) is separated by its cell membrane (*CMP*) from the nucleus (*N*) of the host cell (*A*), which contains many dictyosomes (*D*), osmiophilic bodies (*oB*) and mitochondria (*Mi*). The latter (*Mi*)

are presumably also present in the parasite. Pulsatile vacuoles (*PV*) are present in the host cell (*A*) and probably also in the cytoplasm of the parasite. 119-day-old culture of *E. fluviatilis*. TEM

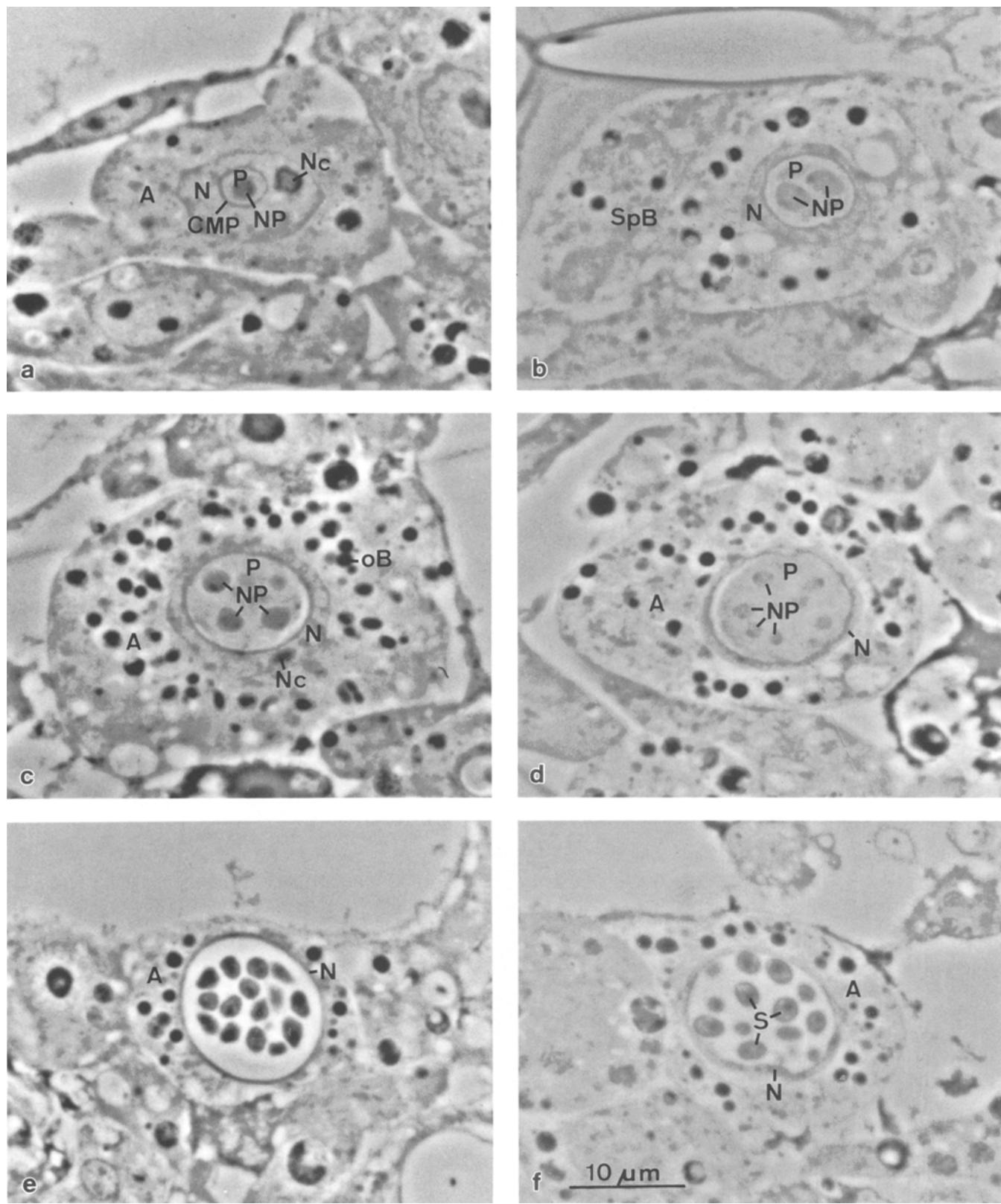
become rounded, so that finally they have the appearance of spores (*S* in Fig. 5f). The cytoplasm of the infected host cells contains osmiophilic bodies (*oB*), only a few at first but later in large numbers.

The parasite (*P*) has recently invaded the nucleus (*N*) of an archaeocyte (*A* in Fig. 6a). The section does not pass through the nuclear material of the parasite but shows profiles of its endoplasmic reticulum (*ERP*) and an osmiophilic body (*oBP*). The cell membrane of the parasite (*CMP*) is unusually osmiophilic. The nucleolus (*Nc*) of the host cell nucleus is in contact with the parasite. Several dictyosomes (*D*) of the archaeocyte are distributed around the nucleus. The host cell cytoplasm

includes a well-developed *ER*, both small and large osmiophilic bodies (*oB*), and mitochondria (*Mi*). The section passes through the system of pulsatile vacuoles (*PV*).

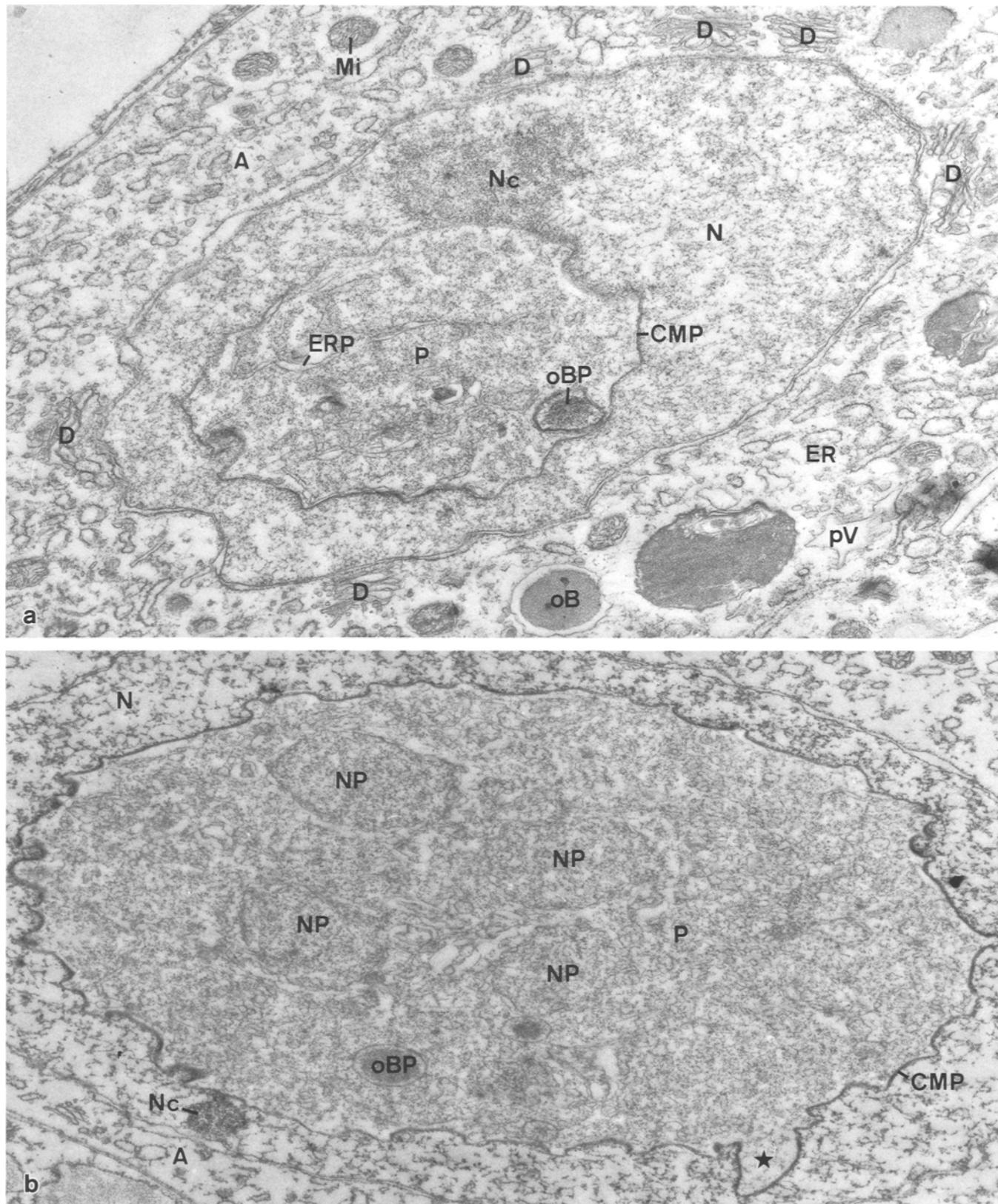
The section in Fig. 6b shows at least four parasite nuclei (*NP*). The cytoplasm of the plasmodium contains canals and cisternae as well as membrane-bounded osmiophilic bodies (*oBP*). The surface of the parasite is evaginated in many places (\*).

The plasmodium in Fig. 6c has become subdivided into a number of individual cells, some of which are still joined together (→). Each new cell contains a nucleus (*NP*) with a nucleolus (*NcP*). The cytoplasm of the new cells is so densely structured that its only prominent



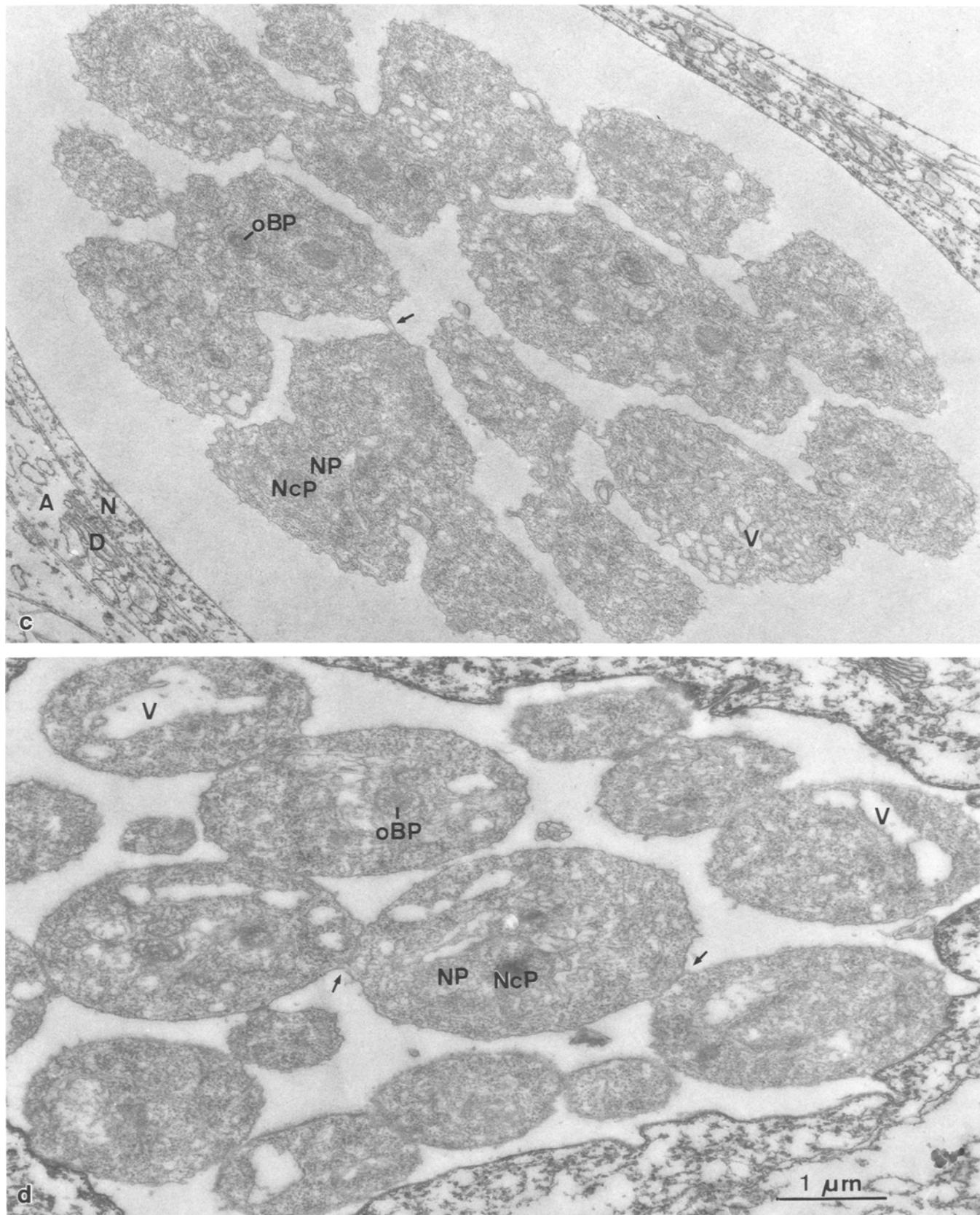
**Fig. 5a–f.** Six different developmental stages of the parasite in five archaeocytes (*A*) and a spongioblast (*SpB*) of *E. fluviatilis*. **a** The parasite (*P*) within the nucleus (*N*) of the archaeocyte (*A*) has a prominent cell membrane (*CMP*) and a nucleus (*NP*). As it grows, the parasite develops into a multinucleate plasmodium (**b–d**) and occupies progressively more space in the nucleus of the host

cell. The plasmodium then fragments cytokinetically into many cells (**e**), which eventually (**f**) have the appearance of spores (*S*). The infected archaeocytes are characterized by numerous osmophilic bodies (*oB*). **a–d, f** 78-day-old, **e** 216-day-old culture of *E. fluviatilis*. PhM



**Fig. 6a-d.** Four different developmental stages of the parasite in archaeocytes of *E. fluvialis*. **a** The section through the parasite (*P*) in the nucleus (*N*) of an archaeocyte (*A*) does not include the nuclear region of the parasite but shows its osmiophilic cell membrane (*CMP*), an osmiophilic body (*oBP*) and endoplasmic reticulum (*ERP*). The nucleolus (*Nc*) of the host cell nucleus touches the parasite. Around the cell nucleus, six dictyosomes (*D*)

are visible. *ER* endoplasmic reticulum; *oB* osmiophilic body; *Mi* mitochondrion; *pV* pulsatile vacuole of the host cell. 78-day-old cultures of *E. fluvialis*. TEM. **b** Plasmodial state of the parasite (*P*). At least four nuclei (*NP*) appear in the section. The parasite has a bizarre surface, with many evaginations (\*); *N* nucleus; *Nc* nucleolus of the host cell; *CMP* cell membrane of the parasite. 119-day-old culture of *E. fluvialis*. TEM. **c** Cytokinetic subdivi-



sion of the plasmodial parasite into individual cells, which contact one another over large surfaces in some cases and only by bridges ( $\rightarrow$ ) in others. NP nucleus of the parasite; NcP nucleolus of the parasite nucleus; oBP osmiophilic body of the parasite; V vacuoles of the parasite. 119-day-old culture of *E. fluviatilis*. TEM. **d** Final phase of cellular subdivision of the parasite plasmodium. The new

cells have become egg-shaped but still touch one another ( $\rightarrow$ ). Soon they will become completely isolated and will develop a thick cell wall. V vacuole system with cisterna-like structure; oBP osmiophilic body; NP nucleus; NcP nucleolus of the sporelike cells. 119-day-old culture of *E. fluviatilis*. TEM

feature is the numerous vacuoles (*V*). The nucleus (*N*) of the host cell (*A*) has been displaced into a restricted peripheral region.

The cells in Fig. 6d have continued to develop, smoothing their surfaces and becoming ovoid in shape, though some contact sites ( $\rightarrow$ ) remain. Later a sturdy cell wall forms. The expanded vacuole system (*V*) now appears more cisternal and is still the dominant feature of the densely structured cytoplasm, which also contains membrane-bound osmiophilic bodies (*oB*).

In the later stages of infection the caryo- and cytoplasm of the host cells, most of which are archaeocytes, is quite labile in its response to the influences of preparation and fixation. Furthermore, it is more osmiophilic than that of the parasite cells, the fine structure of which is always well preserved.

Now the disruptive influence of the parasite on gemmule formation in *E. fluviatilis* can be considered. Gemmulation was induced by theophylline (see Materials and methods), so that gemmules in all desired developmental stages were obtainable. As a result, parasite infection was discovered in the spongioblasts that form the shell of the gemmule. Figure 7 shows a parasite (*P*) in the plasmodium stage, quite distinct from the nucleus (*N*) of the shell-forming spongioblast (*SpB*). The latter contains small and large osmiophilic bodies (*oB*). The gaps (\*) in the gemmule shell are artefacts of the preparation. An osmiophilic layer ( $\rightarrow$ ) forms the boundary between the unfinished shell (*S*) and the cellular contents of the gemmule (*G*).

The spongioblasts required for construction of the shell are known to be recruited from the depot at the base of the sponge and in the spicule-spongin complexes (Langenbruch 1981; Weissenfels 1989). It is especially significant, therefore, that spongioblasts at these sites of origin are found to be infected. For example, in Fig. 8 a spongioblast (*SpB*) at the surface of the spicule (*Si*)-spongin (*Sp*) complex contains a parasite (*P*), in which two nuclei (*NP*) appear. The nucleus (*N*) of the spongioblast appears annular in this section. Hence the evidence indicates that the spongioblasts involved in building the gemmule shell had been previously infected.

The amphidisks (*AD*) that form part of the shell are also evidently affected by the infestation, because the amphidiskoblasts (*ADB*) are also found to have been invaded by parasites (Fig. 9). Here, again, the parasite (*P*) infects the nucleus (*N*) of the amphidiskoblast, where it goes through a plasmodial phase. Such stages of infection have rarely been encountered previously. Although the structure of this preparation is not well preserved, the incompletely developed amphidisk (*AD*) and parts of its axial filament (*AF*) are visible.

It is highly significant that the parasite (*P*) also invades the cellular interior of the gemmule anlage (*G* in Fig. 10). The plasmodial stage shown here lies within the nucleus (*N*) of a young thesocyte, the outline of which is obscured by the numerous yolk globules (*Yo*).

## D. Discussion

There are two problems to discuss: the infection itself, in which *E. fluviatilis* is parasitized by an intranuclear eucaryotic organism, and the resulting malformations of the shells of gemmules produced by the infected spongillids. As a rule, infection becomes manifest in sponge cultures a few months old, when growth stagnates and the body of the sponge then regresses. Finally, the remainder of the sponge degenerates altogether.

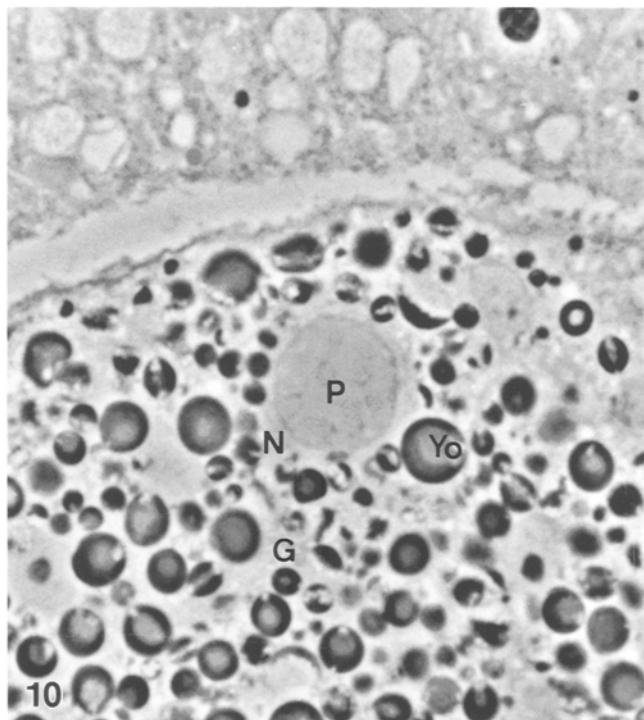
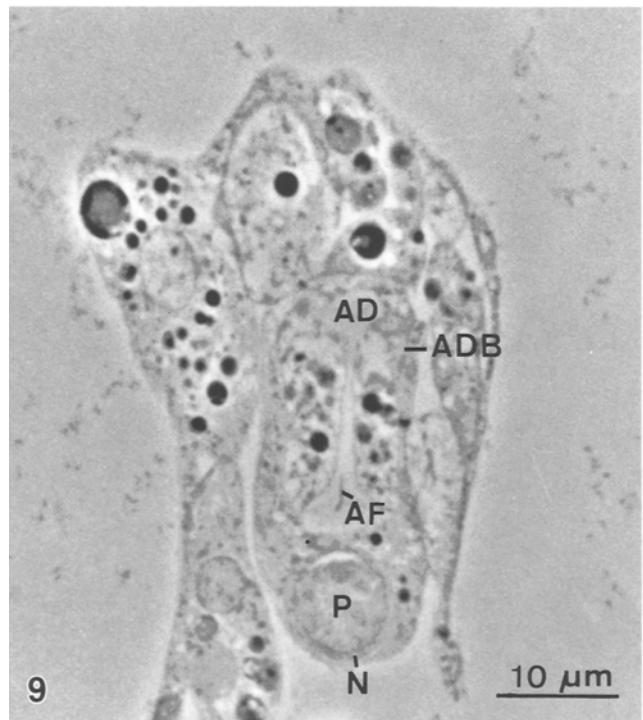
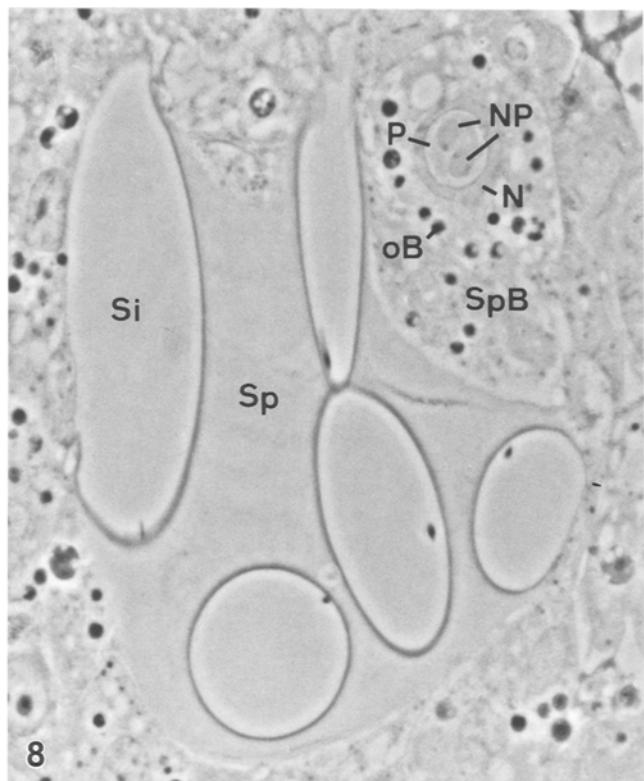
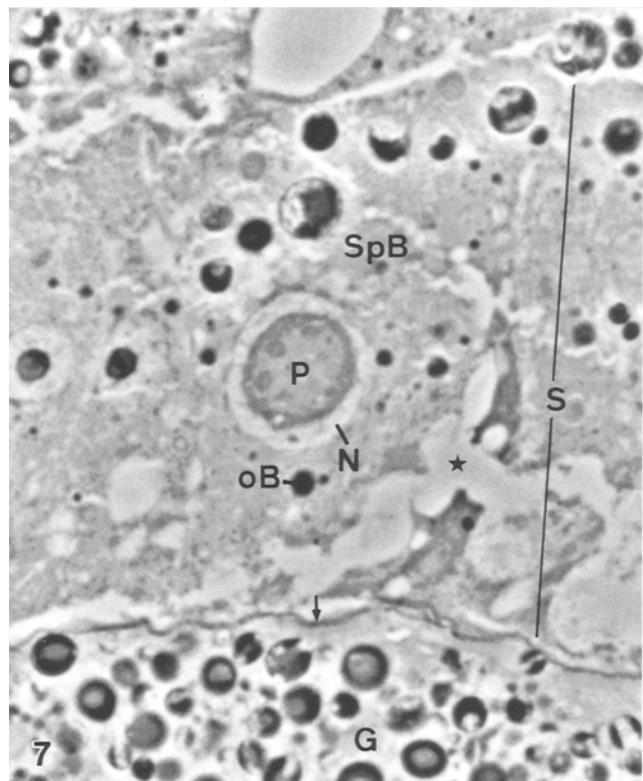
Recently the parasite has also been found in specimens of *E. fluviatilis* collected in the field. However, the infection in these cases was very mild and, in my observations, did not lead to death of the sponges. This finding suggests that the course of infection is facilitated by our culture conditions. An aspect of biological interest is that we are dealing here with a geologically very ancient parasite-host relation that has been revealed by our "advances" in the technology of animal culture.

The parasites infect the archaeocytes first, later spongioblasts and amphidiskoblasts, and finally other types of cells and pinacocytes. It is especially significant with respect to the host-parasite relationship that the host cells give a rapid, profound response to the infection; the number of dictyosomes rises dramatically and many osmiophilic granules appear, while the mitochondria decrease in number. The abundance of osmiophilic granules is associated with the elevated number of dictyosomes, which are probably responsible for production of the granules. At present no interpretation can be given for the disappearance of mitochondria.

To reproduce itself, the parasite invades the nucleus of the infected cell. It undergoes a series of caryokineses, producing a plasmodium with many nuclei, and then by cytokinesis separates into individual cells. The result is a group of new parasites with a spore-like appearance. After the death of the host cell, the propagative phase of the parasite begins, which has a devastating effect in the closed system of a large aquarium. The manner and route by which the new infection occurs remain to be investigated, but presumably involve sponge activities previously described – the intake of particulate food and defecation (Weissenfels 1976).

The presence of parasites in the spongioblasts that construct the shell of the gemmule can be ascribed to the parasitic infection of the spongioblasts in the spicule-spongin complexes of the adult sponge (Langenbruch 1981). The infection of amphidiskoblasts explains the reduction in number of amphidisks in the gemmule shell that forms later. Parasites are also present in the thesocytes of the gemmule anlage, which suggests that the infection is passed on to subsequent sponge generations.

There have been occasional previous reports of diseases in marine sponges. The pathogens involved in those cases were fungi, bacteria, and viruses (Vacelet and Gallissian 1978). The parasite described here differs from bacteria, which are commonly encountered in Demospongiae and Calcarea, and also from symbiotic Cyanophyceae and Chlorophyceae (Vacelet and Donadey 1977).



**Fig. 7.** Parasitic infection of a spongioblast (*SpB*) participating in gemmule shell construction. *P* parasite in the plasmodial stage, with diverse nuclei. *N* nucleus; *oB* osmiophilic body of the *SpB*; *S* incomplete gemmule shell; *G* cellular contents of the gemmule; → osmiophilic layer between *S* and *G*; \* spaces caused by factors in preparation. 119-day-old culture of *E. fluvialis*. PhM

**Fig. 8.** Spongioblast (*SpB*) of a spicule (*Si*)-spongin (*Sp*) complex, infected by a binucleate (*NP*) parasite (*P*). *N* nucleus; *oB* osmiophilic body of the spongioblast. 78-day-old culture of *E. fluvialis*. PhM

**Fig. 9.** Amphiidiskoblast (*ADB*) infected by a plasmodial parasite (*P*). *N* remaining peripheral region of the nucleus of the amphiidiskoblast; *AF* axial filament of the developing amphiidisk (*AD*). 119-day-old culture of *E. fluvialis*. PhM

**Fig. 10.** Plasmodial parasite (*P*) in the nucleus (*N*) of a thesocyte of a young gemmule (*G*). *Yo* so-called yolk granule of a thesocyte. 119-day-old culture of *E. fluvialis*. PhM

No evidence was found of structures such as an endospore, exospore, polar filament, or polaroblast, which distinguishes the organism described here from typical microspora (Levine et al. 1980). We have sent original electron micrographs to Dr. J. Vavra (Weissenfels et al. 1989) and although he cannot precisely identify the organism, his feeling is that it might be a microsporidian of the primitive type, close to the genus *Metchnikovella* (Hildebrand and Vivier 1971; Vivier and Schrevel 1973), which typically occurs in gregarines.

There are several indications that the parasites – originally unicellular, then plasmodial and ultimately, after cytokinesis, multicellular – develop a system of pulsatile vacuoles similar to that of the infected sponge cells.

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