A Proteomyxan Amoeba Stage in the Development of *Labyrinthomyxa patuxent* (Hogue) Mackin and Schlicht, With Remarks on the Relation of the Proteomyxids to the Neoplastic Diseases of Oysters and Clams

J. G. MACKIN and FRANK G. SCHLICHT

Hogue (1921) described *Vahlkampfia patuxent*, an amoeboid protist parasite of oysters (*Crassostrea virginica*) from Chesapeake Bay. Studies of these parasites have shown that early stages of development involve a proteomyxan amoeba, which, under certain environmental conditions, causes lethal epizootic disease in host populations. This report is intended to describe these amoebae. It is believed that all serious students of molluscan pathology have observed these protistans, but have failed to recognize their significance. This report will be concerned only with the early amoeboid stages of the development of *Labyrinthomyxa patuxent*. Later development follows closely the steps described for *Labyrinthomyxa marina*, for which see Mackin (1962). *Labyrinthomyxa marina* passes through all the developmental stages here described for *L. patuxent* including the amoebae, but differs quantitatively, as indicated below.

Earliest uninucleate amoebae, as removed from the haemolymph of oysters in advanced disease, are rounded to oval, about 6 to 12 μm in length, and may have one or two polar pseudopods (Fig. 1), which are curved, lamellate, and capable of a characteristic lateral movement. Growth and further development of these cells results in: 1) Production of endogenous buds (Fig. 2a, b) ranging in number from one to many depending on the size of the amoebae; 2) growth to giant amoebae (Fig. 3a, b, c) which may be more than 200 μm in length when extended; and 3) production of multibranched and anastomosing pseudopodia (Fig. 4) originating from flattened ectoplasmic lamellae. Endogenous buds may produce secondary gemmae (Fig. 2b), and rarely even more. Endogenous buds are extruded from the amoebae, leaving a rounded cavity, which may persist for a period (Figs. 5, 10) in the mother cell. Early buds are rounded uninucleate cells which resemble oyster leucocytes when in stained sections (Fig. 6) and which can reproduce by binary fission to produce massive concentrations in the host tissues and haemolymph (Fig. 7). Later endogenous buds may form small plasmodia, sporangia, and presporangia, often in the same amoeba (Fig. 8). *Labyrinthomyxa patuxent* differs from *L. marina:* 1) In its incomplete response to Ray's (1954) thioglycollate diagnostic culture method, apparently not responding in early ameoboid stages, but responding positively only to presporangia or sporangia; 2) in the relatively greater prominence of early amoeboid stages; 3) presporangia are on the average slightly smaller than the same stage in *L. marina*; and 4) spindle cells of *L. patuxent* are spindle shaped when grown in culture, while those of *L. marina* are round to oval. Both contain a large lipid granule in an eccentric vacuole, and an eccentrically placed nucleus.

Labyrinthomyxa patuxent has the characteristics of the theoretical neoplasms of oysters and clams described by Couch (1969), Farley (1969a, b), Christensen, Farley, and Kern (1974), and others. Sparks (1972) summarized these as: 1) Nuclear enlargement; 2) an unusual number of mitoses in stained preparations, of which some appear to be multipolar; 3) an excess of chromosome number over those in host cells;

J. G. Mackin is with the Department of Biology, Texas A&M University, College Station, TX 77843. Frank G. Schlicht is with Houston Lighting and Power, Houston, TX 77001.

Figure 1.—Two uninucleate cells with polar flattened pseudopods, which can be moved laterally.

Figure 2a.—Early development of endogenous buds in a small proteomyxan amoeba. Figure 2b.—A later stage, with several endogenous buds, one of which has produced a secondary bud of its own.

Figure 3a.—A giant proteomyxan amoeba, contracted into a spherical mass, but with short pseudopods around the periphery. This amoeba contains a large number of endogenous buds in the center. Figure 3b.—Same as Figure 3a, but partially extended, showing the clear ectoplasmic lamellae. Figure 3c.—A fully extended amoeba, except for the pseudopods, photographed at 450 diameters. This amoeba measured about 150 μm in length.

Figure 4.—A small amoeba with fully extended pseudopods, which measure about 75 μm. The pseudopods are branched and subbranched, and arise from one of the extended lamellar ectoplasmic projections.

Figure 5.—A proteomyxan amoeba showing two large vacuole-like cavities, which were occupied by buds which have escaped.

Figure 6.—A proteomyxan amoeba in stained section of the host oyster, *C. virginica*. Here the thin layer of cytoplasm is hardly noticeable around the endogenous buds.

Figure 7.—Stained section taken at 450 diameters to show the large number of amoebae in a heavily infected oyster.

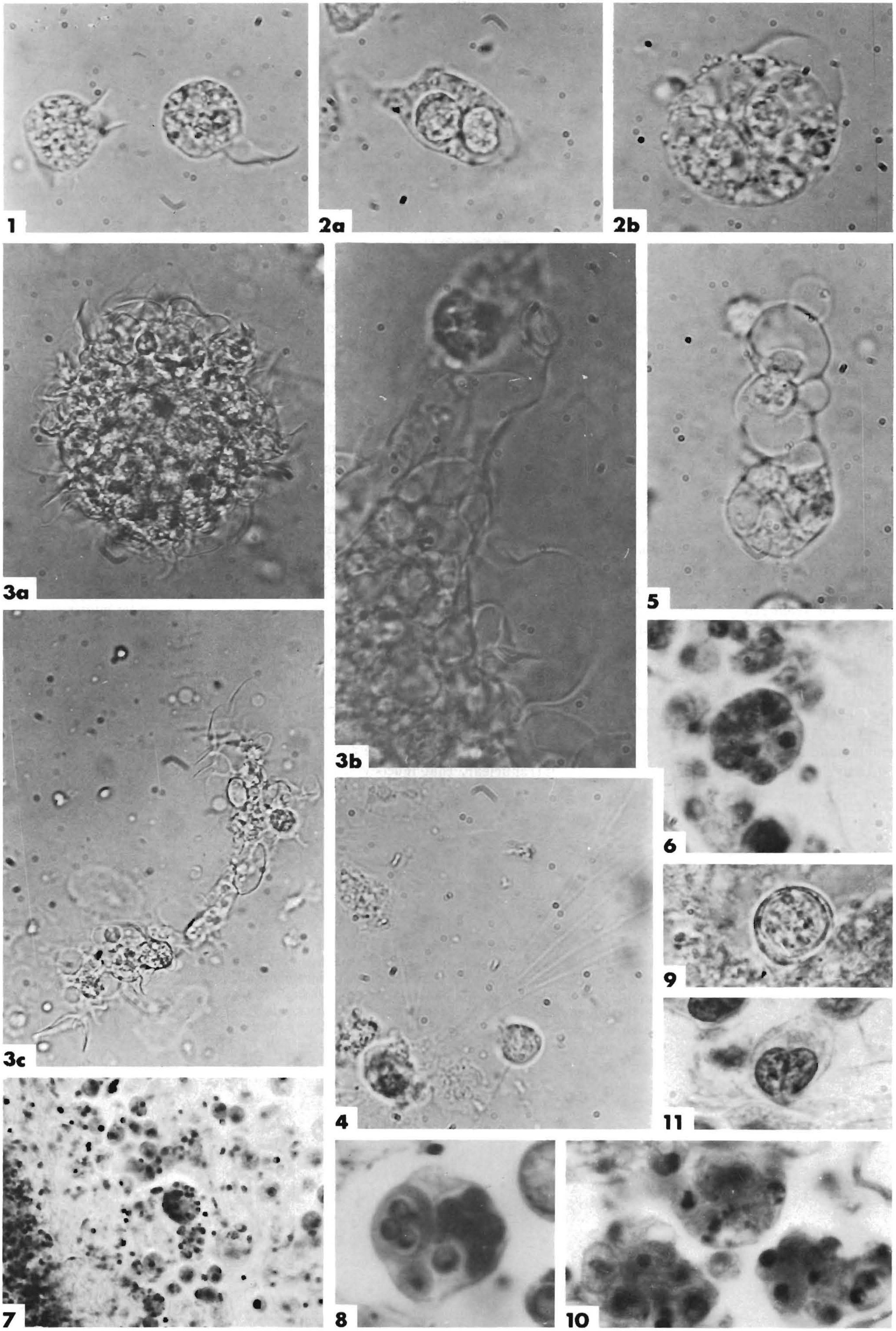
Figure 8.—A proteomyxan amoeba in late stages of disease. The rounded vegetative cells are now replaced as endogenous buds by presporangia (left side of amoeba section), small sporangia with tightly appressed nuclei (right of section), and only one vegetative cell.

Figure 9.—A live cyst attached to gill tissue (note the double wall).

Figure 10.—Several large myxamoebae with vegetative cells, most of which are ready to leave the mother cell, and show the vacuoles in stained preparation, which are shown in vivo in Figure 5.

Figure 11.—An enlarged nucleus of *Mytilus edulis*, from Yaquina Bay, Oreg., stained by C. A. Farley. This nucleus is in process of dividing after an intra-nuclear mitosis. The median plate is clearly shown. (Courtesy of C. A. Farley.)

All photomicrographs were taken at 1,125 diameters, except for Figures 3c and 7. All stained sections were stained with Wolbach's modification of the tissue giemsa. All in vivo photomicrographs were taken using amoebae removed from the hemolymph of oyster number 6-25-75+2 (*Crassostrea virginica*) from West Bay, Tex.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4) frequent lobed nuclei; 5) a prominent nucleolus, and sometimes two or more of these in a single nucleus; and 6) a sparse but highly basophilic cytoplasm, bi- and multinucleate cells. It was assumed that no known protist had these characteristics (Sparks, 1972). The cells, assumed to be of haemopoietic origin, also have certain other characteristics. These are as follows: 1) The mitoses are intranuclear, a characteristic of protistans; 2) the cells frequently become encysted (Fig. 9); 3) they may be formed as endogenous buds; and 4) in addition to "normal" mitoses, some of the mitotic figures are of the cruciform type characteristic of the Plasmodiophorales (see Karling, 1944) and the problematic *Phagomyxa algarum* (Karling, 1944), which the author believes is a species of *Labyrinthula* Cienkowski (1867).

Only one group of the protista combines all of the characteristics described for the assumed neoplastic diseases by the various authors. The list of characteristics was added to by the author from study slides contributed for use of participants at the shellfish pathology conference. This group is the Labyrinthulales Cienkowski (1867). Plasmodiophorales apparently has all characteristics excepting only the capacity to reproduce by endogenous budding.

Analysis of the papers of proponents of the neoplasm theory shows that their case is based on the fallacious assumption that mitoses observed in the enlarged cells are abnormal. Particularly singled out is the apparent presence of polycentric mitoses. These are, in fact, not polycentric. In the enlarged nuclei, the product of the first mitosis is two nuclei, both occupying the area of the original one nucleus, and flattened against each other (Fig. 11). On initiation of the second division in the two daughter nuclei, division of the nucleoli precedes the formation of the equatorial plate. A peculiarity of the group is that the poles thus established by the nucleoli may lie at right angles to each other in the two daughter nuclei. In this manner simultaneous mitoses in the two daughter nuclei may be interpreted as one polycentric mitosis. Because the tetrad of small nuclei, still confined to the area of the original large nucleus, may initiate further crowded mitoses, the confusion is compounded.

The burden of proof lies with the proponents of the "neoplasm" theory. They must prove that carcinogenic or radiational damage to leucocytes of shellfish, or spontaneous changes in leucocytes, can produce alteration of chromosome number ending in a larger number by a significant margin. They must also prove that leucocyte mitoses are intranuclear, that leucocytes may encyst, and that they may be multinucleate.

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