A Disease of Blue Crabs (Callinectes sapidus) Caused by a Parasitic Dinoflagellate, Hematodinium sp.

Martin W. Newman; Charles A. Johnson

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has the high value of 1.26. The patterns are different from those obtained for NADH dehydrogenase (marker for the nonsedimentable portion of the cytoplasm) and for acid phosphatase and β-N-acetylglucosaminidase (markers for the particles containing hydrolases). The results thus indicate that, as in *T. foetus* and *Monocercomonas* sp., there is a particle population with the enzymatic composition and equilibrium density that characterize hydrogenosomes.

Our electron microscopic studies of the fractions comfirmed the results of Brugerolle and Metenier (loc. cit.) who demonstrated that the particles equilibrating at a density of 1.26 and containing malate dehydrogenase correspond morphologically to the microbodylike granules of *T. vaginalis*.

It is known that homogenates of *T. vaginalis* are capable of metabolizing pyruvate with the formation of H_2 and CO_2 (Edwards and Mathison, 1970, J. Gen. Microbiol. **63:** 297–302). In the present communication we demonstrated that the anaerobic enzymes participating in this process are present in the hydrogenosomes of *T. vaginalis*. Pyruvate synthase decomposes pyruvate in a CoA dependent reaction yielding CO_2, acetyl-CoA, and high-energy electrons. Hydrogenase produces molecular hydrogen by combining the electrons with protons. The fate of the acetyl-CoA formed is presently under investigation. Both enzymes can reduce the antitrichomonad drug, metronidazole (Lindmark and Müller, 1974, J. Prot. **21:** 430). This process is assumed to play an important role in the action mechanism of the drug (Edwards et al., 1973, J. Gen. Microbiol **76:** 135–145).

In conclusion, in three trichomonad species the microbodylike paracostal and paraxostylar granules could be characterized biochemically as hydrogenosomes. This finding makes it likely that these organelles are hydrogenosomes in all trichomonads.

Donald G. Lindmark, Miklós Müller, and Helen Shio, The Rockefeller University, New York, New York 10021. Supported by Grant AI-11942 from the National Institutes of Health and by Grant GB-35258X from the National Science Foundation.

A Disease of Blue Crabs (*Callinectes sapidus*) Caused by a Parasitic Dinoflagellate, *Hematodinium* sp.*

During investigations of the occurrence of *Paramoeba perniciosa* in the blue crab, *Callinectes sapidus* (Newman and Ward, 1973, J. Invert. Path. **22:** 329–334), a new disease was found. In 1968 and 1969, the disease occurred in samples of crabs from North Carolina, Georgia, and Florida. Samples from South Carolina examined during those years did not contain any crabs with the disease. The maximum monthly prevalence was seen in one Florida sample, in which 30% of the crabs were infected.

The disease was characterized in its advanced stages by an abundance of peculiar cells in the hemolymph, most of them in various stages of mitosis. The cells were the approximate size of crab hemocytes, stained in a similar manner, and did not resemble any well-known parasites of decapod Crustacea (Sprague and Couch, 1971, J. Prot. **18:** 526–537). These characteristics, plus the apparent fatal outcome of the disease, led Newman (1971, Bibl. Haematol. **36:** 648) to suggest that a possible neoplastic condition of the hemocytes was involved.

In September 1974, a special attempt was made to obtain infected crabs and fix tissues for examination by electron microscopy. An area near Beaufort, North Carolina, was chosen because numerous diseased crabs had recently been found there.

Methods of collection and examination of crabs in which the disease was first found were described by Newman and Ward (1973, loc. cit.).

Crabs for subsequent studies were collected using an otter trawl, and were transported to the Duke University Marine Laboratory for examination. Smears of hemolymph were prepared by forcing the crab to autotomize one of its walking legs, and smearing a drop of the exuding hemolymph onto a microscope slide.

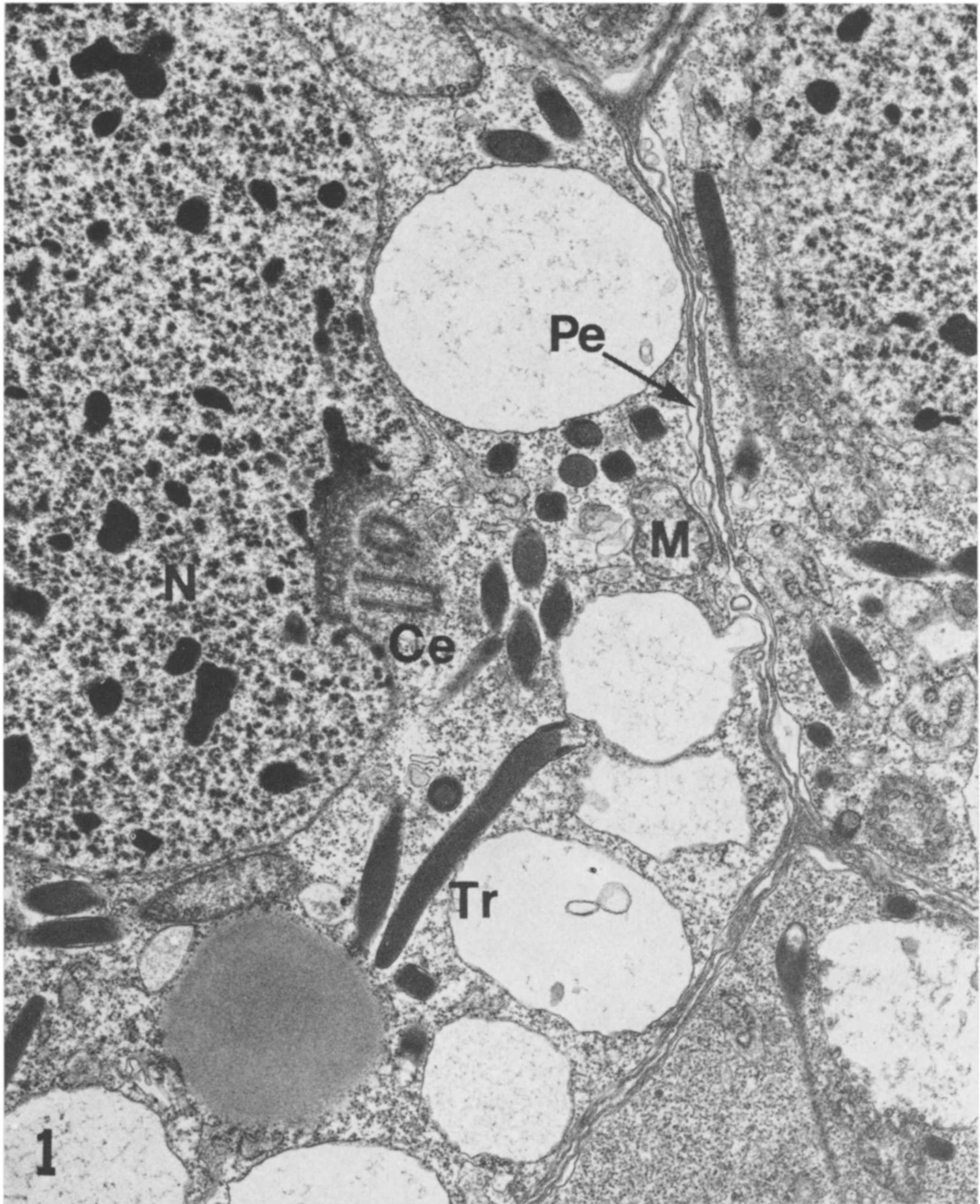
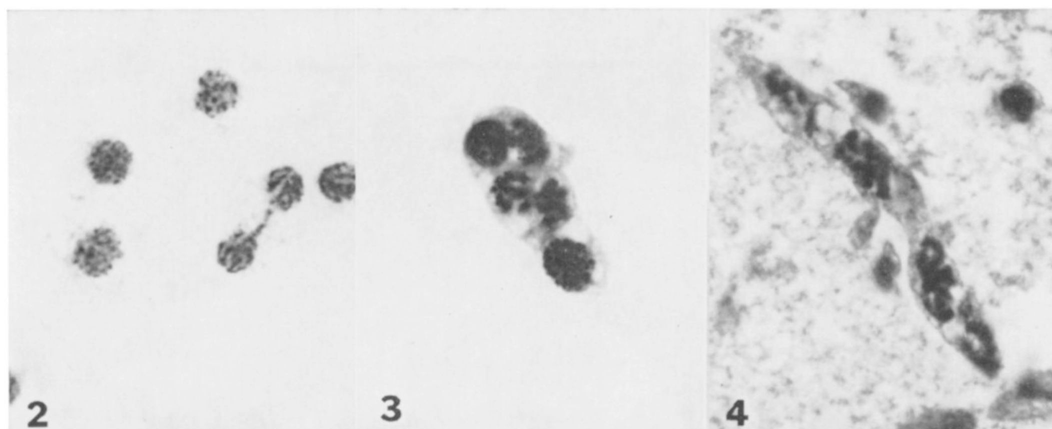


FIGURE 1. Ultrastructure of parts of four adjacent organisms is shown in this electron micrograph. × 23,000. N, nucleus; Ce, centriole; M, mitochondria; Pe, pellicle; Tr, trichocyst.

Smears thus prepared were routinely stained with Giemsa's stain. Additional smears and tissues from crabs found to be infected were fixed in 10% neutral-buffered formalin or Davidson's alcohol–formalin–acetic–seawater fixative (Shaw and Battle, 1957, Can. J. Zool. **35:** 325–347).

Pelleted cells from hemolymph and samples of gill, hepatopancreas, and epidermis were fixed in cold 3% glutaraldehyde for 2 hr, then



FIGURES 2–4. Appearance of the parasite in smears and tissue sections. × 900. **2.** Several uninucleate forms and an organism undergoing division. Note the V-shaped chromosomes. **3.** A single cell containing five nuclei. **4.** Section of gill tissue containing an elongate form of the parasite. Four nuclei are visible in the plane of section.

rinsed, and stored in pH 7.4 sodium cacodylate buffer until they could be postfixed in 1% OsO_4. They were then embedded in Epon, sectioned, and stained with lead citrate.

The disease is most prevalent in the fall. Animals from salinities ranging from 0 to 36‰ have been examined but, to date, infected crabs have been found only in areas of above 11‰ salinity. Both male and female crabs from 70 to 170 mm in width have been found to be affected. In advanced stages of the disease, crabs are moribund and most die before reaching the laboratory. The internal tissue of these crabs is a milky-white color. Hemolymph, when withdrawn, also appears milky-white, is slow to clot, contains few if any hemocytes, and is filled by the peculiar cells.

One moribund crab collected in the Beaufort area in September 1972 was literally filled with the uninucleate cells. Hemolymph and tissues from this and one less heavily infected animal were fixed for electron microscopy. Figure 1 illustrates the structure of these cells as seen at × 2,400. These micrographs show the pellicle, tubular cristae in the mitochondria, and trichocysts, and clearly indicated that we were dealing with a protistan parasite, not an aberrant hemocyte. Parasites from the hemolymph were unicellular and measured 6.4 to 10.4 μm in diameter ($\bar{x} = 8.1$; SD = 1.1) after fixation in Davidson's fluid. Nuclei were 4.6 to 8.1 μm in diameter ($\bar{x} = 6.2$; SD = 0.9) and did not have distinct nuclear membranes or nucleoli. Chromatin was distinct, granular, and Feulgen-positive (Fig. 2). It often occurred as distinct strands, having a beaded appearance. Dividing cells were common (Fig. 2), but there was a notable absence of anything resembling a metaphase nucleus. In wet mounts of fresh tissue, these cells were nonmotile. The cells have been found in the vascular spaces of all tissues examined histologically by light microscopy.

In some affected crabs, large multinucleate cells containing up to eight nuclei or division figures were seen (Fig. 3).

Some animals, assumed to have been only recently infected because of the largely normal appearance of their hemolymph and hemocytes, contained vermiform multinucleate bodies up to 8 by 64 μm with as many as 12 nuclei (Fig. 4). These forms were highly motile when seen in freshly prepared wet mounts, sometimes assuming comma or dumbbell shapes, and were enclosed within a sheath or pellicle.

The structures revealed by the electron microscope, along with the unusual division figures exhibited by the parasite, enable Dr. Phyllis Bradbury of North Carolina State University to suggest a possible affinity with parasitic dinoflagellates of the family Blastodidiidae. Most members of this family are parasites in the coelom of pelagic copepods (Chatton, 1910, C. R. Acad. Sci. Paris **151:** 654–656;

1920, Arch. Zool. Exp. Gen. **59:** 1–475). One genus in this family containing only one species, *Hematodinium perezi* (Chatton and Poisson, 1931, C. R. Seances Soc. Biol. Fil. **105:** 553–557), has been described from the hemolymph of two European crabs, *Carcinus maenas* and *Portunus* (*Macropipus*) *depurator*. Both of these species and *C. sapidus* belong to the family Portunidae. In one of the infected European crabs, it was noted by Chatton and Poisson that the hemolymph appeared opalescent. The milky appearance of the tissues which was observed in this study is probably caused by the opalescence of the parasite-laden hemolymph.

Chatton and Poisson were not able to study the complete life cycle of *H. perezi*, and did not describe a flagellated stage. Their identification was based on the similarities noted between *Hematodinium* and *Syndinium*, a genus found in copepods, and for which the free-swimming flagellated stages were known (Chatton, 1920, loc. cit.). Six characteristic similarities were noted by Chatton and Poisson as follows:

1) The plasmodial nature of the organism in its host
2) The presence of trichocysts in its cytoplasm
3) The dinokaryon-type of nucleus containing five chromosomes arranged in V-shapes, and the apparent absence of a nuclear membrane
4) The identical appearance of the chromosomes of the two genera
5) The continual state of mitotic activity of the nucleus
6) The type of mitosis exhibited (dinomitosis).

The parasite found in *Callinectes sapidus* agrees in all characteristics with *Hematodinium perezi*. The size of the unicellular stages of *H. perezi* is given as 8 to 9 μm in diameter, which agrees with the organism in the blue crab. In contrast to Chatton and Poisson, who found the organism in only three of over 3,500 specimens of *Portunus* (*Macropipus*) and *Carcinus*, we have found *Hematodinium* with great regularity in *C. sapidus* in all but the late winter–early spring period.

To the the best of our knowledge, this is the first report of this parasite in a decapod other than *Carcinus* and *Portunus* (*Macropipus*), and the first time this genus has been found in the western Atlantic. Our observations of infected crabs held in captivity lead us to believe that this organism is probably pathogenic and may cause large mortalities among blue crabs in enzootic areas along the east coast of North America.

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Martin W. Newman, U. S. Department of Commerce, National Marine Fisheries Service, Middle Atlantic Coastal Fisheries Center, Pathobiology Investigations, Oxford, Maryland 21654, **and Charles A. Johnson,** Duke University Marine Laboratory, Beaufort, North Carolina 28516. This research was supported in part by Grant No. GA-30943 from the National Science Foundation.

Use of Counterelectrophoresis to Detect Infections of *Fasciola hepatica*

Immunodiffusion studies show that precipitating antibody is found in the serum of rodents and man infected with *Fasciola hepatica* (Sewell, 1964, Immunology **7:** 671–680; Dawes and Hughes, 1964, Adv. Parasit. **2:** 97–168; Sinclair and Kendall, 1969, Res. Vet. Sci. **10:** 483–485; Everall, 1970, J. Clin. Path. **23:** 636–639). Precipitins against extracts of adult worms are observed in the serum of rabbits about 3 or 4 weeks after infection using Ouchterlony double immunodiffusion tests. Capron et al. (1964, Presse Med. **72:** 3103–3107) demonstrated these antibodies by immunoelectrophoresis and recommended the technique for the immunodiagnosis of fascioliasis.

In this research note I wish to report that another technique which also measures pre-