cleus and is reported from the fresh-water annelid *Limnodrilus udekemianus*.

3) *M. nemertis* (Debaisieux, 1919) (*7, 13*) has a spore with an excentric nucleolus and a conical operculum. It is found in the nemertean *Lineus bilineatus*.

4) *M. cernosvitovi* (Jirovec, 1936) (*7, 8*) has a central chromatin mass in the spore nucleus and was described from the fresh-water oligochaete *Opistocysta flagellum*.

5) *M. dentali* (Arvy, 1949) (*7, 14*) has a spore with a conical operculum and is found in the scaphopod *Dentalium* from Europe.

6) The spore of *M. pickfordae* (Barrow, 1961) (*7, 15*) possesses a collar and an excentric nucleolus and is found in fresh-water snails.

7) *M. louisiana* Sprague 1963 (*16*) has a larger spore than *M. nelsoni* and 3 to 4 peripheral chromatin granules in the nucleus. It was described from one specimen of the mud crab, *Panopeus herbstii*.

8) *M. ascidiarum* (Duboscq and Harrant, 1923) (*17*) has a central or slightly excentric nucleolus in its spore nuclei and was described from the ascidian *Amaroucium proliferum*.

Association of spores with vegetative stages formerly known only by the cryptic label "MSX" provides information about the sequence of life-cycle stages of *Minchinia nelsoni* in the oyster and further strengthens the placing by Haskin *et al.* of the organism in the genus *Minchinia*. However, the possibility of an alternate or intermediate host has not been eliminated, and spores identified in this study may provide workers with another stage to be used in future experiments on transmission. Such experiments and the determination of ecological conditions necessary for sporulation should enhance understanding of the epizootiology of this serious disease of American oysters.

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Fluorescent-Antibody Studies of Haplosporidian Parasites of Oysters in Chesapeake and Delaware Bays

Abstract. *A fluorescent antibody was produced against a plasmodial oyster parasite thought to be a haplosporidian,* Minchinia nelsoni, *which was termed MSX. The antibody reacted with this type of plasmodia from oysters,* Crassostrea virginica *(Gmelin), from all sources, and it also reacted with its sporulating and spore stages. This reaction indicates cospecificity of the stages. No reaction occurred with any stage of another oyster haplosporidian,* Minchinia costalis, *indicating that the* M. nelsoni *and the* M. costalis *are antigenically distinct species.*

A multinucleate, usually spherical organism, MSX (*1*), has been studied during the past 8 years because of its incrimination in considerable mortalities of oysters, *Crassostrea virginica*, of the Middle Atlantic Coast. The observed stages have suggested that the organism is a haplosporidian, and it was named *Minchinia nelsoni* on the basis of plasmodial morphology (*2*). When the spore stage, necessary for morphological characterization of the organism, is known, the organism can be readily identified (*1*). When the taxonomic position of the organism is not known, all but epizootic and pathological studies are hampered—particularly when the parasites have not yielded to laboratory transmission. Recently, however, Couch *et al.* (*3*) described a spore other than *Minchinia costalis* (*1*) in the oyster, which they associated with the plasmodial infections of *M. nelsoni*.

We have prepared a fluorescein-conjugated antibody from horse serum which is highly specific for *M. nelsoni*. This reagent can be used to diagnose and relate microorganisms from different hosts in the life cycle, to seek new stages, and to improve recognition of the parasites, at the same time eliminating other known parasites and associates from involvement in the study.

Cape Cod oysters (thought to be free of *M. nelsoni*) were homogenized, dehydrated, and frozen to produce a powdered oyster antigen that included antigenic substances and organisms from the mantle cavity and digestive system as well as from the oyster. It was used (i) as an antigen to produce antiserum for removal of nonspecific antigens from an extract of homogenized oyster tissue infected with *M. nelsoni*, providing a relatively pure antigen for a second host reaction; and (ii) to absorb oyster antibodies from antiserum to *M. nelsoni*. Six heavily infected oysters from Pocomoke Sound, Chesapeake Bay, were the source of the *M. nelsoni* antigen. The parasite anti-

gen was never dehydrated but was homogenized, extracted to remove oyster reactivity, and divided into dosage quantities which were frozen until needed.

The first injection was a 2-ml mixture of equal parts of one of the above antigens and Freund's adjuvant into the shoulder muscles of two horses. The *M. nelsoni* antigen was always diluted 1:10 in antiserum to oyster, to reduce the nonspecific reaction of the antiserum to *M. nelsoni*. The oyster antigen was mixed undiluted with adjuvant for the initial injection and diluted 1 : 2.5 in 0.85 percent saline for all other injections. The schedule of injection was the 10th, 20th, and 35th days after the first inoculation. Booster injections became necessary on the 100th and approximately 200th days. Horses were bled for serum at intervals of about 3 weeks, the first bleeding being on the 40th day.

Serum for conjugation was extracted aseptically from jugular blood and was immediately reacted with fluorescein isothiocyanate by the direct conjugation method (*4*). The preparation time was reduced by substituting extraction on a DEAE cellulose column for the recommended 4- to 5-day purification by dialysis. One to ten parts of phosphate buffer, *p*H 7.4 (*4*), were mixed with conjugate before applying it to the tissue, and all reactions were carried out at 37°C, for 1 hour.

The effects of fixatives and other histological procedures on the reactions obtained with the conjugate were examined in preliminary studies. The preferred fixatives were absolute methanol or 70 to 95 percent ethanol. Zenker's and Schaudinn's fixatives without acetic acid were slightly inferior to the alcohol, and poorest results were obtained with Davidson's fixative or any other combination containing formaldehyde and acetic acid (*5*). The conjugate reacted best with parasites after the tissues were sectioned on a cryostat or exposed as smears and smashes, rather than after other types of imbedding—although both celloidin and paraffin imbedding techniques gave dependable and reproducible results. Controls included the use of tissues with known sensitive parasites and the use of unconjugated antiserum to block the reaction. In some experiments the reaction specificities were also controlled by tests with normal horse serum, as well as with antiserum to *M. nelsoni* absorbed with dehydrated oyster antigen.

Intensity of autofluorescence was relatively high in all of the oyster tissues, but it was not sufficient to interfere with reading and interpretation of reactions with the parasites. It was practically absent in the "smash" preparations. The only other autofluorescence was observed in some of the dermal cells of an occasional oyster parasite, the larval trematode *Bucephalus* sp., which fluoresced at an intensity equal to that of the plasmodia. An undescribed haplosporidian hyperparasite of the trematode did not show autofluorescence; nor did any other parasite studied (*7*).

The conjugated antiserum to *M. nelsoni* was tested for specificity and cross reaction with 15 different disease entities, including two classes of Platyhelminthes and three subphyla of protozoa (Table 1). Also included were tests with three fungi, "Actinomycete" (*9*), "Mycelial" disease (*6*), and *Dermocystidium marinum* (*8*), a "rickettsia-like" organism, and tissue from a "necrosis-like" condition of the gut and a "lymphocytosis" of unknown etiology (*9*), all of which gave negative results. *M. nelsoni* plasmodia from widely separated geographic populations of oysters isolated by considerable land barriers were also tested to determine the presence of cross reactions within these populations using the conjugate created from one specific population of *M. nelsoni*. We also tested plasmodia and spores of *Minchinia costalis*, recognized as a separate but probably closely related species, and a haplosporidian hyperparasite (*7*) in *Bucephalus* sp. (*6*).

Reactions in this series involved only the sporozoa. *Minchinia costalis* (Fig. 1, *A* and *B*) from Chincoteague Bay showed negative reaction with the conjugate. The conjugate gave the strongest reaction with the plasmodial stages of *M. nelsoni* (Fig. 1*E*) in oysters from Lewes, Delaware, on Delaware Bay; from Franklin City, Virginia, on Chincoteague Bay; from Manokin River (Fig. 1*D*) and Marumsco Bar, Pocomoke Sound, Maryland, in the Chesapeake Bay; and from Wreck Shoal, Virginia, in the James River. Plasmodia and sporulating stages of *M. costalis* did not react with the conjugate in mixed infections with the similar stages of *M. nelsoni* even though the latter gave exceptionally strong reac-

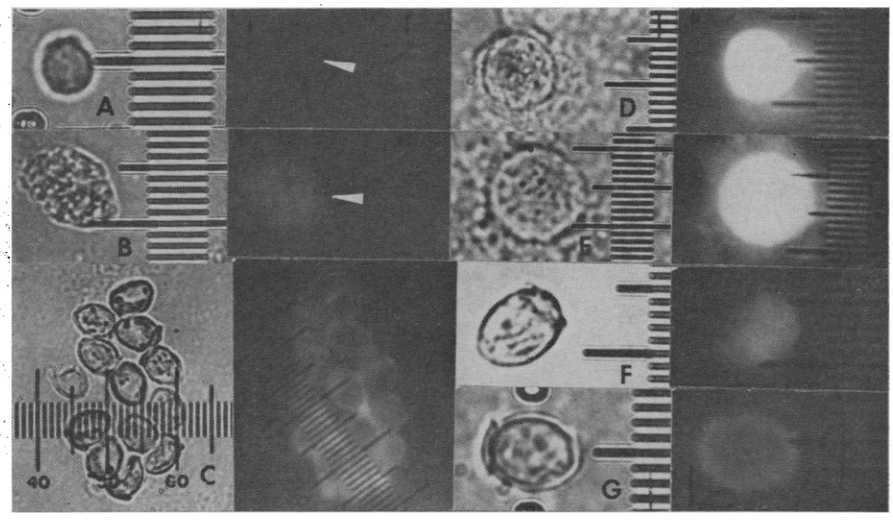

Fig. 1. Reaction specificity of the anti-*Minchinia nelsoni* fluorescent conjugate with available haplosporidians from oysters. Each pair of pictures is an identical magnification of the same specimen that has been reacted with the same stock of antiserum to *Minchinia nelsoni*; those to the left are specimens seen through full-spectrum transmitted light, and those to the right are identical specimens viewed through the fluorescent microscope. (Oil immersion with the included scale divisions = 1.07 μ in every case.) Identical exposure times of the original fluorescent negatives were used (10 minutes); Kodak contrast process film was used for the negatives. *A*, Spore of *Minchinia costalis; B*, plasmodium of the same—note absence of fluorescense in the above (arrows); *C*, mature sporocyst of *Minchinia nelsoni*—note fluorescence in the cyst wall as well as in the capsule of the spores; *D* and *E*, plasmodia of *Minchinia nelsoni* showing the same intensity of reaction in samples from Chesapeake Bay (*D*) and Delaware Bay (*E*); *F*, immature spore of *Minchinia nelsoni*—both the capsule and sporoplasm reacting; *G*, the mature spore—only the capsule reacting. Sporogonic stages through the sporoblast could not be distinguished from *D* and *E* in their fluorescence.

Table 1. Reactions of various recognized parasites and disease entities with fluorescent antibody to *Minchinia nelsoni*. ++++, Maximum fluorescence, brilliant yellow green; +++, slightly less fluorescence; ++, definite fluorescence, yellow green, less brilliant; —, no fluorescence.

Test organism	Ref.	Type of reaction
Platyhelminths		
Trematoda		
Bucephalus sp.	(9)	—
Cestoda		
Tylocephalum sp.	(9)	—
Protozoa		
Sarcomastigophora		
Hexamita sp.	(8)	—
Sporozoa		
Nematopsis ostrearum	(6)	—
Minchinia costalis	(1,2)	—
Minchinia nelsoni		++++
Same, Delaware Bay	(1,2)	++++
Same, Chincoteague Bay	(3)	++++
Same, Chesapeake Bay	(1,3)	++++
(Also amoebula in specimens)		++++
Same from Wreck Shoal, James R., Va.*	(1)	+++
Haplosporidium sp. (Hyperparasite of *Bucephalus* sp.)	(7)	++
Ciliophora		
Ancistrocoma sp.	(6)	—
Thallophyta		
Fungi		
"Actinomycete" disease	(9)	—
"Mycelial" disease	(6)	—
Dermocystidium marinum	(8)	—
Diseases of unknown etiology		
"Rickettsial" disease	(9)	—
"Necrosis" of the gut	(9)	—
"Lymphocystosis"	(9)	—

* Not ideal fixation for this study.

tions. It was possible to follow a series of equally strong reacting stages of *M. nelsoni* through schizogony and sporogony (Fig. 1*C*). The intensity of reaction of the prespore stage was equal to the plasmodial stages (Fig. 1, *D* and *E*) and was not included in the illustration. Fluorescence occurred in the cytoplasm of sporoblasts and in the sporoplasm and capsule of the immature spore (Fig. 1*F*). The conjugate appeared unable to penetrate the mature spore wall and stained only the capsule (Fig. 1*G*). The only other organism in the oysters that fluoresced in the conjugate was an amoebula, which was either part of the schizogony cycle that has not been recognized or a rhizopoda. It was present in two of the infected oysters. Since whole oysters were used in these studies, the antigens included any organisms in the mantle cavity and the digestive system. When the distance separating the origins of these shellfish is considered, the degree of specificity is truly remarkable. Until further studies can demonstrate the relationships of this amoebula in the oysters, it is even possible that this reaction represents unabsorbed rhizopodal antigen. The amoebula was recognized in the gills, fluorescing as brightly as the plasmodia. The *Haplosporidium* sp. (*7*) hyperparasite of the trematode, *Bucephalus* (*6*), reacted in all stages of its life cycle with much less intensity than the *M. nelsoni*.

Since the intensity of reaction of the stages of sporogony tested in these studies of conjugated antiserum to *M. nelsoni* is unlike the reaction of any plasmodia stage of *M. costalis* with the same conjugate, since the reactions with the prespore stages are identical to those with the plasmodial stages in oysters from several geographic areas, and since Couch *et al.* (*3*) have demonstrated morphological bases for describing this as a distinct species, we believe that the foregoing evidence establishes the spore of Couch *et al.* (*3*) as the spore of *Minchinia nelsoni* and serves as further evidence that at least two species of haplosporidian exist in the oyster of the mid-Atlantic estuaries.

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Oxygen as a Primary Species in Radiolysis of Water

Abstract. *Recent results are summarized of γ-radiolysis of dilute nitrate solutions at neutral* p*H in the presence of oxygen and hydrogen as radical scavengers. Complete analysis of the system leads to values of the primary yields showing a net deficit of oxidized products of ~0.4. Analysis of results already available for the oxygen yield shows that oxygen originated from the radiolysis of water with a* g *value of ~0.1* (g *values are derived values for yields of primary species for 100 electron volts). This finding gives material balance and provides evidence of oxygen being a product of water radiolysis.*

Radiolysis of water and aqueous solutions is commonly described in terms of primary species produced in yields independent of the solute (*1*). Well-established primary species are H_2, H_2O_2, OH, H, and e^-, the hydrated electron. However, recent quantitative determinations of these yields indicate a material-balance deficit of 0.6 ± 0.2 based on $\Sigma\, g(\mathrm{OH}) + 2g(\mathrm{H_2O_2})$ (*2*) (*g* signifies derived values for yields of primary species for 100 ev; *G* signifies the experimentally measured yield of product for 100 ev absorbed energy). To account for this deficit, Allen (*3*) suggested that there may be another, hitherto unnoticed, oxidizing species produced in water radiolysis, perhaps the oxygen atom. We now report experimental evidence that oxygen is a product of water radiolysis and indicating that it may originate as O atoms.

Recent work (*4*) on the γ-radiolysis of dilute nitrate solutions enables one to propose a mechanism that completely accounts for the experimental observations. The mechanism is based on the reduction of NO_3^- to NO_2 by e^- and H, NO_2 undergoing dismutation to yield NO_2^-; OH radicals reoxidize NO_2^- to NO_2. In the absence of added scavengers, $G(NO_2^-)$ is essentially given by $\frac{1}{2}\,(ge^- + g\mathrm{H} - g\mathrm{OH})$. Molecular hydrogen acts as an OH scavenger, the nitrite yield then becoming $\frac{1}{2}\,(ge^- + g\mathrm{H} + g\mathrm{OH})$. This aspect of the mechanism is confirmed by our evaluation of the rate-constant ratio: $k(\mathrm{OH} + \mathrm{H_2}) : k(\mathrm{OH} + \mathrm{NO_2^-}) = 0.8 \times 10^{-2}$. Determination of $g(e^-)$ is based on the competition between oxygen and nitrate for the solvated electron, for which we obtain $k(e^- + \mathrm{O_2}) : k(e^- + \mathrm{NO_3^-}) = 2.5$. The mechanism thus involves no unusual spe-