A LONG-TERM STUDY OF "MICROCELL" DISEASE IN OYSTERS WITH A DESCRIPTION OF A NEW GENUS, *MIKROCYTOS* (G. N.), AND TWO NEW SPECIES, *MIKROCYTOS MACKINI* (SP. N.) AND *MIKROCYTOS ROUGHLEYI* (SP. N.)

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ABSTRACT

Continuing long-term studies of oyster disease problems have been carried out over the past 26 years using field monitoring, gross, histologic, and ultrastructural pathologic methods.

A microorganism of uncertain taxonomy was discovered in 1963 by J. G. Mackin in association with lesions and mortalities of Japanese oysters, *Crassostrea gigas*, from Denman Island, British Columbia, Canada. Mackin coined the term "microcell" for this organism and described the parasite as 1–3 μm cells with small nuclei which occurred within vesicular connective tissue cells adjacent to characteristic abscesses. We are describing this organism as *Mikrocytos mackini* sp. n. in his honor. Similar appearing organisms were seen by the senior author in flat oysters, *Ostrea edulis*, from Milford, Connecticut, on three different occasions: 1) in oysters transferred from Milford, Connecticut, to Chincoteague Bay, Virginia; 2) in oysters transferred from Milford to Elkhorn Slough, California; and 3) in oysters transferred from Milford to Oxford, Maryland, and held in recirculated sea water. The causative organism in these three episodes has been shown by electron microscopy to be *Bonamia ostreae*, the parasite that was implicated in recent mortalities in flat oysters in Europe. Similar organisms have also been seen in Olympia oysters, *Ostrea lurida*, from Oregon and in the Sydney rock oyster, *Saccostrea commercialis*, from Australia. Presence of the organism in the latter species is associated with the winter mortalities originally described by T. C. Roughley, and the pathogen is here described as *Mikrocytos roughleyi* (sp. n.) in his honor.

"Microcell" type parasites of oysters are associated with a complex of diseases that occur in Japanese oyster, *Crassostrea gigas*; Sydney rock oyster, *Saccostrea commercialis*; flat oyster, *Ostrea edulis*; and Olympia oyster, *O. lurida*, in North America, Europe, and Australia. Severity of disease varies from an acute, highly lethal form to a chronic, seasonally recurring disease that does not produce massive mortalities. The etiologic agents are small, morphologically simple, and very difficult to compare and characterize taxonomically at light microscope levels of resolution. Associated lesions vary according to species affected and provide some of the differences that may be used to distinguish the agents involved. The complexity of this group and the difficulties involved in achieving an understanding regarding whether we are dealing with one or a group of organisms and how they were transferred to new locations, the long time span involved in answering these questions, and the continuing dissemination of unpublished privileged information shared in informal workshop gatherings of scientists with common interests, make it necessary to use unpublished anecdotal information in order to provide as complete a story as possible.

The first oyster mortality known to be associated with "microcell" disease was reported in *C. gigas* from Denman Island, British Columbia, Canada by Quayle (1961). Quayle's report documents the epizootic aspects from 1956 to 1960 and demonstrates the gross appearance of the disease in the Pacific oyster. A causative agent was not identified until several years later, when the late J. G. Mackin[4] (unpubl. data) discovered a small intracellular organism intimately associated histologically with tissue abscesses in diseased oysters (*C. gigas*) from Denman Island and called this organism "microcell". He demonstrated this material at the 1963 Shellfish Mortality Conference held at Oxford, MD.

Mackin's demonstration provided us with the insight to identify similar organisms in histologic sec-

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Manuscript accepted May 1988.
FISHERY BULLETIN: VOL. 86, NO. 3, 1988.

tions of Denman Island oysters in our laboratory collection and further led to the discovery of microcell disease in *O. edulis*, *O. lurida*, and *S. commercialis* from Australia ("Australian winter disease") described by Roughley (1926). In 1979, the characteristics of microcell disease were demonstrated at a microscopic diagnosis workshop held at the Ministry of Agriculture and Fisheries Laboratory in Weymouth, England. This workshop was attended by molluscan pathologists from several European countries including France. In the late 1970s, serious mortalities of *O. edulis* in France, associated with microcell infections, were described in a paper by Pichot et al. (1979), in which the organism was named *Bonamia ostreae*. Reference to the earlier work on microcell by Mackin and others (Katkansky et al. 1969) was not included in their report.

Since the taxonomic relationship and status of these similar parasites have not been described, it is the purpose of this paper to present a complete background on microcell related epizootics and morphological information which, in North America, preceded the French report (Pichot et al. 1979). Furthermore, microscopic and ultrastructural comparisons of the microorganisms are provided and a new genus, *Mikrocytos* g. n., and two new species, *Mikrocytos mackini* sp. n. and *Mikrocytos roughleyi* sp. n., are described.

MATERIALS AND METHODS

General Procedures

Oyster tissues were collected from a variety of sources as follows:

Code WWC were *C. gigas* collected from Henry Bay, Denman Island, British Columbia, Canada on a periodic basis from April 1968 to June 1969 by D. B. Quayle (spring 1969 samples were collected by N. Bourne). Live oysters were sent by air freight to the Oxford Laboratory where clinical and gross features were recorded and they were processed for histological and, in some cases, ultrastructural studies.

Code S-124-A were *C. gigas* from Hawaii collected in September 1972.

Code S-41 were *S. commercialis* collected by Peter Wolf from 22 July to 23 July 1965 from the Georges River, Woolooware Bay, New South Wales and shipped to the Oxford Laboratory for processing.

Code FK were progeny of *O. edulis* from Boothbay Harbor, ME that were spawned at Milford, CT in April 1961. Seed oysters were transplanted to Chincoteague Bay, VA in May 1961 and processed at subsequent intervals (FK-1-1 to FK-2-5, August 1961; FK-3-1, August 1961; FK-4-1 and FK-4-2, February 1962).

Code WAC were *O. edulis* bred in the Milford, CT hatchery from 1963 to 1965, and introduced into California bays as follows:

WAC-1, Milford 1963 seed oysters planted in Morro Bay, CA in December 1964 and sampled on 7 December 1965 during a heavy mortality.

Sample WAC-21-28 consisted of 38 oysters from a Milford 1963 stock shipped to California in 1964 and held at Pigeon Point Laboratory, Pigeon Point, CA until heavy mortality occurred and sampled on 11 May 1966.

WAC-3-1-10 were 1963 Milford oysters placed in Morro Bay in 1964. Heavy mortality was noted and samples were taken. WAC-3-11-15 were 1963 Milford stock placed in Morro Bay in 1964. Mortality was low. Oysters were necropsied and fixed on 1 May 1966. WAC-3-16-26 were Milford 1962 stock placed in Morro Bay in 1963. They experienced 40% mortality and were examined and fixed on 1 May 1966. Oysters WAC-4-1 through 4-5 were from Milford 1963 stock placed in Tomales Bay, CA in 1964; low mortality was observed. They were examined and fixed on 1 May 1966.

Code FMT were *O. edulis* used in an experimental holding study at the Oxford Laboratory. Ten 2-yr-old *O. edulis* from the Milford hatchery were placed in each of three tanks receiving 0.45 μm membrane filtered 26‰ seawater on 23 February 1968; pH, temperature, salinity, and mortality were monitored daily until 10 October 1968. *Crassostrea virginica* from the Mispillian River, Delaware Bay, were placed in each tank on 28 March. Tanks were designated A (control), B (fed tissues of moribund *O. edulis* from Pigeon Point, CA), and C (fed tissues from Denman Island *C. gigas* infected with Denman Island disease). Seawater in each tank was recirculated through a glass wool, charcoal, calcium flow-through filter via an airlift system moving from C to B to A, respectively. Oyster codes were FMT-A-1-20, FMT-B-1-20, FMT-C-1-20, numbered as they were fixed (species were designated at time of fixation).

Code WAO-A were 50 specimen samples of native oysters (*O. lurida*) from Yaquina Bay, OR, sampled

monthly from February 1969 to January 1971. Samples were coded from WAO-7A to WAO-22A.

Tissues were fixed in Zenker's acetic, Davidson's fluid (Shaw and Battle 1957), or McDowell's fixative (McDowell and Trump 1976) as modified by Farley et al. (1986) (1% glutaraldehyde/4% formaldehyde; pH 7.2–7.4 in one-half ambient seawater). Six μm sections were stained with Harris' hematoxylin-eosin, Ziehl's fuchsin, periodic acid Schiff reagent (PAS) with malt diastase digestion, Feulgen picromethyl blue (Farley 1969), or Giemsa (Howard and Smith 1983).

Electron Microscopy Procedures

Lesions from *C. gigas* from Denman Island were fixed in 2% glutaraldehyde in pH 7.2 seawater, postfixed in 1% osmium tetroxide in phosphate buffer, pH 7.2, and embedded in Epon-Araldite (Feng et al. 1971). Fifty to 100 nm sections, selected on the basis of interference color (silver), were cut and stained with lead citrate and uranyl acetate. *Ostrea edulis* sections from the WAC material (WAC-2-19) were deparaffinized, postfixed in 2% glutaraldehyde and 1% osmium tetroxide, plastic embedded, ultrasectioned, and stained in the same fashion as the *C. gigas* materials. Sections were examined in a Zeiss[5] EM 9 electron microscope.

RESULTS

"Denman Island Disease" Studies

Table 1 presents the seasonal prevalence of gross features of samples collected in this study. Visual condition index remained high (most oysters in medium to fat condition). Mantle recession occurred most commonly from April through June and was most prevalent in June. Pale digestive gland was present in up to 24% of the oysters in the spring and was also seen in up to 16% of the oysters in fall samples. Shell pustules (Fig. 1), abscesses, and ulcers as described by Quayle (1961) (Fig. 2) were present from April through June.

Microscopical examination (Table 2) revealed granular hemocyte infiltration of vesicular connective tissue (VCT) in most samples with high prevalences of microcell infections occurring sporadically throughout the years. Abscesses (Fig. 3) in the VCT of the mantle and gonad consisted of apparently viable granular hemocytes at the periphery with phagocytosis of moribund cells deeper in the lesion and coagulative necrosis in the center. Microcell organisms were associated with these abscesses. The parasite (Fig. 4) was 1–3 μm in diameter, contained a small (1 μm) Feulgen-positive nucleus, and occurred cytozoically in VCT cells and extracellularly adjacent to and within abscesses. Microcells occurred in 60% of the abscesses found in histologic sections. However, microcells were never found outside of abscesses.

A similar disease was discovered by F. Kern[6] in *C. gigas* from Hawaii. Microcell parasites were similar in morphology and size (Figs. 5, 6) to the Denman Island organism, but infections were more

[5]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[6]F. G. Kern, Northeast Fisheries Center Oxford Laboratory, National Marine Fisheries Service, NOAA, Oxford, MD 21654, pers. commun.

TABLE 1.—Gross pathology in oysters from Denman Island, B.C.

Code	Date	Number in sample	% fat condition	% medium condition	% watery condition	% mantle recession	% pale digestive gland	% shell pustules	% with abscesses
WWC-1	22 May 1967	27	88	11	0	11	0	7	7
WWC-2	5 June 1967	28	100	0	0	11	7	14	7
WWC-3	10 Apr. 1968	25	88	4	8	78	24	0	16
WWC-4	22 Apr. 1968	25	100	0	0	28	0	4	12
WWC-5	24 May 1968	25	100	0	0	0	0	0	4
WWC-6	24 June 1968	25	92	8	0	8	0	4	0
WWC-7	5 Aug. 1968	25	48	48	7	8	0	0	0
WWC-8	14 Oct. 1968	25	100	0	0	0	0	0	0
WWC-9	18 Nov. 1968	25	76	24	0	0	16	0	0
WWC-10	27 Jan. 1969	25	44	56	0	0	4	0	0
WWC-11	26 Feb. 1969	18	33	61	6	0	0	0	0
WWC-12	21 Mar. 1969	25	96	7	0	0	0	0	0
WWC-13	11 Apr. 1969	25	100		0	0	4	16	16
WWC-14	12 May 1969	25	72	28	0	0	0	12	12
WWC-15	20 June 1969	25	88	12	0	12	0	4	0
WWC-18	9 June 1980	42	95	2	2	19	0	17	17

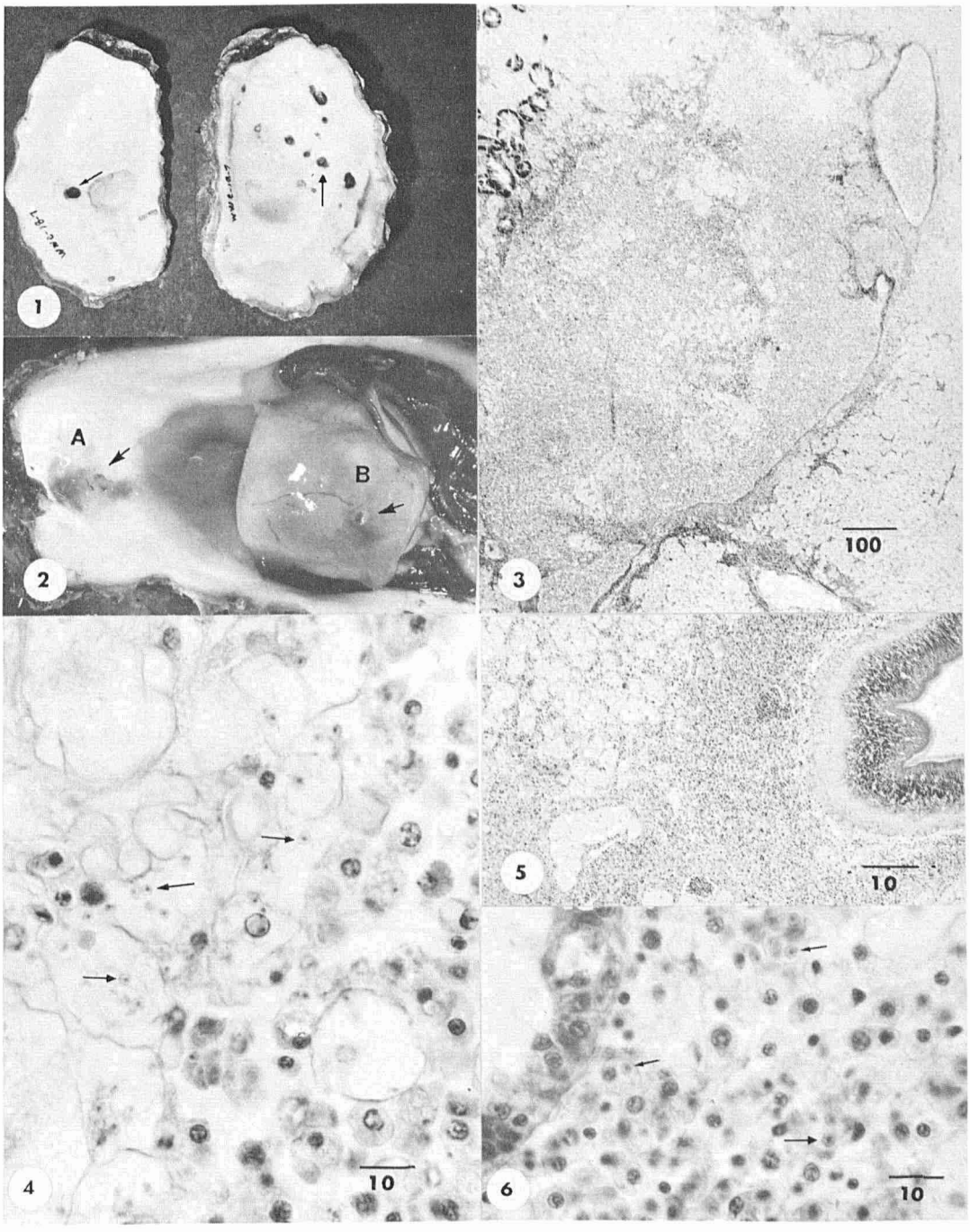
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TABLE 2.—Percentage of prevalance of lesions and parasites in Denman Island oysters.

Code	Date	Granulocyte infiltration (%)	Tissue abscess (%)	Hyaline hemocyte infiltration (%)	Neoplasms (%)	Microcell infection (%)	Mytilicola (%)	Ciliates	
								Gill (%)	Other (%)
WWC-1	22 May 1967	33	7	0	0	7	7	4	4
WWC-2	5 June1967	4	18	0	0	14	4	4	0
WWC-3	10 Apr. 1968	40	16	0	0	4	0	0	0
WWC-4	22 Apr. 1968	0	16	0	0	16	0	0	4
WWC-4	24 May 1968	8	4	0	0	4	0	0	4
WWC-6	24 June1968	32	4	24	0	0	0	0	0
WWC-7	5 Aug. 1968	0	0	0	0	0	0	0	0
WWC-8	14 Oct. 1968	36	0	0	4	0	0	0	0
WWC-9	18 Nov. 1968	12	7	36	8	0	0	0	0
WWC-10	27 Jan. 1979	20	0	8	0	0	0	0	0
WWC-11	26 Feb. 1969	4	0	0	0	0	0	0	0
WWC-12	21 Mar. 1969	8	4	0	0	4	8	0	0
WWC-13	11 Apr. 1969	20	20	8	0	12	0	4	4
WWC-14	12 May 1969	24	32	0	8	16	0	0	0
WWC-15	30 June1969	8	12	0	0	0	0	0	0
WWC-18	9 June1980	4	20	0	0	16	8	0	0

systemic with diffuse inflammatory infiltration of connective tissue (Fig. 5) associated with the presence of microcell parasites (Fig. 6). Microcells were cytozoic in hemocytes and VCT. Focal abscesses were present but not nearly as prominent in the Hawaiian oysters as the lesions in Denman Island oysters.

Australian Winter Disease Studies in *Saccostrea commercialis*

Australian winter disease was characterized by pustules, ulcerations, and abscesses (Fig. 7). Seven individuals, consisting of six females and one male, displayed ulcerations in the gonad and mantle. Ulcerations of the gills were also common and frequently occurred near the adductor muscle. Impaired adductor muscle contraction was characteristic of the disease.

Histologically, the animals contained abscesses (Fig. 8) with intense phagocytic infiltrations in the connective tissue and varying degrees of necrosis. The abscesses contained a small (1–2 μm) organism (Figs. 9, 10) which contained a nucleus >1 μm that was spherical with bipolar or eccentric nucleolar structures. The size and cytozoic location of these organisms suggest a strong similarity to other microcell type parasites seen in other species of oysters. Four of the six females had gonads in a state of resorption and digestive diverticular epithelium was slightly metaplastic in two of the seven oysters.

Microcell Disease Study in *Ostrea edulis*

Episode 1

Microcell disease in *O. edulis* from progeny from Boothbay Harbor, ME brood stock spawned at Milford, CT and transferred to Chincoteague Bay, MD (Code FK).

Two of the 13 oysters fixed between August 1961 and July 1962 had heavy infection of microcells (Fig. 13) which were 1–3 μm in diameter with a Feulgen-positive nucleus (1 μm diameter) and found intracellularly within hemocytes. In three of the other oysters, moderate infiltrations of hemocytes were observed but without parasites.

FIGURE 1.—Right and left valves of *Crassostrea gigas* from Denman Island, British Columbia. The darker rounded lesions are conchiolinous shell pustules characteristic of the Denman Island disease.

FIGURE 2.—Scale units in Figures 2-15 are in micrometers. Macrophotograph showing shell pustule (A) and adjacent tissue ulcer (B).

FIGURE 3.—Histologic section from Denman Island oyster. A large abscess-type lesion is apparent in the connective tissue-gonad region of the section. 100×. Feulgen picromethyl blue (FPM) stain (specimen WWC-2-7).

FIGURE 4.—Higher magnification photomicrograph taken at the edge of the lesion in Figure 3. Many microcell protistan parasites (*Mikrocytos mackini* sp. n.) are evident in vesicular connective tissue cells adjacent to necrotic inflammatory cells deeper in the lesion. 1,000×. Harris' hematoxylin and eosin (HHE) stain (specimen WWC-2-7).

FIGURE 5.—Lesion caused by microcell infection in *C. gigas* from Hawaii. 100×. HHE stain (specimen S-124A-45).

FIGURE 6.—High magnification photomicrograph showing microcell parasites intracellular in hemocytes in vesicular connective tissue of the Hawaiian oyster. 1,000×. HHE stain (specimen S-124A-45).

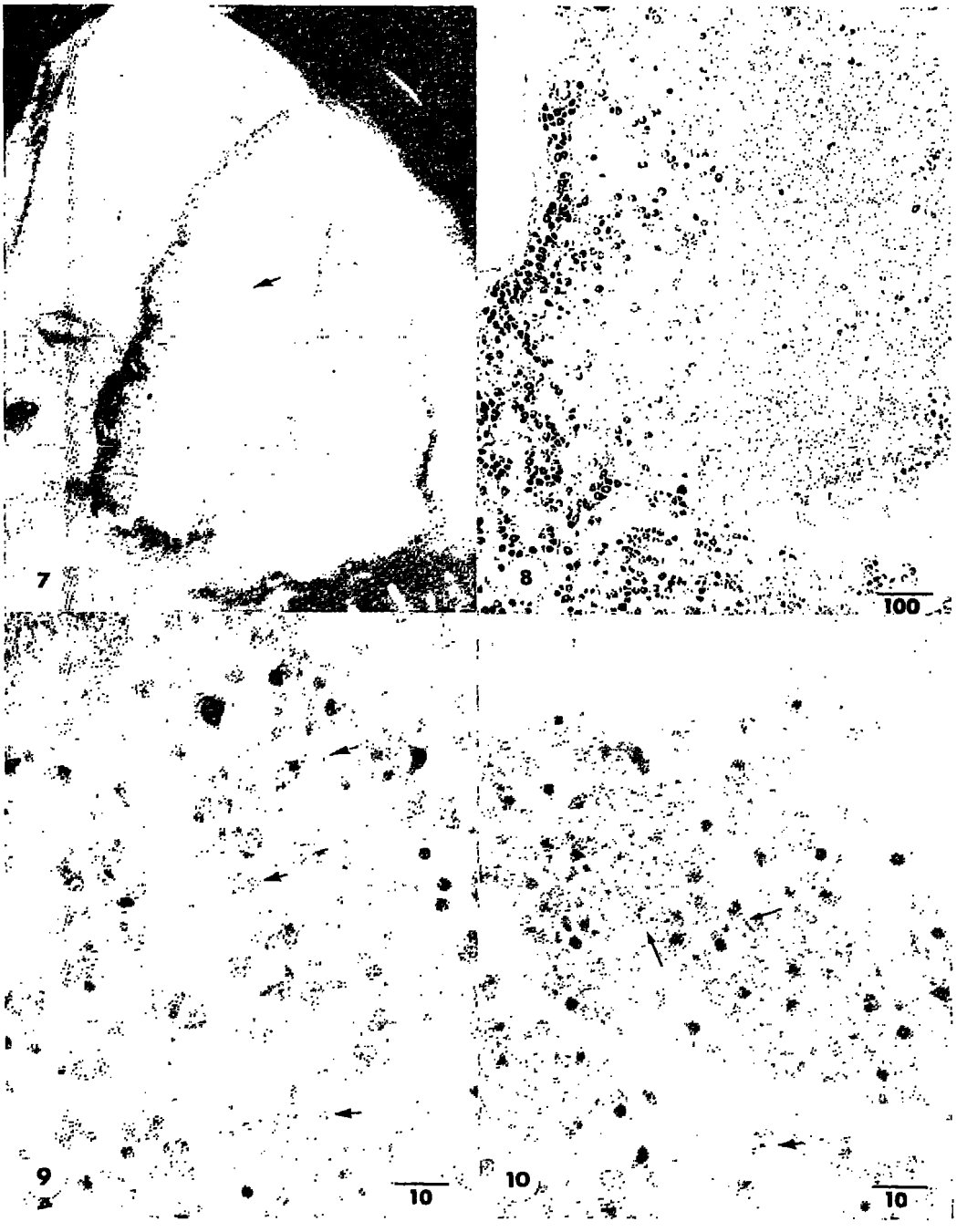


FIGURE 7.—Ulceration in the mantle of *Saccostrea commercialis* with Australian winter disease.

FIGURE 8.—Large abscess-type lesion in the connective tissue-gonad region of *Saccostrea commercialis* (code S-41-7) from Australia with Australian winter disease. 100×. HHE stain (specimen S-41-7).

FIGURE 9.—Higher magnification photomicrograph from lesion in Figure 8. Note intracellular microcell parasites (*Mikrocytos roughleyi* sp. n.) with hemocytes. 1,000×. HHE stain (specimen S-41-7).

FIGURE 10.—Heavy infection of Australian winter disease; microcell parasites in the gonad of S-41-4. 1,000×. HHE stain.

Episode 2

Microcell disease in *O. edulis* transferred from Milford, CT to Elkhorn Slough, CA (Code WAC).

Epizootiological gross and histopathologic data are presented in Table 3. Clear association is apparent between high mortality, emaciation (watery condition), mantle recession, hemocytic infiltration, and heavy microcell infection.

Infections were particularly intense in gill and GI tract epithelia which contained dense infiltrations of infected hemocytes (Fig. 11). Infections were characterized by the intracellular (in hemocytes) and extracellular presence of "microcell" organisms (Fig. 12) similar to those seen in the Chincoteague Bay (FK) samples.

Episode 3

Microcell disease in *O. edulis* transferred from Milford, CT to aquaria in Oxford, MD (Code FMT).

The experiment ran from 20 February 1968 until 9 October 1968. No transmission was noted. However, tank C drained accidentally on 19 March and the filtered seawater was replaced with unfiltered seawater from Chincoteague Bay, VA. A combined total of 9 of 11 animals died between 22 March and 27 March and the remaining 2 live oysters were fixed on 28 March.

The first six that died were from tank C and had moderate to heavy cases of microcell disease with associated hemocytic infiltration. Histologic examination of both species, either after mortalities, or when they were sacrificed in October, failed to reveal any more infections. The parasites and disease characteristics were identical to the previously described cases (Fig. 14).

Microcell Disease Study in *Ostrea lurida*

Microcell disease in *O. lurida* from Yaquina Bay, OR (Codes WAO-7-A and WAO-12-A).

Monthly to bimonthly samples of 50 specimens were examined grossly and for histopathology for a 2-yr period. A 24% prevalence of microcell disease was seen in WAO-7-A samples collected in February 1969, and 12% prevalence was seen in the WAO-12-A samples collected in February 1970. No microcell infections were seen in any of the other samples. However, neoplasms and *Mytilicola orientalis* infections were seen commonly throughout the study.

Microcell infections tended to be less intense than in *O. edulis*, but organisms were similar in size and appearance to the organisms seen in flat oysters (Fig. 15). Intracellular infections were in VCT cells and not hemocytes.

Ultrastructural Studies

Electron microscopy revealed *O. edulis* parasites that were usually intracellular with up to three organisms per cell. Parasites were 1–3 μm in diameter, and contained an eukaryotic nucleus about 1 μm in diameter. A crescent-shaped, peripheral nucleolus was evident in most organisms (Fig. 16). The cytoplasm contained numerous ribosome-like organelles and spherical dense bodies, presumably membrane bound, which were 90–130 nm in diameter, and generally resembled the "haplosporosomes" (Fig. 17) described in other haplosporidan

TABLE 3.—Epizootiology of microcell disease in *O. edulis* from California.

Code	Stock origin	Sample location	Date fixed	Mortality	*N*	Condition			Mantle recession	Hemocyte infiltration	Microcell infection
						Fat	Med	Watery			
WAC-1 (1–20)	Milford 1964	Morro Bay	7 Dec. 1965	Heavy	20	no gross data				18	18
WAC-2 (1–25)	Milford 1964	Pigeon Pt.	1 May 1966	Heavy	38	1	8	19	22	25	25
WAC-3 (1–10)	Milford 1964	Morro Bay	1 May 1966	Heavy	10	2	6	2	6	8	8
WAC-3 (11–15)	Milford 1963	Morro Bay	1 May 1966	Low	5	4	1	0	0	0	0
WAC-3 (16–26)	Milford 1963	Morro Bay	1 May 1966	40%	10	6	4	0	1	6	3
WAC-3 (27–31)	Milford 1965	Morro Bay	1 May 1966	0	5	5	0	0	0	0	0
WAC-4 (1–5)	Milford 1964	Tomales Bay	1 May 1966	Low	5	5	0	0	1	0	0

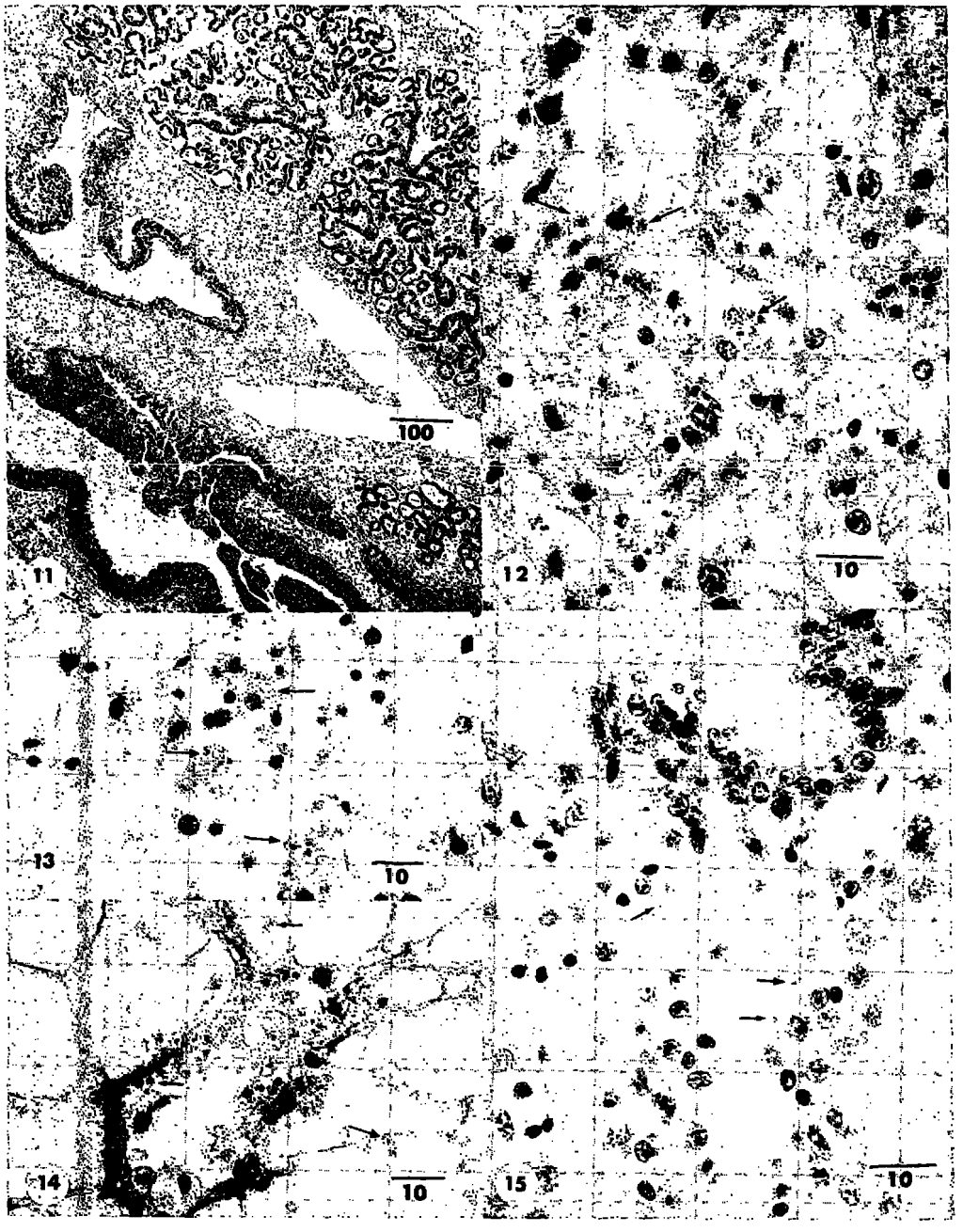


FIGURE 11.—Low magnification view of *Ostrea edulis* from California (WAC-2-29). Note diffuse inflammatory infiltrate in vesicular connective tissue. 100×. HHE stain.

FIGURE 12.—Microcell infection in *Ostrea edulis* (specimen WAC-2-19) from population of oysters introduced into California waters from Milford, CT. 1,000×. HHE stain.

FIGURE 13.—Microcell infection in hemocytes in *Ostrea edulis* transported from Milford, CT to Chincoteague Bay, VA in 1962 (first diagnosed case of *Bonamia ostreae*). 1,000×. HHE stain (specimen FK-5-1).

FIGURE 14.—Microcell infection in *Ostrea edulis*. Transferred from Milford, CT to Oxford, MD recirculated seawater aquarium. Cells of the vesicular connective tissue are infected. 100×. HHE stain (specimen FMT-B-1-3).

FIGURE 15.—Microcell infection in vesicular connective tissue and hemocytes of *Ostrea lurida* from Yaquina Bay, OR. 1,000×. FPM stain (specimen WAO-7A-41).

parasites (Perkins 1979). Larger "membrane" bound structures (500 nm) consistent with the appearance of mitochondria were also present in the cytoplasm; however, cristi could not be distinguished.

Ultrastructural studies were also performed on positively diagnosed oysters (*C. gigas*) from Denman Island, British Columbia (sample WWC-18, collected 9 June 1980). Microcells were always associated with focal abscesses, but their occurrence was restricted to the periphery. Parasites were found as cytozoic organisms primarily in VCT cells (Fig. 18). Microcells were 3–4 μm in diameter, had nuclei 1 μm in diameter, and nucleoli 250–300 nm in diameter. Nucleoli were spherical, eccentrically located within the nucleus, but never peripheral (Fig. 19). One to many parasites occurred within the cytoplasm of vesicular cells. None were ever found within hemocytes. Figure 20 shows an organism possibly undergoing division. The cytoplasm was densely packed with free ribosomes and contained a variety of organelles as follows: double membrane bound dense bodies 50–185 nm in diameter (Fig. 19A); double membrane bound, dumbbell-shaped structures (Figs. 20, 21h), approximately 37 × 18 nm to 85 × 260 nm; and dense bodies 40–45 nm in diameter that appeared to be membrane bound, and a suggestion of six- and five-side angularity (Figs. 19, 20, 21v). Endoplasmic reticulum was extremely sparse if present at all. The plasma membrane complex consisted of possibly two membranes with the external membrane containing dense material. An electronlucent zone was present around the cell, suggestive of a glycocalyx.

Taxonomic Descriptions

The information acquired on these diseases and the organisms associated with them and outlined previously allows us to propose taxonomic descriptions of them.

Mikrocytos g. n. (*Protista incerta sedis*) (little cell) — Definitive life cycle stages that would permit higher classification of this protistan parasite have not been observed. Named after the term microcell as coined by the late John G. Mackin. Small (1–4 μm), unicellular, protistan, cytozoic parasite normally infecting VCT cells of oysters. Always associated with abscess-type focal inflammatory lesions.

Type species - *Mikrocytos mackini* sp. n.

Bonamia, the other closely similar genus, infects hemocytes of ostreid oysters only and is associated with systemic non-abscess type disease manifestations.

Mikrocytos mackini sp. n. — Named in honor of the late John G. Mackin who discovered this parasite in the early 1960s.

Type specimen - A 6 μm thick hematoxylin-and-eosin-stained histologic section of an infected oyster, *C. gigas* (WWC-2-7), was deposited in the Registry of Marine Pathology, Northeast Fisheries Center, Oxford, MD 21654.

Host - *Crassostrea gigas*

Type locale - Henry Bay, Denman Island, British Columbia, Canada.

Range - Occurrence confined to above site. (A closely related candidate for inclusion within this species was found in *C. gigas* from Hawaii.)

Morphologic characteristics - Small, 1–4 μm intracellular parasites of VCT cells; infections always associated with focal inflammatory tissue abscesses. Parasites are unicellular and contain a small 1 μm nucleus that has an eccentric nucleolus 250–300 nm in diameter. The cytoplasm contains dense, double membrane bound, dumbbell-shaped haplosporosome-type organelles 50–180 nm in diameter and 40–45 nm membrane bound five- and/or six-sided dense bodies.

Mikrocytos roughleyi sp. n. — Named in honor of T. C. Roughley who published the initial study of the Australian winter disease in the 1920s.

Type specimen - A 6 μm thick hematoxylin-and-eosin-stained section of an infected oyster, *S. commercialis* (S-41-7), was deposited in the Registry of Marine Pathology, Northeast Fisheries Center, Oxford, MD 21654.

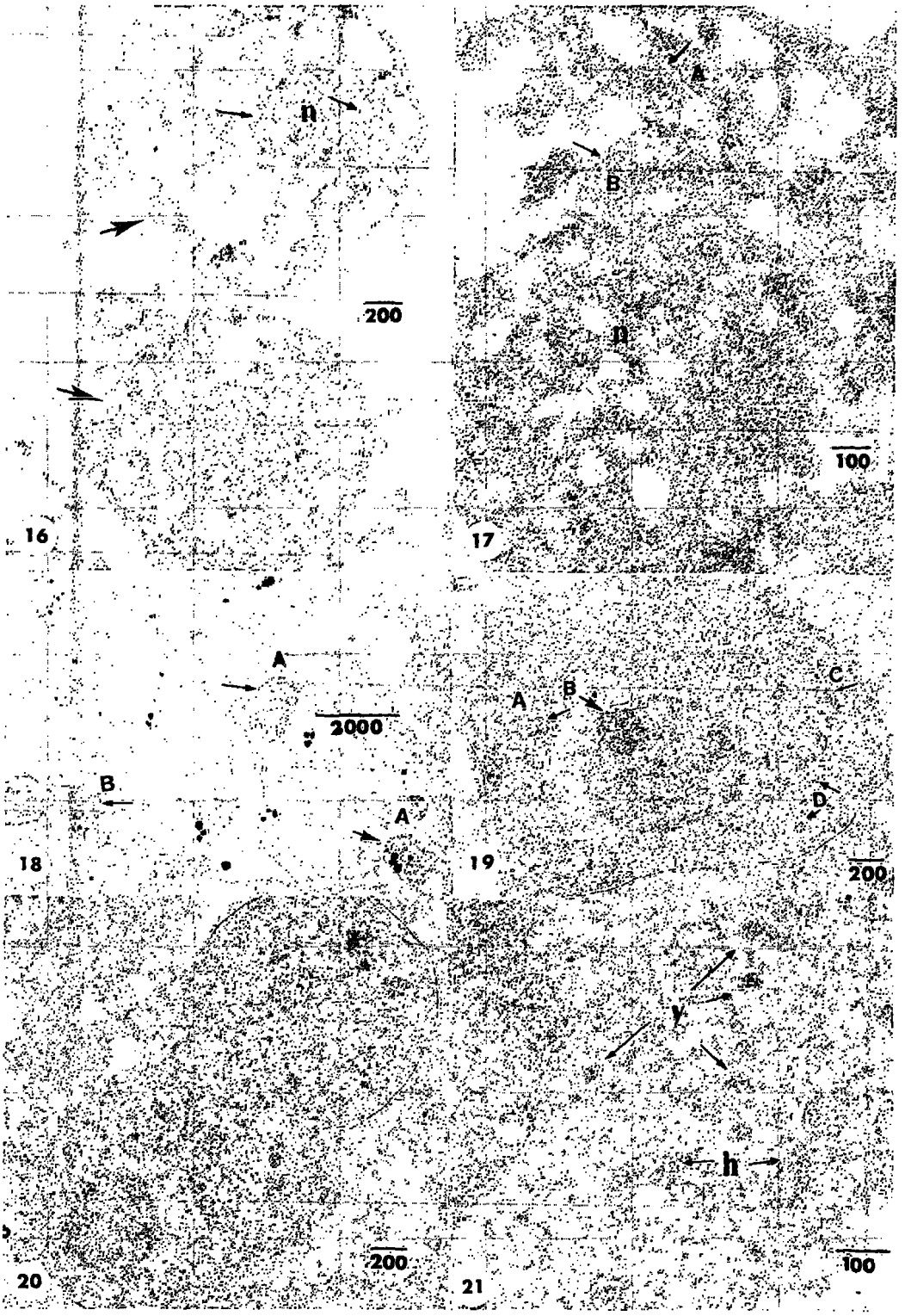
Host - *Saccostrea commercialis*

Type locale - Georges River, Woolooware Bay, New South Wales, Australia.

Range - Known only from the above location and other high salinity estuaries in this region of New South Wales.

Morphologic characteristics - Infections occur in hemocytes and are associated with focal abscess-type lesions in the gill, connective, and gonadal tissues. Organisms are small 1–3 μm cells that contain an eccentric nucleus and a cytoplasmic vacuole. Ultrastructural characteristics are not known.

Comparisons — *Mikrocytos* g. n. is always associated with focal abscesses and occurs in crassostreid oysters. *Bonamia* is always associated with generalized infections and only occurs in ostreid oysters.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Mikrocytos mackini sp. n., which has eccentric nucleoli, and *M. roughleyi* sp. n., which has a cytoplasmic vacuole and a nucleus that is displaced to the periphery of the cell, are morphologically distinct from *B. ostreae* (the only closely related species). All three species occur in separate host species and all appear to be host specific.

DISCUSSION

Denman Island Disease

Quayle's original study (1961) documented heavy mortality (40%) clearly associated with surface tissue pustules in *C. gigas* in an area of British Columbia in May. Studies of *C. gigas* by Mackin resulted in the discovery of the "microcell" organism and its association with tissue abscesses. Subsequent histological examinations done by the senior author, in cooperation with D. Quayle and N. Bourne, confirmed the association of mortality, pustules, tissue abscesses, and microcell infections that have continued to occur during each May and June to the present time.

Histopathologically, this disease (caused by *Mikrocytos mackini*) is characterized by acute inflammatory abscesses which remain focal until the oyster dies or resolution occurs. While microcell organisms are not always found in abscesses, they are never found in oysters that do not have abscesses, indicating at least an associational relationship.

Electron microscopy has demonstrated only one stage of a small protistan organism that contains organelles resembling haplosporosomes. No clear demonstration of mitochondria has been accomplished. The haplosporosome-like organelles often tend to be elongated and contain layers of membranes. Internal structure of these organelles is not nearly as dense as that seen in *Bonamia* or other haplosporidans.

The small 45 nm dense bodies also seen in the cytoplasm have characteristics suggestive of virus structure; namely, uniform size, abundant occurrence in the cytoplasm only, and a suggestion of icosahedral symmetry. The cytoplasmic occurrence, 46 nm size, icosahedral symmetry, and the presence of an envelope are characteristics of the family Togaviridae. The lack of paracrystalline arrays, strategy of development, empty capsids, and extracellular occurrence prevent conclusive identification of these particles as virus at this time, and these may prove to be an exclusive organelle that is a characteristic of this protistan group. The presence of a lytic virus in the parasite could also explain the self-limiting nature of the focal abscesses characteristic of the Denman Island disease.

Australian Winter Disease

Since Roughley described this disease in an Australian *S. commercialis* in 1926, little progress has been made toward identification of the etiologic agent. Careful examination of the tissues of affected animals, collected by Peter Wolf, has revealed apparent small cytologic and histozoic organisms associated with abscesses. These organisms have features such as size, morphology, and tissue location remarkably similar to organisms present in other oyster microcell diseases. Peter Wolf (unpubl. data) has stated that this disease thrives in high (30–35‰) salinity (and is unknown in lower salinities); the incubation period is about 2½ months and mortality does not occur in animals less than 3 years old. The occurrence of shell lesions and focal tissue abscesses appears to be common features in *Saccostrea* and *Crassostrea*. This may indicate a degree of resistance characterized by the ability of the animal to isolate parasites in a focal lesion and to eliminate them by either rupture of the abscesses or diapedesis through the mantle epithelium to the shell surface, with subsequent calcification via shell pustule formation, or it may indicate a host parasite relationship response. This mechanism was reported in oysters that had acquired resistance to *Haplosporidium nelsoni* (Farley 1968).

Kern (fn. 6) found microcell infections in *C. gigas* from Hawaii (Fig. 6) that were not always associated with focal abscesses. General systemic infection and inflammatory infiltration were noted in these cases (Fig. 5), but the organisms appear

FIGURE 16.—Scale units in Figures 16–21 are in nanometers. Electron micrograph of deparaffinized *Ostrea edulis* from California. Two intracellular microcells showing prominent nuclei with peripheral nucleoli. 36,000×.

FIGURE 17.—Higher magnification of California microcells showing haplosporosome-like bodies (A) and a probable mitochondria (B). 108,000×.

FIGURE 18.—Electron micrograph of several microcells in vesicular connective tissue of *Crassostrea gigas* (A) from Denman Island, British Columbia. Probable degenerate microcells can also be seen in the cytoplasm of phagocytic hemocytes (B). 7,320×.

FIGURE 19.—Electron "micrograph" of microcell from Denman Island *Crassostrea gigas*. Note the eccentric nucleolus (A), myelin bodies (B), haplosporosome-like bodies (C), and virus-like entities (D). 36,900×.

FIGURE 20.—Electron micrograph of *Crassostrea gigas* microcell with structure suggestive of division. 36,900×.

FIGURE 21.—Higher magnification of electron micrograph showing haplosporosomes and virus-like dense bodies. 108,000×.

to be identical to the Denman Island microcell organisms.

Microcell Infections in *Ostrea edulis*

Microcell infections were first seen in *O. edulis* in moribund oysters which had been transferred from Milford, CT to Chincoteague Bay, VA in 1962. Two animals from the FK sample had developed clinical manifestations of the disease. Other cases appeared in animals transferred from Milford to California, and Milford to Oxford. Morphology of the infectious organisms was identical in all of these episodes and the histopathology always consisted of acute inflammatory infiltration and systemic involvement. All episodes were associated with movement of oyster stocks originating in Milford, and all experienced severe mortality. No differences were noted between any of the *O. edulis* epizootics in regard to morphology of the organisms. Comparison of the American *O. edulis* infections with tissues of French oysters experiencing *B. ostreae* infections (Pichot et al. 1979; Balouet et al. 1983) revealed no morphologic or histologic differences at the light or electron microscope level. Indeed, ultrastructural comparisons demonstrate close similarities. Size comparisons of organisms are identical and the nucleus contains a peripheral nucleolus, and identical haplosporosome-like organelles are present in the cytoplasm. The major difference is in epizootic occurrences. The French epizootic occurred in natural or feral populations of European flat oysters. Since introduction of oysters to French sites from locations outside of France was a common event in the past, the source of the index case may have originated from an introduction of oysters from Elkhorn Slough, CA in the late 1970s (Elston et al. 1986). Contagious spread (Tige et al. 1981) is well documented in many locations in France and also the Netherlands. With the exception of the established breeding populations of flat oysters in the central Maine coast region, no natural or feral populations of *O. edulis* exist in the United States. The State of Maine carefully controls imports into the state; the disease has not been established in this population. The discovery of microcell infections in *O. lurida* in Oregon suggests that this may be a naturally occurring disease in that species. Infection intensity and prevalences suggest that some animals may die from the disease. The disease appears to be enzootic in the Oregon location. The lack of ultrastructural studies prevents close comparison of the *O. lurida* disease with the *O. edulis* disease. However, the disease in *O. lurida* tended to infect VCT while *B. ostreae* is primarily a disease of hemocytes.

CONCLUSIONS

There is a complex of oyster diseases caused by a group of protistan parasites of several species. These small intracellular and extracellular organisms designated originally as microcells have been found in association with serious disease in two species of *Crassostrea* and two species of *Ostrea*. It appears that the disease in *C. gigas* and *S. commercialis*, while exhibiting some similarities in types of lesions, are probably caused by different species of microcell type parasites.

A new genus, *Mikrocytos* g. n., and two new species have been described for the organisms causing disease in oysters: *Mikrocytos mackini* sp. n. in *C. gigas* from British Columbia, Canada, and *Mikrocytos roughleyi* sp. n. in *S. commercialis* from Australia.

Disease that has struck *O. edulis* in France is identical to the microcell disease seen in *O. edulis* in three episodes in the United States. The organism causing the disease in *O. edulis* is *Bonamia ostreae* and is clearly different from the microcell organism found in *C. gigas* in British Columbia and Hawaii. Finally, additional ultrastructural studies are needed for more complete characterization of the organisms from *O. lurida* and *S. commercialis*.

ACKNOWLEDGMENTS

We thank Cecelia Smith, Dorothy Howard, and Gretchen Roe for preparation of histologic materials; Jane Wade for preparation of the ultrastructural material; and Muriel McNelis, Karen Hayman, and Jane Swann for manuscript preparation. The senior author would like to acknowledge the help of the late John Mackin for his expert advice through the years; Daniel B. Quayle and Neil Bourne for assistance with samples of oysters from British Columbia; Fred Kern for allowing us to use his Hawaiian material; and Albert K. Sparks and Inke Sunila for critical review of the manuscript without implying agreement with interpretations herein. Partial support from the Department of Energy under Contract DE-AC06-76RLO 1830 to Battelle Memorial Institute is acknowledged.

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