

***Hartmannella tahitiensis* sp. n., an Amoeba Associated with  
Mass Mortalities of the Oyster *Crassostrea commercialis*  
in Tahiti, French Polynesia<sup>1</sup>**

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*Hartmannella tahitiensis* sp. n. is described from moribund specimens of the oyster *Crassostrea commercialis* from Vahi tarua, Port Phaeton, on the southern shore of Tahiti. Histopathological and cytopathological alterations in the oysters are described to reveal their similarities with those reported as postmortem changes by Sparks and Pauley (1964). Although *H. tahitiensis* trophozoites have been observed intermingled with the epithelial lining of the alimentary tract, Leydig tissue, gonadal trabeculae, and ctenidia, these amoebae are not believed to be the cause of the mass oyster mortalities. Rather, it is proposed that *H. tahitiensis*, soil amoebae, are deposited in the bay through soil erosion and they become secondary invaders of oysters which have been rendered moribund by pollution. Although generalized, nonfocal leukocytosis occurs in the oysters examined, there is no apparent cellular reaction to the amoebae. Two types of blood cells, granular and hyaline leukocytes, are described from healthy *C. commercialis*. In addition, a third type, the so-called multinucleate macrophages, occurs in oysters harboring amoebae. The PAS-positive condition of *H. tahitiensis* trophozoites renders this staining procedure a useful diagnostic tool.

INTRODUCTION

Because of the continuous demand for fresh oysters, especially among the French population in Tahiti, French Polynesia, several commercial ventures to establish an oyster industry have been initiated in a number of the estuaries along the southern coast of this South Pacific island. The beds of *Crassostrea commercialis*, the Sydney rock oyster or commercial oyster, established on the mud flats at Port Phaeton, Afaahiti District, on the southern shore of Tahiti (Fig. 1) represent one of these. These beds were initially established at some time prior to 1964 and became of

limited commercial value during that year. During the building of a road in 1968, at Vahi tarua, where one of the beds exists, not only was the direct connection between the estuary and the open bay cut off, except for a number of man-made drainage pipes, but building materials were dumped over the oyster beds. Thus, not only was the water circulation greatly disrupted, but the water was polluted. Consequently, it was not surprising that a rapid decline of the oyster population at Vahi tarua, resulting from mass mortalities, began shortly after completion of the road.

Through the courtesy of Mr. Charles Walters of the University of Hawaii and Monsieurs S. Stein and Sylvain Millaud of the Tahitian Service de la Pêche, 14 specimens of *C. commercialis* from the Vahi tarua bed were flown to Honolulu, Hawaii, where they were immediately examined by

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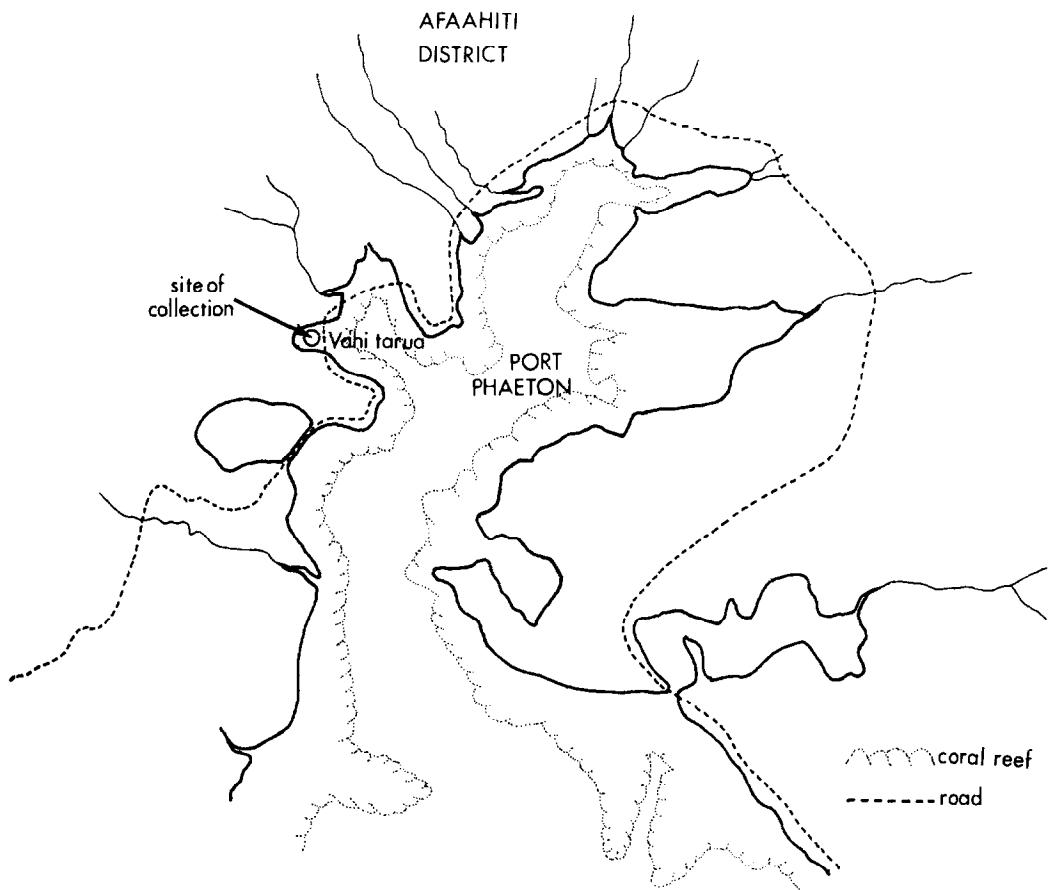


FIG. 1. Map showing Port Phaeton region of Tahiti, French Polynesia, and site from which the oysters were collected. The dotted line represents the newly built road.

the author. All of these oysters were alive when examined.

According to Messrs. Stein and Walters, the oyster bed at Vahi tarua is in water ranging from 5 to 10 feet, depending on the tide. The salinity in this area ranged from 29.8–35.4 ‰ prior to the building of the road but has dropped to about 15–18‰ due to the damming and dilution by rain and freshwater runoffs from the adjacent shore. The temperature ranges from 27.4–39°C all year round. No major sewage outlets occur in this region.

#### MATERIALS AND METHODS

In addition to gross examination, all of the specimens of *Crassostrea commercialis*

were fixed in 10% seawater-formalin, embedded in Tissumat, and serially cross-sectioned at 9 µ. Representative sections were stained with the following histological and histochemical stains: (1) Harris' hematoxylin and eosin (H and E); (2) Mallory's triple connective-tissue stain; (3) periodic acid-Schiff's reaction (PAS), with selected sections pretreated with a 0.6% aqueous malt diastase solution for 3 hr at 37°C and others exposed to Schiff's reagent without prior oxidation with periodic acid.

For comparison, 16 specimens of *C. commercialis* collected from concrete embankments in the vicinity of Coconut Island, Kaneohe Bay, Oahu, Hawaii, were similarly fixed, sectioned, and stained.

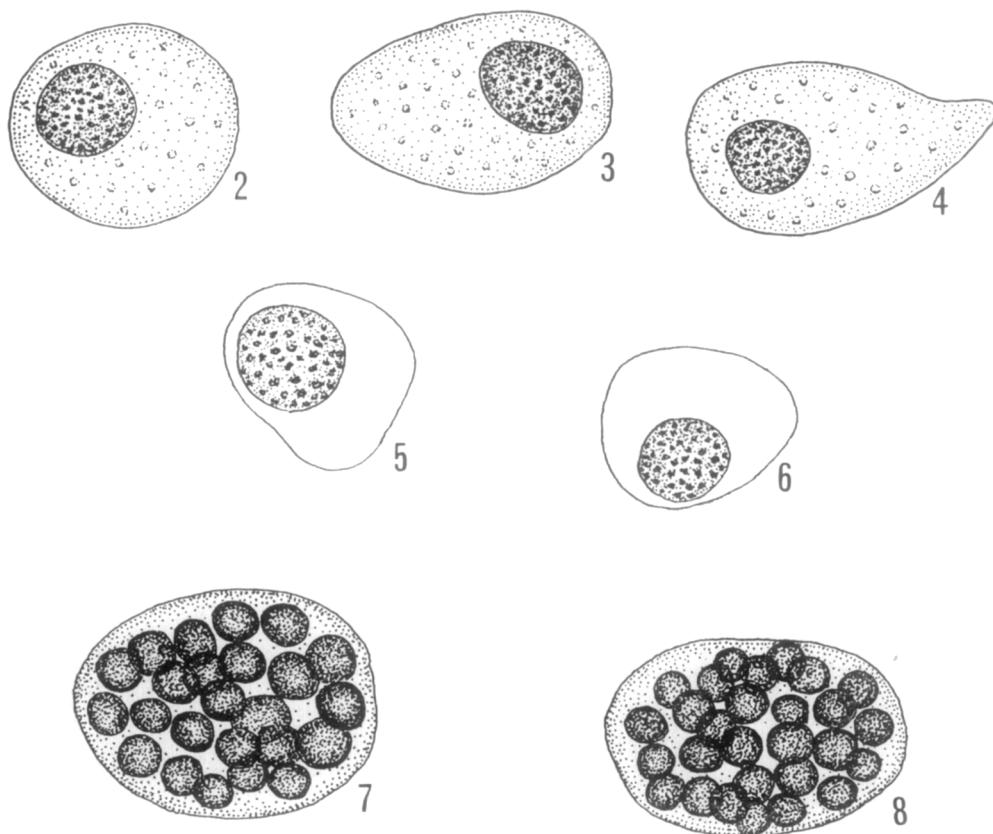
## OBSERVATIONS

*Blood Cells and Amoebae*

Examination of histological sections of oysters from Tahiti has revealed not only severe histopathological changes associated with various tissues but also the presence of an unusual type of amoeboid organism. In order to ascertain that this organism does not represent a type of leukocyte, a concerted effort was made to determine the types of leukocytes normally present in *C. commercialis*. Observations of living cells from intracardially collected blood examined with conventional and phase-contrast microscopy and of tissue sections

of healthy Hawaiian oysters were made. These were confirmed in sections of Tahitian oysters. As the result, two types of leukocytes, granular and hyaline cells, have been identified. These are described below. All measurements were made with a calibrated ocular micrometer.

*Granular leukocytes* (Figs. 2-4). These cells occur both intra- and extravascularly. They are capable of movement by producing lobopodia. Each cell averages 6  $\mu$  (5-8  $\mu$ ) in greatest diameter and includes a conspicuous nucleus of the compact type, i.e., the chromatin granules are more or less evenly distributed within the nuclear membrane. Each nucleus averages 3  $\mu$



Figs. 2-4. Granular leukocytes of *Crassostrea commercialis* showing compact nuclei and cytoplasmic granules. Drawn from living cells. (See text for dimensions). Figs. 5,6. Hyaline leukocytes of *Crassostrea commercialis* showing compact nuclei. Drawn from living cells. (See text for dimensions).

Figs. 7,8. Multinucleate macrophages of *Crassostrea commercialis* from Tahiti showing compact nuclei. Drawn from cells in histological sections. (See text for dimensions).

(2–3  $\mu$ ) in diameter. The most characteristic feature of these cells is the occurrence of more or less evenly distributed cytoplasmic granules.

*Hyaline leukocytes* (Figs. 5,6). The second type of leukocyte in *C. commercialis* also occurs both intra- and extra-vascularly. It is smaller, averaging 4  $\mu$  (4–5  $\mu$ ) in greatest diameter. The spherical nucleus, also of the compact type, averages 3  $\mu$  (2–3  $\mu$ ) in diameter. Hyaline cells can usually be distinguished from granular cells by the absence of cytoplasmic granules although occasionally hyaline cells have been encountered with a few granules, no more than three or four per cell, in their cytoplasm. Hyaline cells are less motile than granular cells when observed in fresh preparation but they are capable of producing blunt lobopodia, usually no more than one at a time.

In addition to the two types of leukocytes occurring in normal specimens of *C. commercialis*, a third type has been encountered in sections of Tahitian oysters. These are identical to the so-called "multinucleate macrophages" originally described by Sparks and Pauley (1964) in moribund and dead *Crassostrea gigas*. A description of this type of cell as encountered in *C. commercialis* from Tahiti is given at this point.

*Multinucleate macrophages* (Figs. 7,8). These spherical to ovoid cells, averaging 11  $\mu$  (8–11  $\mu$ ) in greatest diameter, are multinucleate, usually with 20–30 compact nuclei. Each nucleus measures approximately 2  $\mu$  in diameter. In H and E stained sections, the cytoplasm of these cells are eosinophilic while the nuclei are weakly hematoxyphilic and in Mallory's stained sections, all of the constituents of each cell are intensely red. The entire cell is PAS-negative. Multinucleate macrophages are found primarily associated with the epithelial lining of the alimentary tract although a few have been observed in other areas of the body.

It has become quite apparent with the identification of the two types of blood cells present in the oysters from Hawaii and the three types in the oysters from Tahiti that the molluscan leukocytes all include nuclei of the compact type. On the other hand, the amoeboid organisms encountered in Tahitian oysters are readily distinguished by their inclusion of vesicular nuclei, among other characteristics. A detailed study of these organisms has convinced me that they represent a hitherto undescribed species of amoeba belonging to the genus *Hartmannella*. A description of this organism follows.

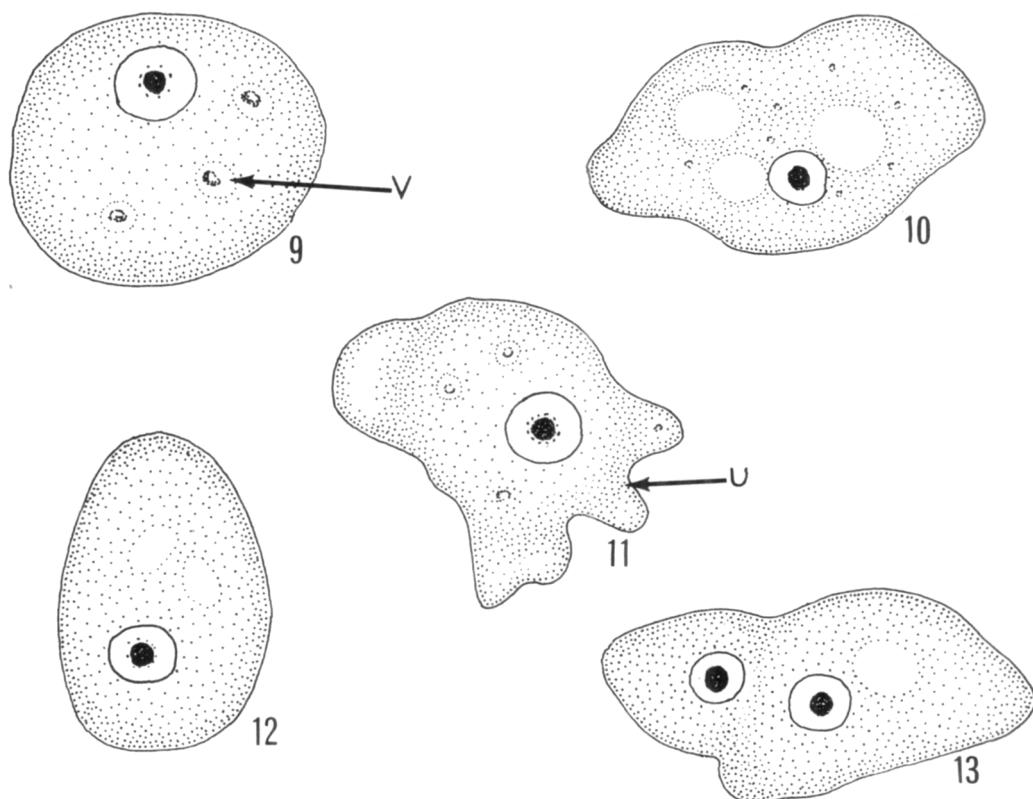
*Hartmannella tahitiensis* sp. n.  
(Figs. 9–13).

*Description.* Limax-type amoeba. Body asymmetrical, 10  $\mu$  (8–13  $\mu$ ) in greatest diameter, commonly spherical or ovoid when not producing pseudopodia; ectoplasm and endoplasm indistinct; cytoplasm may include one or more vacuoles of varying sizes, some enclosing cellular debris, bacteria, and minute granules; few extravacuolar cytoplasmic granules may also be present; pseudopodia of lobopodial type, commonly monopodial although several pseudopods may be present simultaneously; vesicular nucleus, 2  $\mu$  (1–2  $\mu$ ) in diameter, with conspicuous nucleolus (endosome) measuring 0.8–1  $\mu$  in diameter; nucleolus with or without peripheral fine chromatin granules; uroid, when present, bears three to four lobose projections (Fig. 11). Trophozoites capable of division as specimens with two nuclei and zone of fission have been observed (Fig. 13).

*Deposition of slides.* Center for Pathobiology, (CPUCI-192AAB), University of California, Irvine, and author's collection (P-1001), Department of Biology, Lehigh University.

*Type habitat.* In tissues of *Crassostrea commercialis*.

*Type locality.* Vahi tarua, Port Phaeton, Afaahiti District, Tahiti, French Polynesia.



Figs. 9-13. Trophozoites of *Hartmannella tahitiensis* sp. n. showing typical vesicular nuclei. Notice uroid with blunt lobopodia in FIG. 11 and binucleate, dividing amoeba in FIG. 13. Drawn from amoebae observed in histological sections of oysters from Tahiti. (See text for dimensions). U = uroid; V = cytoplasmic vacuole.

In H and E stained sections, the cytoplasm of *H. tahitiensis* appears homogeneously, faintly eosinophilic except for the chromophobic vacuoles. In Mallory's stained sections, the cytoplasm is blueish although some of the granules stain red. The cytoplasm of these amoebae are PAS-positive and the positively stained material is for the most part diastase labile and hence can be considered to include glycogen. The absence of free aldehydes is indicated by the absence of a positive reaction to Schiff's reagent without prior oxidation with periodic acid.

#### *Histopathology*

Gross examination of the Tahitian oysters revealed the typical lean, watery, and semitransparent appearance of spec-

imens in poor condition. The hearts of all 14 specimens were faintly beating when the valves were opened, thus indicating that they were alive. No silt-containing mucus was observed on the mantle surfaces and no macroparasites and/or other symbionts were observed.

Histological examination of the H and E and Mallory's stained sections revealed most of the features considered by Sparks and Pauley (1964) to represent post-mortem changes.

The following descriptions of the histo- and cytopathological changes associated with cells comprising the epithelial lining of the alimentary tract, digestive gland, Leydig tissue, gonads, and ctenidia are being presented to emphasize the similarities between the changes observed and

what have been reported as postmortem changes (Sparks and Pauley, 1964).

*Alimentary tract epithelium.* As Shaw and Battle (1957) have reported, the columnar epithelial cells lining the luminal borders of both the stomach and intestine

of oysters are ciliated. In sections of control oysters from Hawaii, leukocytes of both the hyaline and granular types have been observed intermingled among these epithelial cells, as well as in the lumen, adhering to the cilia (Fig. 14).

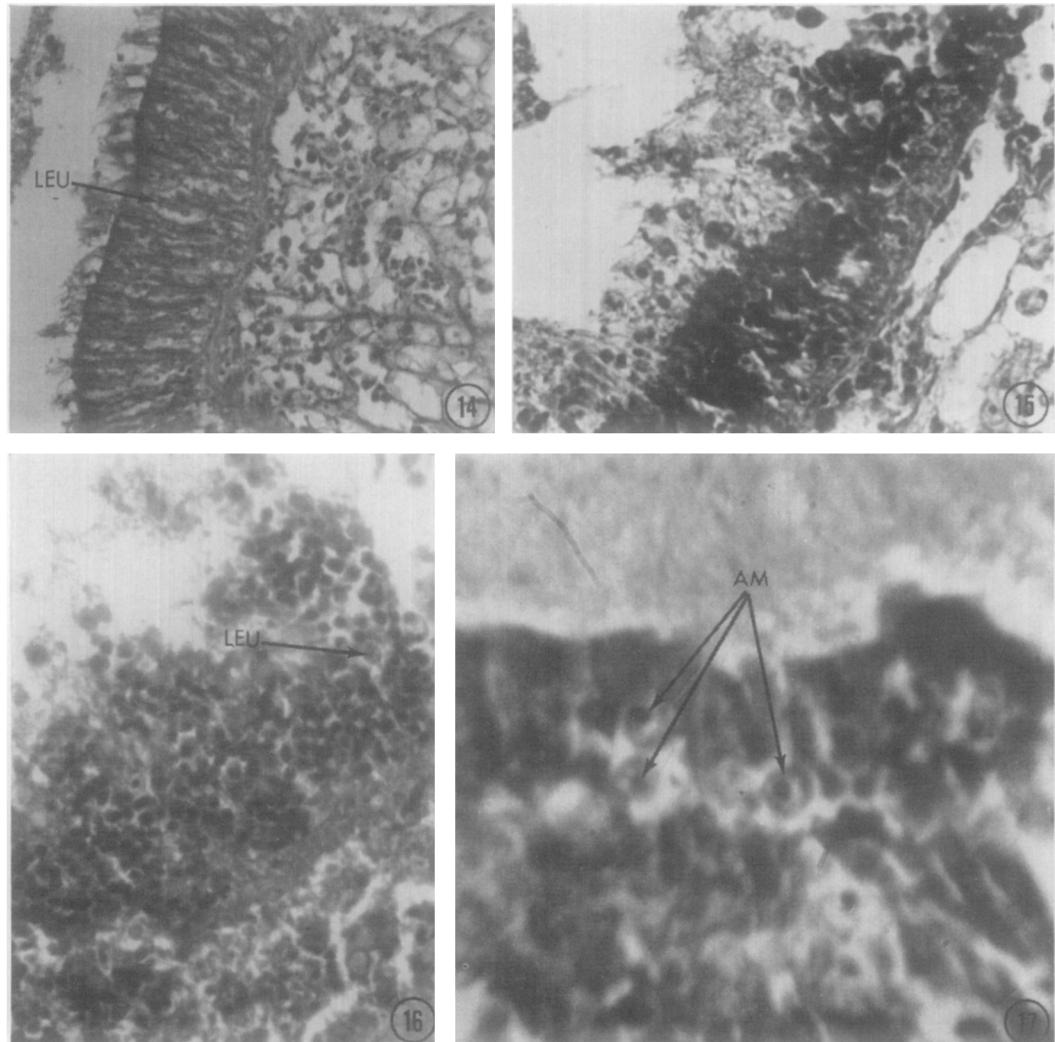


FIG. 14. Photomicrograph of section of intestinal tract of normal *C. commercialis* showing lining of ciliated, columnar epithelium and leukocytes intermingled with epithelial cells. LEU = leukocyte. (H&E; 40 $\times$  obj.). FIG. 15. Photomicrograph of section of intestinal tract of *C. commercialis* from Tahiti showing disorganized and necrotic epithelial cells with intermingled leukocytes. (H&E; 40 $\times$  obj.). FIG. 16. Photomicrograph of section of intestinal tract of *C. commercialis* from Tahiti showing severely disorganized necrotic epithelial cells with conspicuous pyknotic nuclei most of which are detached from the surrounding cytoplasm. LEU = leukocyte. (H&E; 10 $\times$  obj.). FIG. 17. Photomicrograph of section of intestinal tract of *C. commercialis* from Tahiti showing disorganized necrotic epithelial cells with *H. tahitiensis* trophozoites situated intercellularly. AM = amoebae. (H&E; 90 $\times$  obj.).

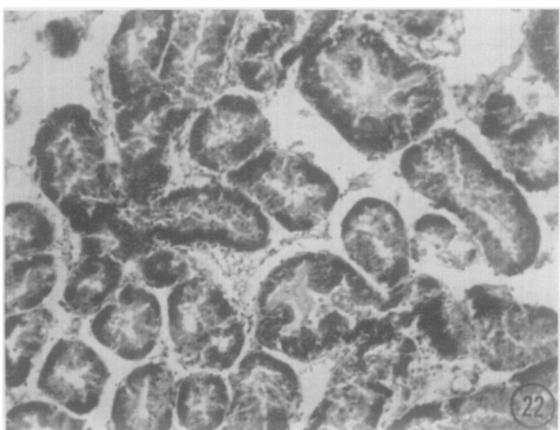
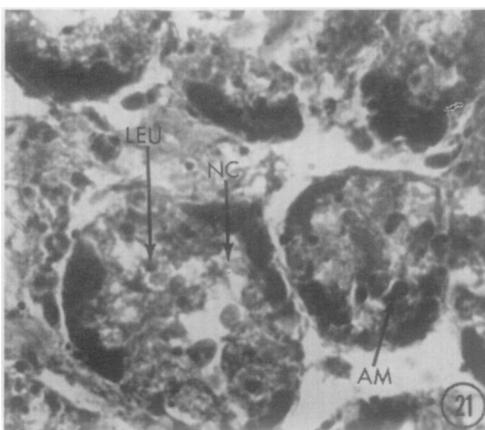
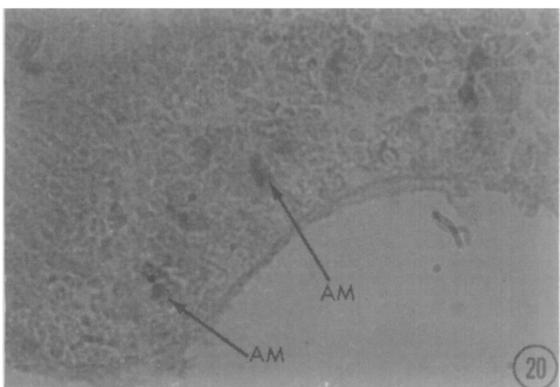
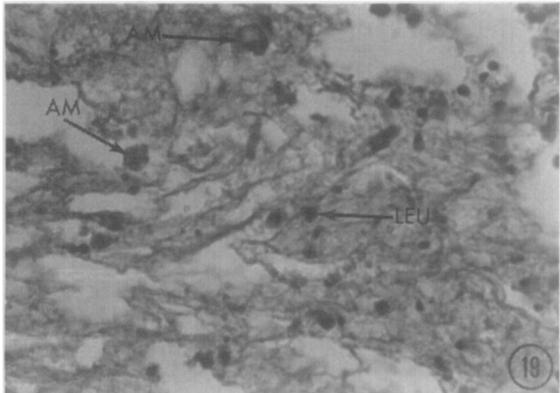
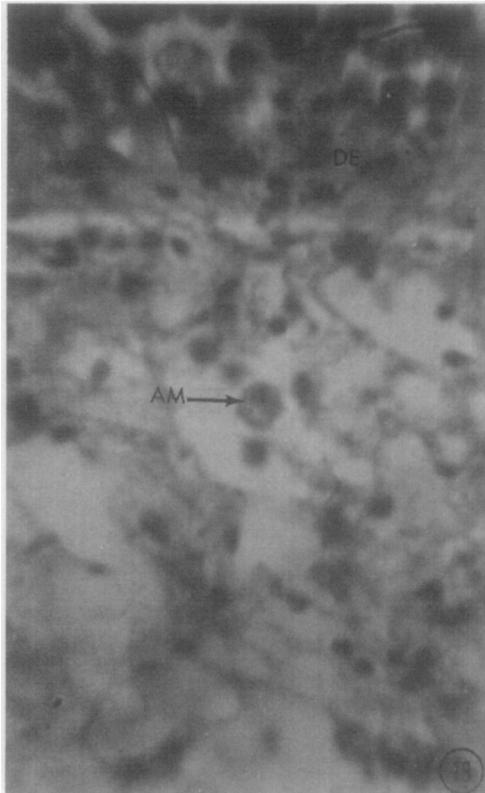


FIG. 18. Photomicrograph of section of *C. commercialis* from Tahiti showing *H. tahitiensis* trophozoite situated in Leydig tissue beneath epithelial lining of digestive tract. (H&E; 90 $\times$  obj.). FIG. 19. Photomicrograph of section of *C. commercialis* from Tahiti showing *H. tahitiensis* trophozoites intermingled with slightly disarranged Leydig cells in subepithelial region of digestive tract. Notice the presence of randomly distributed leukocytes in same region. (H&E; 40 $\times$  obj.). FIG. 20. Photomicrograph of section of intestinal wall of *C. commercialis* from Tahiti showing conspicuous PAS-positive *H. tahitiensis* trophozoites intermingled with disorganized epithelial cells. Notice that the oyster cells are PAS-negative. (PAS reaction; 40 $\times$  obj.). FIG. 21. Photomicrograph of section of digestive gland of *C. commercialis* from Tahiti showing acinar cells. Notice the absence of cilia and the vacuolated, necrotic state of the luminal portion of each cell and the presence of infiltrated leukocytes (H&E; 40 $\times$  obj.). FIG. 22. Photomicrograph of portion of digestive gland of *C. commercialis* from Tahiti showing scarcely arranged acinar tubules. Notice accumulation of randomly distributed leukocytes in interacinar spaces. (H&E; 10 $\times$  obj.). AM = amoeba; DE = disorganized and necrotic epithelial lining of digestive tract; LEU = leukocyte; NC = necrotic portion of acinar cell.

Most of the epithelial cells lining the stomachs and intestines of the oysters from Tahiti appear disorganized and necrotic. Specifically, the cells are more basophilic, with many being detached from the underlying basement membrane (Figs. 15, 16). Their nuclei are pyknotic, with many being either completely dissociated from, or are surrounded by, disintegrating cytoplasm (Fig. 16). A large number of leukocytes, primarily of the granular type, is found to have infiltrated the region of the necrotic cells (Figs. 15, 16). In addition to mononucleate leukocytes, multinucleate macrophages are found intercellularly between the lining epithelia. Another striking change is the mass sloughing of cilia (Figs. 15, 16). Only in a few restricted areas in each oyster are these organelles intact.

Associated with the necrotic lining epithelia of the stomach and intestine are numerous amoebae. These are situated primarily between the epithelial cells (Figs. 17, 23), although some are attached to the luminal borders. In addition, some are found beneath the basement membrane, intermingled with Leydig cells (Figs. 18, 19). There does not appear to be an intimate association between invading amoebae and molluscan blood cells. It is of interest to note that in eight of the Tahitian oysters, large numbers of trophic amoebae occur within the gastric and intestinal lumina.

In normal oysters, the lining epithelium of the alimentary tract includes PAS-positive granules which are diastase labile and hence may be considered to be glycogen deposits. The underlying basement membrane is also PAS-positive but it is diastase resistant. The blood cells are PAS-negative. In the Tahitian oysters, there is an almost complete absence of PAS-positive material in the lining epithelia, although the basement membrane is PAS-positive. The multinucleate macrophages are PAS-negative.

The cytoplasm of amoebae include PAS-positive material and hence in sections of Tahitian oysters treated with the PAS reaction, the amoebae are readily recognized as distinct red bodies in an essentially unstained field (Fig. 20). The PAS-positive material in the amoebae is diastase labile and hence can be considered to be stored glycogen.

*Digestive gland.* The columnar cells comprising each acinus of the digestive gland in oysters are ciliated along their luminal borders (Shaw and Battle, 1957). This pattern is quite apparent in the control oysters although in the Tahitian oysters the arrangement is drastically altered. In the latter, not only are the cilia absent, but approximately one-fourth or more of the luminal border of each cell appears highly necrotic (Fig. 21). This region is highly vacuolated and the normally discrete cell boundary is no longer apparent. Most of the nuclei of the acinar cells are pyknotic.

Another striking pathological change present in Tahitian oysters is the shrinkage of the individual acinar cells resulting in a reduction in the diameter of each tubule. As a result, the acinar tubules are sparsely positioned (Fig. 22), whereas in healthy oysters these are closely packed within the digestive gland.

Not only are numerous leukocytes found infiltrating the intertubular and intercellular spaces within the digestive gland (Figs. 21, 22), but amoebae also have been observed between the disarranged tubules (Fig. 25) and infiltrating acinar cells (Figs. 21, 24). The digestive vacuoles of these amoebae are usually filled with cellular debris. Again, no apparent affinity between leukocytes and amoebae has been observed.

The cells forming the digestive gland acini of oysters serve as one of the major storage sites for glycogen (Bernard, 1853; Bizio, 1866; Creighton, 1899; Pekelharing, 1901; Russell, 1923; Yonge, 1926; Bargeton, 1941, 1945; Cheng and Burton, 1966).

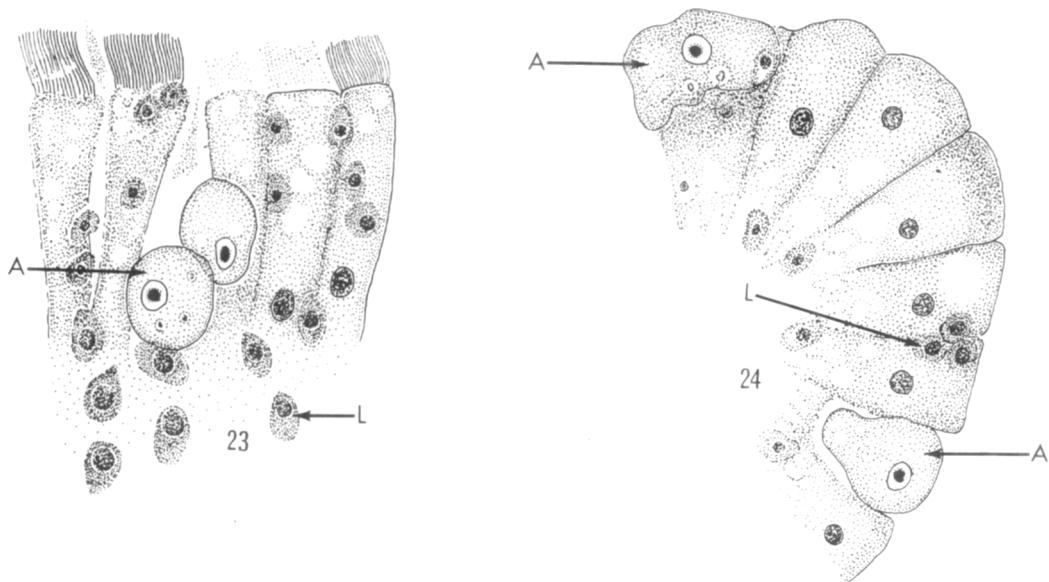


FIG. 23. Drawing showing *H. tahitiensis* trophozoites invading intercellular space between ciliated columnar epithelial lining of intestine of *C. commercialis* from Tahiti. (Drawn from stained section). FIG. 24. Drawing showing *H. tahitiensis* trophozoites destroying acinar cells of digestive gland tubule of *C. commercialis* from Tahiti. (Drawn from stained section). A = amoeba; L = leukocyte.

This has been verified in sections of control oysters treated with the PAS reaction, with sections pretreated with diastase serving as controls. In the oysters from Tahiti, there is a dramatic decrease in the amount of stored glycogen in acinar cells. In fact, sections treated with the PAS reaction have revealed almost a total absence of glycogen granules in these necrotic cells.

As shown in the wall of the alimentary tract, the PAS-positive inclusions of the amoebae associated with the digestive gland render these organisms conspicuous against an essentially PAS-negative background.

**Leydig tissue.** The normal cytology of the large, vacuolated, vesicular connective tissue cells, known as Leydig cells, of oysters has been reviewed by Galtsoff (1964) and Cheng and Rifkin (1970). In H and E stained sections, the nuclei are hematoxyphilic while the vacuolated cytoplasm is faintly eosinophilic. In Mallory's stained sections, the cell boundaries and

cytoplasmic inclusions tend to be blueish while the nuclei are red.

Normal Leydig cells are richly packed with glycogen granules (see reviews by Galtsoff, 1964, and Cheng and Rifkin, 1970). This has been confirmed in the control sections. On the other hand, not only is glycogen almost completely absent within Leydig cells of the Tahitian oysters, but conspicuous, pathologic alterations occur. Specifically, most of the cells are detached and shrunken, with many having undergone partial autolysis. Most of the nuclei are pyknotic. A conspicuously large number of leukocytes and a few multinucleate macrophages are consistently found in all areas where Leydig tissue occurs (Fig. 26). These blood cells are not limited to the intercellular spaces but many are also found in the process of phagocytizing autolyzed cells. Amoebae also occur in Leydig tissue, especially in the subgastric and subintestinal zones.

**Gonads.** All of the Tahitian oysters were

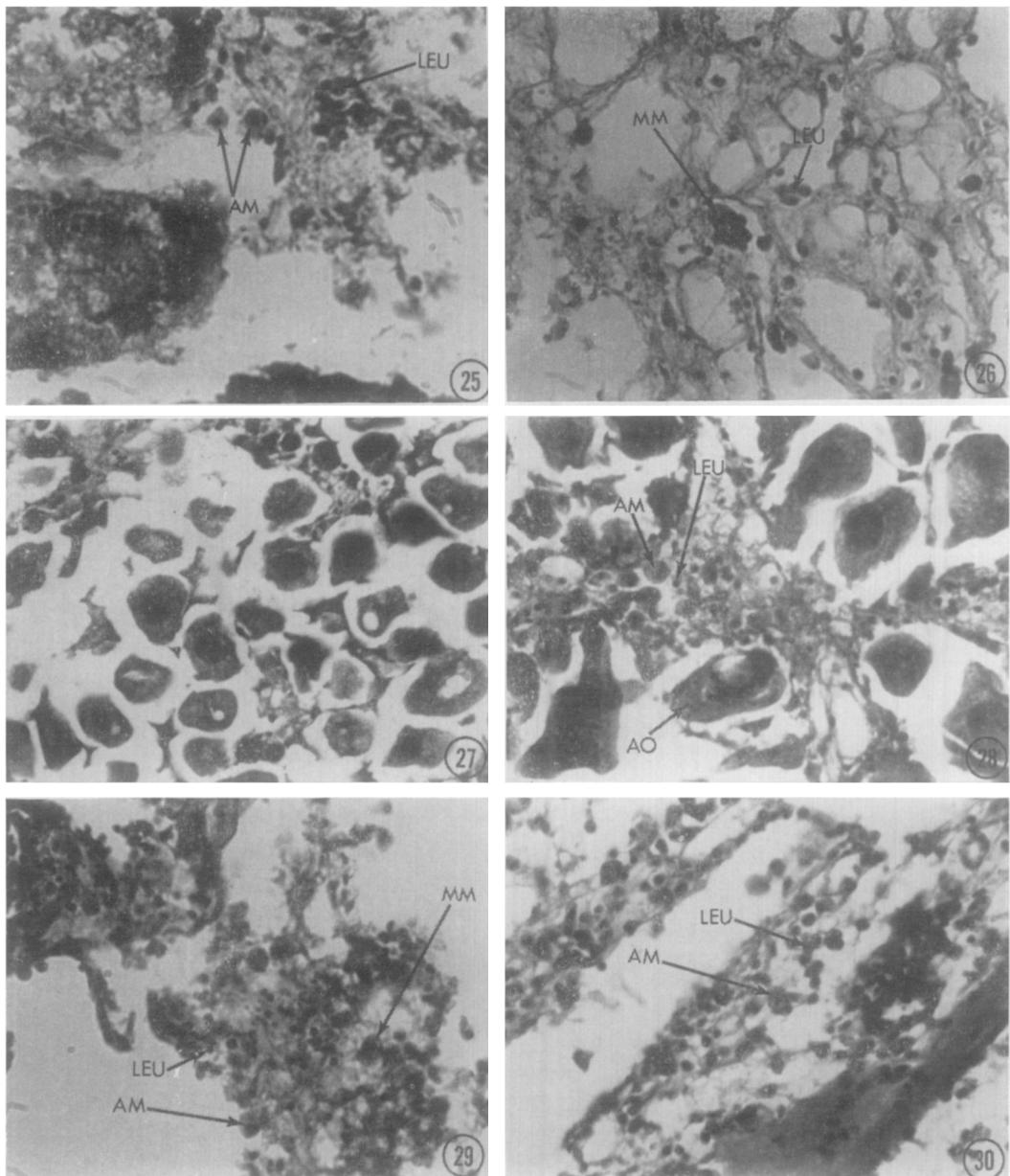


FIG. 25. Photomicrograph showing occurrence of *H. tahitiensis* trophozoites and leukocytes associated with intertubular connective tissue elements in digestive gland of *C. commercialis* from Tahiti. (H&E; 40 $\times$  obj.). FIG. 26. Photomicrograph showing occurrence of multinucleate macrophage and leukocyte intermingled with Leydig cells in *C. commercialis* from Tahiti. (H&E; 40 $\times$  obj.). FIG. 27. Photomicrograph showing abnormal, shrunken ova of *C. commercialis* from Tahiti. (H&E; 40 $\times$  obj.). FIG. 28. Photomicrograph showing abnormal ova of *C. commercialis* from Tahiti with leukocytes and amoebae associated with trabecula. (H&E; 90 $\times$  obj.). FIG. 29. Photomicrograph showing leukocytes, multinucleate macrophages, and amoebae associated with interfollicular trabecula in *C. commercialis* from Tahiti. (H&E; 40 $\times$  obj.). FIG. 30. Photomicrograph showing leukocytes and amoebae associated with the gill filaments of *C. commercialis* from Tahiti. (H&E; 40 $\times$  obj.). AM = amoeba; AO = abnormal ovum; LEU = leukocyte; MM = multinucleate macrophage.

in the mature, ova-producing stage. Unfortunately, none of the Hawaiian oysters were; hence, a direct comparison has not been possible. However, based on a comparison of the normal histology of oyster gonads as given by Galtsoff (1964) and on personal experience, it is apparent that the ova are undergoing degenerative changes comparable to the postmortem changes as described by Sparks and Pauley (1964). The ova are generally shrunken so that there is considerable empty space between the ova and the surrounding follicular trabeculae (Fig. 27). Upon examination at higher magnifications, many of the ova are found undergoing extensive autolysis. Consequently, many are abnormal in shape and structure (Fig. 28). Although comparatively few leukocytes appear to have infiltrated the ovarian follicles, large numbers are found associated with the necrotic Leydig cells situated between follicles (Fig. 28). In addition to granular and hyaline leukocytes, primarily the former, multinucleate macrophages and amoebae are also found in this area in Tahitian oysters (Figs. 28, 29).

Normal ova include cytoplasmic glycogen deposits (Cheng and Burton, 1966). The abnormal ova present in Tahitian oysters are essentially devoid of glycogen. Only a small percentage, approximately 6%, includes PAS-positive and diastase labile material and in these, this material is greatly reduced.

*Ctenidia.* The histology of normal oyster ctenidia is well known (see review by Galtsoff, 1964). In the oysters from Tahiti, not only are the surface cilia sloughed but the lining cells are necrotic and shrunken. Their nuclei are pyknotic. Furthermore, large numbers of leukocytes, amoebae, and occasionally multinucleate macrophages are found associated with all areas of each gill filament (Fig. 30). In addition to blood cells, amoebae are found adhering to the gill surfaces.

The amount of PAS-positive material is also greatly reduced in the ctenidial tissues of Tahitian oysters and consequently, in sections treated with this reaction, the amoebae, which are PAS-positive, are quite conspicuous.

#### DISCUSSION AND CONCLUSIONS

*Blood cells.* There is yet no agreement as to how many types of blood cells occur in mollusks (see reviews by Cheng et al., 1970; Cheng and Rifkin, 1970) although there appears to be a consensus that two types of leukocytes, granular and hyaline, occur in oysters (Galstoff, 1964; Cheng and Rifkin, 1970). This pattern apparently is true in *Crassostrea commercialis*, the blood cells of which, as far as I have been able to determine, have not been examined prior to this report. It is of interest to note, however, that as Sparks and Pauley (1964) have found in the tissues of unhealthy and dead *C. gigas*, multinucleate macrophages also occur in moribund *C. commercialis*. The function(s) of these multinucleate cells remains undetermined although their presence appears to be associated with degenerative changes, be these pre- or postmortem.

In the case of *C. commercialis*, harboring *Hartmannella tahitiensis*, although leukocytes and multinucleate macrophages commonly occur at the same sites, no cellular reaction to the amoebae has been observed.

*The amoeba.* Only two species of amoebae, *Vahlkampfia calkensi* and *Vahlkampfia patuxent*, have been described from oysters (Hogue, 1915, 1921). Both were found in the contents of the digestive tract of the American oyster, *Crassostrea virginica*, collected along the Atlantic coast of the United States (see Cheng, 1967 for review). In addition, Sawyer (1966) has reported the presence of other yet unidentified "parasitic amoeboid organisms"

in the same species of oysters from Maryland. The trophozoites found in tissue sections of *C. commercialis* from Tahiti, upon which the description of *H. tahitiensis* is based, do not satisfy either the specific or generic characteristics of *V. calkensi* and *V. patuxent*.

The morphological evidence obtainable from trophozoites occurring in the tissues of moribund *C. commercialis* permits the assignment of this amoeba to the family Amoebidae Bronn as defined by Kudo (1966) or to the family Hartmannellidae Volkonsky as defined by Page (1967a). This, however, does not constitute a paradox since both Hall (1953) and Kudo (1966) have included in the Amoebidae those genera which are considered by Volkonsky (1931) and Page (1967a,b) to be members of the Hartmannellidae. Thus, the latter family is in part synonymous with the Amoebidae.

Because of the absence of encysted amoebae in the tissues of the oysters examined and the fact that trophozoites enclosing identifiable mitotic figures have not been encountered, it has been extremely difficult to decide as to whether this species of amoeba should be assigned to the genus *Acanthamoeba* Volkonsky or *Hartmannella* Alexeieff. It is noted that several investigators (Singh, 1952; Ray and Hayes, 1954; Adam, 1964) have expressed the opinion that the original diagnosis of *Acanthamoeba* given by Volkonsky (1931) is unsatisfactory and prefer to consider the members of both genera as representatives of the broader genus *Hartmannella*. This practice has been adopted by many subsequent investigators. Page (1967a,b), however, has emended the original generic diagnoses and according to him, the members of *Acanthamoeba* can be distinguished from those of *Hartmannella* by the production of slender, hyaline projections (acanthopodia) from the surface of a single hyaline lobopodium, among other

characteristics. Since the trophozoites found in *C. commercialis* are devoid of acanthopodia, they are being assigned to *Hartmannella*. Furthermore, certain differences between the amoeba under consideration and the previously described ones have led me to consider it as representative of a new species, *Hartmannella tahitiensis* sp. n.

The trophozoite of *Hartmannella tahitiensis* is most similar in morphology and dimensions to that of *Hartmannella quadriparia* as described by Richards (1968). In fact, their dimensions are almost identical. The other known species are all considerably larger. *H. quadriparia* is found in the tissues of several species of freshwater gastropods which are listed at a later point, with the type being from *Biomphalaria pellida*. *H. tahitiensis*, however, can be distinguished from *H. quadriparia* by the absence of "fine, acute projections" protruding from the lobopodium. Furthermore, Richards (1968) has reported that when present in the molluscan host, *H. quadriparia* occurs intracellularly within a cytoplasmic vacuole and multiplies therein, producing four daughter amoebae. This does not occur in the case of *H. tahitiensis*. Finally, *H. quadriparia* is reported to possess a "variable uroid consisting of a group of small finger-like projections." The uroid of *H. tahitiensis*, when present, bears thicker pseudopodial projections (Fig. 11).

Amoebae of the hartmannellid group are usually found in soil and freshwater. A few, however, are known to invade tissues. King and Taylor (1936) have described *Malpighamoeba locustae* from the lumina of the Malpighian tubes of grasshoppers. Later (Taylor and King, 1937), as the result of finding trophozoites of this amoeba within epithelial cells living in the midgut, they changed the name to *Malameba locustae*. Richards (1968) has described two other species, *Hartmannella biparia* and *Hartmannella quadriparia*,

which are tissue invaders. Interestingly, he has found that both of these species infect freshwater gastropods. *H. biparia* occurs in various tissues of six species of *Bulinus*, seven species of *Biomphalaria*, *Helisoma* sp., *Indoplanorbis exustus*, and *Bythinia* sp. *H. quadriparia* occurs in various tissues of four species of *Biomphalaria*, *Drepanotrema simmonsi*, *Bythinia* sp., and *Physa* sp. The fact that both of these species of *Hartmannella* are also found freeliving suggests that their presence in mollusks may represent facultative parasitism. Richards' findings serve to negate the earlier report by Getz (1961) that "Acanthamoeba" (in the broad sense, thus including *Hartmannella*) will not infect mollusks.

The hartmannellid amoebae are of considerable interest as the result of their occurrence as contaminants of tissue cultures. Jahnes et al. (1957) have reported an unidentified species of *Acanthamoeba* contaminating monkey kidney tissue cultures. These amoebae are believed to have been present in the monkey kidney at the time it was excised. Since this initial report, Culbertson (1961) and Culbertson et al. (1958, 1959) have reported *Acanthamoeba* in tissue cultures and also that these amoebae are pathogenic to mice and monkeys if inoculated intracerebrally and intraspinally or by intranasal instillation. The pathological significance of these amoebae has increased since Pereira et al. (1966) and Armstrong and Pereira (1967) have reported that an infection in man originally known as "Ryan virus" has been subsequently identified as being a species of *Hartmannella* and Wang and Feldman (1967) have isolated 54 species (or strains) of *Hartmannella* from human throats. In addition, Butt (1966), Patras and Andujar (1966), and Callicott (1968) have reported species of *Hartmannella* can cause meningoencephalitis in man.

*Histopathology.* Because the changes in

the Tahitian oysters were severe, nonfocal, and degeneratively histopathological, even in areas where amoebae are not found, it is believed that these changes have resulted primarily from the adverse environmental conditions reported earlier rather than from the presence of the amoeba although the latter may be contributive. Richards (1968) is of the opinion that the presence of *H. biparia* and *H. quadriparia* in *Biomphalaria globosus* may adversely affect growth and reproduction and may even cause death. The lack of adequate water circulation, pollution, and the drastic decrease in ambient salinity are believed to be the primary causes of the mortality. Nevertheless, it is of interest to note that the degenerative changes do occur in moribund rather than truly dead oysters. The more rapid necrotic alterations in the specimens of *C. commercialis* from Tahiti may be due to the higher ambient temperatures (27.4–39°C). In the experimental studies conducted by Sparks and Pauley (1964) to determine postmortem changes in *C. gigas*, the oysters maintained in the laboratory, after one valve had been removed, were exposed to ambient temperatures of 14–16°C. In the case of the oysters maintained in the field after injury, their ambient temperatures ranged from 15.6 to 18.1°C. Thus, as one would expect, necrosis in oysters occurs much more rapidly at higher temperatures.

Although the necrotic changes in *C. gigas* described by Sparks and Pauley (1964) are without doubt postmortem changes, whether all of these alterations can be considered exclusively to be characteristic of these changes is now in doubt, inasmuch as many of them also occur in *C. commercialis* which, although moribund, were not dead at the time of fixation as indicated by their weak cardiac activity and tightly closed valves. It would appear that certain degenerative changes, such as those described as associated with

the lining epithelium of the digestive tract, ovary, digestive gland, Leydig tissue, and ctenidia of *C. commercialis*, represent generalized changes which commence with the onset of deterioration leading to death. The speed of such changes depends, at least in part, on the ambient temperature.

*Possible source of amoebae.* The terrain at Port Phaeton, Tahiti, allows for almost continuous freshwater runoff and soil erosion in the area where the specimens examined were collected. Thus, it is conceivable that soil amoebae could have been washed into the low salinity estuary. It is also possible that as the result of the pumping activity of the oysters, amoebae washed into the estuary are concentrated within their bodies. The routes of entry appear to be the alimentary tract and ctenidial surfaces where large numbers of trophozoites have been found. Once within the oyster, the degenerating tissues serve as a concentrated source of nutrients and the amoebae thrive. If this hypothesis is correct, then *H. tahitiensis* should be considered as a facultative parasite of moribund oysters or, more appropriately, as a secondary invader of necrotic tissues rather than as a true obligatory parasite. That *H. tahitiensis* is not a parasite that invades healthy oysters prior to their undergoing degenerative changes appears to be supported by the conspicuous absence of host cellular reactions. In the case of both *H. biparia* and *H. quadriparia* in gastropods, Richards (1968) has reported that both are found intracellularly in host amoebocytes which in turn are surrounded by fibroblasts. When a cluster of such cells occur together, a nodule results. Thus, conspicuous host cellular reactions occur in healthy mollusks invaded by these species of amoebae. The point being made is that the oyster hosts of *H. tahitiensis* are believed to be in the terminal phase of lethal degeneration prior to invasion by the amoebae and hence they

are most probably physiologically incapable of responding to the invasion.

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