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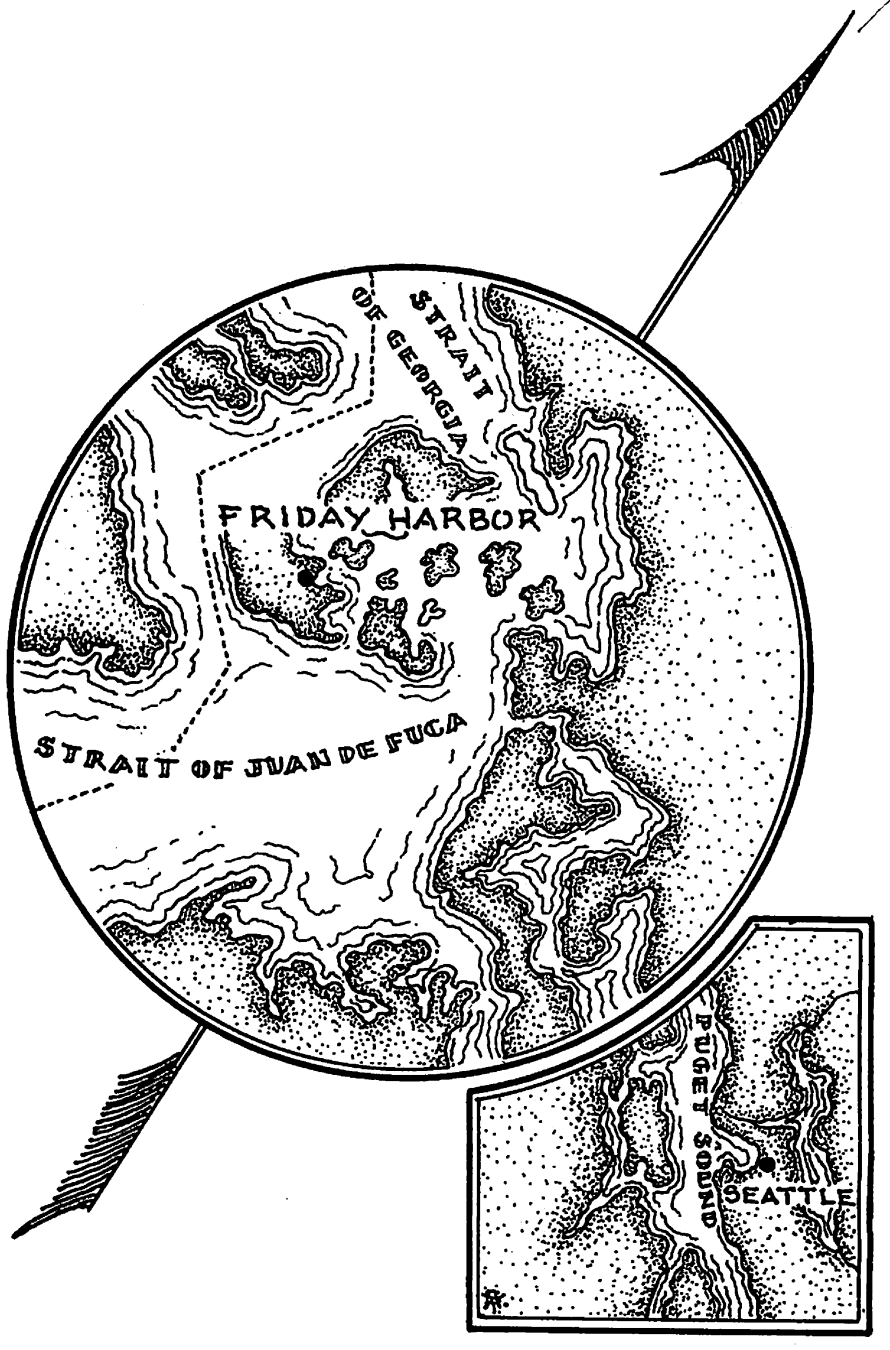
STUDIES ON LABYRINTHULA

Technical Report No. 3



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STUDIES ON LABYRINTHULA

by

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I. INTRODUCTION

Labyrinthula is a little studied and poorly understood aquatic microorganism with distinctive morphological characteristics. The major distinguishing features of Labyrinthula, as reported in the literature, are its peculiar type of motility, its vegetative "net-plasmodial" stage, and its communal phase of fructification.

The original description of Labyrinthula was made by Cienkowski in 1863. Other publications on Labyrinthula prior to 1935 were few in number and dealt primarily with the organism itself. No widespread interest was shown in this organism until Renn in 1935 reported Labyrinthula as the etiological agent of wasting disease in Zostera marina (eel-grass). As Renn has pointed out: "The plant is a prominent member of the shallow water community, sheltering a variety of larval and small sea animals, serving substantially as food for migratory game birds, and checking erosion of the bottom, and because the leaves are used for packing, upholstering, insulating, and other commercial purposes for which they are often peculiarly desirable, the disappearance of eel-grass became a problem of practical concern." Hence study of Labyrinthula seemed warranted from an economic standpoint as well as from a scientific interest in the biology of the organism.

All previous investigations of this organism were carried out using grossly contaminated enrichment cultures of this

microorganism. In no case were reproducible laboratory cultures obtained. The primitive cultural techniques used by the earlier investigators contributed to the conflicting and fragmentary nature of the reports on the morphology of Labyrinthula and served to confuse the reader. It was thought that the confusion concerning this microorganism could be eliminated by a critical review of the literature and by additional observations on Labyrinthula growing in pure culture.

This paper is a report of the isolation of Labyrinthula in pure culture and an investigation of the organism with special emphasis on the morphology, life cycle and nutritional requirements.

II. CULTIVATION AND ISOLATION

Previous investigators succeeded in obtaining natural enrichment cultures of Labyrinthula, primarily for observational purposes, from a variety of aquatic plants but failed to obtain reproducible laboratory cultures.

In this laboratory the original cultures of Labyrinthula were obtained from phytoplankton and zooplankton tows. The cultural technique employed by this laboratory for the original cultures of Labyrinthula was as follows: a drop of the plankton tow was spread with a sterile bent glass rod upon a solid agar medium composed of 0.9 per cent agar and aged sea water without any added nutrients.

The petri plates containing the cultures were then sealed with rubber tape and stored at 18° C for a period of two weeks. Upon examination, Labyrinthula was found growing in association with various species of bacteria. Labyrinthula grew within and around the periphery of the bacterial colonies, but migration from the edge of the bacterial colonies did not exceed 2 mm.

Subcultures of Labyrinthula were made by transferring a piece of agar 1 to 2 cm square containing a growing culture of this microorganism onto a fresh non-nutrient sea water agar plate. Growth continued but always in association with bacteria (Fig. 1). Subcultures were usually made at two-week intervals, but in a few instances subcultures were made from some of the original plates after a period of six months' incubation.

Many attempts were made to isolate Labyrinthula in pure culture. Nutrients were added to the non-nutrient sea water medium, but addition of nutrients merely increased the bacterial population to such an extent that Labyrinthula was crowded out or overrun. It was not until a medium containing 5 per cent human blood, 0.9 per cent agar, and aged sea water was made that a successful cultural medium was found for Labyrinthula. Labyrinthula crept away from the bacteria when placed on this medium. Additional subcultures were made every 24 hours from areas that appeared to be free of bacteria. After a series of such subcultures,

it was believed that a pure culture* of Labyrinthula had been obtained. In order to insure having a pure culture, subcultures were made on a series of sea water blood agar plates with the addition of 200 units of penicillin and 100 units of streptomycin per cc. Bacteria were never observed on repeated direct examinations or when the cultures were streaked on various nutrient agars. It was thus concluded that a pure culture* of Labyrinthula had been obtained.

There appeared to be no ingestion of the blood cells on a blood agar medium, but there was definite beta hemolysis of the red blood cells. It was likewise shown that good growth of this microorganism could be obtained on a medium which contained non-nutrient sea water agar and 10 per cent ox serum. On this medium Labyrinthula has been grown by periodic subculturing for a period of 15 months.

III. MORPHOLOGICAL STUDIES

Introduction:

The organisms placed in the genus Labyrinthula are easily distinguished from all other described microorganisms by their

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* When a pure culture of Labyrinthula is spoken of in this paper, it refers to a culture free of all other microorganisms. A single cell isolation of this organism has not been accomplished; therefore, a pure culture in the sense of a genetically homogenous stock cannot be claimed.

characteristic morphological features. The vegetative cells are fusiform-shaped with a flexible cell membrane and exhibit a gliding type of motility, the mechanism of which is unknown. The motility may be either an individual or group affair; however, the cells usually appear to be dependent in some manner upon a tube or track-like structure which they secrete prior to their migration. Cells of Labyrinthula vary in size. The internal structure of the cell consists of a vesicular nucleus, many small granules and vacuoles. The most striking morphological features are the filaments and pathways secreted by the cells. The filaments anastomose with filaments from adjacent cells or with adjoining cells to form a lacy network. As the cells migrate along the filaments, they constantly modify the shape and size of the filaments until broad riverlike channels are formed along which numerous individual organisms flow abreast.

Cell Morphology:

The cell shape of Labyrinthula is variable. In young cultures the predominant form is fusiform-shaped (Fig. 2), though cells are commonly found which differ from this form. Many times cells acquire a spherical shape with the ends of the cells curling toward each other. This is not a permanent form; the cells may uncurl and assume their normal fusiform shape. Cells also assume a spherical shape by lateral expansion in senescent cultures or in cultures exposed to unfavorable environmental conditions.

Young (1937) described the round bodies as cysts formed

by the curling process just described. Other investigators (Cienkowski, 1867; Zopf, 1892; Dangeard, 1910, 1932) mentioned the presence of round bodies but failed to indicate how they were formed. It is the author's opinion that the round bodies observed by these workers were formed by lateral expansion of the cell described above. Although earlier workers described these round bodies to be cysts, they were never able to observe the germination of these so-called "cysts". Repeated examination of these spherical shaped cells in this laboratory did not reveal any type of an external membrane nor was germination of the round bodies ever observed. The author feels therefore that the interpretation that these forms are cysts is a very debatable question.

Another modification in shape of Labyrinthula occurs when a cell passes a mechanical barrier or conforms to the contour of the track upon which it glides. This change in shape is best illustrated when one cell passes another cell along a narrow track (Fig. 3) or when a cell passes through any type of orifice which has a smaller diameter than that of the cell. What occurs is quite similar to pushing an inflated balloon through an orifice smaller than the balloon. As the cell approaches the place of constriction, it seems to stretch out and constrict laterally until it is small enough to pass through the opening. As each portion of the cell passes the place of constriction, it resumes its normal shape or becomes slightly inflated as a balloon would under the same circumstances. When finally

the whole cell passes the point of constriction, the normal fusiform shape is again resumed. It appears that the changing shape of the cell, rather than being caused by some function of the cell, is controlled by some external factor.

Additional observations also showed the ability of this organism to change its shape. Cells migrating on the tracks conformed to both the shape and direction of the tracks. Often cells temporarily distorted their bodies to conform to a bend in the track as shown in Fig. 4.

It is apparent from the foregoing description that the cells do not possess a rigid cell wall, and all previous workers have come to this same conclusion. It was impossible in the present investigation to demonstrate any type of structural membrane with staining, phase microscopy, or electron microscopy. It is recognized however that, although the cell cannot possess a rigid cell wall, some type of structural membrane must be present.

Cells of Labyrinthula vary considerably in size. Previous investigators (Cienkowski, 1867; Renn, 1935-1936; Young, 1943; Valkanov, 1929) reported the length to be from 8 mu to 30 mu. The fusiform-shaped Labyrinthula cells observed in this investigation measured 10 to 20 mu in length and 3 to 5 mu in width. The spherical cells measured 7 to 9 mu in diameter.

The internal structure of cells of Labyrinthula seems relatively

simple. With present-day optical aids, it was possible to demonstrate only a vesicular nucleus, granules, and vacuoles in these cells.

The many granules (Fig. 2) are the most conspicuous feature of the cell. The number and size of these granules vary. Cells were rarely found void of the granules; usually between 60 to 100 granules were present. The size of the granules ranged from 0.5 mu to 1.5 mu. However when the cells died the granules often fused to form one or more solid masses which then filled the cell completely.

There are conflicting reports in the literature on the nature of these granules. Jeppe (1930) stated that she found the granules to be of a lipoid composition while Young (1937) was unable to obtain a positive test for fat. In the present investigation granules gave a positive test for the presence of fats when stained with sudan black. In addition, a positive test for polysaccharides was obtained by this investigator using Pennington's (1949) polysaccharide stain. The function of these particles cannot be explained at this time.

A single vesicular-type nucleus with a central nucleolus (Fig. 2) is always present. The nucleus may be seen clearly with the aid of a phase contrast microscope as shown in Fig. 2. Although the nucleus is usually located near the center of the cell, it may occasionally be at either end of the cell, and it was shown in this investigation with the aid of a phase contrast microscope that the nucleus did not occupy a stationary position in the cell. Often

the movement of the cell caused the nucleus to change position in the cell. Although it was impossible to demonstrate the mechanism of nuclear division, it was possible to demonstrate that karyokenesis precedes cytokenesis, since two or more nuclei were often present in a cell prior to cellular division.

All previous investigators have reported the presence of vacuoles in the cells of Labyrinthula. Only Young (1937) however has attempted to explain the function of these vacuoles. He arrived at the conclusion that they were contractile vacuoles and served some type of an excretory function.

In the present investigation, vacuoles were not generally present in young healthy cultures, but were found when cells were senescent or were subjected to unfavorable environmental conditions such as changes in osmotic pressure, heat, and desiccation. In one instance, however, vacuoles were observed in an apparently healthy culture of Labyrinthula (Fig. 5). It has not been possible to demonstrate in this investigation the characteristic diastolic and systolic movements which are characteristic of contractile vacuoles in other microorganisms, and therefore it does not seem logical to conclude that these vacuoles are contractile in nature. Rather it is the author's belief that the vacuoles present in Labyrinthula are merely symptoms of a degenerative process. The same phenomenon of vacuolation in degenerating cells is common in other microorganisms (Osterud 1950).

Plasmodium morphology:

A morphological feature which has perplexed and fascinated investigators since the discovery of this organism is the network which connects the cells and appears to control the motility of these microorganisms. Previous investigations on Labyrinthula have dealt primarily with this interesting structure, for it is this net-plasmodium and its relation to motility that makes this organism so strikingly different from all other microorganisms.

The net-plasmodium is formed when the cells in some way secrete a mucoid substance which forms the characteristic filaments, which in turn anastomose with adjacent filaments and cells to form the initial net-plasmodium. The filaments are modified with the migration of the cells until they are broad track-like* structures (Fig. 6). The formation and physical and chemical nature of these filaments and tracks will now be examined for a clearer understanding of the nature of this net-plasmodium.

Previous investigators believed the filaments were a direct secretion of the cells. In contrast, the author believes that the filaments are not direct secretions of the cells but are formed from

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* The pathways on which the cells migrate will for convenience usually be referred to as tracks. The writer does not wish to preclude the possibility that on some occasions these pathways may be of a different nature. This problem will be discussed in detail later in this paper.

mucoid lamella which has been secreted from the cell. The following evidence supports this hypothesis: (1) filaments formed from the edge of a mucoid lamella (the edge of this lamella was 60 mu from the nearest cell) in which the cells at the periphery of the colony were embedded. (2) Although many cells traversed the locus of secretion during the filament formation, filaments were constantly being formed from the edges of the pathways making it impossible to believe that any one cell was responsible for the secretion of any one filament. (3) micromanipulation studies showed that beneath the surface of the cell was a mucoid sheet and that the filaments were merely prolongations of the lamella. (4) Filaments were observed to form from lamella which extended beyond the surface of the cell.

The filaments, when they first form, are very fine colorless homogeneous strands. These filaments grow in length, bifurcate, and anastomose with cells and adjacent filaments until a highly reticulated network is formed (Fig. 7). Once this network is formed, the cells migrate along it. With the migration along the filaments, these fine strands widen until they are broad channels along which numerous organisms flow abreast (Fig. 6). These channels range in size from a few microns to as wide as 1 mm.

Previous investigators (Cienkowski, 1867; Zopf, 1892; Mangenot, 1910, 1932; Young, 1937) stated that these structures were formed by many fine filaments coming to lie close to one another to

give a track-like appearance. Although tracks were never observed forming in this manner, there are certain indications that tracks may likewise be formed in this fashion (Watson, 1951).

The nature of the pathways has perplexed all workers since the discovery of Labyrinthula by Cienkowski (1863). Prior to Young (1937) workers were uncertain as to whether the pathways represented communicating tubes in which the organism flowed or tracks on which the organism moved. Young (1937) concluded from his investigation that the cells moved on the outside of a solid track. This question was further investigated by the present author.

Evidence obtained by the use of carmine particles indicates that the cells migrate on an open track rather than within a tube. In this experiment carmine particles were blown upon actively migrating cultures of Labyrinthula growing on a serum sea water agar medium. The carmine particles often fell on the surface of pathways along which they were moved by the migrating cells along the pathways. This movement of the carmine particles would not be possible if the cells were moving within a tubelike structure.

The above evidence seems to indicate that the pathways were track-like rather than tube-like in nature. Other observations however indicate that the filaments and pathways might also be tube-like structures. For instance, the cells as they migrate on the fine filaments appear to move within a mucoid tube. The filaments expand just anterior to the cells and contract partially just posterior to the cells. This

phenomenon appears analogous to pushing a ball through a pliable tube of a smaller diameter.

The observations discussed thus far have been concerned with the organisms growing on the surface of agar plates. The cells of Labyrinthula have the ability to grow and to migrate beneath as well as upon the surface of the agar. In this case the organisms must certainly form some type of "subway" or tunnel-like structure in which to migrate. In some instances organisms were observed which migrated in the normal manner along pathways on the surface of the agar but which disappeared into holes in the agar in a fashion analogous to gophers disappearing into their holes. Upon further examination it was found that the pathways on which these organisms migrated extended into the agar, often at an angle of 90°. The shape of the organisms migrating beneath the surface of the agar appeared to be quite different from the shape of the cells observed on the surface of the agar. The tube-like structures appeared to be smaller in diameter than the cells themselves, causing the cells to elongate and constrict laterally to conform to the diameter of the "subway" in which they migrated.

The above observations indicate that the cells of Labyrinthula migrate through a tube-like structure when growth occurs beneath the surface of the agar. It is not necessarily true, however, that the pathways beneath the surface of the agar are formed from the ectoplasmic secretion of the cells, though it is undoubtedly true that at

least some portions of these "subways" are lined with this mucoid ectoplasmic secretion. Possibly this question could be settled by making cross-sections of an agar block with Labyrinthula growing beneath the surface of the agar.

The formation of filaments and tracks with the eventual formation of a highly reticulated network is accomplished very rapidly. It was shown (Watson, 1951) that the network and tracks re-formed within 30 to 60 minutes after being completely destroyed, given the proper conditions.

Little is known about the chemical nature of the filaments and pathways. Previous investigators were unsuccessful in their attempts to stain these structures. In this laboratory it was possible only to stain with one specific stain, Pennington's (1949) polysaccharide stain, thus indicating that these structures contained a polysaccharide complex.

All previous investigators except Cienkowski (1867) considered the filaments to be pseudopods. It was on this basis that Labyrinthula was classified among the Sarcodina. The validity of the theories regarding pseudopodial nature of these filaments was doubted early in this investigation. It was noted that when cells of Labyrinthula died, the filaments were not retracted. It is well known that organisms possessing pseudopods nearly always retract their pseudopods prior to the death of the organism (Osterud, 1950). Such a retraction of the filaments of Labyrinthula was reported by Zopf (1892),

Dangeard (1910, 1932), and Young (1937). Obviously this conflict in findings must be explained. One possibility is that the earlier investigators observed the disappearance of the filaments and assumed that they were retracted by the cells. This may be observed in a culture undergoing dehydration, with the accompanying disintegration of the filaments and subsequent transformation of the mucoid material to fine balls. Gradually even these mucoid balls seem to dissolve in the medium.

Additional observations show that the shape and size of filaments may change. This does not mean, however, that the filaments are being retracted by the cells. Rather, it appears that the mucoid material which constitutes the filaments is being redistributed in the filaments or in the lamella. For example, various filaments were observed to abort to form lamellae (Fig. 8), whereupon these lamellae would frequently disappear. There was no evidence that the material in these mucoid sheets was being retracted by the cells. Rather, it seemed that the mucoid material in the lamellae was being redistributed to form new filaments and to enlarge old filaments.

Single cells isolated from other cells were watched for any evidence of retraction of the filaments. However, in no case did the cells retract filaments.

To summarize, the cells of Labyrinthula secrete a mucoid material which is never retracted by the cells. This substance once secreted may change its form by a process which is probably controlled

only by physical or chemical factors rather than directly by the cells. According to Kudo (1947), "a pseudopodium is a temporary projection of part of the cytoplasm". Therefore if our observations and conclusions are correct, these filaments cannot be considered to conform to this definition. It is our belief that these filaments do not represent pseudopodial structures.

Colonial Morphology:

When colonies of Labyrinthula are examined with the naked eye, it is noted that they are white and opaque. The organisms grow either within or on the surface of the agar with occasional growth in both places.

Little detail of the colonial morphology may be seen when the colonies of Labyrinthula are examined without optical aids. However, it is seen even with the naked eye that the colonies of Labyrinthula are quite unlike bacterial colonies in that they do not have the solid appearance so characteristic of many bacterial colonies (Fig. 1). In addition the periphery of the colonies of Labyrinthula is more irregular than the periphery of most bacterial colonies. Colonies of Labyrinthula are, when seen grossly, more sililar to colonies of molds, though with experience they are easily differentiated.

Many of the details of construction of a colony of Labyrinthula become apparent upon examination with a wide field binocular microscope. With low magnification, the surface growth of the colony appears as a lacy network (Fig. 7). This reticulated structure, as previously shown,

consists of many bifurcated tracks which join one another in a fashion analogous to tributaries joining a river. More details of the surface growth may be seen with 70x magnification (Fig. 7). With this magnification single organisms may be distinguished and the tracks appear to be composed of solid masses of cells. However, as shown previously in this paper, the cells are merely migrating on the mucoid tracks. Likewise, it may be seen that a massing effect, as previously discussed, occurs at the edge of a colony (Fig. 7, 9).

Thus far the discussion of the colonial appearance has been limited to the growth of the organism on one plane. However, the tracks are not always limited to one plane when this organism grows within the agar. When the growth occurs within the agar, oftentimes the colony is bush-like in appearance, shaped much like a piece of coral (Fig. 1). It must be emphasized that this type of growth never extends above the surface of the agar and only occurs within the agar.

In some respects this bush-like colony (Fig. 10) has a mold-like appearance. However, a mold colony appears to be much more regular and has a stiff appearance which is lacking in colonies of Labyrinthula.

In summary it may be said that growth of Labyrinthula occurs within and upon the surface of the agar. Although the

colonial appearance is to some degree similar to that of other microorganisms, it differs enough so that Labyrinthula is distinguishable from all other microorganisms.

Motility:

The movement of Labyrinthula is perhaps the most perplexing feature of this organism. It has a gliding type of motility which usually appears to be dependent upon the filaments and pathways secreted by the cell.

The mechanism of motility is unknown. It has been shown in this investigation by means of electron microscopy and phase microscopy that no flagella, cilia, or pseudopodial appendages are present. Likewise it was shown (Watson, 1951) that the motility is not similar to that of the naviculoid diatoms.

In addition it was shown (Watson, 1951) that the motility is an active type of motility rather than a passive type and is not caused by some motion of the filaments or pathways on which it migrates. This was demonstrated by placing carmine particles on the tracks and filaments and observing that the carmine particles moved only when the migrating cells dragged or pushed them along the tracks. Never was movement of the particles observed independent of the cells. Additional evidence was found when three cells were observed to migrate in the absence of any observable filaments,

tracks, or any type of mucoid material.

Although the above observations indicate that the cells are actually motile and are not being moved by some external force, it still appears that the cells are in some way aided in their migrations by the presence of the tracks and filaments. If the cells are not dependent in some way upon the tracks for their peculiar type of motility, one would expect motility to occur in the same fashion free of the filaments and tracks. This is not the case. As was previously stated, only three cells have been observed to migrate in the absence of observable tracks and filaments. In contrast there is an abundance of evidence to indicate that the cells are in some unknown way dependent upon the mucoid secretions. Cells which are pushed or for other reasons leave the tracks do not migrate further. Even more convincing evidence of the cells' dependency upon the tracks and filaments is the effort which the cells exert to stay on the filaments. Cells often meet physical barriers on the tracks but distort their bodies to pass any such barrier rather than leave the track during their migration. Likewise a distortion of the shape of the cell is seen when the cells migrate upon the narrow filaments. In this case the cell apparently attempts to conform to the shape of the narrow filament upon which it migrates. In general it must be concluded that the cells are in some way aided in their migrations by the presence of a mucoid

track or filament.

The tracks also exert a guiding influence upon the migrating cells. One of the most striking examples of track-guided cells is seen when a cell comes to the junction of two tracks. The cell will continue to migrate on one of the two tracks depending on the angle of approach to the junction (Fig. 11). The guiding influence of the tracks is likewise indicated when cells are seen to conform to a bend in a pathway along which the protist migrates (Fig. 4).

The general direction of movement of these organisms is always away from the point of inoculation. However, the cells often take devious pathways resembling winding rivers to reach the goal of their wanderings.

The maximum rate at which these organisms migrate has been recorded by Young (1937) as 150 mu per minute. The organism's maximum rate of migration as recorded in this laboratory was 143 mu per minute. This measurement was made on organisms migrating upon agar while Young's was made on organisms migrating in a wet mount.

IV. PHYSICAL FACTORS AFFECTING THE GROWTH OF LABYRINTHULA

It was found during the course of investigation (Watson 1951) that certain physical factors were important in the growth of Labyrinthula. These factors were as follows: (1) a high

moisture content, (2) an obligate surface requirement, (3) a temperature range from 18° C to 22° C, (4) an obligate salt requirement, as growth was not obtained on substituting distilled water or distilled water with 3 per cent sodium chloride added, (5) pH range of the medium used for growth was pH 7.0 to pH 8.9 with the maximum growth obtained at pH 7.8, (6) an obligate aerobic requirement.

V. ECOLOGY

It would seem that Labyrinthula, with its interesting morphological features which were described over 60 years ago (Cienkowski, 1867), would have been studied by many workers, but this is not the case. Apparently few investigators, to judge from the literature, have studied Labyrinthula. One possible reason for the lack of interest in this organism might be the inadequate cultural techniques used by earlier investigators. However, another factor may be the relative scarcity of Labyrinthula.

The following reports are to be found in literature concerning the hosts of Labyrinthula. Cienkowski (1867) found Labyrinthula associated with algae in the harbor of Odessa but failed to mention the genera. Zopf (1892) described a fresh water species found associated with Vaucheria sessilis. Dangeard (1910)

described Labyrinthula zopfi as being associated with species of Chlamydomonas. Later Dangeard (1932) found the organism he named Labyrinthula chattonii to be parasitic on Cladophora refracta. In addition he was able to parasitise two additional species of Cladophora, Cladophora laetevirens, Cladophora flavescens, with Labyrinthula chattonii. Jepps (1930) found Labyrinthula associated with diatom cultures and indicated that Labyrinthula was a parasite on the diatoms. In addition Jepps (1930) was able to infect Laminaria sp. with this microorganism. Sparrow (1936) found Labyrinthula as a saprophyte in the cells of Rhizosolenia and was likewise able to demonstrate Labyrinthula in association with Cladophora sp.. Renn (1936) was the first worker to demonstrate Labyrinthula to be a parasite of Zostera marina. Young (1937, 1943) confirmed Renn's findings and in addition was able to parasitise the following plants: Cladophora hirta, Chaetomorpha linum, Chaetomorpha Melagonium, Fucus furcatus, Ectocarpus confervoides, and Cystoclonium purpureum. Young likewise discovered four new natural hosts of Labyrinthula, two of which were algae and two others which were species of Naiadaceae.. The two algae, Cladophora hirta and Chaetomorpha linum, had previously been shown to be susceptible to infection. The two members of the Naiadaceae were Zannichellia palustris var. major and Ruppia maritima var. rostrata.

In this laboratory, as has been previously stated, the original cultures of Labyrinthula were obtained from phytoplankton and zooplankton tows. The predominant organisms in the phytoplankton tows were species of the Chaetoceros, Rhizosolenia, Nitzchia, and Coscinodiscus genera. The predominant organisms in the zooplankton tows were various species of Copepods. All plankton tows were made in the vicinity of San Juan Island.

Other marine plants: Fucus sp., Nereocystis luetkeana, Zostera marina, Laminaria sp., and Ulva sp., were likewise examined for the presence of Labyrinthula. Labyrinthula could be cultured from Ulva and Zostera marina. The Ulva was found attached to the docks at the Oceanographic Laboratories at Friday Harbor, Washington. The first culture of Labyrinthula from Zostera was obtained from floating pieces of Zostera in front of the Oceanographic Laboratories at Friday Harbor. These blades of Zostera were darkish brown in color. It was not possible, however, to culture Labyrinthula from the apparently healthy beds of Zostera which grew in front of the Oceanographic Laboratories. Labyrinthula was consistently cultured from the beds of Zostera at Mitchell Bay, San Juan Island. Zostera in these beds were brown and discolored, which gave them an unhealthy appearance. In addition they were covered with hydroids and naviculid-type diatoms. The above examinations were made in the summer of 1949.

Cultures of Labyrinthula were again obtained from the Zostera beds at Mitchell Bay in the summer of 1950. In addition, cultures of Labyrinthula were obtained from the Zostera beds at Squaw Bay, Shaw Island, likewise located in the San Juan Islands.

It must be concluded from the above reports that growth of Labyrinthula does not seem to be restricted to any one geographic locality since it has been found in various places both in Europe and the United States. Likewise, this organism does not seem to have a fastidious host requirement. However it is not possible at the present time, although the above facts are true, to show a great abundance of the organism in any given area. More work is needed on the ecology of this organism to determine if it is actually a rare organism, or if it is merely that very few investigators have shown interest in this microorganism.

In view of the occurrence of Labyrinthula in association with a variety of marine plant forms, the question may be raised whether Labyrinthula is actually the primary pathogen for Zostera marina or is merely a secondary invader. Renn (1935) was able to infect healthy plants of Zostera marina with diseased plant tissue of Zostera marina. This is not conclusive evidence that Labyrinthula is the primary pathogen since other microorganisms including bacteria were present in the diseased tissues and some of them may have been the primary pathogen. Isolation of Labyrinthula in pure culture

provides an opportunity to determine whether Labyrinthula is actually responsible for the wasting disease of Zostera marina.

VI. LIFE CYCLE

The life cycle of Labyrinthula has been reported to consist of alternate stages of fructification and vegetation. Most of the previous investigators have reported both of these stages in their studies of Labyrinthula. However, their conclusions were based upon observations of various cultures in different stages, since these investigations were limited by crude cultural techniques.

The writer was unable to demonstrate conclusively the existence of a fructification stage. In the following section, his observations on the vegetative stage will be summarized and evidence obtained in this laboratory suggestive of a fructification stage will be presented.

The first stage is the vegetative state or the net-plasmodial state which has been discussed in the section on morphology. In this stage the fusiform cells of Labyrinthula extrude a mucoid ectoplasmic substance which forms a flat plate beneath the cells. From this lamella filaments are formed which connect with other cells or other filaments to form a lacy network. Once this network is formed, the cells migrate along the filaments away from

the point of inoculation. As the cells of Labyrinthula migrate, they gradually modify the filaments upon which they move until a wide track-like structure is formed upon which a few or many such cells may flow abreast. As the cells migrate the whole colony is constantly being changed; cells are increasing by division, new filaments are being formed and old filaments are being widened. The cells continue to migrate until they reach the edge of the colony at which point a massing of cells occurs. Each group of cells at this point is invariably imbedded in a granular mucoid layer. Frequently, filaments may be seen to extend from such a lamella.

The vegetative state is the only one which presented itself when Labyrinthula was grown bacteria-free on agar plates. Most of the previous workers, however, described a stage of fructification. According to Cienkowski (1867), Zopf (1892), Dangeard (1932) and Young (1937), the vegetative cells mass together to form sori. The vegetative cells in this mass become spherical and are held together by a mucoid material. These workers have also reported that the cysts of the sorus divided into several parts, each of which developed into a vegetative cell after germination.

In the present investigation it was impossible to demonstrate the formation of sori using pure cultures of Labyrinthula. However, this observation does not preclude the formation of a sorus by Labyrinthula. On the contrary, it might indicate that

certain nutritional requirements were lacking in the medium upon which it was grown. A similar phenomenon is commonly found among the myxobacteria (Watson, 1951).

Structures suggestive of a fructification tendency were often seen in the pure culture studies in the present investigation. These structures are formed by a massing of cells but differ from the massing of cells in sori formation as observed by earlier investigators. The first indication of this structure was seen when a few cells massed together and revolved counter-clockwise. These cells continued to rotate in this circle while more cells flowed into the mass. This phenomenon is much like a coiling of rope with the incoming cells forming the periphery of such a structure. After several hours the originally small structure would contain several hundred cells. The rotation of the cells eventually ceased and the cells remained in a dormant condition. However, maturation of this structure to form a sorus was never observed.

The above observations were made on pure cultures which were kept growing by serial transfers for fifteen months. Recently a new strain of Labyrinthula was obtained from Zostera marina but this strain was not isolated in pure culture. Upon the first serial transfer of this impure culture, a yellow structure about 0.5 mm in diameter was found on several agar plates. This structure was composed mostly of spherical shaped cells (Fig. 12) 10 mu in diameter plus a few normal vegetative Labyrinthula cells, all of them

yellow in color. It was impossible to demonstrate the germination of the individual spherical cells. However after ten hours of incubation on a fresh plate, the characteristic net-plasmodium was found around the periphery of the structure with vegetative cells migrating out of the yellow mass of cells. In addition there were many amoeboid cells around the periphery of the structure. It is too early to draw a definite conclusion concerning these amoeboid cells but it would be interesting if they were shown to be a part of the life cycle of Labyrinthula. This work is in progress at the present time.

In conclusion, it is possible to say only that Labyrinthula did not form fruiting bodies when grown in pure culture, although there is certain evidence to indicate that Labyrinthula is capable of forming such a structure when environmental conditions are favorable.

SUMMARY

1. A species of Labyrinthula was obtained free of contaminating organisms other than bacteria on a sea water agar medium without added nutrients, and was isolated in pure culture using a sea water agar medium containing blood.

2. The morphology of the individual cells of Labyrinthula was investigated. Following is a summary of the pertinent features. Although usually fusiform, the cells may modify their shape when passing a mechanical barrier or when conforming to the contour of a filament or pathway on which they are migrating. In addition spherical cells are occasionally found which are formed by the ends of the cells folding over or by lateral expansion of the cells. The latter type of spherical body is found when the organisms are subjected to unfavorable environmental conditions. No demonstrable cell wall is present but from necessity some type of a flexible cell membrane must be present.

The internal structure of the cell was found to be relatively simple. A vesicular nucleus and numerous uniform granules were characteristically present. It was shown that karyokinesis characteristically preceded cytokinesis. It was not possible, however, to reconstruct the details of nuclear division from stained specimens. The function of the uniform granules characteristically

present in the cell is unknown, though it was possible to demonstrate that these granules contain polysaccharide and lipoid. Vacuoles are rarely present in healthy cultures of Labyrinthula although they occur frequently in senescent cultures. No evidence was obtained which might indicate that these vacuoles are contractile in nature.

3. The cells of Labyrinthula exhibit a high degree of motility, the mechanism of which is unknown. No flagella or cilia are present. The motility appears dependent upon the filaments and pathways produced by the cells though the motility is of an active rather than a passive type.

4. The formation and morphology of the filaments and pathways were investigated. It was definitely shown that the filaments are not pseudopods, a finding which contradicts reports in the literature. The filaments are not direct secretions of the cell but are formed from flat mucoid lamellae secreted by the cells. The pathways over which the cells travel are formed by expansion of the filaments. Polysaccharide was found present in the mucoid material of the lamellae, the filaments and the pathways. Evidence was obtained indicating that in many instances the pathways are open tracks over which the organisms migrate. Further evidence however, indicated that in other instances pathways are tubelike structures.

5. The colonies of Labyrinthula on agar are easily distinguishable from colonies of other microorganisms. Labyrinthula grew upon, in but never above the surface of the agar. On the surface of the agar the growth is fan-like in appearance, while in the agar the growth is bush-like. This is undoubtedly due to the highly reticulated network on which or in which the organisms migrate.

6. A variety of environmental factors affected the growth of Labyrinthula in pure culture. Growth occurred on agar in concentrations of 1 per cent or less. Growth was never obtained in conventional liquid media, probably because the organism has an obligate surface requirement. Synthetic sea water proved to be an acceptable substitute for natural sea water, but growth was not obtained on media containing either 3 per cent sodium chloride or fresh water as a base. The pH optimum for growth on sea water media containing serum was pH 7.8. On this medium, the growth range was pH 7.0 to pH 8.9. The optimum temperature proved to be in the range of 18°C. to 23°C. The maximum temperature of growth was 30°C. The minimum temperature was not specifically determined but growth occurred at icebox temperatures.

7. Although alternate stages of vegetation and fructification have been reported to occur in Labyrinthula, it was not possible to

demonstrate without qualification a stage of fructification in pure cultures. Survival of Labyrinthula in impure cultures was suggestive of the existence of a resistant stage, but no supporting cytological evidence was obtained.

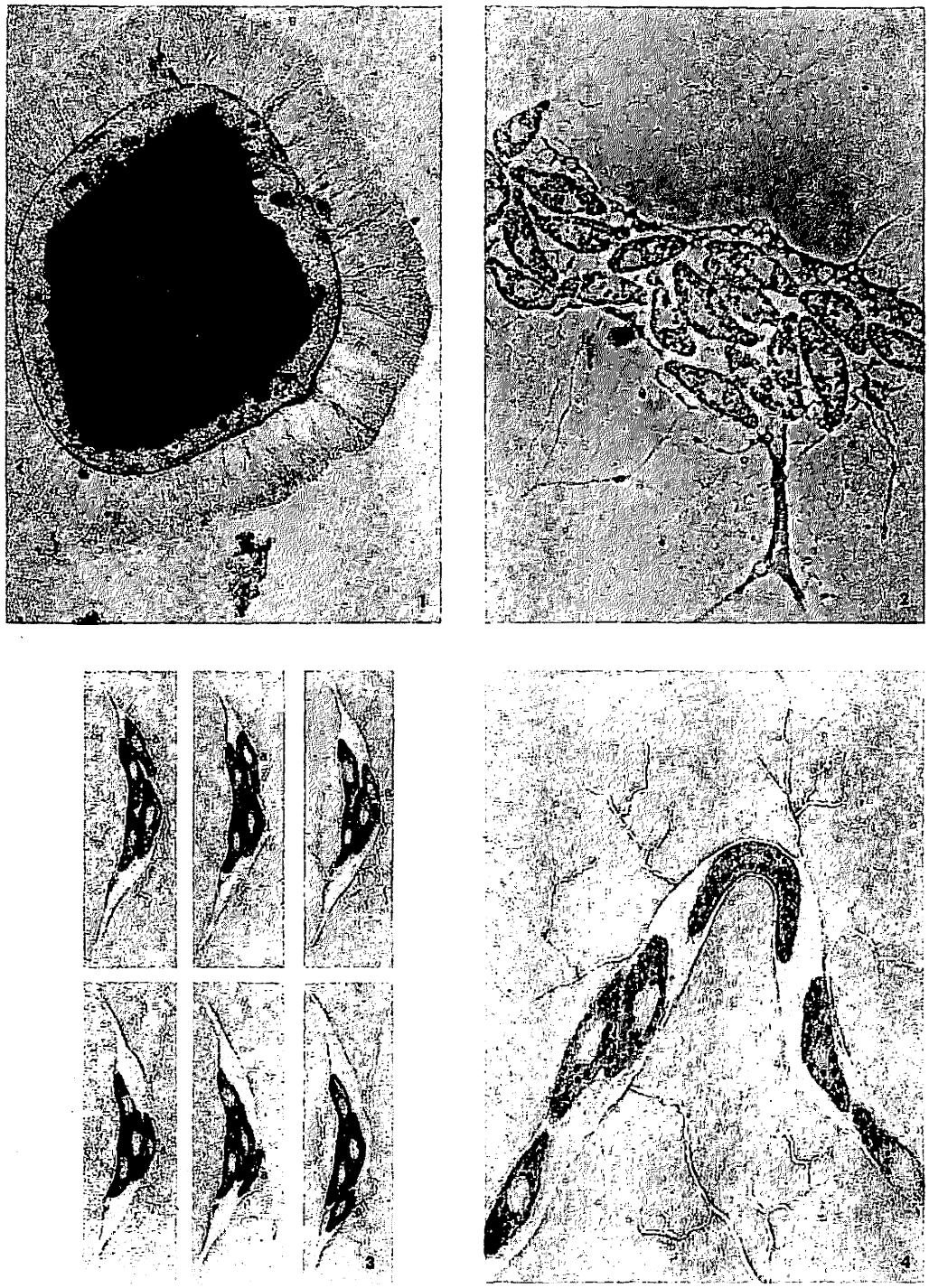


Fig. 1 A photomicrograph showing a culture of Labyrinthula extending beyond the periphery of a bacterial colony. 10X.

Fig. 2 A phase of photomicrograph showing the shape and internal structure of a Labyrinthula cell. 1150X.

Fig. 3 A drawing illustrating a cell "squeezing" past other cells which are not moving. 600X.

Fig. 4 A drawing illustrating a cell conforming to a bend in the pathway. 2300X.

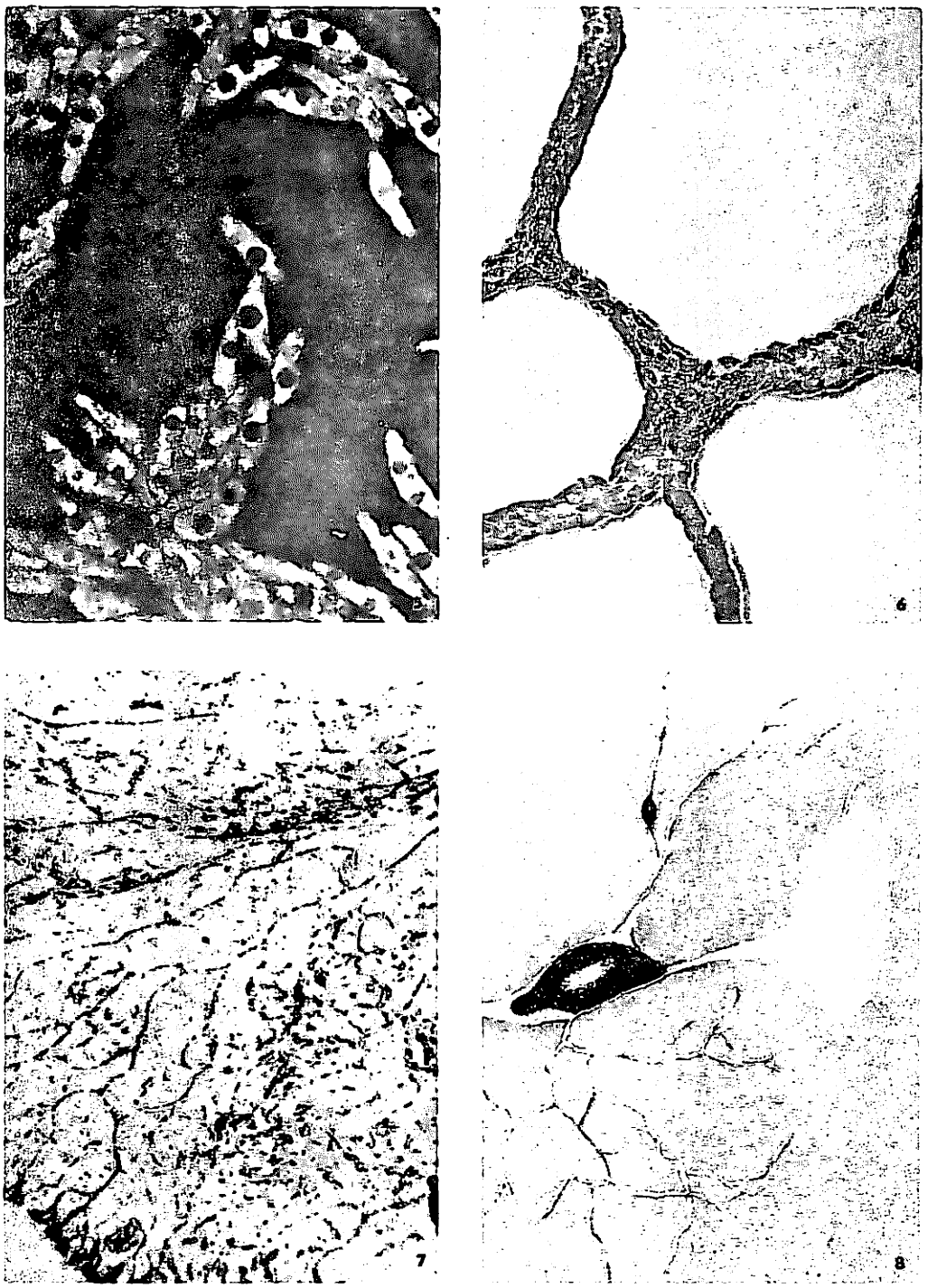
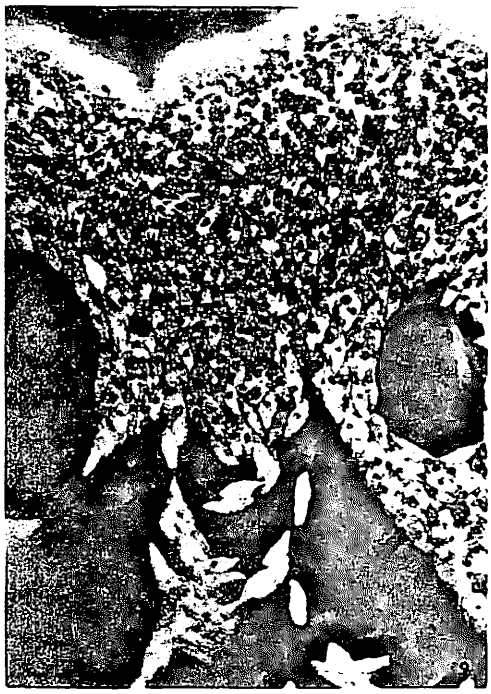


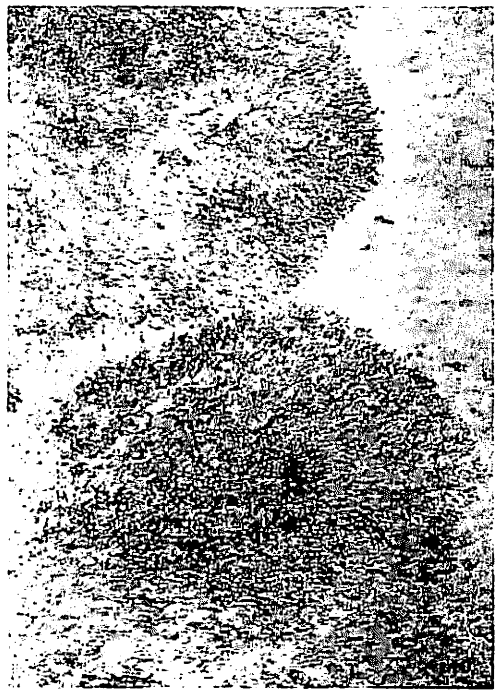
Fig. 5 A phase photomicrograph showing vacuolated cells of an apparently healthy culture of Labyrinthula. 650X.

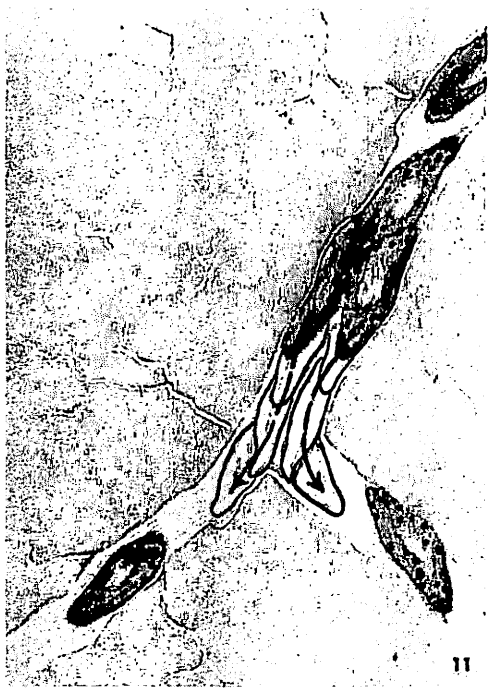
Fig. 6 A phase photomicrograph showing medium-size pathways of Labyrinthula. 650X.

Fig. 7 A phase photomicrograph showing the lacy network structure of a colony and the massing effect at the periphery of the colony. 70X.

Fig. 8 A drawing illustrating a broad lamella which is occasionally formed from a filament. 1350X.







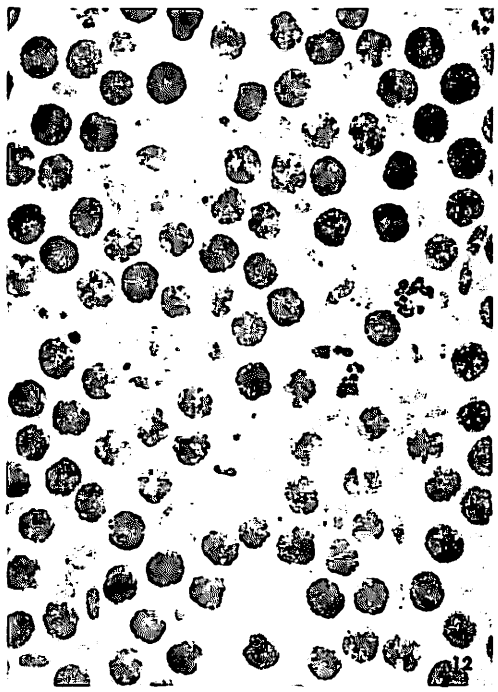


Fig. 9 A phase photomicrograph showing the massing of cells at the periphery of a colony. 450X.

Fig. 10 A photomicrograph showing the "bushlike" appearance of colonial growth which occurs in the agar. 15X.

Fig. 11 A drawing illustrating the angle at which a cell approaches a Y determines the branch of the Y on which it will continue to migrate. 1000X.

Fig. 12 A photomicrograph showing the spherical shaped cells found in a crushed "sorus." 500X.

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