1959

# Observations on the biology of Pelomyxa palustris Greeff, collected under polysaprobic conditions

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# OBSERVATIONS ON THE BIOLOGY OF *PELOMYXA PALUSTRIS* GREEFF COLLECTED UNDER POLYSAPROBIC CONDITIONS

by

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A Thesis Presented to the Graduate School of
the University of Richmond in Partial Fulfillment of
the Requirements for the Degree of Master of Science

University of Richmond

May, 1959

# ACKNOWLEDGEMENTS

It is a pleasure to express my gratitude to the members of the faculty of the Department of Biology for the opportunity of conducting this investigation. I am especially grateful to Dr. Nolan E. Rice for his suggestion of the problem and for his guidance throughout its course. I am indebted to Dr. John C. Strickland and Dr. Wilton R. Tenney for their advice and criticism and for use of personal equipment.

Grateful acknowledgement is made to the other members of the staff of the Department of Biology, Dr. Jack D. Burke, Dr. Warwick R. West, and Dr. William S. Woolcott, for their patient guidance and many considerations.

The kindness of the Southampton Quarry Club of Richmond for permitting free access to the quarry is appreciated.

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# INTRODUCTION

*Pelomyxa palustris* Greeff, the largest rhizopod of which we have knowledge, was described in 1870 by Greeff who named the organism *Pelobius*. According to Penard (1902) it was redescribed by Archer in 1871 and reviewed by Greeff (1874) who then gave it the name *P. palustris*.

## Habitat

*P. palustris* is polysaprobic. It is found in river beds, stagnant ponds, artificial basins, slow-flowing streams, and in lakes. It is limited to the top or upper layers of the bottom mud or silt in an environment of decaying vegetable and animal material. Dissolved oxygen in this sapropel layer is low, but there may be high concentrations of one or more of the following gases: carbon dioxide, hydrogen sulphide, nitrogen, ammonia, hydrogen, or methane (Bott, 1906; Gicklhorn and Dejdar, 1931; Kudo, 1957). The organism has been reported to occur on the continent of Europe, in the British Isles, and in North America. Kudo (1957) lists the collecting sites by country. The discovery of the organism in the Southampton Quarry in Richmond, Virginia, by Rice (1957) appears to be the first record of the occurrence of *P. palustris* in a quarry.

## Morphology and Physiology

*P. palustris* is simple in outline, with a thin hyaline plasmalemma and usually opaque undifferentiated cytoplasm, granular in structure, highly alveolated, and often filled with mud, sand, algae, and tests of small crustacea (Plate I).

In addition numerous nuclei, refringent bodies (Glanzkörper), and rod-shaped bacteria are present. The animal is characterized by a uroid located posteriorly (Greeff, 1874; Leidy, 1879; Penard, 1902; Minchin, 1922; Gicklhorn and Dejdar, 1931; Fortner, 1934; Mast, 1934; Doflein, 1949; Kudo, 1957).

The organisms range in size from 70 microns to 3 mm. in diameter (Greeff, 1874; Leidy, 1879; Penard, 1902; Mast, 1934; Kudo, 1946; Doflein, 1949; Kudo, 1957), and there is one report of large animals measuring 3.5 to 4 mm. in diameter (Gicklhorn and Dejdar, 1931).

Locomotion studies by Blochmann (1894) demonstrated rates of travel to be 20 cm. in 24 hours or 1/3 to 1/2 mm. per minute. Locomotion is by means of pseudopodia, usually a single lobose pseudopod, blunt and rounded, located anteriorly (Mast, 1934). Veley (1905) reported the occurrence of reticulose pseudopodia, but she alone has seen them.

Formerly, a number of races of P. palustris were admitted on the basis of color (Hollande, 1945). Three main color types were recognized: white, grey, and yellow; but a number of other colors including green, brown, black, red, red-orange, and intermediates have been described (Greeff, 1874; Leidy, 1879; Leiner, 1924; Okada, 1930; Gicklhorn and Dejdar, 1931; Fortner, 1934; Kudo, 1946). Differences in color were attributed to age (Okada, 1930), difference in species (Leiner, 1924), and difference in metabolic state (Fortner, 1934). Greeff (1874), Leidy (1879), Veley (1905), Hollande (1945), and Kudo (1957) believe color to be a result of cytoplasmic inclusions.

Since its discovery workers have extensively investigated the bacterial flora of *P. palustris*. Greeff (1874) believed the bacteria to be rods of some organic material. Penard (1902) noted "sticks" (*baguettes*) 10 to 20 microns in length. Veley (1905) considered them to be bacteria and named them *Cladothrix pelomyxae*. Keller (1949) identified two additional species, *Myxococcus pelomyxae* and *Bacterium parapelomyxae*. Leiner, Wohlfeil, and Schmidt (1954) found only one species of bacterium, while Kudo (1957) reported two distinct types of rod-shaped bacteria which he did not identify. These bacteria have been variously considered to be parasitic, symbiotic, or commensal on the host (Leiner, 1924; Kudo, 1957).

Parasites have been recorded for *P. palustris*. These include *Amoebophilus destructor* (Hollande, 1945) and *Entamoeba sp.* (Leiner, Wohlfeil, and Schmidt, 1954). Kudo (1957) found similar organisms.

Greeff (1874) described refractory bodies (*Glanzkörper*) from which he believed small amoebae to be formed. Penard (1902) believed the *Glanzkörper* to be "spores" of *Pelomyxa*. Stolc (1900), Bott (1906), and Leiner, Wohlfeil, and Schmidt (1954) agree that these refractory bodies contain reserve food in the form of a glycogen. Leiner (1924) noted the presence of glycogen in the cytoplasm outside of the *Glanzkörper*.

Reproduction in *P. palustris* is by plasmotomy. Division is usually into two individuals of equal size. As many as sixteen have been reported to originate from a single individual, although these were found to arise from one animal that had not been observed over the entire period of reproduction

(Schulze, 1875; Schirch, 1914; Leiner, 1924; Fortner, 1934; Hollande, 1945; Kudo, 1957).

P. palustris is a voracious feeder. Greeff (1874) and Leiner and Wohlfeil (1953) state that the organism ingests crustacea, rotifers, testacea, and phytomastigidians. Kudo (1957) reports that he has never observed this feeding. Various workers suggest that the organism has different food preferences. Bott (1906) considers the principle food source to be diatoms and decaying plant parenchyma; Dejdar (1931) noted filaments of Oedogonium; Fortner (1934) suggests that particulate organic material, chlorophyllous plants, and animal remains are food sources. Hollande (1945) counted 500 green pelomyxae feeding on the chlorophyllous parenchyma of a leaf. Kudo (1957) found that he was able to culture his animals on Spirogyra and other filamentous algae.

Until the work of Kudo (1957) no-one had successfully cultured P. palustris in the laboratory. Kudo's success and the subsequent discovery of P. palustris in the Richmond area by Rice (1957) suggested the present investigation of their culture requirements and the factors affecting their occurrence under natural conditions.

## MATERIALS AND METHODS

P. palustris was found in the Southampton Quarry on Riverside Drive, approximately 1.1 miles east of the Huguenot Bridge near Williams Island Dam on the James River at Richmond, Virginia. The organisms were collected on the bottom of the quarry on the accumulated debris at a depth of 50 to 55 feet in the western part of the quarry, and at a depth of 30 to 35 feet in the eastern part (Plate II). These two areas are connected by a narrow channel. The long axis of the Southampton Quarry roughly parallels the James River which is less than 200 feet to the north. The organisms were more plentiful at a depth of 50 to 55 feet in the western portion of the quarry. They were collected near the center by means of a 300 cc. B. O. D. bottle contained in a sewage water sampler (Precision Scientific Company, Chicago; Plate III).

Temperatures at various depths were determined by using a maximum-minimum Fahrenheit thermometer. Determinations of the amount of oxygen dissolved in the water at various depths from the bottom to the surface were made by using the Alsterberg modification of the Sodium Azide Method (American Public Health Association, 1955). Hydrogen ion concentrations in terms of pH were ascertained at the same time for the same depths using a Hellige comparator.

When each collection of organisms was brought to the quarry surface, the top of the water sampler was removed and the glass stopper of the B. O. D. bottle was inserted below the water level in the sampler and placed in the mouth of the bottle so that no direct contact between the water in the bottle

and the atmosphere occurred. After capping, the bottle was removed from the water sampler for transport back to the laboratory. It was placed in a gallon picnic jug containing ice and water at a temperature approximating that of the quarry water at the bottom (40 - 44° F.).

Upon returning to the laboratory the B. O. D. bottles were placed in the refrigerator. They were examined immediately, or in some instances were left in the ice water until the ice melted and the water temperature approached that of the laboratory (22 - 25° C.). The contents of the bottles were prepared for examination by placing them in finger bowls (4" x 1½") or in Carrel tissue culture flasks of two sizes (35 mm. in diameter x 11 mm. deep; 50 mm. in diameter x 14 mm. deep). Examinations of the pelomyxae were made using a dissecting microscope or the low power of the compound microscope. When necessary, single organisms were placed on a well slide and examined under the low and high powers of the compound microscope.

Collection of algae for use as a food source for the pelomyxae was begun in early June of 1958. Collecting sites included Laurel Golf Course on U. S. 33 northwest of Richmond, Pocahuntas State Park in Chesterfield County, the farm of L. O. Morrow at Glendale in eastern Henrico County, Lakeside Country Club at Lakeside, Virginia, and several sites in Richmond including Bryan Park, Byrd Park, Maymont, and the campus of the University of Richmond.

Attempts to culture Spirogyra, Oedogonium, Ulothrix, Mougeotiopsis, and Zygnema as potential food sources for the

pelomyxae were based on a fishmeal medium. Spring water from Byrd Park in Richmond, Virginia, was used in all media and for washing all glassware. The fishmeal medium was prepared by placing 0.2 grams of fishmeal in 1 liter of spring water in an Ehrlenmeyer flask. The medium was heated to 90 - 95° C., but not to boiling, and was filtered while hot. After filtering 1/2 c. c. of ferric chloride solution ($FeCl_3.6H_2O$) was added by pipette, if desired, and the medium was covered and left to cool. The ferric chloride solution was prepared fresh each week by dissolving 1 gram in 100 ml. of distilled water.

After cooling 200 c. c. of medium, fishmeal with or without ferric chloride, was poured into a clean finger bowl. One of the species of algae to be cultured was added, and each bowl was covered with another finger bowl to prevent evaporation of the medium and airborne contamination. Some of the cultures were placed in a darkroom at 22 - 24° C. under 20 watt cool white fluorescent lamps located 15.5 inches above the culture bowls. Other cultures were placed in an east window which permitted illumination by direct sunlight and diffused daylight. Additional illumination was provided by 20 watt cool white fluorescent lamps located 15.5 inches above the culture bowls.

For three months a fertilizer medium was also used in attempts to culture the algae. This medium was prepared by heating 1 liter of spring water containing 1 gram of commercial fertilizer (4-12-4) in an Ehrlenmeyer flask to 90 - 95° C. After filtering the solution while hot and cooling it to room temperature, the alga was added.

Daphnia used in culture experiments on Spirogyra were

cultured in spring water containing a suspension of a small amount of the yolk of a hard-boiled chicken egg, the culture vessel being placed in dim light in a darkroom at a temperature of 20 - 24° C.

In certain experiments, Ankistrodesmus sp., a small desmid (Smith, 1933), was used as one of the sources of food for the pelomyxae.

# OBSERVATIONS AND EXPERIMENTS

## General Observations

P. palustris appears to be restricted to the bottom of the Southampton Quarry. Samples obtained throughout both the eastern and western parts revealed no specimens from the leaf-choked shallows or from the sheer granite walls. The eastern part of the quarry is shallower than the western part. Pelomyxae were usually found in the eastern part but not in the quantities occurring in the deeper areas of the western part. The best area for collection appeared to be the center of the western portion at a depth of 50 to 53 feet. Specimens here varied in diameter from 140 microns to 2.75 mm.

Collections during late June and early July of 1958 contained few pelomyxae; all specimens measured less than 600 microns in diameter. After the second week in July the animals were larger and more numerous throughout the collecting period, until collections were discontinued in May of 1959.

Pelomyxae collected from the bottoms of both the eastern and western parts of the quarry were examined under the light microscope for cytoplasmic inclusions. These were seen to be the same color as the sediment from the quarry bottom and were composed of the same materials: sand, mud, bits of algae, plant debris, and the tests of crustaceans.

From early June through August, daily collections of pelomyxae were made, and the animals were brought back to the laboratory in tightly capped B. O. D. bottles. The bottles were uncapped and placed in the refrigerator until they could be examined at which time the contents were

divided between two finger bowls. After examination the organisms were left in the finger bowls, and they died in 24 to 72 hours. It was observed that the sediment in the bowls or uncapped B. O. D. bottles changed color when exposed to the atmosphere, from the black of the newly collected material to a reddish or orange brown, which apparently resulted from an oxidative change. This assumption seemed logical, because later determinations (Table I) showed the oxygen content of the water on the quarry bottom at a depth of 50 to 53 feet to be less than 1 ppm.

Table I

Date: November 8. 1958

| Depth In Ft. | Oxygen; P.P.M. | pH | Temperature; Degrees F. |
|---|---|---|---|
| 51 | 0.000 | 6.9 | 43.0 |
| 48 | 0.000 | 6.7 | 43.5 |
| 42 | 0.000 | 6.7 | 43.5 |
| 36 | 0.000 | 6.7 | 44.0 |
| 30 | 0.000 | 6.6 | 44.0 |
| 24 | 0.834 | 6.4 | 48.0 |
| 18 | 4.632 | 6.6 | 53.0 |
| 12 | 4.876 | 6.8 | 54.0 |
| 6 | 4.879 | 6.6 | 55.0 |
| 1 | 4.951 | 6.8 | 55.0 |

Date: February 7, 1959

| Depth In Ft. | Oxygen; P.P.M. | pH | Temperature; Degrees F. |
|---|---|---|---|
| 51 | 0.000 | 6.7 | 40.0 |
| 48 | 0.000 | 6.8 | 40.5 |
| 42 | 0.168 | 6.6 | 40.5 |
| 36 | 1.734 | 6.5 | 40.5 |
| 30 | 2.588 | 6.5 | 40.5 |
| 24 | 3.141 | 6.4 | 40.5 |
| 18 | 3.750 | 6.5 | 40.5 |
| 12 | 4.688 | 6.5 | 40.5 |
| 6 | 5.222 | 6.5 | 40.5 |
| 1 | 5.513 | 6.5 | 41.0 |

Date: May 9. 1959

| Depth In Ft. | Oxygen; P.P.M. | pH | Temperature; Degrees F. |
|---|---|---|---|
| 51 | 0.00 | 6.5 | 43.0 |
| 48 | 0.00 | 6.4 | 43.0 |
| 42 | 0.47 | 6.6 | 43.0 |
| 36 | 0.86 | 6.5 | 43.0 |
| 30 | 1.73 | 6.5 | 43.0 |
| 24 | 2.64 | 6.4 | 43.0 |
| 18 | 4.88 | 6.5 | 44.0 |
| 12 | 8.13 | 6.8 | 48.0 |
| 6 | 9.87 | 7.5 | 58.0 |
| 1 | 6.94 | 7.3 | 64.0 |

## Factors Affecting Culture

In August several B. O. D. bottles of pelomyxae were collected and placed in the refrigerator at 8° C. without removing the glass stoppers of the bottles as had been done previously. When opened a week later, pelomyxae were numerous and apparently healthy. Previously the majority of the organisms in B. O. D. bottles without stoppers or in open finger bowls died in less than a week, while those remaining were greatly diminished in size as well as number.

In order to determine the effect of exposure to the atmosphere upon the viability of the pelomyxae, a number of collections were made at a depth of 50 feet in the center of the western part of the quarry. The stoppered collection bottles were transported back to the laboratory and stored for two weeks in the refrigerator at 8° C., after which the bottles were opened and poured into finger bowls for examination. These showed many viable pelomyxae. Each finger bowl was covered with another bowl and replaced in the refrigerator. Twenty-four hours later, all organisms were dead. The organic matter had changed color from black to reddish orange. On several occasions the pelomyxae in the finger bowls remained viable longer than 24 hours, but never longer than 72 hours. This experiment would seem to indicate that the animals cannot remain viable for any length of time in the presence of measurable quantities of oxygen.

In order to investigate the effects of temperature on the organisms 10 pelomyxae were placed in each of two capped B. O. D. bottles containing water from a depth of 45 feet in the quarry. One bottle was placed in the dark at 8° C., and the other in the dark at temperatures ranging from 22 to 25° C.. No debris from the quarry bottom was present in either bottle to act as a food source. Upon examination eight weeks later the bottle stored in the refrigerator was seen to contain nine pelomyxae, each of which was greater than 700 microns in diameter. The bottle stored in the cabinet at room temperature contained six small animals, each less than 500 microns in diameter.

Extension of these observations seemed appropriate because of the differences in number and size of the animals observed under different conditions. Therefore the following experiment was set up. Forty similar B. O. D. bottle collections of P. palustris were made from the bottom of the western part of the quarry at a depth of 50 to 53 feet. The collections were divided into four groups of 10 B. O. D. bottles each and four different experimental conditions were established as in Table II. The bottles were left for four weeks after which the contents were poured into finger bowls and the maximum, minimum, and average number of pelomyxae per bottle per group were determined.

Table II

| Group-Designation | Maximum No. Per Bottle Per Group | Minimum No. Per Bottle Per Group | Average No. Per Bottle Per Group | Experimental Conditions |
|---|---|---|---|---|
| A - Ten B.O.D. Bottles | 269 | 30 | 86.9 | Refrigerator; Capped; 8 Degrees C. |
| B - Ten B.O.D. Bottles | 19 | 0 | 8.4 | Refrigerator; Uncapped; 8 Degrees C. |
| C - Ten B.O.D. Bottles | 58 | 8 | 29.4 | Cabinet; Capped; 22 - 25 Degrees C. |
| D - Ten B.O.D. Bottles | 13 | 0 | 4.2 | Cabinet; Uncapped; 22 - 25 Degrees C. |

The results in Table II seem to indicate that the pelomyxae maintain themselves most successfully at low temperatures in

containers, the contents of which are not exposed to atmospheric oxygen. It appears that atmospheric gases are more important factors in the maintenance of the organisms than were the water temperatures tested, because more animals remained viable under conditions of room temperature and nonexposure to atmospheric gases than under conditions of low temperature and exposure to atmospheric gases. Some collections within a group contained more debris from the quarry per bottle than did others. The number of pelomyxae per bottle appears to be related to the amount of debris present, such that the greater the quantity of debris, the larger the number of organisms. It was often necessary to add additional oxygen deficient water from lower depths in the quarry to the contents of the bottle to be examined in order to dilute the debris so that sufficient light could pass through it to permit examination. Often as many as 15 finger bowls containing approximately 200 cc. of material per bowl were used to determine the number of animals present in an undiluted sample originally contained in one 300 cc. B. O. D. bottle.

In order to determine the length of time that pelomyxae could exist in the laboratory in capped B. O. D. bottles, two collections were made and one was placed in the refrigerator in the dark at 8° C., the other into a darkened cabinet at 22 - 25° C. The bottles were opened once each month, and the contents were poured into finger bowls in order to observe the presence of living animals. The contents of the two bottles were never exposed to the atmosphere long enough to count the individuals present in each one, but scanning

indicated a greater number of pelomyxae in the bottle that had been kept in the refrigerator. After the monthly examination the contents of the bottles were raised to the full mark with distilled water to replace that left in the finger bowls after examination. Both bottles contained viable organisms for a period of five months. Examination at the end of the sixth, seventh, and eighth months showed living animals only in the bottle that had been kept at 8° C.. The pelomyxae remained viable for a considerable length of time at room temperature, but temperatures approximating those of the natural habitat seem to aid in maintaining them over a longer period.

Attempts were made to maintain pelomyxae in culture by dividing a collection of organisms between two finger bowls and placing these in airtight containers sealed with Vaseline. In some experiments finely ground pyrogallol was used to remove oxygen; candles were burned in the vessels to remove oxygen more quickly then could be done with pyrogallol and to add carbon dioxide to the atmosphere inside the containers; and calcium hydroxide was utilized to remove carbon dioxide from the atmosphere inside the vessels. All experiments were set up in duplicate and yielded similar results. The containers were prepared according to Table III ('Pres.' or 'Abs.' indicating, respectively, the presence or absence of the substance in question).

Table III

| Culture | Pyrogallol | Calcium Hydroxide | Candles | Temperature | Viability of Pelomyxae |
|---|---|---|---|---|---|
| A | Abs. | Abs. | Abs. | 22 - 25° C. | 2 days. |
| B | Abs. | Abs. | Pres. | 22 - 25° C. | From 2 to 3 days. |
| C | Pres. | Abs. | Pres. | 22 - 25° C. | From 2 to 3 days. |
| D | Pres. | Pres. | Pres. | 22 - 25° C. | From 2 to 3 days. |
| E | Pres. | Pres. | Pres. | 8° C. | From 2 to 3 days. |

The containers at room temperature (22 - 25° C.) were placed in cabinets in the dark. One vessel (E) was placed in the refrigerator in darkness at 8° C. The contents of the debris in the containers turned a reddish-orange within 48 hours after the beginning of the experiment. The color of the debris in container E was less brilliant than that of the contents of the containers at room temperature, seemingly signifying less oxidation. Attempts to culture P. palustris under the conditions indicated in Table III failed.

As a result of a suggested culture method (Lackey, 1959) for organisms living in sewers under conditions assumed to be similar to those experienced by the pelomyxae, i. e., microquantities of oxygen, large amounts of decaying organic matter, and the presence of noxious gases, attempts were made to culture the organisms in finger bowls under oil. Several collections from the bottom of the western part of the quarry were brought back to the laboratory and examined in finger bowls (150 cc. per bowl) for the presence of

P. palustris. If the organisms were present, mineral oil was immediately poured over the surface of the water, and the bowls were placed in the refrigerator at 8° C. The debris in the bowls changed from black to brownish-orange in color within 48 hours, and no living organisms were detected upon examination after 72 hours. New collections were made and the contents of the B. O. D. bottles were poured into finger bowls, the surfaces of which were covered with mineral oil immediately, without prior examination to determine the presence of the animals. The bowls were placed in the refrigerator with the same results as before. The color shift occurred within 48 hours, and only dead pelomyxae with blistered pellicles were observed at the end of 72 hours. Attempts to culture the organisms in finger bowls under mineral oil met with failure.

In order to determine the adaptability of the pelomyxae from the bottom water of the quarry to the higher temperatures and increased oxygen of the surface water, a series of water samples were collected at various depths and placed in 35 x 11 mm. Carrel flasks. Pelomyxae collected from the quarry bottom at the same time were added to a flask containing water from the lowest depth and transferred 24 hours later to a flask containing water which had been collected from a lesser depth. The lowest depth was 45 feet. The next depth was 40 feet, followed by depths of 35 feet, 30 feet, 25 feet, 20 feet, 15 feet, 10 feet, 5 feet, and 1 foot, as shown in Table IV.

Table IV

| Water Sample Depth Below the Surface | Temperature; Degrees F. | No. of Pelomyxae | No. of Hours After Beginning of Series |
|---|---|---|---|
| 45 feet | 43 | 19 | 1 |
| 40 feet | 43 | 17 | 24 |
| 35 feet | 43 | 21 | 30 |
| 30 feet | 43 | 21 | 48 |
| 25 feet | 43 | 3 | 60 |
| 20 feet | 43 | 0 | 72 |
| | | Date: September 3, 1958. | |
| 45 feet | 43 | 22 | 24 |
| 40 feet | 44 | 23 | 48 |
| 35 feet | 44 | 35 | 72 |
| 30 feet | 44 | 25 | 96 |
| 25 feet | 46 | 27 | 120 |
| 20 feet | 52 | 21 | 144 |
| 15 feet | 64 | 16 | 168 |
| 10 feet | 65 | 0 | 192 |
| | | Date: October 21, 1958 | |
| 45 feet | 43 | 15 | 24 |
| 40 feet | 43 | 14 | 48 |
| 35 feet | 43 | 13 | 72 |
| 30 feet | 43 | 0 | 96 |
| | | Date: February 7, 1959 | |

It should be observed that in the process of transfer the medium containing the pelomyxae was exposed for a brief period to the atmosphere, at which time it may have absorbed oxygen and this, in addition to the originally higher oxygen content of the medium, may account for the failure of the pelomyxae to adjust to quarry water collected at lesser depths.

A further attempt to adapt P. palustris to conditions at lesser depths in the Southampton Quarry was made by devising a method for elevating the animals to lesser depths

without removing them from the quarry water. A one-gallon glass jug was utilized as a float and two three-pound lead weights at the end of a 55-foot line were attached to the jug handle to prevent the jug from drifting. A collection with the water sampler was made at the anchorage site of the jug near the center of the western part of the quarry. The B. O. D. bottle was removed from the sampler and tied to an additional 55-foot line without being exposed to the air. This was done by keeping the entire sampler and contents just beneath the water's surface. The B. O. D. bottle was gently lowered to the quarry bottom as a control. Upon reaching the bottom the control was raised an inch to keep it upright. Knots were tied every six feet in a third line, the sampler was sent to the bottom, and a collection was made as before. The collection bottle was removed from the sampler without exposing the bottle's contents to the atmosphere, tied to a line with knots every 6 feet, and the bottle was returned slowly to the bottom. It too was raised an inch off the quarry floor to keep it upright, after which it was raised through the following depths, one step every 48 hours: bottom (51 feet), 48 feet, 42 feet, 36 feet, 30 feet, 24 feet, 18 feet, 12 feet, 6 feet, and 1 foot. The water in the quarry being quite clear, it was noted that the debris in the bottle being raised had turned from black to brownish-orange before the 1 foot level was reached. Because the line tied around the bottle's neck caused it to tilt slightly from the vertical, a small amount of debris remained black in color as the bottle was raised toward the water surface, because

it was covered by a layer of oxidized debris 4 mm. thick. Forty-eight hours after the bottle had reached a depth of 1 foot, both it and the control that had been left on the quarry bottom were removed from the water after being stoppered beneath the water surface, so that the contents of the bottles were not directly exposed to the atmosphere. Both bottles were returned to the laboratory for examination. The pelomyxae in the bottle that had been raised periodically were small, averaging 100 to 150 microns in diameter; the largest was 240 microns in diameter. These organisms were rounded and the cytoplasm contained large black granules. The animals moved very little. A few showed blistered projections of the pellicle. Ostracods, copepods, and infusoria were present. In the bottle that had remained on the quarry bottom as a control, pelomyxae were numerous and appeared healthy. Diameters ranged from 750 microns to over 1,000 microns. The animals, both the control and experimental, were replaced in their respective bottles which were then capped and placed in the refrigerator at 8° C. When reexamined after 24 hours, organisms in the bottle that had been raised periodically were dead. All controls died within 72 hours, perhaps due to prolonged exposure to the atmosphere during the initial examination.

All of the experiments with P. palustris thus far described have dealt with the influences of gases, temperature, and culture water from various sources upon the survival of the organisms, as well as with the maintenance of the animals in an active state.

Experiments were also performed in order to study the utilization of certain algae for food. Several different genera of algae in different physical states were used as potential food sources for the pelomyxae.

Noting that Kudo (1957) successfully cultured P. palustris in Carrel flasks using chopped Spirogyra as the food source, an experiment was devised to cultivate the organisms following his procedure. Five organisms were collected from a depth of 50 feet in the western part of the Southampton Quarry and were placed in spring water in a 50 x 14 mm. Carrel flask. The flask was stoppered with a cork and placed in the refrigerator in darkness at 8° C. The animals were dead within 24 hours. Similar results were obtained using previously boiled spring water. Duplication of this experiment yielded negative results.

In view of the fact that Kudo (1958) successfully cultured the organisms in finger bowls with Spirogyra filaments or chopped Spirogyra as the food source, an attempt was made to duplicate his results, to observe the success of culture in water from various sources, and to note the preference of the animals, if any, for chopped or filamentous algae. Nine cultures of six or more pelomyxae each were set up in finger bowls at 21° C. in dim light with Spirogyra filaments and chopped Spirogyra for food. The chopped alga was prepared by placing the filaments in a few drops of water in a small evaporating dish and cutting with a sharp scalpel, or by grinding up the filaments with powdered glass in a mortar and pestle; the latter proved

less satisfactory than the former. Cultures were set up as shown in Table V.

Table V

| Culture | Source of Water Used in Medium | Depth of Water in Finger Bowls | Condition of Spirogyra | Result |
|---|---|---|---|---|
| A | Spring water | 14 mm. | Entire filaments | Death within 24 hours |
| B | | | No filaments | |
| C | | | Chopped filaments | |
| D | Surface water- from Southampton Quarry | 14 mm. | Entire filaments | Death within 24 hours |
| E | | | No filaments | |
| F | | | Chopped filaments | |
| G | Water from a depth of 45 - feet in Southampton Quarry | 14 mm. | Entire filaments | Death within 24 hours |
| H | | | No filaments | |
| I | | | Chopped filaments | |

Culture of the pelomyxae using finger bowls containing water from several sources including the natural habitat of the organisms, and chopped or filamentous Spirogyra for food, met with failure.

Ten pelomyxae were placed in each of three capped B. O. D. bottles of quarry water from a depth of 45 feet and stored in darkness at 8° C. in order to determine the viability of the organisms in B. O. D. bottles when fed with different genera of chopped algae as shown in Table VI.

Table VI

| Culture | Genus of Chopped Alga | No. of Days Since Beginning of the Study | No. of *Pelomyxae* Present |
|---|---|---|---|
| A | *Spirogyra* | 0 | 10 |
| | | 6 | 3 |
| | | 13 | 0 |
| B | *Oedogonium* | 0 | 10 |
| | | 21 | 8 |
| | | 22 | 0 |
| C | *Mougeotiopsis* | 0 | 10 |
| | | 11 | 16 |
| | | 21 | 0 |

The animals in the B. O. D. bottle with *Mougeotiopsis* and *Oedogonium* appeared to ingest some of the algae. In Culture C, the number of pelomyxae increased temporarily. No *Spirogyra* was noted inside the animals of Culture A.

Carrel flasks (35 x 11 mm.) were filled with water collected at a depth of 45 feet in the quarry and a different alga was added to each in an attempt to study the relative merits of several kinds of chopped algae as food sources for pelomyxae being cultured in Carrel flasks. The genera of algae used included *Ankistrodesmus*, found as a surface contaminant in finger bowls of algae being cultured on fishmeal-ferric chloride medium, *Oedogonium*, and *Mougeotiopsis*. Pelomyxae, collected several days before and stored in the refrigerator at 7° C., were added to each Carrel flask, five organisms to a flask, and the flasks were placed in the refrigerator in darkness at 7° C.

All animals died in three days. Ten additional newly collected pelomyxae were added to each of the same flasks, and the flasks were again placed in the refrigerator in darkness at 7° C. The flask containing 10 pelomyxae and Mougeotiopsis cracked, and, although the water leaked out very slowly, it was possible for some atmospheric oxygen to enter the water retained in the flask. The pelomyxae therein soon became black in color. Their pellicles blistered, and they died in a week. The Carrel flask containing 10 organisms and Oedogonium showed no living animals at the end of two weeks. However, the flask containing 10 animals and Ankistrodesmus contained two live and apparently healthy animals, each with ingested alga, at the end of seven weeks.

# DISCUSSION

Pelomyxa palustris Greeff is a characteristic inhabitant of the sapropel layer, an area composed of mud or ooze consisting almost entirely of the remains of dead organisms, both plants and animals, and inorganic materials such as sand (Minchin, 1922). The life of the pelomyxa is bound to this environment, and the organism is found nowhere else (Leidy, 1879; Schirch, 1914; Mast, 1934; Hollande, 1945; Doflein, 1949; Kudo, 1957).

The presence of pelomyxae has been reported throughout the year in various areas (Greeff, 1874; Leidy, 1879; Leiner, 1924; Fortner, 1934; Leiner, Wohlfeil, and Schmidt, 1954). Greeff (1874) found the animals most plentiful in the spring of the year; Bott (1906) collected more in the autumn, and collected only a few animals in winter. Kudo (1957) also found a plentiful source in autumn, but he noted that the well fed individuals collected in April lived longest. Pelomyxae were collected in the Southampton Quarry in an active state from June, 1958, through May, 1959, without appreciable variation in number.

Bott (1906) maintained P. palustris in a tank in winter, but the number diminished in eight weeks. He notes that the animals were found on or near the top of the bottom mud in the tank when the water was fresh, but that they were found creeping on the sides of the container when the water had not recently been changed. With water plants in the vessel the animals were never found on the wall, but with no plants and unchanged water, they were never seen

on the floor of the container. The observations of Blochmann (1894) agree with those of Bott. When Elodea canadensis was put into the containers in the laboratories of Bott and Blochmann to restore oxygen, the pelomyxae remained on the bottom of the vessel. It appears that the organisms of Bott and Blochmann faired better in the presence of some oxygen, because they remained in the mud layer only when green plants were present.

Determinations of the oxygen content of the water of the Southampton Quarry on three occasions yielded results that would appear to be inconsistent with those of Blochmann and Bott; that is, the absence of oxygen in measurable quantities from the sapropel of the quarry in which the organisms live (Table I). Attempts to adapt the pelomyxae to lesser depths, with the accompanying increase in temperature and amount of oxygen present, met with failure, both in the laboratory (Table IV) and in the natural habitat. The temperatures in Tables I and IV were not corrected for pressure increase due to increase in depth. The local organisms seem to maintain their existence only in an environment low in oxygen. However, the large number of bubbles that arose whenever an object was dragged across the quarry floor indicated a considerable quantity of unidentified gas or gases in the debris.

Results of the writer's gas and temperature studies (Table II) appear to indicate that both temperature and atmospheric gases affect the successful cultivation of P. palustris in the laboratory. Although the animals were

found to live in unstoppered bottles, they were fewer in number than in capped containers kept under similar conditions. Likewise, collections maintained at room temperature contained fewer and smaller organisms than controls maintained in the refrigerator at 7 - 8° C.

Leiner (1924) and Kudo (1957) assert that pelomyxae cannot live anaerobically. The writer's observations indicate that the animals, if not anaerobes, are certainly microaerophilic. The unanswered question is how microaerophilic? It would appear from the foregoing discussion that the oxygen tolerance of the organisms studied by others may vary in the different habitats. This variation may indicate race differences, since all attempts to adjust the local organisms to an environment containing measurable quantities of oxygen failed.

Dejdar (1931) analyzed the conditions in the artificial pool in a cement basin from which pelomyxae were collected. Oxygen measured 4.74 mg. per liter. Free carbon dioxide measured 18 mg. per liter; hydrogen sulfide, 0.11 - 0.13 mg. per liter; and ammonia, 0.6 mg. per liter. What relation these gases might have to the pelomyxae of the Southampton Quarry was not determined.

Depth <u>per se</u> seems to be a negligible factor in culture of the pelomyxae. In nature the animals tolerate depths from several centimeters to 40 meters (Hollande, 1945). Several workers (Gicklhorn and Dejdar, 1931; Kudo, 1957) observed that the organisms lived only two or three days in shallow vessels, but that they were viable for two

months under this condition. Whether increase in numbers occurred in the capped cultures is unknown, because the animals were not counted when collected in order to avoid prolonged exposure to the atmosphere. Similar capped B. O. D. bottle collections contained from 30 to 269 pelomyxae per bottle after being kept for one month in the refrigerator at 8° C.

Fortner (1934) attempted to culture the animals on an artificial medium prepared from finely ground meat-peptone and gelatin. He used water from the collecting site of the organisms and to this added mud and quartz sand loosened with cotton fibers. The meat-peptone and gelatin were placed on top of the sand, and water was poured over the peptone-gelatin layer. Pelomyxae that were placed in this medium died in a few days. Fortner suggested unbalanced decomposition and the overproduction of noxious material as reasons for the failure of the culture procedure. He believed it most unlikely that a suitable nutrient medium could be found for such a specialized heterotrophic protozoon, especially if not only the nutrient but the mechanical environment is also important.

Kudo (1957) successfully cultured P. palustris on Spirogyra ground in a tissue mill and placed in Carrel flasks. He (1958) has even successfully cultured the organisms in covered finger bowls, again utilizing chopped algae as the food source. Duplication of his methods met with no success. Exposure of the animals to the atmosphere in transferring them from the collecting bottles to finger

bowls for determination of their presence and isolation of individual organisms, and exposure to the atmosphere in transferring the organisms to Carrel flasks or finger bowls containing the alga may have been detrimental. In addition the shortest filaments obtained by the writer's manipulations were 5 - 6 cells in length and most were longer. These may have been too long for the pelomyxae to ingest. Some success was achieved in culturing the organisms on Ankistrodesmus, a smaller species of alga, in Carrel flasks containing bottom quarry water, indicating that the size of algal food material may be important.

## SUMMARY

1. *P. palustris* Greeff was collected at a depth of 50 - 53 feet in the Southampton Quarry in the sapropel layer, where it exists under polysaprobic conditions.

2. Culture of *P. palustris* with *Spirogyra* for food according to the method of Kudo (1957) proved unsuccessful. However, partial success was obtained when the organism was cultured on *Ankistrodesmus sp.* or organic debris from the quarry floor.

3. Viability of the organisms in the laboratory could be maintained for eight months only in tightly capped containers filled with water from the habitat.

4. A microquantity of oxygen and a low temperature are believed to be important factors in the successful maintenance in the laboratory of the pelomyxae from the Southampton Quarry.

5. Comparison of these observations with those of other investigators suggests that there are different races adapted to different conditions of oxygen and / or temperature.

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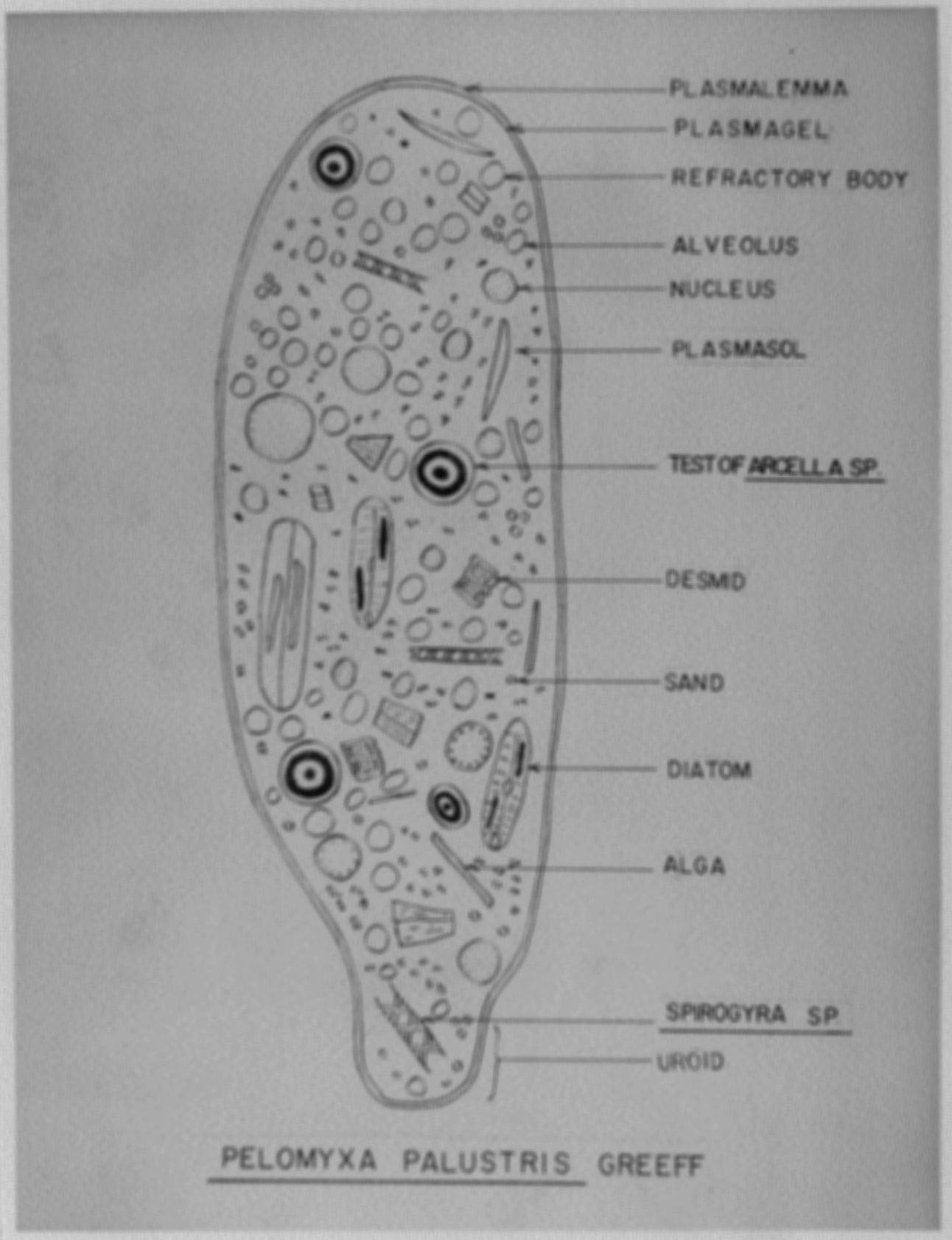
# PLATES

# PLATE I

*Pelomyxa palustris* Greeff

The drawing was prepared from living and fixed material.

PLATE I




PELOMYXA PALUSTRIS GREEFF

PLATE II

The Southampton Quarry

Upper Figure: The eastern part of the quarry is shown in the foreground, and the western part is shown on the far side of the foot-bridge.

Lower Figure: The western part of the quarry is shown. The location of the rowboat marks the approximate site at which the majority of samples were collected, at a depth of 50 - 53 feet.

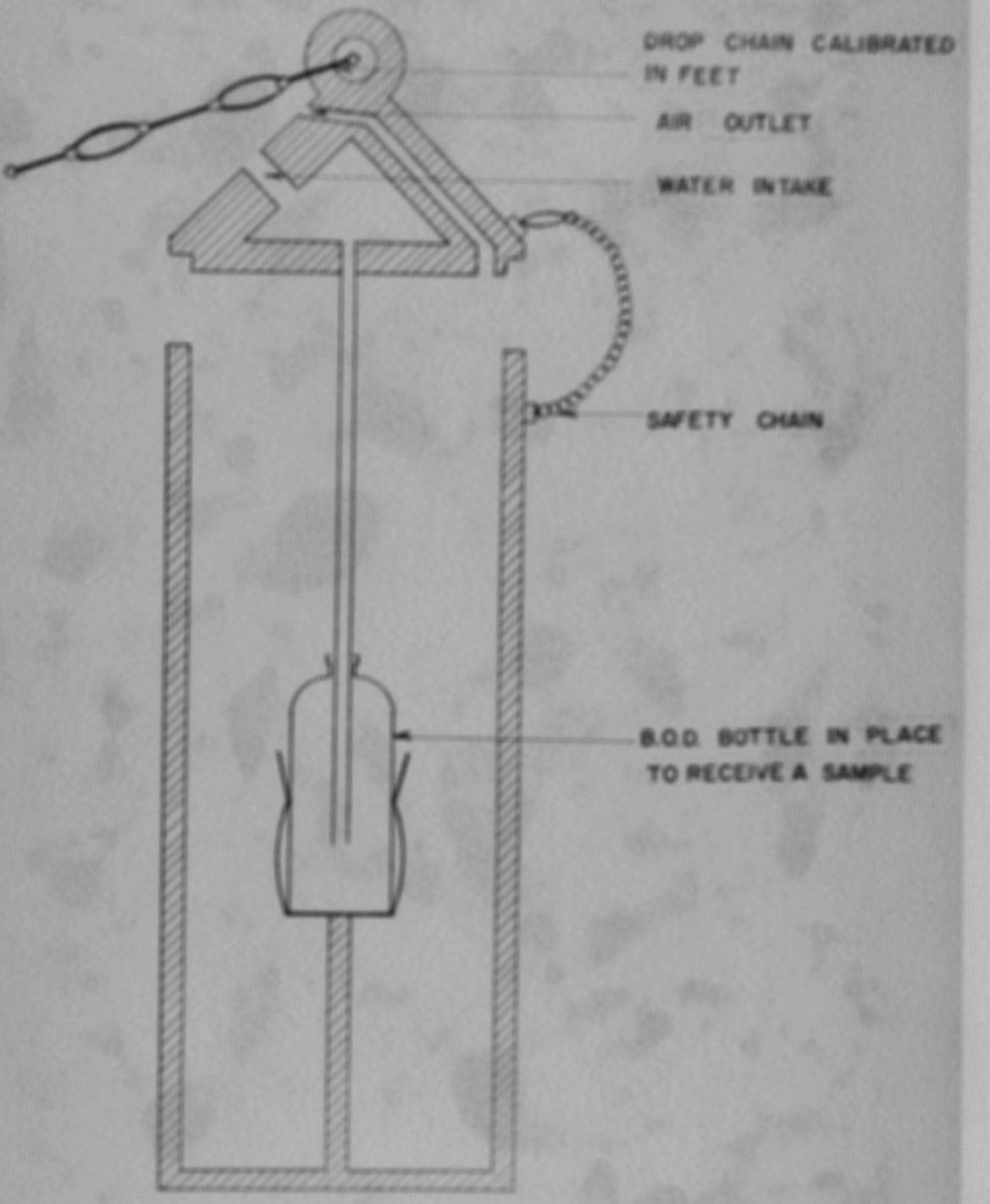
PLATE II

## PLATE III

Diagrammatic Section of a Sewage Sampler.

(Precision Scientific Company, Chicago.)

PLATE III




DIAGRAMMATIC SECTION OF A SEWAGE SAMPLER

( THE TOP OF THE SAMPLER HAS BEEN RAISED A SHORT DISTANCE )

# VITA

Daniel Henry Stern was born June 18, 1934, in Richmond, Virginia. He attended John Marshall High School and was graduated in 1951, receiving the Bausch and Lomb Science Medal. He entered Oberlin College in Septembber, 1952, and transferred to Richmond College of the University of Richmond in September of 1953. Following graduation from Richmond College with the degree of Bachelor of Science in Chemistry in 1955, he entered the Medical College of Virginia. He became a student in the Graduate School of the University of Richmond in 1956 to pursue a course of study toward the Master of Science degree in Biology.

While at the University of Richmond he served as a laboratory assistant in General Chemistry and in General Biology. During the 1958-1959 session he acted as president of the local chapter of Beta Beta Beta National Honorary Biological Society. He is a member of the Society of Protozoologists and the Association of Southeastern Biologists. In 1959 he was awarded a fellowship from the National Science Foundation for summer study at the Mountain Lake Biological Station of the University of Virginia. He has received a Teaching Assistantship in the Department of Zoology at the University of Illinois for the 1959-1960 session, at which institution he will undertake a program of studies leading to the doctorate.