PART I. METHODS OF MAINTAINING PROTOZOAN CULTURES

PART II. A MICRO-RESPIROMETER FOR DETERMINING THE METABOLISM OF PARAMECIUM CAUDATUM EHREN. AND PELOMYXA CAROLINENSIS (WILSON)

by

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PART I. METHODS OF MAINTAINING PROTOZOAN CULTURES

INTRODUCTION

The purpose of these experiments was to select protozoa and satisfactory culture methods which would maintain the protozoa for use in experiments dealing with the effects of certain hormones on one celled animals.

Protozoa that were of large size and could be maintained in a strong culture for several weeks were selected, and a method of culture which was adapted to local conditions was sought.

Since Paramecium caudatum Ehren. is easily obtained in culture, and since it is of sufficient size to isolate readily, it was selected as one of the organisms to be used in these experiments.

A second organism of large size though not so constant in culture is Stentor coeruleus Ehren., which was selected as a second organism of study.

Finally the large Pelomyxa carolinensis (Wilson) became available commercially, and it was decided to include it in the problem. This is the same organism that is commonly marketed as Chaos chaos (Schaeffer).

REVIEW OF LITERATURE

Paramecium caudatum

Media Preparation. Straus (1923) cultured paramecia on a medium consisting of 3 gm. of desiccated sheep's thyroid to 2500 ml. of spring water. He observed that the paramecia were scattered throughout the culture, and the ring at the surface was not formed. Lechsenring (1925) used dried lily pond leaves and tap water. Since non-sterile methods were used, many kinds of bacteria were present. The effects of thyroid and thyroxine were investigated by Torrey, Riddle and Brodie (1925). They used an infusion of hay and a mixed bacterial content with a green monad. Parpart (1928) made up a 0.7 per cent infusion of pure timothy hay and autoclaved it for 20 minutes at 15 pounds pressure. The paramecia were grown under sterile conditions.

Glaser and Coria (1933) grew pure cultures of Paramecium caudatum in a medium of liver extract and pure killed yeast cells. They filtered the bacteria out and added the 2 mg. of sterile fresh rabbit kidney. A later experiment (1935) showed that 0.2 mg. of rabbit kidney was better for this particular medium. Smith (1932) used

15 gm. of cut oat straw and poured 900 ml. of boiling water over it. The pH was adjusted to 7.8 with NaOH, and after standing for two hours the medium was inoculated with paramecia. Phelps (1931) grew Paramecium aurelia on dried lettuce leaves. The leaves were dried in an oven and ground to a powder. He used 1.4 gm. of powder to make 1000 ml. of the culture medium. The pH was adjusted to M/600 with KH_2PO_4.

Brandwein (1935) formulated a general culture solution composed of 1.20 gm. of NaCl, 0.03 gm. of KCl, 0.04 gm. of $CaCl_2$, 0.02 gm. of $NaHCO_3$, and 50 ml. of phosphate buffer solution. Distilled water was added to make 1000 ml. Brandwein prepared the culture dish for amoeba by adding 1-2 mm. of one per cent agar solution to the bottom of a finger bowl, and imbedded five preheated rice grains in the agar before it hardened. The dish was then filled about one-third full of the general culture solution which was first diluted one to ten with distilled water.

Loefer (1936) made up a buffered Difco Bacto-tryptone solution to which he added a little dextrose.

Sterile methods were used, and the paramecia were grown on a single species of yeast, Saccharomyces ellipsoideus.

Sterilization of Paramecia. Many successful methods have been devised for the separation of paramecia from bacteria. Filtering out the paramecia, and killing the bacteria with X-rays have been used. Hargitt and Fray (1917) sterilized paramecia by washing them in sterile media. They used seven washings and sterile pipettes to transfer them from one wash to the next. Parpart (1928) improved this method by adding three more washings and leaving them in the fifth wash for five hours at 25° C.

Glaser and Coria (1935) used two methods. One method consisted of a long tube filled with a sterile medium. The paramecia swam to the top faster than bacteria and other forms of protozoa and were removed by breaking the end of the tube off before the other organisms reached the top. Their second method was to fill a "V" tube with a semi-solid medium, and as the paramecia were capable of more rapid progress than the other forms, they were successful in swimming through this medium. In the struggle to get through the dense medium, the bacteria were thrown off the cilia, hence the paramecia were free of bacteria.

Secondary Organisms. Most investigators used a mixed unknown bacterial content for the food supply of

paramecia. Bacillus subtilis seems to be an old favorite. Hetherington (1934) used Erythrobacillis prodigiosis. Loefer (1936) used a Parke Davis and Company yeast, Saccharomyces ellipsoideus with a tryptone medium. Torrey, Riddle and Brodie (1925) used a mixed bacterial content.

Stentor coeruleus

Turner (1937) developed a special medium for stentors. To 1000 ml. of pond water, which had been heated to boiling, he added a handful of timothy hay. This was cooled and diluted with another 1000 ml. of pond water and inoculated with paramecia. After the paramecia had developed for a week, the stentors were added along with about a gram of timothy hay. He kept the culture in a window but not in direct sunlight. The culture kept for several weeks in good condition.

Pelomyxa carolinensis

Schaeffer (1937) rediscovered this giant amoeba in a marsh in Virginia. By daily transfers into clean watch glasses and feeding paramecia to the amoebae, he was able to keep the culture going. There seems to be some doubt

as to whether the organism is correctly named by Schaeffer. Because of the lack of exactness and detailed description on the part of early investigators, Rosel von Rosenhoff (1755), Leidy (1878), and Wilson (1900), it is difficult to determine whether the giant amoeba described by these workers is the same organism. Mast and Johnson (1931) prefer Wilson's nomenclature, Pelomyxa carolinensis.

MATERIAL AND METHODS

Paramecium caudatum

Media. Several different media were prepared and investigated to see if they were suitable for this experiment. The first to be tried was Glaser and Coria's (1933) medium. This medium was made up of 500 ml. of killed yeast cells and one-half gram of filtered liver extract diluted to 100 ml. with water. The liver extract and yeast cells were autoclaved to kill the bacteria since a pressure bacteriological filter was not available. One gram of rabbit kidney was added to the flasks of media. The medium was kept in 100 ml. Erlenmeyer flasks plugged with cotton.

The second medium prepared was Loefer's (1936) buffered medium. This was prepared by adding 5 gm. Difco Bacto Tryptone, 2 gm. dextrose, 0.02 gm. $Ca(NO_3)_2$, 0.02 gm. NaCl, 0.002 gm. K_2HPO_4, 0.002 gm. $MgSO_4 \cdot 7H_2O$ and a trace of $FeCl_2$ to a liter of distilled water. This was put into Erlenmeyer flasks and autoclaved at 15 pounds pressure for 20 minutes. The flasks were inoculated the next day with either Bacillus subtilis or a commercial yeast.

A third medium was a combination of Phelps' (1931) solution made from powdered lettuce and Brandwein's (1933) general culture solution. The lettuce leaves were dried in the sun until they were nearly dry and then put into a ventilated oven heated by a 50 watt. lamp. When they were brittle, the leaves were removed and ground into a powder. One gram of this powder was added to 1000 ml. of Brandwein's general culture solution. The general culture solution was prepared by diluting a stock solution one to ten.

The stock solution was composed of the following: 1000 ml. of distilled water, 1.20 gm. NaCl, 0.03 gm. KCl, 0.04 gm. $CaCl_2$, 0.02 gm. Na_2HCO_3 and 50 ml. of phosphate buffer solution. The buffer solution was prepared by dissolving in 1000 ml. of distilled water one gram of a mixture of KH_2PO_4 and Na_2HPO_4 in such proportions as to give a pH of 6.9 to 7.0.

Another medium tried was cut oat straw. Fifteen grams of cut oat straw was cut into a liter flask and 900 ml. of boiling spring water was poured over it. After standing until cool, the pH was adjusted to 7.8 with NaOH and the culture was set aside for two days. Paramecia were inoculated into 50 ml. of the solution.

A fifth medium tried was made from one cm. of the tip of a cigar, broken and placed in a 450 ml. Brandwein's (1935) general culture solution. The cigar was used when lettuce leaves were unavailable. Because of the heavy growth of paramecia with this method, cigarette and cigar tobacco were tried, varying the amounts of these in the culture. The pH of culture solutions was made with a glass electrode potentiometer.

Following the procedure of other investigators, rice grains, hay extract, oak leaves, yeast cells, and wheat grains were also used to culture protozoa.

Sterilization of Paramecia. Paramecia were obtained from a general culture at the Kansas State College Zoology Department laboratory. They were sterilized by the method outlined by Parpart (1928). Several pipettes were made with a very small opening in the point by drawing them out. They were wrapped in newspaper and heated to 130° C. for one hour. Several depression slides were sterilized in a similar manner. A rich culture of paramecia was placed on one side of a watch glass containing a sterile beef extract wash. As the paramecia appeared on the other side of the glass, they were removed with a sterile

pipette and placed on the edge of a second watch glass. The watch glasses used in the first four washings were not sterile but were simply cleaned in alcohol and dried in an oven. The paramecia were left in the fifth wash in a depression slide for five hours and then washed through five more washings before they were used to inoculate the lettuce-leaf medium.

Other methods of sterilization were tried but were not found to be superior to Parpart's.

Selection of Secondary Organism. Commercial yeast was tried, but the bacterial growth which resulted spoiled the cultures. Bacillus subtilis was selected as a secondary organism for the paramecia to feed upon. Bacillus subtilis was selected because it was easy to obtain and produced a dense culture without souring the medium. The Bacillus subtilis was obtained from the Department of Bacteriology at Kansas State College. The bacteria were placed into the medium with a sterile wire or pipette.

Culture of Stentor coeruleus

The stentors were cultured by the method used by Turner. One thousand ml. of water from a rock garden

pool was heated to boiling, and a handful of dried timothy hay was added. This mixture was allowed to stand for 24 hours, and the hay was then strained out. The liquid was diluted with 1000 ml. of the fresh water from the pool, and the paramecia were added. Five days later the Stentor coeruleus were added to the culture, along with a gram of cut timothy hay.

Culture of Pelomyxa carolinensis

Brandwein's culture method for amoeba as modified by Cohen (1938) was used to grow Pelomyxa carolinensis. Two rice grains were imbedded in a thin sheet of one per cent agar solution in a 25 ml. Erlenmeyer flask. When the agar hardened, it was covered to a depth of one cm. with Brandwein's general culture solution. Pelomyxa carolinensis were introduced into this medium, in addition to a supply of paramecia. Into some of the cultures two amoebae were placed, while to other cultures much of the original culture was added. When Pelomyxa carolinensis were found in abundance in the Erlenmeyer flasks, the culture was transferred to finger bowls. The supply of Pelomyxa carolinensis was obtained from the General Biological Supply House in Chicago.

RESULTS AND DISCUSSION

Paramecium caudatum. After trying oak leaves, yeast cells, wheat grains, oat straw, timothy hay, tobacco, Glaser and Coria's special medium, and Loefer's tryptone medium with varying degrees of success, it was decided to use lettuce leaves as prepared by Phelps for growing paramecia. Loefer's solution may have given better results if the special yeast could have been secured. For the growing of cultures on one species of bacteria, Erlenmeyer flasks were found to be very useful. For large cultures, Florence flasks seemed to give better results. When a large culture of paramecia growing on *Bacillus subtilis* was wanted, the animals were introduced into a small amount of the medium containing the bacteria in a 25 ml. Erlenmeyer flask. After four days the entire culture was introduced into the large flask of the same medium. Best results were obtained by using a new sterile pipette whenever organisms were to be removed from a culture. Several cultures were kept running on unknown secondary organisms. For these, separate pipettes were used for each culture and kept separate by placing them in a pasteboard rack.

Temperature plays an important part in keeping a culture of paramecia going. When the temperature rose

above 26° C., the culture deteriorated very rapidly. During the hot summer days a culture left at room temperature deteriorated from 500 paramecia per ml. to 10 per ml. within three days.

When the temperature of the room reached 30° or above, the only paramecia that lived in the culture were near the bottom of the culture dish. The culture that was kept at 24° had well over 500 paramecia per ml. at the end of the nine-week summer school session. Keeping cultures clean seemed to keep them in good condition. This was done by straining out the sediment or drawing it out with a long pipette.

When grown on one species of bacteria, the cultures developed better if the medium was changed whenever a new culture was started. The pure cultures on one species of bacteria (B. subtilis) were kept pure by inoculating a flask of sterile medium before the culture was used for any other purpose. The pure cultures were very slow to build up after inoculating them with a few paramecia, but when 10 ml. of a strong culture were added to sterile media, the culture built up in about six days.

Stentor. A large bluish stentor, Stentor coeruleus, was obtained from a culture at the Kansas State College. This stentor grew and multiplied when put into a solution

of small protozoa but disappeared when put into a solution of Paramecium caudatum. Since the organism could not be cultured in large numbers under laboratory conditions, it was not considered further.

Pelomyxa carolinensis. Brandwein's culture method for amoebae, as modified by Cohen, produced weak cultures. This was probably due to unfavorable organisms in the mass cultures and also partly to the fact that the temperature could not be reduced to the optimal temperature of 19° to 20° C. and maintained at this temperature. However, when 11 organisms were placed in the cup of a micro-respirometer for two days with a culture of paramecia, 21 amoebae were found at the end of the determination.

The cultures made up of two washed organisms, and fed paramecia from the Bacillus subtilis cultures, were much stronger than the cultures in which a liberal supply of the original solution was used.

SUMMARY

As a phase of protozoan metabolism studies, various culture methods were investigated to determine which would be most suitable for culturing in volume (a) Paramecium caudatum, (b) Stentor coeruleus, and (c) Pelomyxa carolinensis.

1. For strong cultures of Paramecium caudatum, leaf solutions were found to be as satisfactory as other general culture media, and in addition were easier to prepare. Leaves used with equal success were lettuce, timothy, oats, oak, clover, bermuda grass, and tobacco (obtained from a cigar).

2. During the experiments, temperature was found to be a very important factor in the length of life of a culture. At 30° C. the cultures were destroyed.

3. It was found more practical to inoculate the new culture than to try to rebuild the old culture by the addition of more food substances. Better results were obtained when the type of culture medium was changed every time a new culture was started.

4. Stentor coeruleus was not adapted for further experimentation because of the inability to develop a strong culture.

5. Pelomyxa carolinensis developed into weak cultures, but because of their size were retained for the micro-respirometer tests. These organisms were large enough to be seen plainly with the unaided eye.

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PART II. A MICRO-RESPIROMETER FOR DETERMINING THE METABOLISM OF *PARAMECIUM CAUDATUM* EHREN. AND *PELOMYXA CAROLINENSIS* (WILSON)

INTRODUCTION

The purpose of this study was to find a method of measuring the metabolism of certain protozoa, so that the effect of hormones on them could be determined. There are two methods of measuring the metabolism of animals. In one method the carbon dioxide liberated is absorbed with an alkali and the amount determined by weight or by some other suitable means. Another method is the determination of the amount of oxygen used by the organism. The latter method was the one which was used in this problem.

Since most respirometers are either expensive or complicated, it was thought feasible to attempt to devise a simple yet inexpensive one which would be sensitive enough for the purpose of the problem.

REVIEW OF LITERATURE

There are two types of respiration apparatus for the determination of the metabolic rate in organisms, the Pettenkofer (1862) or air current type and the Regnault (1849) or the closed space type.

In the first type the animal is inclosed in a suitable container, and a current of air is passed through the animal chamber. Knowing the amount of air passed through, the composition of the air that enters, and the composition of the exhaust air, one can calculate the oxygen used and the carbon dioxide given off. This type is especially adapted to larger animals, although it is used by some to measure the carbon dioxide given off by small organisms, especially bacteria.

In the second or closed space type the culture is sealed in an air-tight container. The composition of the original air is known. By determining the amount of O_2 and CO_2 in the apparatus at the beginning and at the end of the experiment, the amount used can be found.

Thunberg (1905) and Krogh (1906) made use of a compensating flask to construct a micro-respirometer.

Krogh (1906) improved the apparatus of Thunberg (1905)

by arranging the equipment so that the results would be more accurate. He used capillary tubing and ground glass stoppered flasks for the culture and the compensating vessel. Krogh used a "U" type manometer with kerosene to measure the oxygen consumption.

Winterstein (1905, 1906) improved the arrangement of Krogh so that the respirometer would be more sensitive. The culture flask was connected to the compensating flask by means of a straight capillary tube, 0.25 mm. in diameter. As the organisms used oxygen, the pressure of the gas would decrease, causing the indicator to move along the scale.

Winterstein (1912) developed a micro-respirometer that could be read directly. He also used the compensating vessel, but he connected them together by a straight tube with a short plug of kerosene as an indicator. Both vessels were connected to the outside by means of a tube and glass stopcock. The vessel that contained the culture was connected to a mercury manometer. The other end of the manometer was connected to a short rubber tube. By using a tube-clamp on this end, he could set his instrument at any point desired, and so bring the indicator back to the starting point. The displacement of the mercury indicated the amount of oxygen used by the

organisms, providing the vessels were of equal temperature.

The Krogh (1914) modified apparatus used two compensating vessels connected by a manometer. Colored kerosene was used as an indicator. Each side of the manometer was connected to the outside by a tube with a common clamp to seal out the air. As the organism used oxygen out of one vessel, the kerosene was forced toward that side of the manometer. By correcting for pressure he was able to calculate the respiration rate of protozoa and small animals. He recommended that the weight of the animal used be less than two grams.

The effect of thyroxine and thyroid substance on paramecia was investigated by Torrey, Riddle and Brodie (1925). They found that thyroid substance caused the doubling of the rate of contraction of the contractile vacuole.

Straus (1923) found that paramecia would live on a medium made from desiccated thyroid. Lechsenring (1925) found an increase of 13 per cent in respiration of paramecia after thyroxine had been added to a 0.5 ml. centrifuged culture.

Tables and formulae from Standard Methods of Water Analysis (1925) were used to find the effect of temperature

and air pressure on the accuracy of the micro-respirom-
eter.

MATERIALS AND METHODS

Design of Micro-Respirometer

After careful consideration of Krogh's and Winterstein's micro-respirometers, it was decided to simplify the Winterstein type so that it would be practical for the study of cultures of protozoa.

Several types were devised, each of which had disadvantages. A type was finally constructed which would eliminate the two one-way stopcocks and the four-way stopcock and use only the "T" bore three-way stopcock. By bending the connecting capillary (of 0.5 mm. bore) near the ends, the vessels were brought closer together, thereby making it easier to maintain them at the same temperature. This capillary was calibrated by placing a 15 cm. celluloid rule, graduated in millimeters, under it ("A" Plate I). In order to maintain a more constant temperature of the two, a water bath was constructed out of a two-gallon oil can. The upper half of the can was cut away, and brass tubes six cm. long and one cm. in diameter were soldered into holes punched in the can. One tube was soldered near the bottom so that the water coming in would

run between the two flasks, and one was soldered near the top at each end. Water was run into the bath at the bottom tube and let out at the tubes at each end. The bath was leveled so that the same amount of water ran from each tube in the sides. As a further measure to insure an equal temperature, a small electric clock motor, to which a stirrer was connected, was used to stir the water bath.

The stopcock "B" (Plate I) was a "T" (one mm.) bore, which made it possible to connect both vessels to the outside through "E". Either the culture vessel or the compensating vessel could be connected to the outside separately, or the two vessels could be connected to each other without being connected to the outside.

The vessels ("C" and "D" Plate I) and their stoppers were made from 30 mm. standard taper glass joints. The female parts of the joints were made into the flasks, while the male parts were sealed to the ends of the capillary tube. Only Pyrex glassware was used in the construction of the micro-respirometer. Culture dishes were made from the bottom of glass vials. A wood mounting-frame was made to hold the capillary and protect it from breakage.

Technique for Using Micro-respirometer

The indicator was put into the capillary tube by inverting the micro-respirometer and dropping a small drop of the indicator from a pipette into the end of the capillary. It was found that the indicator went into the capillary more readily if the stopcock at "B" were kept closed. The instrument was tilted so that the indicator flowed very slowly down the right end of the capillary into the graduated scale "A". When the drop was allowed to flow too fast through the capillary, there was a tendency for it to carry air bubbles along with it. Also small amounts of fluid were left behind the main drop and formed small drops in the vertical arm of the capillary and thus making the instrument less accurate. Gasoline gauge fluid was used at first, but because it had a tendency to stick to the glass it was not satisfactory, and kerosene, which had been heated for an hour with hot water, was substituted.

Before the special flasks were attached to the instrument the stopcock "B" was opened so that both sides were connected to the outside through the tube "E", and stopcock grease was applied to the joints. The flasks

were held in place by a rubber band and submerged in the water bath. The mechanical stirrer was started, and a small stream of water was turned into the bath. After the instrument had attained equilibrium, readings were recorded hourly.

To test the respiration of a culture, the culture was first cooled to the temperature of the water bath and placed in the special dish. It was then introduced into the flask "C". So that the carbon dioxide produced by the organisms would not interfere with the determinations, a two per cent NaOH solution was put into the bottom of the flask around the dish. NaOH was also added to the compensating vessel to serve as a control. Keeping the culture and the alkali at the same temperature as the water bath saved time. As soon as the micro-respirometer reached equilibrium, the stopcock at "B" was closed, and the position of the indicator was noted. The instrument was read again in about 20 minutes, and further readings depended upon the culture which was being tested.

The micro-respirometer indicator was re-set by tilting the instrument when empty, or, if it was being used, the stopcock was opened so that the culture flask "C" was

connected to the outside. The air pressure forced the indicator back to the other side of the scale. Sometimes neither the first nor the second method was successful, so a pipette bulb was put on "E", and the stopcock was set to connect to "C". By applying a little pressure the indicator was forced across the scale.

Calibration of Micro-respirometer

Calibration of the instrument was accomplished by inserting a small drop of mercury into the capillary tube. Weighing the mercury and noting the amount of the scale it covered gave all the information needed to calibrate the scale.

Wt. of paper and mercury	0.8240
Wt. of paper	0.4833
Wt. of mercury	0.3407

On the left side of the scale the mercury covered from 15.0 to 4.7, or the mercury was 10.3 cm. long. On the right end of the scale the mercury covered from 1.1 to 11.4, hence the column again was 10.3 cm. long and weighed .3407 grams.

$a^2 \times .7854 \times 10.3 \times 13.6 = .3407$

$d^2 \times .7854 =$ area of cross section of tube.

13.6 = density of mercury, or area of cross section x 10.3 x 13.6 = .3407.

$$\text{Area of cross section} = \frac{.3407}{13.6 \times 10.3}$$

Area of cross section = .0024 sq. cm. or .24 sq. mm.

Therefore each millimeter of the scale represents 0.24 c. mm.

Counting Paramecia

To determine the metabolism of paramecia it was necessary to know how many organisms were present in the culture. After investigating several methods, it was decided to use the 20 c. mm. pipette of a Sahli Hemoglobinometer to draw off a definite volume of the culture. The culture was first stirred thoroughly, and 20 c.mm.of it were drawn into the pipette. The pipette was dried off on the outside with a clean towel, and the culture was then streaked on to a clean glass slide. The pipette was rinsed in distilled water, and this was added to the streak. The number of organisms in the streak of water was counted with a low power binocular microscope.

The oxygen consumption of one paramecium was found by stirring thoroughly a strong paramecium culture. The paramecia, being more capable of swimming than other

forms, congregated first. Two ml. of the culture collected from a region where the paramecia occurred in large numbers were placed into one of the test flasks. An equal amount of the culture collected from a region where the paramecia were sparce was introduced into the other flask. The number of paramecia in each flask was determined before they were attached to the micro-respirometer and again as soon as the test was over. From the difference in the average number of organisms in the two flasks and the amount of oxygen used, the oxygen consumption of one paramecium was determined.

DISCUSSION

One of the handicaps of the compensating vessel is the fact that it must be kept at the same temperature as the culture vessel. A fraction of a degree will cause the indicator to move completely across the scale and sometimes out of the capillary into one of the flasks. The upward slant of the tube where the scale portion connects with the side aims to prevent this.

A disadvantage of this micro-respirometer over Winterstein's is the fact that this instrument has to be opened to the outside when re-setting to zero. A corrective factor need not be considered if the atmospheric pressure or temperature at the end of the determination were different from that at the start, since the error in omitting this corrective factor would be only 0.01 part in a million for each millimeter change in pressure, and 0.15 part in a million for each degree change in temperature.

This micro-respirometer, however, has an advantage in that it is much simpler to operate. Winterstein's equipment has four stopcocks to operate, while this has only one. The closer proximity of the two flasks is an aid in keeping the temperature uniform. The flasks are

removable. This fact makes them easy to clean and, in addition, they can readily be sterilized. Having separate culture dishes makes it easy to remove one culture and insert another. Thus another test can be started in a few minutes. Mounting the respirometer, although introducing a slight error by exposing the capillary to temperature variations, makes the instrument more stable and facilitates operation.

Figure 1 shows the first test made with the micro-respirometer. The test was made with the instrument empty and started with the instrument at room temperature. The test indicates that it takes one and one-half hours for the instrument to come to equilibrium.

The broken line in figure 1 shows that it takes only one-half hour for the instrument to come to equilibrium if started at the same temperature as the water bath. This is due to several factors, probably the most important of which was the water vapor in the air at the time the experiment was performed. As the temperature of the water was below the dew point, water was condensed in the instrument, which decreased the volume. This condensation was sure to occur on the culture side because the flask

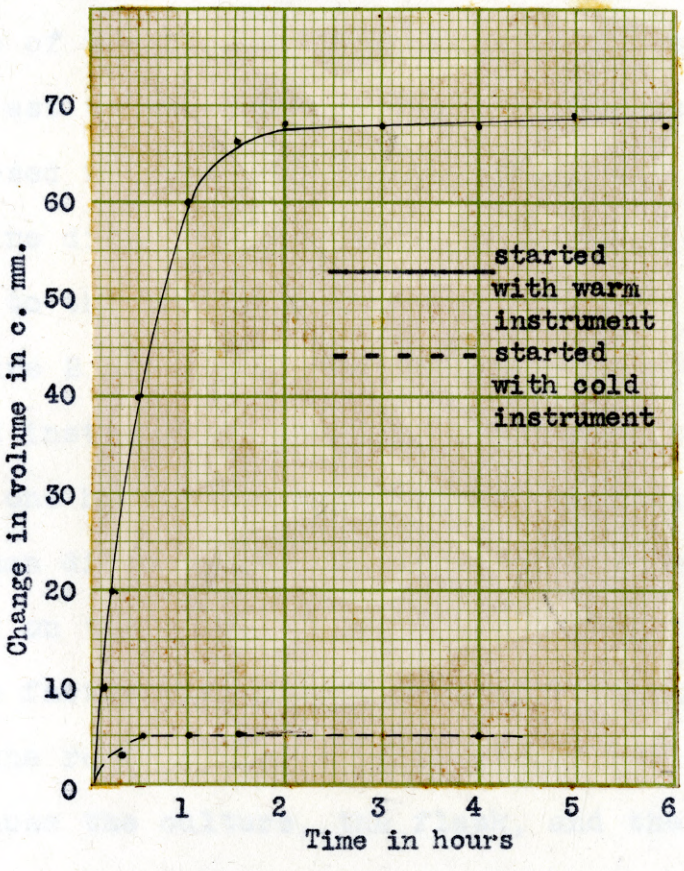

Figure 1. The effect on the equilibrium of the respirometer of cooling the instrument to 22° C.

was warmed some by handling during the introduction of the cultures. If the instrument were cold, much of this water vapor was already removed from the air, thus saving time in running the tests. A second factor was the temperatures of the flasks. They must be the same temperature for the test to run properly. If the flasks were warm and left exposed to room temperature, there was always enough temperature difference to cause an unstable condition when attached to the instrument.

Figure 2 shows the result of placing a warm culture in a cold instrument. The instrument came to equilibrium in about one hour. The warm culture evaporated water faster than did the cold culture, and the water vapor condensed on the sides of the flask caused the instrument to change faster than normal. The dotted line in figure 2 shows the results when a cold culture was used.

Because the culture, the flask, and the alkali were all the same temperature when the test was begun, the instrument attained equilibrium within 15 minutes.

Figure 3 shows the oxygen consumption of two Pelomyxa carolinensis in a culture of paramecia. When the culture was introduced into the flask, about 560 paramecia and 11 amoebae were present, but after the test, which lasted

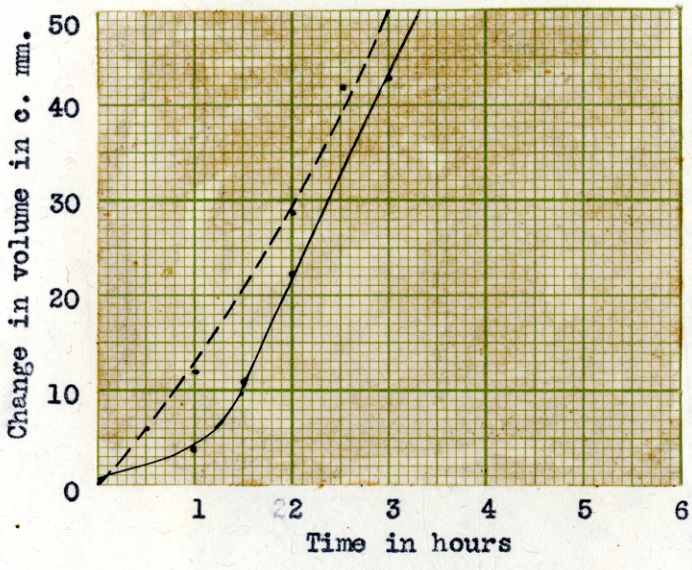

Figure 2. The effect of culture temperature on the equilibrium of the respirometer.

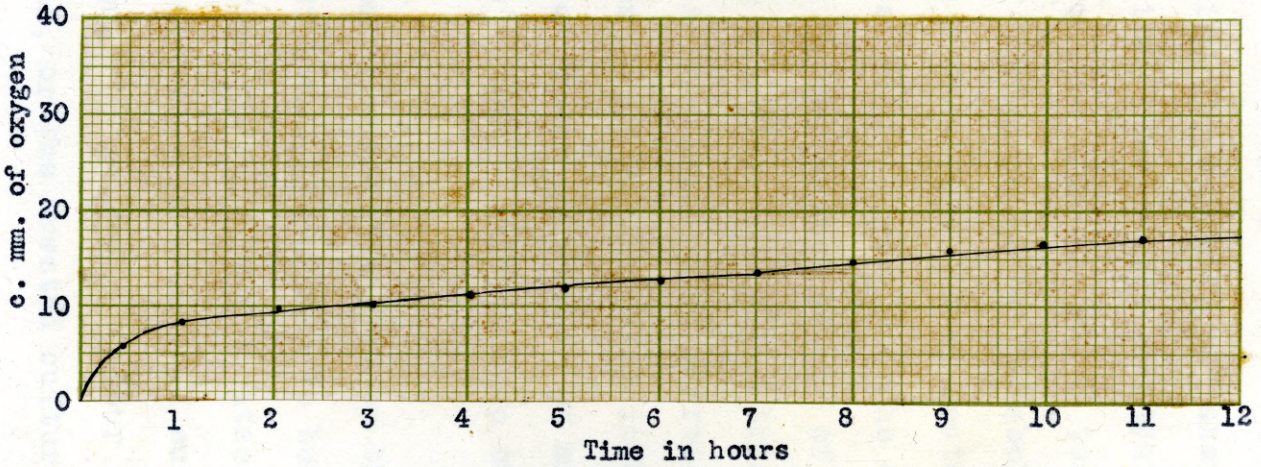

Figure 3. Oxygen consumption by a culture of Pelomyxa carolinensis in a culture of paramecia.

28 hours, 13 paramecia and 21 amoeba were left. As the slope of the curve did not change, the first 12 hours are all that are shown on the graph. This test was made to see how many organisms were needed to make a determination of oxygen consumption. The results indicate that fewer than 20 organisms can be used to make a test.

The effect of thyroxine on the metabolism of Paramecium caudatum in a mixed culture of bacteria and a few small protozoa is shown in figure 4. The dilution of the thyroxine is based on the actual amount of thyroxine in the desiccated thyroid tablets. The first trial was made with two ml. of a solution of desiccated thyroid tablets of a concentration of one part of thyroxine in 500,000, to which was added two ml. of a strong culture of mixed protozoa, thereby making the dilution of thyroxine one part in one million.

The curve from "A" to "B" indicates the normal rate of oxygen consumption of the culture to be 6.5 c. mm. per hour. The curve from "B" to "C" indicates the oxygen consumption after treating with thyroid substance. There is a variation from 10.75 c. mm. per hour to 26 c. mm. per hour of oxygen used, or the treated culture increased the oxygen consumption as much as 300 per cent. The part of the curve from "C" to "D" may have been caused by alkali

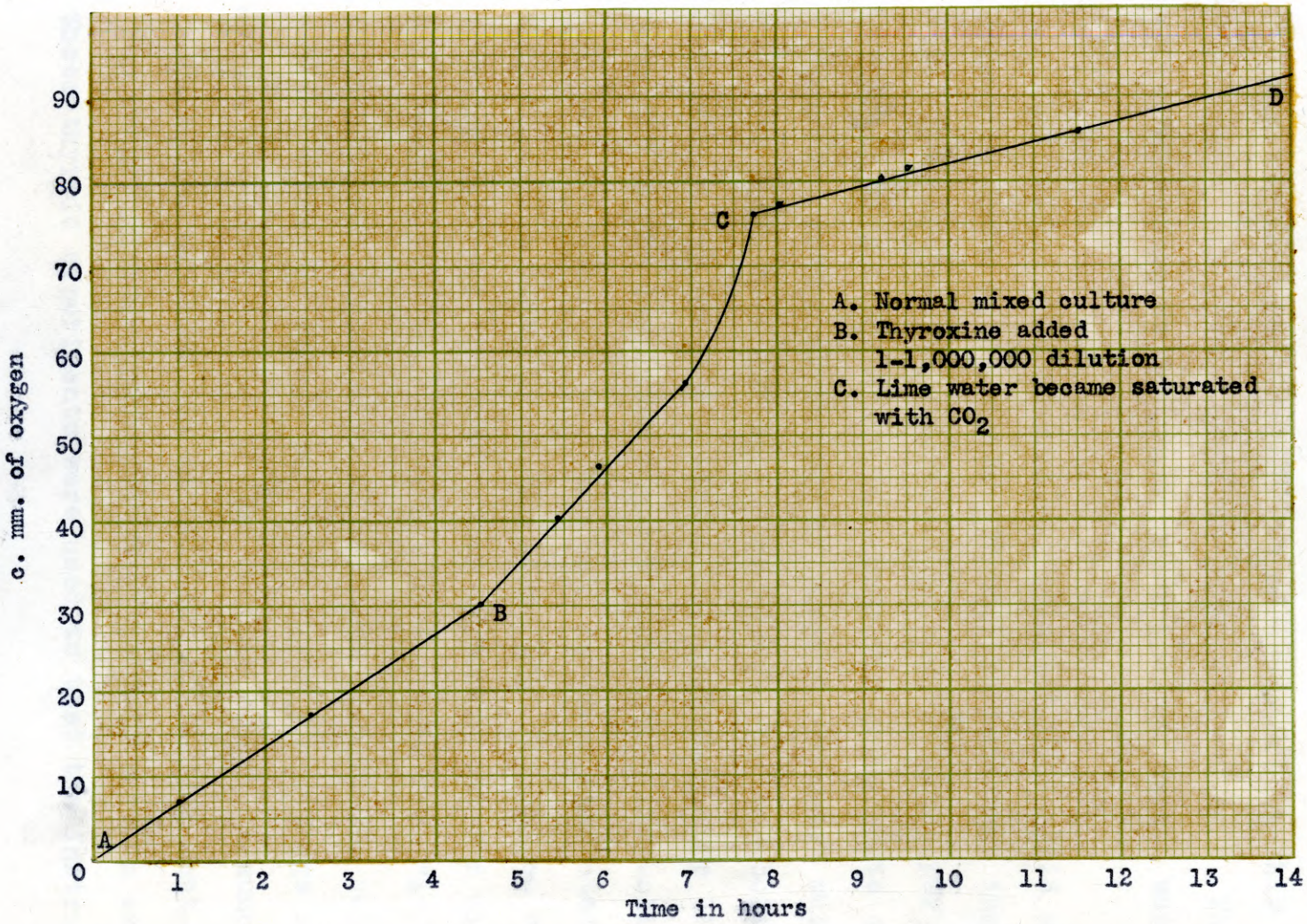

Figure 4. The effect of thyroid substance, dilution 1-1,000,000, on the oxygen consumption of a protozoan culture.

becoming saturated with CO_2. New alkali had to be added to take any more readings. This was not done in this experiment, but it was tried as shown in figure 5. The point "D" indicates the place where the new alkali was added.

The effect of a more dilute solution of thyroid substance is shown in figure 5. It will be seen that the rate of metabolism was increased to a much higher per cent. The graph line from "A" to "B" shows the normal rate of oxygen consumption of two ml. of a mixed protozoan culture.

The graph from "B" to "C" represents the metabolism rate with a 1-1,500,000 dilution of thyroxine. The line from "C" to "D" indicates a great decrease in metabolism that was probably due to the alkali becoming saturated with CO_2. The line "D" to "E" shows that the oxygen consumption returned to normal after replacing the old alkali with fresh NaOH. From the slope of the line from "A" to "B" the oxygen consumption of the normal untreated culture is 3.75 c. mm. of oxygen per hour. From the average slope of the line from "D" to "E" the average oxygen consumption is 21 c. mm. per hour, or an increase of 460 per cent.

Other dilutions were not tried in this problem as these thyroid experiments were used to test the instrument.

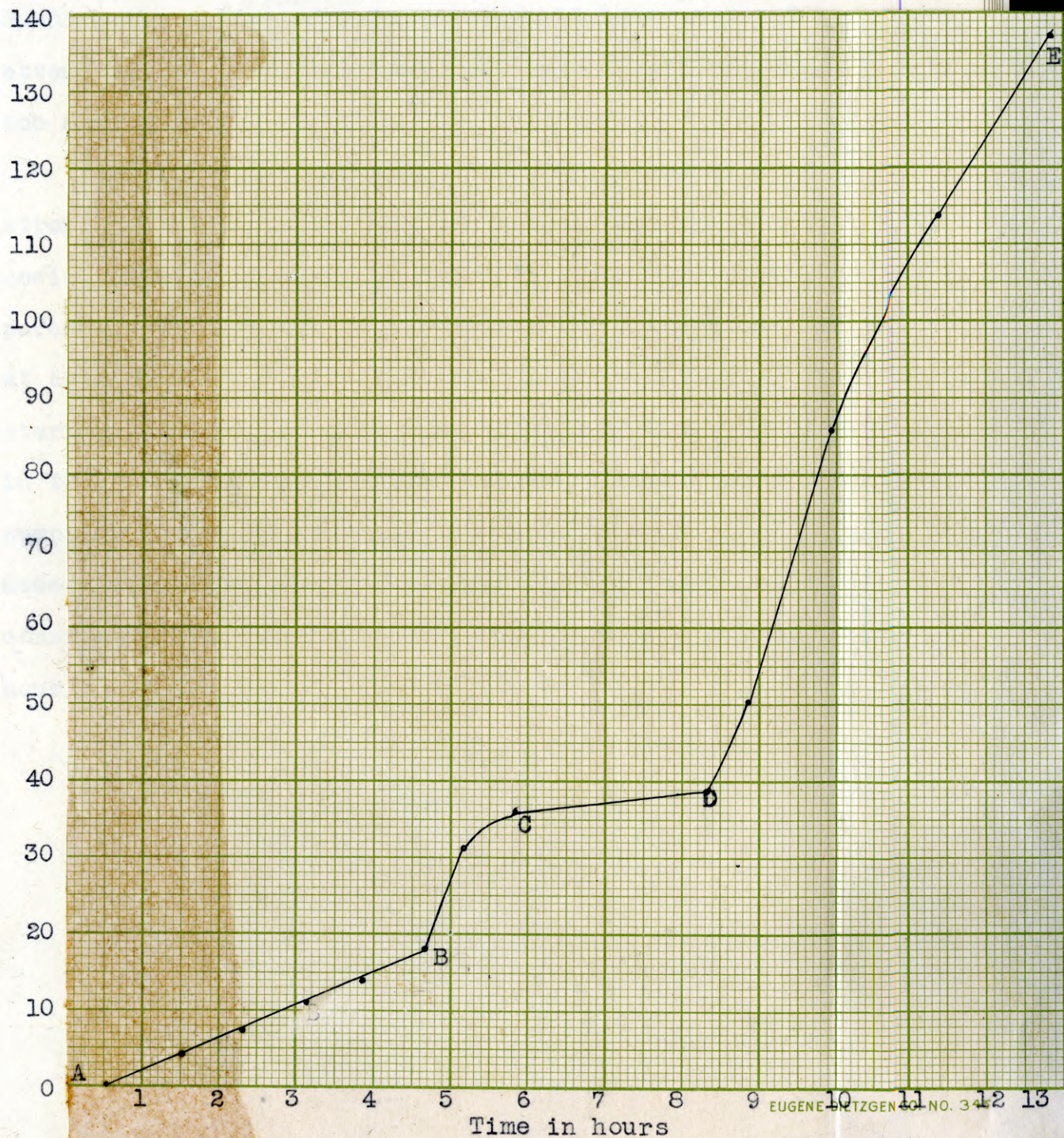

Figure 5. The effect of thyroxine, dilution 1-1,000,000, on the oxygen consumption of a protozoan culture.

Running the culture for longer than 12 hours was not attempted, as it was thought that the culture might change too much to be of any value.

Figure 6 shows the rate of oxygen consumption of a strong culture balanced against a weak culture of paramecia. The flask with the strong culture had 2,100 paramecia (in two ml.) at the start of the test and 2,300 at the end of the test. The weak culture had 100 at the start and 100 at the end. The average effective number in the strong flask was 2,100 paramecia. The oxygen consumption for eight hours of the test was 590 c. mm. This made the consumption for one hour 73.75 c. mm., and the consumption for each organism 0.035 c. mm. of oxygen per hour.

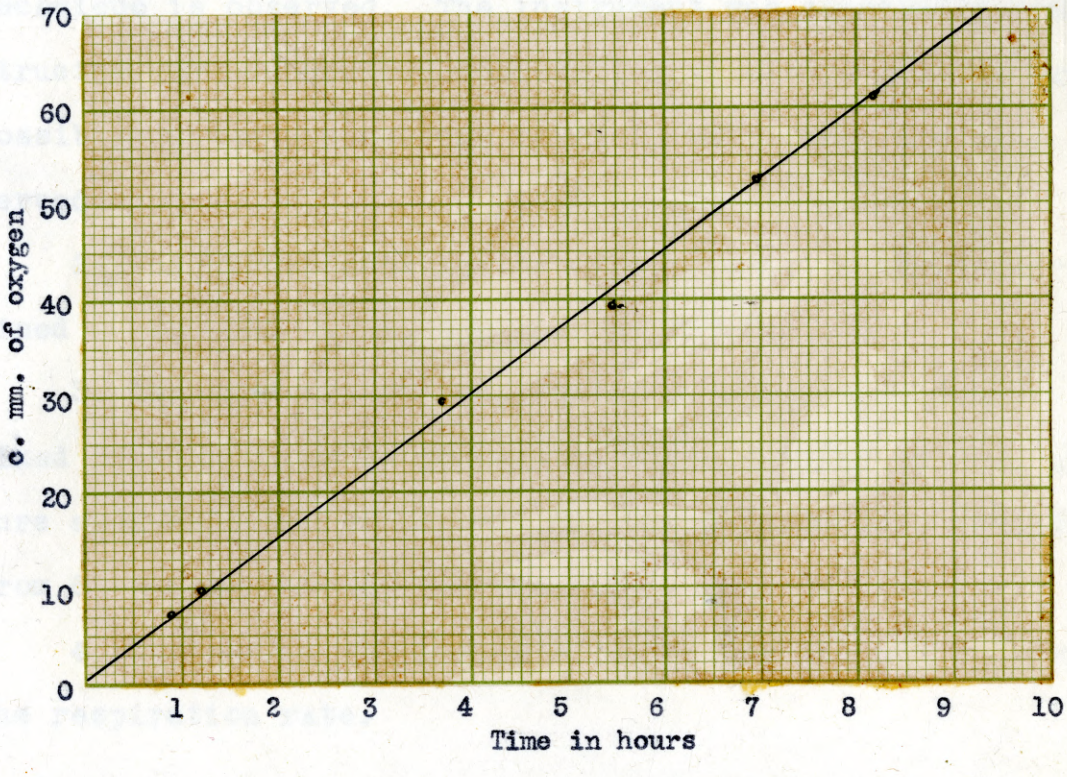

Figure 6. Oxygen consumption of 2,100 paramecia.

SUMMARY

1. A micro-respirometer with a compensating vessel, as shown in Plate I, gives constant results if the proper technique is observed. The instrument was especially constructed for comparative results. Qualitative results are possible if the precautions given in this study are observed.

2. The oxygen consumption of a paramecium was determined to be .035 c. mm. per hour at 23° C.

3. The increase in respiration of a culture of a mixed protozoa, due to desiccated thyroid added to the culture at a dilution of 1-1,000,000 and 1-1,500,000, varied from 61 per cent to 460 per cent.

4. Increasing the amount of thyroxine did not increase the respiration rate.

ACKNOWLEDGMENT

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Plate I. Micro-respirometer

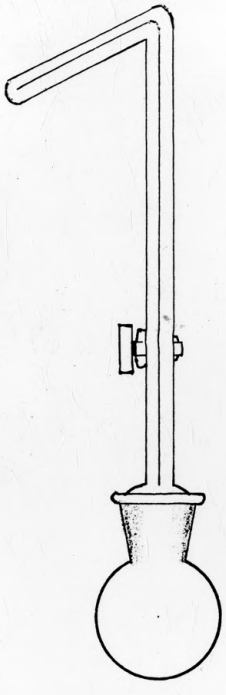

Fig. 1

Side view

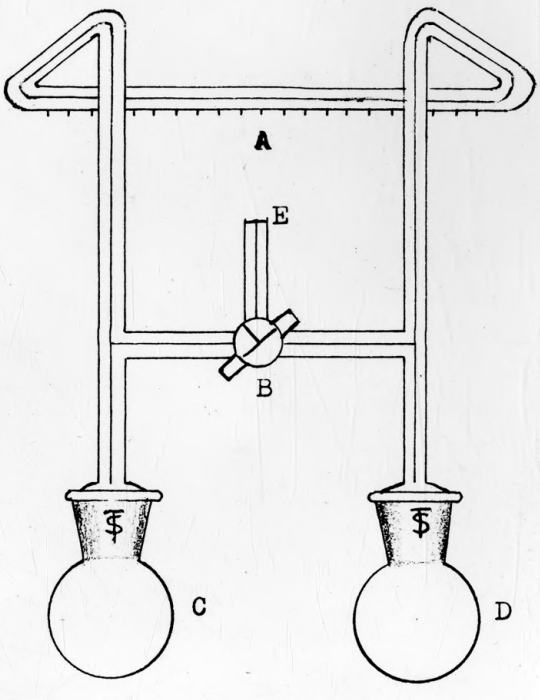

Fig. 2

Front view