7-1-1954

A polarographic study of respiration in a blue-green alga

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A POLAROGRAPHIC STUDY OF RESPIRATION IN A BLUE-GREEN ALGA

BY

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A THESIS
SUBMITTED TO THE GRADUATE FACULTY
OF THE UNIVERSITY OF RICHMOND
IN CANDIDACY
FOR THE DEGREE OF
MASTER OF SCIENCE IN CHEMISTRY

Approved by
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AUGUST, 1954

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ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. T. C. Franklin and Dr. J. C. Strickland. I am indebted to Dr. Franklin for advice on polarography which brought about the development of the technique. I am indebted to Dr. Strickland for advice concerning all phases of phycology and particularly for stock cultures from which the algae used in this research were grown.

I also wish to thank the faculties of the Biology and Chemistry departments for their cooperation and suggestions.

This work was facilitated by the use of a recording polarograph purchased with funds made available by Research Corporation of America. For this I am extremely grateful.

INTRODUCTION

The study of many aspects of physiology is accomplished by the study of respiration under varied conditions. For this purpose, a simple, rapid, and relatively accurate respirometer would be useful. This project was undertaken to investigate the possibility of adapting previously described polarographic techniques to the measurement of respiration.

Because of the difficulty of obtaining a large homogeneous sample, it is desirable to have a method which can be used with micro size samples. Most previous respirometric methods are not adaptable to micro size samples. The few methods which can be used with such small samples are quite tedious in operation.

Of the many possible approaches to this problem, only two were thought to be feasible at this time. These two

methods are electrochemical and manometric. Micromanometric techniques have been developed which are satisfactory but quite difficult to use. Electrochemical techniques, on the other hand, have been employed previously but not with volumes less than 1-2 milliliters as was desired in this study. It was decided to adapt one of these electrochemical techniques to this small volume.

Because of their biological uniqueness, the Myxophyceae were used in the physiological studies after the development of the technique.

HISTORICAL

The techniques which have been or can be adapted to respiration studies on a micro scale can be divided roughly according to the method of measurement. Thus in outline form:

1. Manometric techniques
   a. Barcroft-Warburg apparatus
   b. Microcapillary
   c. Cartesian diver
2. Gasometric techniques
   a. Gas phase analysis
      1. Biometer
      2. Electrical resistance method
      3. Mass spectrometer
      4. Magnetic (paramagnetic) method
   b. Volumetric evacuation analysis
      1. van Slyke apparatus
      2. Roughton-Scholander syringe method
      3. Micrometric gasometer

3. Chemical techniques

a. Titration methods

1. Winkler (thiosulfate) titration

2. Chromous ion titration

3. Amperometric titration

b. Optical methods

1. Colorimetric methods

2. Spectrophotometric methods

c. Electrochemical methods

1. Potentiometric determination

2. Voltammetric techniques

3. Polarographic techniques

In order to discuss the advantages and disadvantages of the polarographic method with respect to those of these other methods, it will be necessary to describe each one briefly.

MANOMETRIC TECHNIQUES

The Barcroft[1] and Warburg[2] methods are quite similar and rely upon the change in volume upon the removal of a gaseous product. Figure 1 shows a typical vessel for use with these methods. For respiration studies, the carbon dioxide evolved

1. Dixon, M., Manometric Methods, 3rd ed., Cambridge, 1951.
2. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism, Burgess, Minneapolis, Minn., 1949.

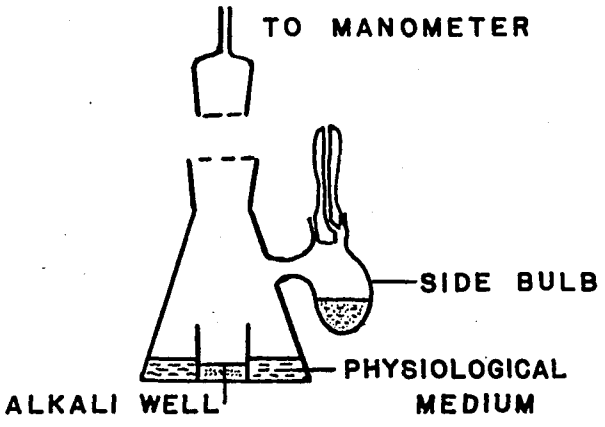


FIGURE I. WARBURG MANOMETER VESSEL

is removed from the gas phase by the alkali in the alkali well. This change in volume is indicated by the manometer connected with the vessel. One objection to these methods involves the time lag in the absorption of the carbon dioxide by the alkali and another[1] is the low carbon dioxide pressures and correspondingly high oxygen pressures which prevail when strong alkali is used. The former can be reduced by shaking the vessels. The latter can be overcome by the use of a weaker alkali or a buffer solution which is accompanied by an undesirable reduction in the speed of response.

Bonner attempted to adapt the Warburg technique to micro work in two different ways. He first[2] used micro vessels

1. Noyons, A. K. M., and van Goor, H., Acta brev neerland, 10, 99-102 (1940).
2. Bonner, J., Jour. Gen. Physiol., 17, 63 (1934).

without alkali and depended upon the difference in the solubilities of carbon dioxide and oxygen for his measurable volume change. He later[1] employed semi-micro vessels with alkali and obtained somewhat better results.

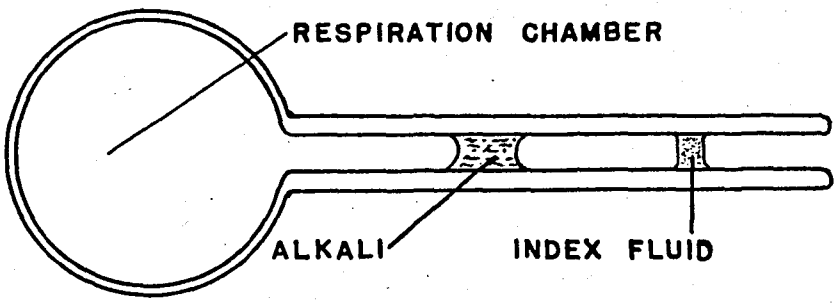


FIGURE 2. MICROCAPILLARY

The simple microcapillary[2-4] of Figure 2 does not possess great accuracy. It has, however, been modified by Cunningham and Kirk[5] to permit the measurement of volume changes as small as 5×10^{-5} cubic millimeters (cmm.). As such, it is the most sensitive respirometer known. The principle of operation is the same as for the other manometric techniques, i. e., the measurement of the volume change caused by the removal of carbon dioxide by alkali. The volume change is observed by

1. Bonner, J., Jour. Gen. Physiol., 20, 1 (1936).
2. Dixon, op cit.
3. Umbreit, Burris, and Stauffer, op cit.
4. Tobias, J. M., Physiol. Rev., 23, 51 (1943).
5. Cunningham, B., and Kirk, P., J. Cell. Comp. Physiol., 20, 119 (1942).

measuring the displacement of the index fluid. Microcapillary methods are unfortunately very sensitive to temperature changes and usually involve an error of a few percent[1].

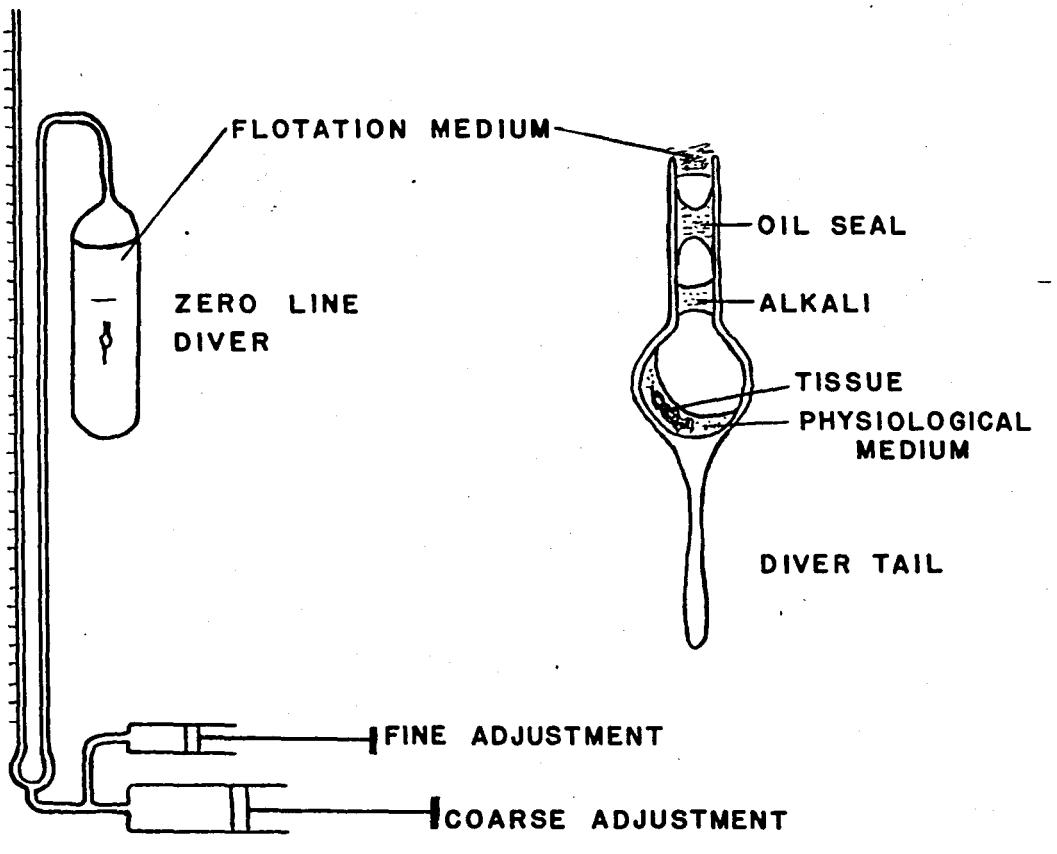


FIGURE 3. CARTESIAN DIVER

The most effective micro adaptation of manometric techniques is the Cartesian diver developed by Linderstrom-Lang[2] and illustrated in Figure 3[3]. This consists essentially of

1. Tobias, J. M., op cit.
2. Linderstrom-Lang, K., Compt. Rend. Lab. Carlsberg, Ser. chim., 25, 229 (1946).
3. Boell, E. J., Needham, J., and Rogers, V., Proc. Roy. Soc. (London), B127, 322-356 (1939).

a modified microcapillary with a more accurate indicator of volume changes. The alkali placed as shown causes a decrease in the volume of the diver as respiration proceeds. This causes the effective specific gravity of the diver to increase and thus the diver falls. By decreasing the pressure in the chamber above the flotation medium, the effective volume and specific gravity of the diver can be returned to the original value. The diver is thus operated as a null point instrument with the required pressure decrease being a measure of respiration.

The divers can be made quite small for the study of a minute amount of tissue. However the apparatus is quite tedious to operate and requires experience on the part of the investigator before the data can be properly interpreted.

Thus, manometric methods in general suffer from the same difficulties, i. e., those of low carbon dioxide pressure and time lag in response. These problems are even more acute in the micro versions of these techniques which are correspondingly more difficult to use.

GASOMETRIC TECHNIQUES

Gasometric techniques involve the measurement or analysis of gas phases. In the broadest sense gasometric techniques include those just considered as manometric techniques. These

were considered separately because of the specialized method of gas analysis which they employ. One aspect of these methods is the analysis of the gaseous phase above the culture medium containing the respiring tissue. The other aspect of these methods consists of removing the gases dissolved in the solution by evacuation at low pressures followed by the analysis, usually volumetrically, of the evacuated gases.

The various methods employed in gas phase analysis rely upon some distinctive feature of the gas to be studied. The biometer, which is historically the oldest respirometer, uses ordinary chemical titration methods. This is difficult to perform and requires special gas handling equipment which largely accounts for its infrequent use in recent years[1].

The electrical resistance method of Noyons[2] makes use of the fact that the resistance of a wire surrounded by a gas mixture varies with the gas concentrations[3]. This method requires that the gas mixture be very constant with respect to gases not being studied as such changes affect the calibration. Only the author has employed the method since its conception in the late 1930s. This would seem to indicate

1. Manning, W. M., Stauffer, J. F., Duggar, B. M., and Daniels, F., J. Am. Chem. Soc., 60, 266 (1938).
2. Noyons, A. K. M., Acta brev. neerland, 5, 23-4 (1935)
3. Heilbrunn, L. V., Outline of General Physiology, 3rd ed. Saunders, 1952)

that other workers have considered the method of little advantage over more conventional approaches.

The mass spectrometer has been used fairly extensively[1-3] because of its ability to distinguish between isotopes of oxygen or carbon dioxide. In practice, a vessel similar to the Warburg manometer vessel without an alkali well is used. Facilities are provided for connecting the leak of the mass spectrometer to the gas phase above the medium. With the use of isotopes, a means must be provided for their introduction under controlled conditions.

The proponents of this method claim that photosynthesis and respiration can be distinguished by the use of heavy isotopes of either carbon dioxide or oxygen. For example, by introducing a known amount of a heavy isotope of oxygen, respiration can be followed by measuring the rate of disappearance of the heavy isotope. Photosynthesis would continue to produce normal oxygen from the normal carbon dioxide already in the environment. Thus the rate of appearance of normal oxygen is the measure of the rate of photosynthesis. This does not take into consideration the possibility of the preferential utilization by one process of the product of

1. Brown, A. H., Hier, A. O., and van Norman, R. W., Plant Physiol., 27, 320 (1952).
2. Brown, A. H., Amer. Jour. Bot., 40, 719 (1953).
3. Brown, A. H., and Webster, G. C., Amer. Jour. Bot., 40, 753 (1953).

the other process. In other words, in the case cited, respiration might consume the normal isotope of oxygen which is being produced by photosynthesis within the same cell. It is also just as likely that the carbon dioxide produced by respiration might be consumed in preference to that which must diffuse from the gas phase through the medium and into the cell.

Thus it appears that the mass spectrometer offers another method of studying respiration with rapid and continuous measurements, although it may not be able to isolate the effects of photosynthesis and respiration as has been supposed.

The Pauling-Beckman Oxygen Analyzer relies upon a unique property of oxygen for its measurement. Molecular oxygen has a much greater magnetic susceptibility than any other common gas and therefore the magnetic susceptibility of a mixture of gases is largely due to the oxygen contained in the mixture. The simple method used to quantitate this property is quite ingenious.

> "The magnetic force acting on a small test body in an inhomogeneous magnetic field is proportional to the product of the field strength, the gradient of the field strength, and the difference in volume magnetic susceptibility of the test body and the gas surrounding it. The equilibrium orientation of the test body, as determined by the magnetic force and the torsional force of the fiber, would accordingly be dependent on and would provide a measure of the magnetic susceptibility of the gas."[1]

1. Pauling, L., Wood, R. E., and Sturdivant, J. H., J. Am. Chem. Soc., 68, 795-798 (1946).

Thus the apparatus shown in Figure 4[1] yields a continuous record of the oxygen content of the gas surrounding the dumbbell or test body.

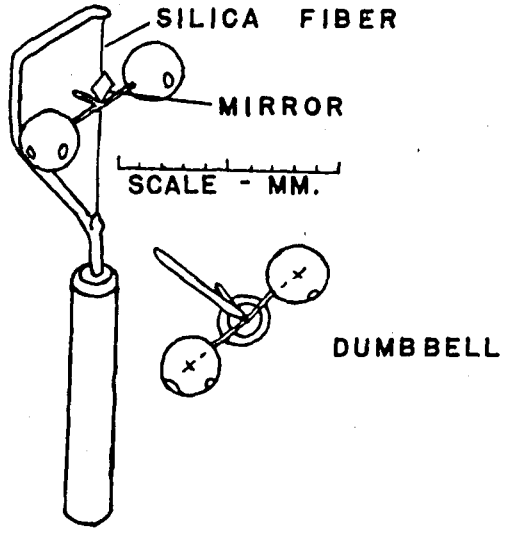
Although this method could be adapted to micro work with no more difficulty than the mass spectrometer, such an adaptation has not been made.

All of these gas phase analytical methods involve a time lag for diffusion of the gases between the medium and the gas phase. This time lag is of the same magnitude for these methods as for those previously discussed. However, since most of these methods are capable of continuous measurement this slow response is more noticeable.

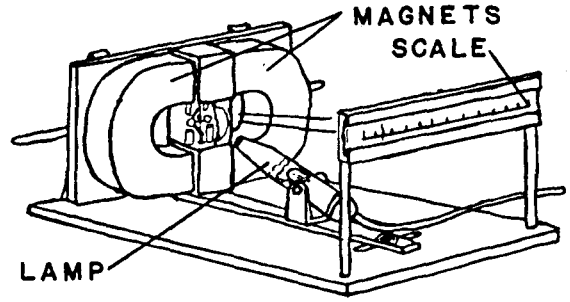
Volumetric evacuation methods are all quite similar and require the removal of a sample for analysis. These methods rely upon the evacuation of all dissolved gases under vacuum. The volume of these gases is then noted and an absorbent for the desired gas introduced. The absorbent is permitted to react for a moment and the volume is again measured. The amount removed being noted as the volume of the desired gas.

The van Slyke method[2] is perhaps the most accurate of all but requires a sample of at least 2 ml., the largest for any of these methods. The Roughton-Scholander syringe method[3]

1. Pauling, Wood, and Sturdivant, op cit.
2. Peters, J. P., and van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II, Williams and Wilkins, Baltimore, 1931.
3. Roughton, F. J.,W., and Scholander, P. F., J. Biol. Chem. 148, 541 (1938).



A. DUMBBELL ASSEMBLY



B. THE INSTRUMENT

FIGURE 4. THE PAULING OXYGEN ANALYZER

requires only 40 cmm. of sample and can be readily adapted. The micrometric gasometer[1] is more convenient to use and requires only 20 cmm. of sample.

Because these methods require the removal and subsequent manipulation of a sample, they cannot provide rapid and continuous measurements. The removal of a sample also prohibits the use of a permanently closed vessel thereby increasing the danger of diffusion from the atmosphere.

CHEMICAL TECHNIQUES

Chemical techniques which involve a titration suffer from the same problems as the gasometric techniques considered above, i.e., those involved in the removal of a sample. These methods have been employed in respiration studies on very small amounts of tissue but, at best, they are quite tedious and time consuming. All of these methods rely upon the oxidation by the dissolved oxygen of some reduced ion present in excess. The oxidized form is then titrated by an accepted method. The chromous ion titration[2] involves the oxidation of this ion to the chromic state whereas the historically important Winkler titration[3] involves the oxidation of the manganous ion to the manganic ion. Both of these methods use an iodometric

1. Lazarow, A., Laboratory Investigation, 2, 22 (1953).
2. Stone, H. W., and Sigal, P., Anal. Chem., 26(7), 1236 (1954).
3. Assoc. of Official Agricultural Chemists, Methods of Analysis, 5th ed. p. 531. A. O. A. C., Wash. D. C., 1940.

titration with a starch iodide endpoint and require the prior oxidation of any nitrites present in the solution. An amperometric titration for small concentrations of iodine has been applied to the determination of extremely low concentrations of dissolved oxygen[1].

Micro versions[2-4] of the Winkler titration have been attempted with varying results. They either require elaborate burettes for microvolumetric titrations or possess a 2% error. These methods obviously cannot afford rapid and continuous measurements.

Optical methods rely upon the change in color of a dye or complex with a change in the oxygen concentration. Since this change in color must be observed in the presence of light, these methods are of no value in studying photosynthetic organisms and are of doubtful value for nonphotosynthetic organisms, since the effects of light on respiration are still being debated in the literature.[5]

Various indicators have been used with the colorimeter for this purpose. Among them are diaminophenol hydrochloride[6], ortho-toluidine[7], indigo carmine[7], tetrammine copper (II) com-

1. Evans, D. P., and Simmons, N. T., J. Soc. Chem. Ind., 63, 29 (1944).
2. van Dam, Jour. Exptl. Biol., 12, 80 (1935).
3. Fox and Wingfield, Jour. Exptl. Biol. 15, 437-45 (1938).
4. Barth, L. G., Physiol. Zoology, 15, 30 (1942).
5. Warburg, O., Burk, D., Schocken, V., and Hendricks, S. B., Arch, Biochem., 23, 330-333 (1949).
6. Brinkman and van Schreven, Jour. Exptl. Biol. 19, 1 (1942).
7. de Hauss, J. L., Eau, 38, 195-197 (1951).

plex[1], and the thiocyanato iron (III) complex ion[2]. The spectrophotometer has been used for this purpose to measure the ultraviolet absorption of the tri-iodide ion, thus avoiding the starch-iodide reaction[3].

The electrochemical methods are of two general types. The first utilizes the relationship between the oxygen concentration and the potential of a polarizable electrode whereas the second relies upon the current drawn by an electrode in a state of concentration polarization.

The potentiometric method[4] of determining dissolved oxygen is good for continuous recording under controlled conditions. It is unfortunately subject to interference from a change in electrolyte, oxidizing agents or surface active substances which renders it less valuable in this application.

Electrochemical techniques of the second type considered here have been collectively designated as voltammetric. However, in this thesis the term voltammetry will be restricted to current-voltage studies with solid electrodes. Polarography will likewise be limited to current-voltage studies with the dropping mercury electrode (d.m.e.).

Voltammetric techniques have been employed since 1940 by a number of workers. Laitenen and Kolthoff did some of the

1. Zan'ko, A. M., Manusova, F. A., and Nikitin, A. D., Zavodskaya Lab., 8, 937-40 (1939).
2. Wickert, K., and Ipach, E., Vom Wasser, 18, 337 (1950-51).
3. Ovenston and Watson, Analyst, 79, 383 (1954).
4. Laitenen, H. A., Higuchi, T., and Czuha, M., J. Am. Chem. Soc., 70, 561-565 (1948).

early work on the development of the stationary[1] as well as the rotating[2] electrode and the application of both to the determination of oxygen[3]. Davies and Brink[4] were the first to apply the technique to respiration studies. All of these workers and those that followed[5,6] experienced poor stability and reproducibility to varying degrees. The rotating platinum electrode of Laitenen and Kolthoff[2] and the flow system of Giguere and Lauzier[7] posess greater stability but involve more elaborate apparatus with disturbing movements.

Recent remarkable improvements in voltammetric techniques by Olson, Brackett, and Crickard[8] have almost completely eliminated these difficulties however. Their rather elaborate apparatus applies an alternating square wave signal of the desired voltage and photographically records the current at the proper phase during the cycle. By this means a time resolution of the order of 2 to 10 seconds has been achieved.

1. Laitenen, H., and Kolthoff, I. M., J. Phys. Chem. 45, 1061 (1941).
2. Laitenen, H., and Kolthoff, I. M., loc. cit 1079 (1941).
3. Kolthoff, I. M., and Laitenen, H. A., Science, 92,152(1940).
4. Davies, P. W., and Brink, F., Rev. Sci. Instr., 13 524(1942).
5. Roseman, E., Goodwin, W., and McCulloch, W. S., J. Neurophysiol., 9, 33 (1946).
6. Giguere, P. A., and Lauzier, L., Can. J. Research, 23B, 76 (1945).
7. Giguere, P. A., and Lauzier, L., loc. cit 23B, 223 (1945).
8. Olson, R. A., Brackett, F. S., and Crickard, R. G., J. Gen. Physiol. 32, 681-703 (1949).

This technique has great possibilities for adaptation to a micro size sample. However, the nature and cost of the apparatus coupled with the little advantage over polarographic techniques discouraged this adaptation.

Polarographic techniques date back to 1923 with most of the pioneer work being done by J. Heyrovsky[1] and other Czechoslavakian workers. The first applications of the technique to the measurement of dissolved oxygen were, in fact, made by Heyrovsky[2,3]. Since that time, numerous workers have used the technique to study the reduction of oxygen at the dropping mercury electrode[4] and to measure the oxygen dissolved in natural waters[5-8] of sewages and industrial wastes[9-15]. The initial biological application was made by Prat[16] in 1926

1. Heyrovsky, J., Phil. Mag., 45, 303 (1923).
2. Heyrovsky, J., Casopis ceskoslovenskeho lekarnictva, 7, 242-51 and 12, 224 (1927).
3. Heyrovsky, J., Archiv. za Hemija Pharmiciju, 5,162-73 (1931).
4. Kolthoff, I. M., and Miller, C. S., J. Am. Chem. Soc., 63, 1013-17 (1941), also Chem. Zbl., 11, 1830 (1941).
5. Manning, W. M., Ecology, 21, 509-12 (1940).
6. Spoor, W. A., Science, 108, 421-2 (1948).
7. Vitek, V., Chem. et Ind., 29, 215-221 (1933).
8. Zan'ko, A. M., Manusova, F. A., and Nikitin, A. D., Savodskaya Lab., 8 937-40 (1939) (Russian).
9. Busch, A. W., and Sawyer, C. N., Anal.Chem. 24, 1887 (1952).
10. Ingols, R. S., Sewage Works J., 13, 1097 (1941).
11. Ingols, R. S., Ind. Eng. Chem., Anal. Ed., 14, 256 (1942).
12. Ippen, A. T., Yoseph, R. S., and Posthill, B. N., Tech. Rept. 3, Public Health Project No. R.G.-863, (1951).
13. Moore, E. J., Morris, C. J., and Okun, D. A., Sewage Works J., 20, 1041 (1948).
14. Rand, M. C., and Heukelekian, H., Sewage and Ind. Wastes, 23, 1141 (1951).
15. Seaman, W., and Allen, W., Sewage and Ind. Wastes, 22, 913 (1950).
16. Prat, S., Biochem. Z., 175, 268 (1926).

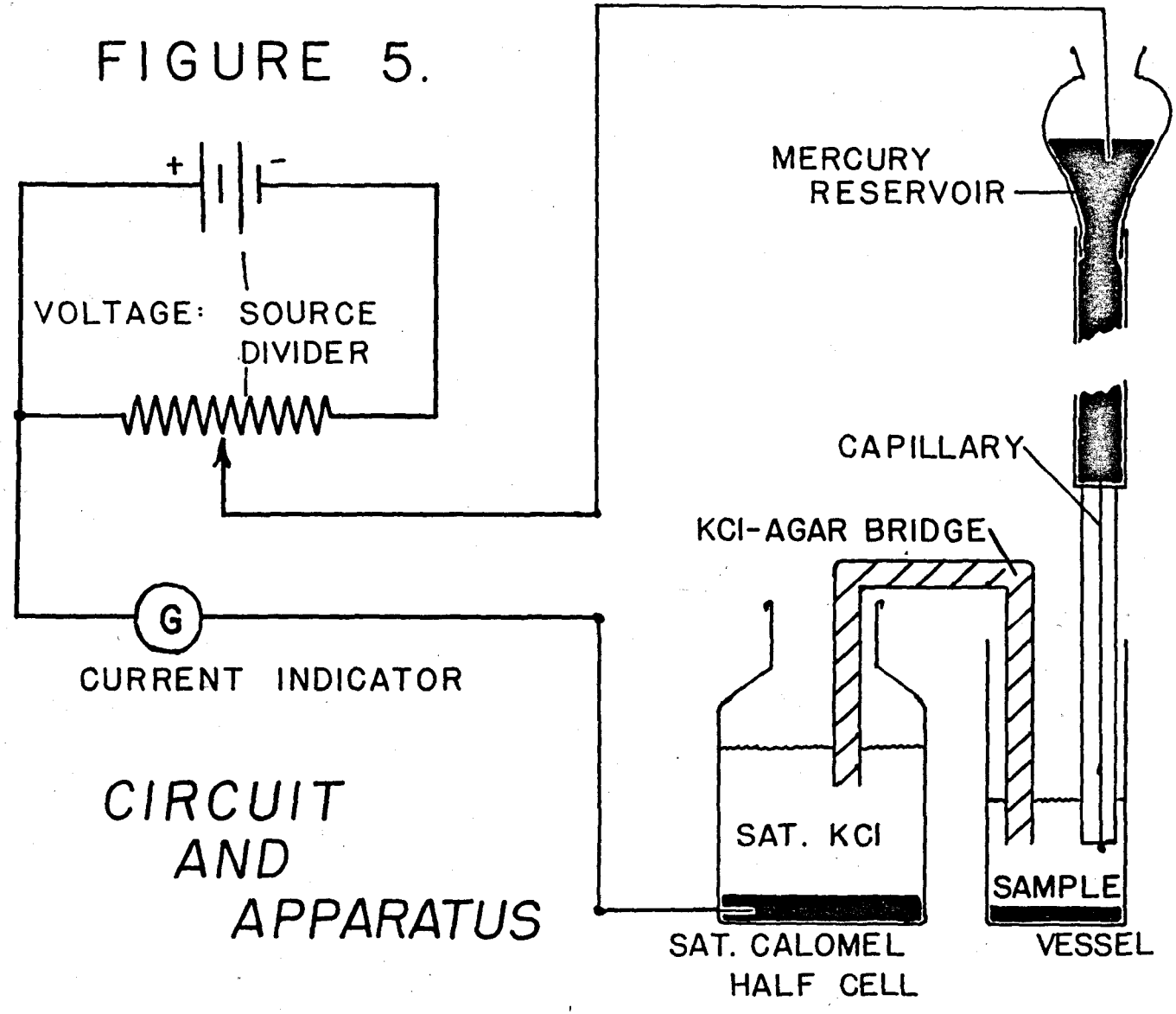
while the first respiration study with the polarograph was made by the Wisconsin group in 1938[1]. This was followed by a number of similar respiration studies[2-6].

In the following discussion of polarographic theory, no references are listed since this literature has been thoroughly reviewed by Kolthoff and Lingane in their monograph[7].

Polarography basically consists of measuring the current drawn at various potentials applied across a dropping mercury electrode and some reference electrode in the solution to be analyzed. A simple but effective circuit is shown in Figure 5. The voltage divider permits the application of any potential up to that of the source while the galvanometer in the circuit indicates the current drawn at that potential. The polarograph actually employed in this research was the Sargent-Heyrovsky Model XXI automatic recording polarograph shown in Figure 6. This consists essentially of the same components with the modifications necessary for automatic variation of the poten-

1. Petering, H. G., and Daniels, F., J. Am. Chem. Soc., 60, 2796 (1938).
2. Karsten, K., Am. J. Bot. 25, Abstr. Section 14 (1938).
3. Baumberger, J. P., Cold Spring Harbor Symp. 7, 195 (1939).
4. duBuy, H. G., and Olson, R. A., Am. J. Bot. 27, 401 (1940).
5. Baker, E. G. S., and Baumberger, J. P., J. Cell. Comp. Physiol., 17, 285 (1940).
6. Winzler, R. J., J. Cell. Comp. Physiol., 17, 263 (1940).
7. Kolthoff, I. M., and Lingane, J. J., Polarography, vols. I, II, Interscience, New York, 1952.

FIGURE 5.



CIRCUIT AND APPARATUS

Figure 6. The Sargent-Heyrovsky Model XXI Polarograph

tial (synchronous motor driven potentiometer) and pen-recording of the current (Brown recording potentiometer).

A typical polarogram for dissolved oxygen is shown in Figure 7. It can be observed that the current is not affected appreciably by a change in the potential initially (at low voltages). However, as the oxidation-reduction potential of some constituent of the solution (in this case oxygen) is approached, the current is observed to rise sharply as the potential is increased. Following this the current again becomes constant: this region is commonly referred to as a "plateau" region. In the case of oxygen, however, two polarographic waves are observed because of the two reductions which oxygen undergoes.

This plateau region is caused by concentration polarization from which the polarograph derives its name and its usefulness. This is, the number of molecules reacting (and hence the current) at the electrode has become limited by the concentration in the immediate vicinity of the electrode. The oxygen at the surface of the mercury drop has all reacted leaving an extremely low concentratinn. The reaction is thus maintained by the diffusion of oxygen from the bulk of the solution. Thus the current drawn or the height of each wave is a function of the concentration.

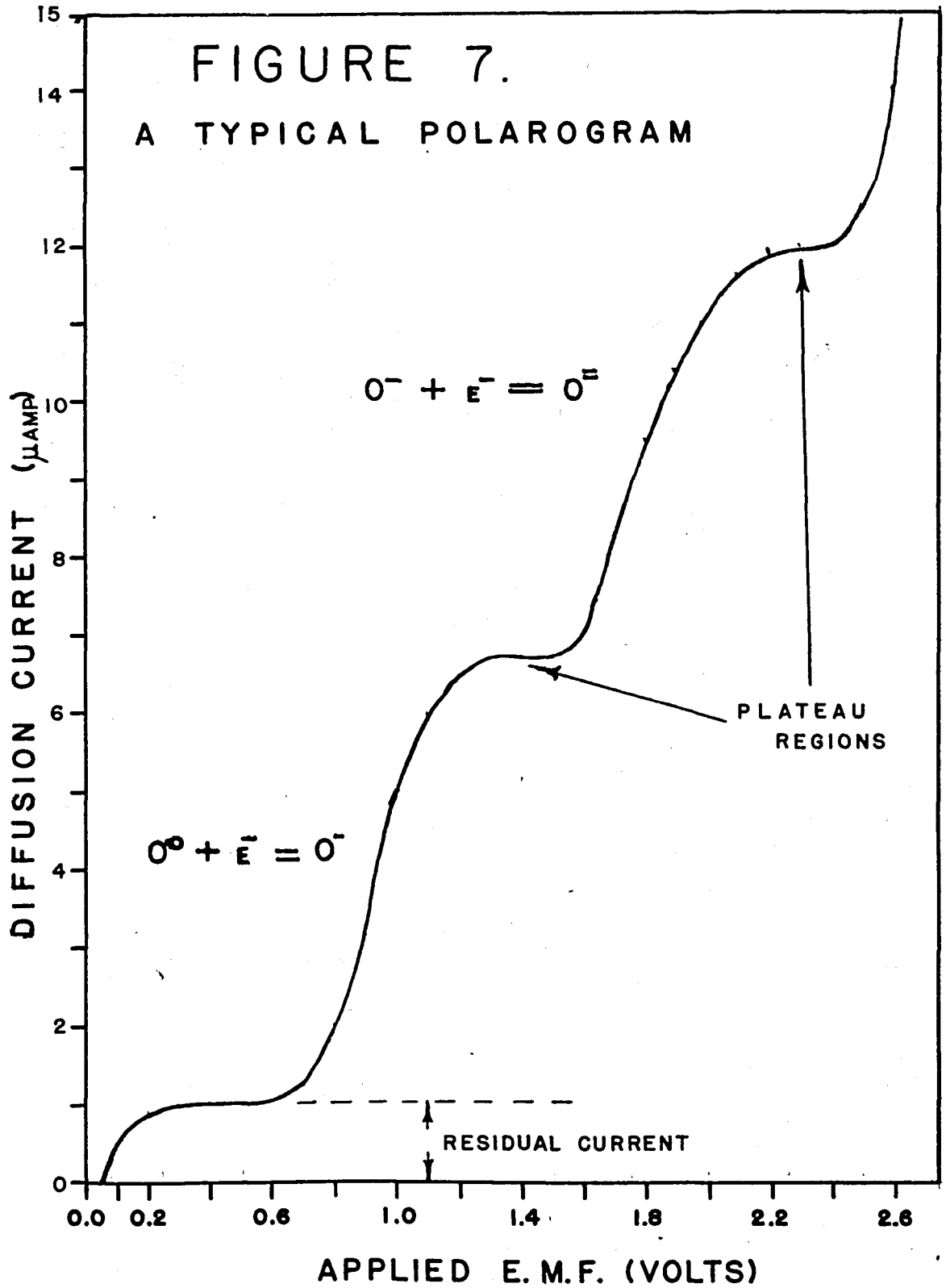


FIGURE 7.
A TYPICAL POLAROGRAM
$O^- + e^- = O^{=}$
$O^{\circ} + e^- = O^-$
PLATEAU REGIONS
RESIDUAL CURRENT
DIFFUSION CURRENT (μAMP)
APPLIED E.M.F. (VOLTS)
0
2
4
6
8
10
12
14
15
0.0
0.2
0.6
1.0
1.4
1.8
2.2
2.6

The following equation, derived originally by Ilkovic from a consideration of diffusion to an expanding sphere, has been found to hold experimentally.

$$i_d = k\, n\, D^{\frac{1}{2}}\, C\, m^{\frac{2}{3}}\, t^{\frac{1}{6}}$$

where i_d = diffusion current.
k = constant.
n = number of electrons involved in the reaction at the electrode.
D = diffusion coefficient of reactant.
C = concentration of reactant in bulk of the solution.
m = weight of mercury passed by electrode in unit time.
t = drop time; average life of drop.

For any given reaction, n and D are constant and for any one electrode m and t are constant. Thus the Ilkovic equation for any one analysis can be simplified to:

$$i_d = K\, C$$

where $K = k\, n\, D^{\frac{1}{2}}\, m^{\frac{2}{3}}\, t^{\frac{1}{6}}$

This expresses the linear relation between the diffusion current and the concentration of the reducible substance.

In comparison with the above considered techniques, the polarograph possesses certain definite advantages. Measurements can be made rapidly and, with some limitations, continuously. The lower limit on the size of the chamber is of the order of 0.1 ml. thus reducing the sample size to the order of 0.02 ml. when desired. Experiments can be conducted in the dark for photosynthetic organisms and over relatively long periods of time when desired. The sensitivity of the polar-

ograph is maintained at low oxygen concentrations, thus providing a means for studying respiration with the same accuracy at low concentrations as at high. The sensitivity of the electrode is affected only by $_{p}H$ changes, some surface active substances, heavy metal ions, or easily reduced substances, and varies only about 2% per Centigrade degree. Thus there is no problem in maintaining all errors to within 1 or ½ %.

A unique feature of the polarograph is the ability to remove any desired amount of dissolved oxygen electrolytically. This is accomplished by drawing current through the electrodes until the oxygen concentration is at the desired level, as indicated by the polarographic wave height. This makes possible the accurate standardization of the apparatus following a method suggested by Tobias[1]. Thus, by knowing the number of coulombs passed, the amount of oxygen removed can be calculated. Knowing this and the volume of solution enables one to calculate the change in concentration which can then be related to the change in the current drawn.

The disadvantages connected with the polarograph involve the use of metallic mercury and the necessity of a total salt concentration of approximately 0.1 M. to act as a supporting electrolyte. Previous workers have tested the tissue of organism which they investigated with regard to the effect of metallic mercury. None has ever noticed any influence on growth over an extended period of time or respiration over a

1. Tobias, J. M., loc. cit.

short time. The ionic forms of mercury however may be toxic and should therefore be avoided. The use of a salt bridge and half cell for the anode makes it impossible for the mercurous ions produced at the anode to get into the medium, thus eliminating any danger of toxicity from that source. Some previous workers have not used such an anode however and have observed no demonstrable effects.

The prerequisite supporting electrolyte coupled with the osmotic relationships found in living organisms can prohibit the use of the polarograph in some investigations. Fortunately, most physiological media contain sufficient salts. In other applications, however, some difficulty might arise in regard to satisfying the osmotic requirements of the organism and the supporting electrolyte requirements of the polarograph. Lower salt concentrations are permissible, but decreased sensitivity and erratic behavior may be observed. Table 1 from Tobias[1] presents an interesting comparison of some of these methods.

Table 1

Instrument	Measures	Smallest measurable volume change (from experiment)	Error %
Biometer	CO_2	2.5×10^{-2}	6
Winkler titration	Dissolved O_2	28	1-2.5
Indicator	Dissolved O_2	5×10^{-1}	10
Cartesian diver	CO_2, O_2, etc.	$1-2 \times 10^{-3}$	50-100
Polarograph	Dissolved O_2	1×10^{-3}	very precise
Microcapillary	CO_2, O_2, etc.	5×10^{-5}	a few

1. Tobias, J. M., loc. cit.

THE MYXOPHYCEAE

The physiological investigation with the modified polarographic technique was undertaken as an exploratory study of respiration in a blue-green alga. It was thought that additional knowledge concerning the physiology of this little known and quite important group of plants would be of value.

The algae are a "heterogeneous assemblage of simple plants"[1] belonging to the lowest division of the plant kingdom, the Thallophyta. This classification is based on the absence of stems and leaves and is thus not completely satisfactory. It does, however, serve to illustrate the morphological simplicity found in this group. The morphological and taxonomical relationships of the algae also illustrate the evolutionary significance of the algae. The algae as a class are believed to be a very early step in evolution which has subsequently evolved very little, being somewhat of an evolutionary "blind alley". The Myxophyceae are considered to be even more primitive than the other algae.

The algae are also important since most of them contain chlorophyll and are therefore capable of photosynthesis. Due to the existence of unicellular forms, algae, particularly the Chlorophyceae, have been used extensively in investigations of photosynthesis.

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1. Smith, G. M., Freshwater Algae of the United States, 2nd ed., McGraw-Hill, New York, 1950.

In recent years, the steady decrease in the ratio of land area being tilled to population has prompted a number of investigations concerning the efficiency of utilization of the sun's energy[1,2]. The Chlorophyceae, particularly Chlorella, have been investigated in this regard much more than any other organism or group of organisms. The algae are especially useful for this purpose since: 1) They are morphologically simple and therefore more efficient. 2) Unicellular forms can be obtained readily. 3) They can be cultivated in mass. 4) They contain over 50% protein (with a low molecular weight and therefore readily digestible) which contains the ten amino acids now considered essential[3].

The Myxophyceae have a number of characteristics which make them even more important for investigation. The condition of the nucleus and chloroplasts is not the same as in other algae. The Myxophyceae lack "a nucleus as found in other algae" and do not have their chlorophyll confined in discrete bodies or plastids as do other algae. These differences are apparently more in degree of organization than in content.

Previous investigators of algal physiology have seldom been concerned with the Myxophyceae per se. A number of

1. Burlew, J. S., ed. Algal Culture, Carnegie Institution of Wash. Pub. 600, Wash., D.C., 1953.
2. Daniels, F., and Morgen, R., Science, 119, 82 (1954).
3. Spohr, H., in Burlew, op. cit.

comparative studies have drawn one or two genera from the Myxophyceae for examples. The literature on their physiology is therefore small and scattered. Only two reviews on the much more general topic of algal physiology have appeared[1,2].

To illustrate the biological uniqueness of the blue-green algae, the two major distinctions observed will be discussed. The first difference is in the pigmentation. The only chlorophyll present is chlorophyll a which, as mentioned previously, is not found in chloroplasts. Present knowledge indicates that the only carotene present is B-carotene. Myxoxanthin and myxoxanthophyll are believed to be the major if not the only xanthophylls present. These have been observed in only one other organism. The major phycobilin found is c-phycocyanin with some c-phycoerythrin present. The c-phycocyanin is responsible for the blueness in the color. Neither of these pigments has been found elsewhere[3].

The second major difference concerns the mechanism of carbohydrate metabolism. The presence of the tri-carboxylic acid cycle is strongly contraindicated by experimental evidence. Webster[4] observed that pyruvic acid was not a suitable

1. Myers, J., in Burlew, op. cit.
2. Blinks, L. R., in Smith, G. M., Manual of Phycology, Chronica Botanica, Waltham, Mass., 1951.
3. Strain, H. H., Chap. 13 in Smith, G. M., Manual of Phycology.
4. Webster, G. C., Am. J. Bot., 37, 682 (1950).

substrate for respiration. Nor did he observe any inhibition of respiration by sodium malonate in Cylindrospermum. This was the only Myxophyceae studied and the only alga satisfactorily observed to participate in either of the above phenomena. Pyruvic acid would be a natural substrate if the tri-carboxylic acid cycle exists in the blue-green algae as described in other organisms. Malonate is considered to be a specific inhibitor for succinic dehydrogenase indicating by the above results that either succinic acid is not converted into fumaric acid or that an alternate pathway is provided. The presence of thiamine[1] however indicates that acetic acid can condense with oxaloacetic acid. Thus the tri-carboxylic acid cycle, if it exists in the Myxophyceae, is altered in a number of enzymes and substrates.

These two distinctions isolate the Myxophyceae with regard to two of the most important metabolic processes--photosynthesis and carbohydrate metabolism. This coupled with numerous other differences substantiate the beliefs concerning the evolutionary uniqueness of the Myxophyceae.

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1. Hutchinson, G. E., Arch. Biochem., 2, 143-50 (1943).

MATERIALS AND METHODS

THE ADAPTATION OF THE TECHNIQUE

The two major problems met in the development of the technique were 1) the provision of a sufficient total salt concentration to act as a supporting electrolyte and 2) the design and construction of a suitable respiration chamber and electrode assembly.

The medium for the study was developed to satisfy two demands. The first was that of the algae for a compatible environment while the second was that of the polarographic method for an adequate supporting electrolyte. Fortunately, the algae used in this work appear relatively insensitive to osmotic changes. Thus the medium can be altered to satisfy the supporting electrolyte requirement.

Thus the half normal Beijerinck's solution in which the

algae were grown[1] could be concentrated to bring the total salt concentration to the desired level of 0.1 M. This was accomplished by dissolving ten times the normal amount of the soluble salts yielding two different modified Beijerinck's solutions according to which ion was omitted. Table 2 tabulates the composition of the various solutions.

Table 2

Composition of the Physiological Media used

Salt	Amount in grams/liter solution		
	Beijerinck's	1st Modification	2nd Modification
NH_4NO_3	0.5	5.0	5.0
K_2HPO_4	0.2	- -	2.0
$MgSO_4 \cdot 7HOH$	0.2	2.0	2.0
$CaCl_2 \cdot 2HOH$	0.1	1.0	- -
$FeCl_3$	trace	none	none

The first modification was observed to cause erratic behavior of the electrode, presumably due to the unbuffered nature of the solution. The second modification was free from this and was therefore employed in this work and is hereafter referred to as modified Beijerinck's solution.

No loss of vigor was observed in samples suspended for as much as 48 hours in modified Beijerinck's solution. This

1. Beijerinck, M. W., Appendix, p. 99 in Brunel, et al., The Culturing of Algae, C. F. Kettering Foundation, 1950.

verifies the assumption made earlier that the algae were relatively insensitive to osmotic changes.

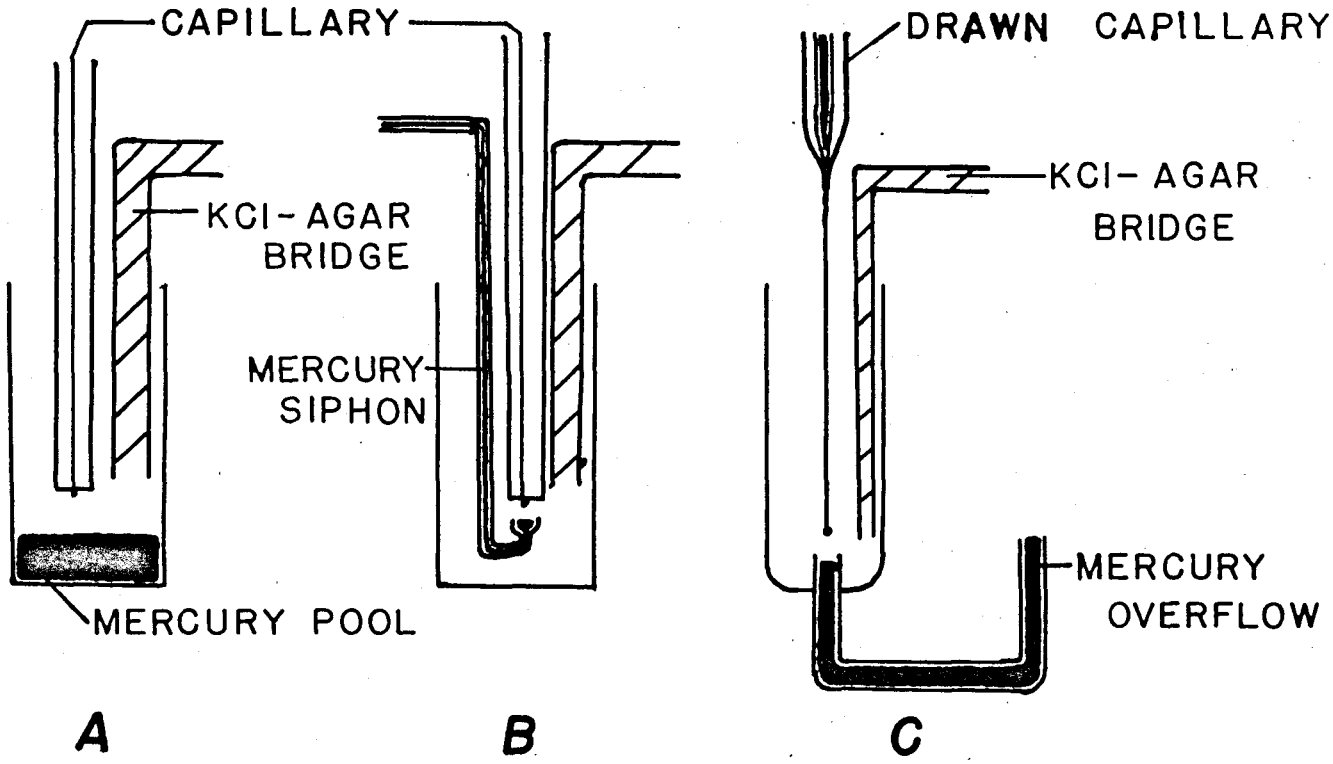
The design of the respiration chamber to be used in this research was the principle instrumental difficulty. Several chambers tested and discarded are illustrated in Figure 8. The difficulties with the simple arrangement pictured at A are: 1) the tendency for the bulk of the mercury pool to trap the algae against the bottom and sides of the chamber, 2)the diffusion of oxygen into the solution from the atmosphere, and 3) the accumulation of a quantity of mercury in the chamber with the subsequent elevation of the solution when a study is desired over a longer period of time.

Difficulties 1 and 3 were successfully overcome by a siphon arrangement in the vessel pictured at B. This however proved to be quite difficult to set up and very tedious in operation. The diffusion from the atmosphere was still quite apparent in this type chamber.

The mercury overflow arranged as in C was equally effective but still permitted the diffusion of oxygen from the atmosphere. The capillary for this and subsequent chamber types was made by drawing barometer tubing (outside diameter 7 mm., inside diameter 2 mm.) down to a very small size. This obtained the desired small (0.05 to 0.09 mm.) inside

FIGURE 8.

EXPERIMENTAL VESSELS



diameter without the 8 to 10 mm. outside diameter of conventional polarographic capillaries.

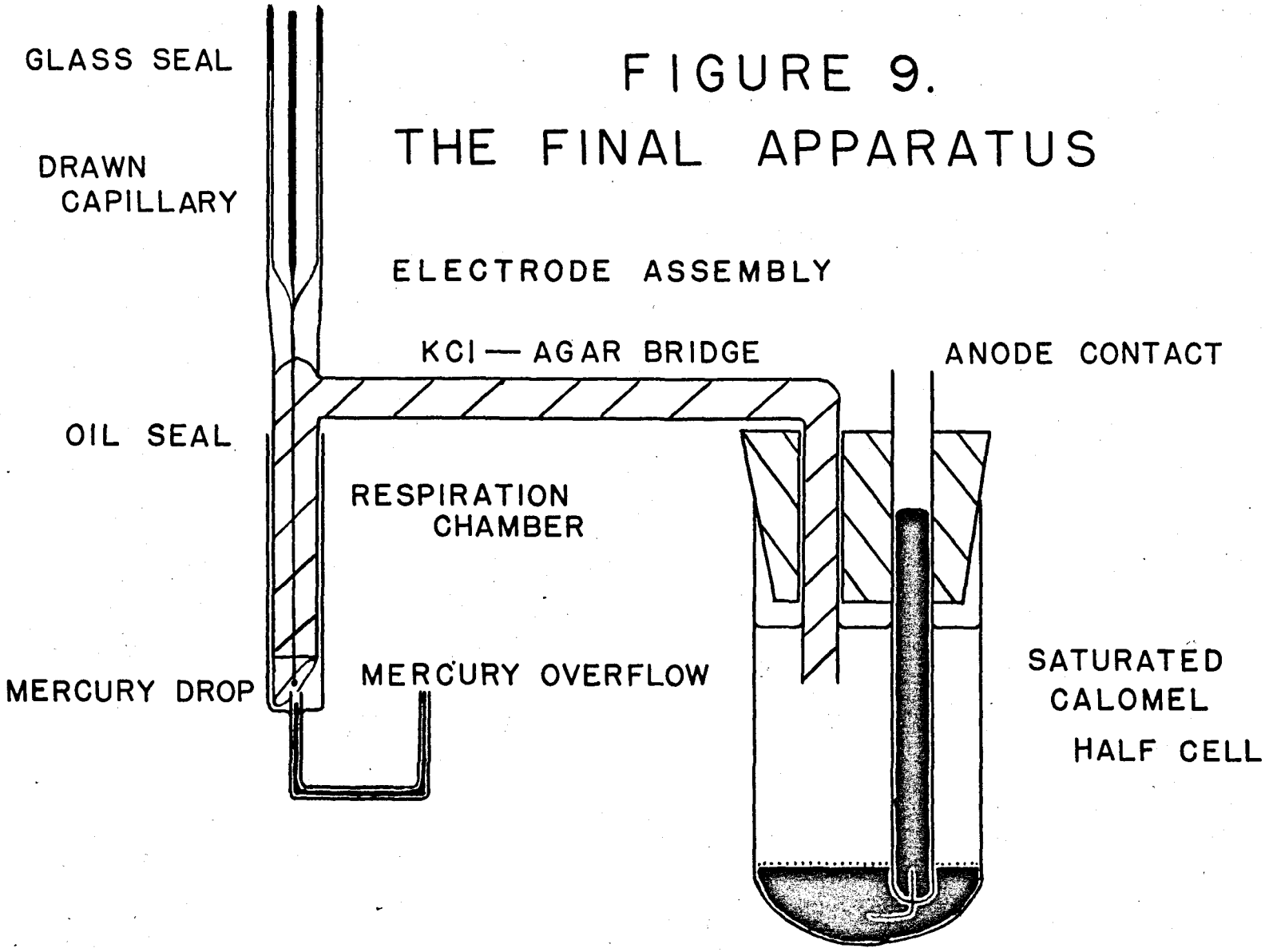
It was necessary to reduce the size of the salt bridge in types B and C. This increased the series resistance (Figure 4) of the electrode circuit and thereby slightly decreased the sensitivity and speed of response of the polarograph.

All of these difficulties were reduced in the final apparatus shown in Figure 9. The same drawn capillary is here placed in the center of the larger salt bridge. By choosing the proper size tubing, the diffusion of oxygen from the atmosphere was sharply reduced. This diffusion was virtually eliminated by placing a ring of paraffin oil about the neck of the respiration chamber when in position on the electrode assembly.

This apparatus also has the distinct advantage of permitting the use of a micro size sample (0.1-0.3 ml. of solution) when desired. The mercury overflow functions as in Figure 8C and maintains the volume of the chamber constant. Thus the electrode assembly can be used with any size respiration chamber.

It should be noted that with this micro size sample, continuous readings with the polarograph are not possible unless

FIGURE 9.
THE FINAL APPARATUS



drastic corrections are to be made for electrode consumption. The usual policy was to take readings at ten minute intervals, being careful to switch the electrodes off between readings.

ALGAL CULTURE AND TRANSFER METHODS

The algae used in this research were subcultivated from stocks supplied by Dr. J. C. Strickland. These stocks have been grown continually under uniform laboratory conditions since their isolation in 1951. The algae are of the order Chroococcaceae and believed to be the genus Gleocapsa. This genus is described in Smith[1].

These cultures were established and subcultivated on Beijerinck's solution diluted 1:1 and containing 5% (final concentration) of a soil decoction. This soil decoction was made by boiling approximately 0.5 l. of top soil with 0.5 l. of deionized water for 3/4 hour. This was then filtered first through coarse filter paper and second with suction through a dense asbestos fiber filter.

All transfers were made in an area decontaminated by an ultraviolet germicidal lamp. This protected the purity of the culture especially with regard to bacteria and permitted the use of samples from the same culture in subsequent studies.

1. Smith, G. M., Freshwater Algae of the United States, McGraw-Hill, New York, 1950.

In practice, 25 ml. of the culture medium was placed in each of a number of 125 ml. Erlenmeyer flasks. These were closed with cotton plugs and capped lightly with aluminum foil. They were then sterilized in the autoclave at 240° F. and 2 atmospheres pressure for 15 minutes. The pressure was released slowly and the flasks permitted to cool before inoculation.

The cooled flasks were placed with the stock cultures in the sterile area for 20-30 minutes after which inoculation was effected by dividing the stock culture equally among the subcultures. These were plugged, covered with aluminum foil, and placed under moderate fluorescent illumination.

EXPERIMENTAL PROCEDURE.

In order to obtain a relatively uniform sample for study from the subcultures, a small pipette was made with a calibrated volume of 0.12 ml. The subculture to be used was placed in the sterile area in a slanted position for a few minutes. This caused the cells to collect in a relatively uniform dense aggregate in the bottom of the flask. The pipette was then introduced under sterile conditions and 0.12 ml. of loosely packed cells withdrawn.

The pipette was then discharged into the respiration chamber to be used. The pipette was then rinsed into the

chamber and the chamber filled with modified Beijerinck's solution. The cells were permitted to settle to the bottom, the supernatant fluid was removed, and the cells were washed with two portions of modified Beijerinck's solution. The cells were then ready for study when the respiration chamber was placed on the electrode assembly.

For those determinations of the effect of potassium cyanide on respiration, the cells were transferred to the respiration chamber in the usual manner. The desired concentration of cyanide was then added and permitted to remain in contact with the algae for the specified exposure. The chamber was shaken at five minute intervals during the exposure to insure uniform treatment of all the cells. The algae were then permitted to settle and the supernatant fluid removed. The cells were then washed three times with modified Beijerinck's solution and placed on the electrode assembly.

Later studies at higher oxygen pressures were achieved by bubbling oxygen through a very fine capillary inserted in the bottom of the respiration chamber. Five minutes usually sufficed to elevate the oxygen pressure to more than twice the normal atmospheric equilibrium value.

To investigate the effects of ultraviolet irradiation on respiration, the cells were exposed to the ultraviolet

source in 50 ml. pyrex beakers with approximately 30 ml. of solution. The source was then placed above the beaker and left on for the specified length of time. Following this, the supernatant solution was removed and the cells were washed into a respiration chamber with modified Beijerinck's solution. The cells were then washed twice with portions of modified Beijerinck's solution and were then ready for study when placed on the electrode assembly.

During the early work, a preliminary polarogram was made routinely after each sample was placed on the electrode assembly. This practice was abandoned for two reasons: 1) The polarograph required 13.5 minutes to make a complete polarogram, thereby depleting the oxygen in the respiration chamber. 2) All of the preliminary polarograms indicated that all studies should be made at the same potential of - 1.48 volts.

All respiration studies were made in total darkness. This was achieved by surrounding the respiration chamber with a one liter beaker painted black. The respiration chamber was then covered with several layers of heavy black cloth.

All chemicals used were of reagent grade except the potassium cyanide, which was technical grade (96%).

DATA AND RESULTS

It was thought necessary to verify experimentally the linear relationship between the oxygen concentration and the diffusion current at the chosen potential. Figure 10 illustrates the effect on the complete polarographic wave of bubbling hydrogen gas through the solution. In subsequent work, the potential was fixed at -1.48 volts for all diffusion current measurements. The plateau region of the first wave was chosen in preference to that of the second wave because of the greater stability of the electrode in the region of the former.

To effect a calibration, the oxygen concentration as determined by the Winkler method[1] was compared with the diffusion current at the chosen potential of -1.48 v. The data for a number of electrodes are tabulated in Table 3. The

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1. Assoc. of Official Agricultural Chemists, Methods of Analysis, 5th ed., p. 531, A.O.A.C., Wash., D.C., 1940.

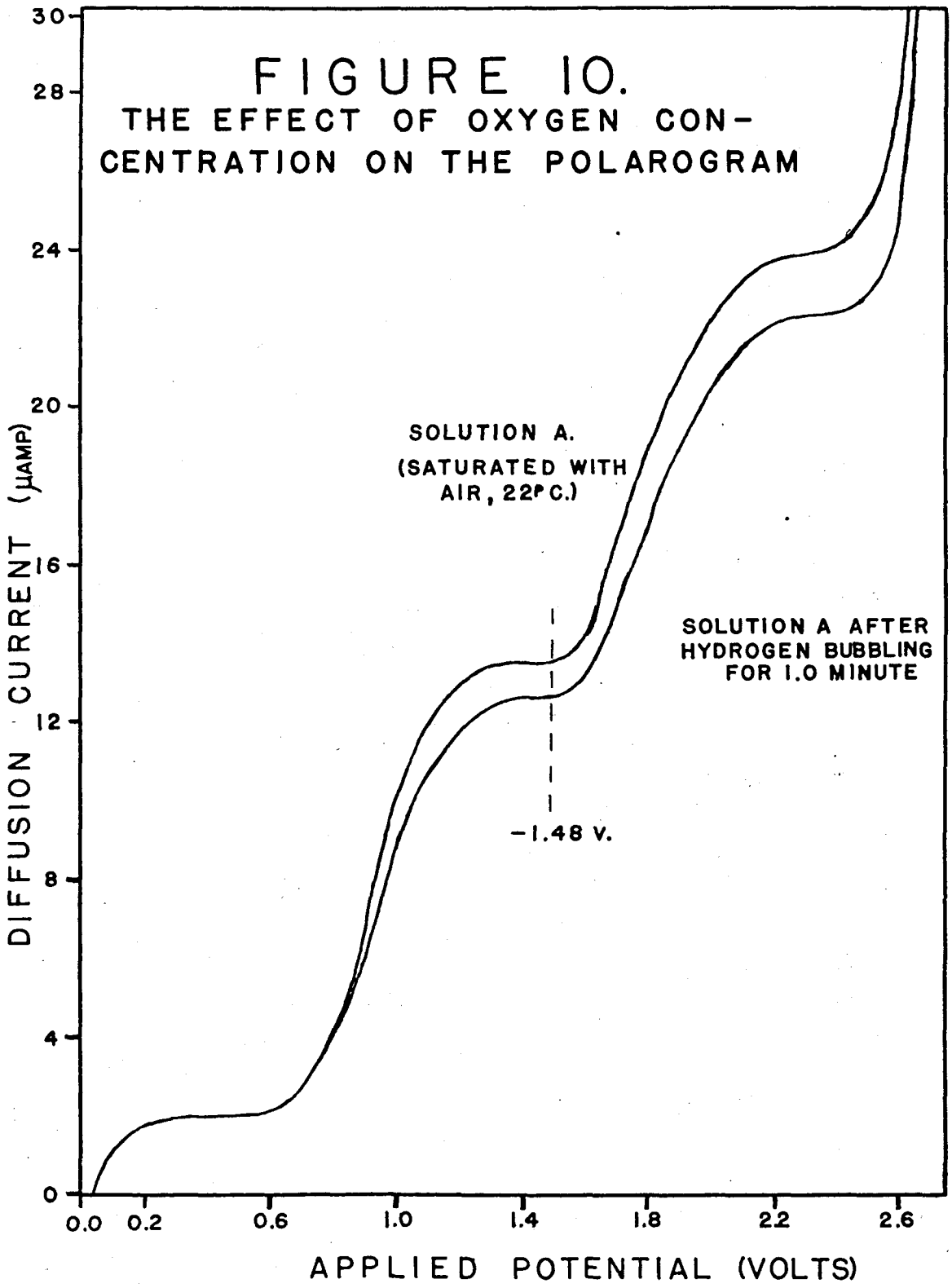


FIGURE 10.
THE EFFECT OF OXYGEN CONCENTRATION ON THE POLAROGRAM
SOLUTION A.
(SATURATED WITH AIR, 22° C.)
SOLUTION A AFTER HYDROGEN BUBBLING FOR 1.0 MINUTE
-1.48 V.
DIFFUSION CURRENT (μAMP)
APPLIED POTENTIAL (VOLTS)
0
4
8
12
16
20
24
28
30
0.0
0.2
0.6
1.0
1.4
1.8
2.2
2.6

Table 3

Electrode Number 1

Oxygen conc. (mg./l.)	Diffusion current (amp.)
3.74	9.75
3.26	8.40
0.06	1.76
2.24	6.28

Electrode Number 2

Oxygen conc. (mg./l.)	Diffusion current (amp.)
3.29	11.85
4.26	15.87
5.01	19.51

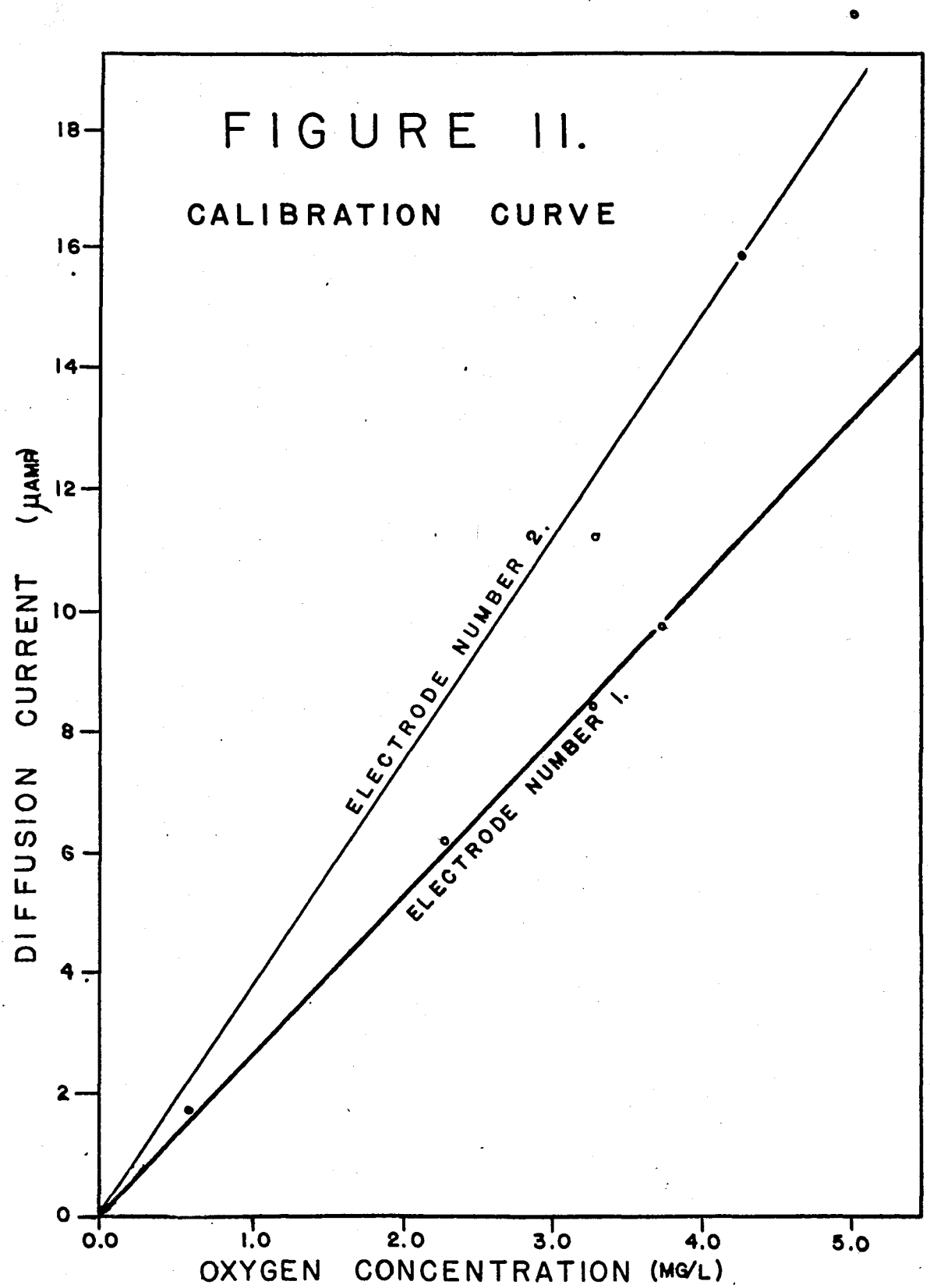


FIGURE II.
CALIBRATION CURVE
DIFFUSION CURRENT (μAMP)
OXYGEN CONCENTRATION (MG/L)
ELECTRODE NUMBER 2.
ELECTRODE NUMBER 1.
0
2
4
6
8
10
12
14
16
18
0.0
1.0
2.0
3.0
4.0
5.0

desired linear relation was obtained as shown in Figure 11.

The diffusion current was recorded as a function of time during the respiration of the algae. This was converted whenever possible to the oxygen concentration by a calibration of each electrode.

The data for the normal cells are shown in Table 4 and plotted in Figure 12.* To verify the fact that the observed reactions were first order with respect to oxygen, the semilogarithmic plot of Figure 13 was made of the same data.

Since cyanide is a recognized inhibitor of certain respiratory enzymes, cyanide poisoning was attempted. The data for this are shown in Table 5 and Figure 14. The semilogarithmic plot of the same data are shown in Figure 15.

Because of the general toxic effect of ultraviolet irradiation on living organisms, the invluence of ultraviolet irradiation on respiration was also investigated. These data are tabulated in Table 6 and plotted in Figures 16 and 17.

* In all of these figures, whenever necessary to avoid crowding, only alternate readings were plotted. Corrections were applied to the raw data only when necessary to correct for an unusually high or low current chain during the measurement period. Hence the data are not corrected completely for electrode consumption, but are rendered comparable.

Table 4 - Normal Algae

Sample A				Sample B	
Raw Data (amp.)	Corrected Data (amp.)	Oxygen conc. mg./l.	Time	Raw Data (amp.)	Oxygen conc. mg./l.
55.47	55.47	12.33	0000	18.97	7.82
54.48	54.48	12.10	0010	16.75	6.90
53.22	53.22	11.83	0020	15.33	6.32
52.32	52.32	11.63	0030	14.59	6.01
51.36	51.36	11.41	0040	14.06	5.79
50.34	50.34	11.20	0050	13.69	5.64
48.99	48.99	10.79	0100	12.59	5.18
47.85	47.85	10.64	0110	12.20	5.03
46.56	46.56	10.38	0120	11.59	4.77
43.17	45.17	10.07	0130	10.88	4.48
42.54	44.54	9.92	0140	10.40	4.28
41.74	43.74	9.74	0150	9.86	4.07
41.22	43.22	9.62	0200	9.35	3.85
-	-	-	0210	9.03	3.72
39.30	41.30	9.19	0220	8.64	3.56
-	-	-	0230	8.00	3.30
38.10	40.10	8.93	0240	7.63	3.14
36.84	38.84	8.64	0300	-	-
-	-	-	0320	6.26	2.58
-	-	-	0330	5.99	2.47
33.84	35.84	7.98	0340	-	-
-	-	-	0350	5.75	2.37
-	-	-	0400	5.54	2.28
-	-	-	0410	5.34	2.20
-	-	-	0420	5.25	2.16
-	-	-	0430	5.09	2.10
30.30	32.30	7.19	0445	4.69	1.93
29.52	31.52	7.02	0450	-	-
-	-	-	0510	4.26	1.76
-	-	-	0520	4.27	1.76
-	-	-	0530	4.26	1.76
-	-	-	0550	4.51	1.86
26.37	27.37	6.07	0610	4.38	1.81
-	-	-	0630	4.24	1.75
-	-	-	0650	4.22	1.74
24.63	25.63	5.70	0705	2.76	0.87
-	-	-	0740	3.67	1.51
-	-	-	0800	3.99	1.64
22.53	23.53	5.22	0810	4.11	1.69
18.66	19.66	4.37	1000	-	-

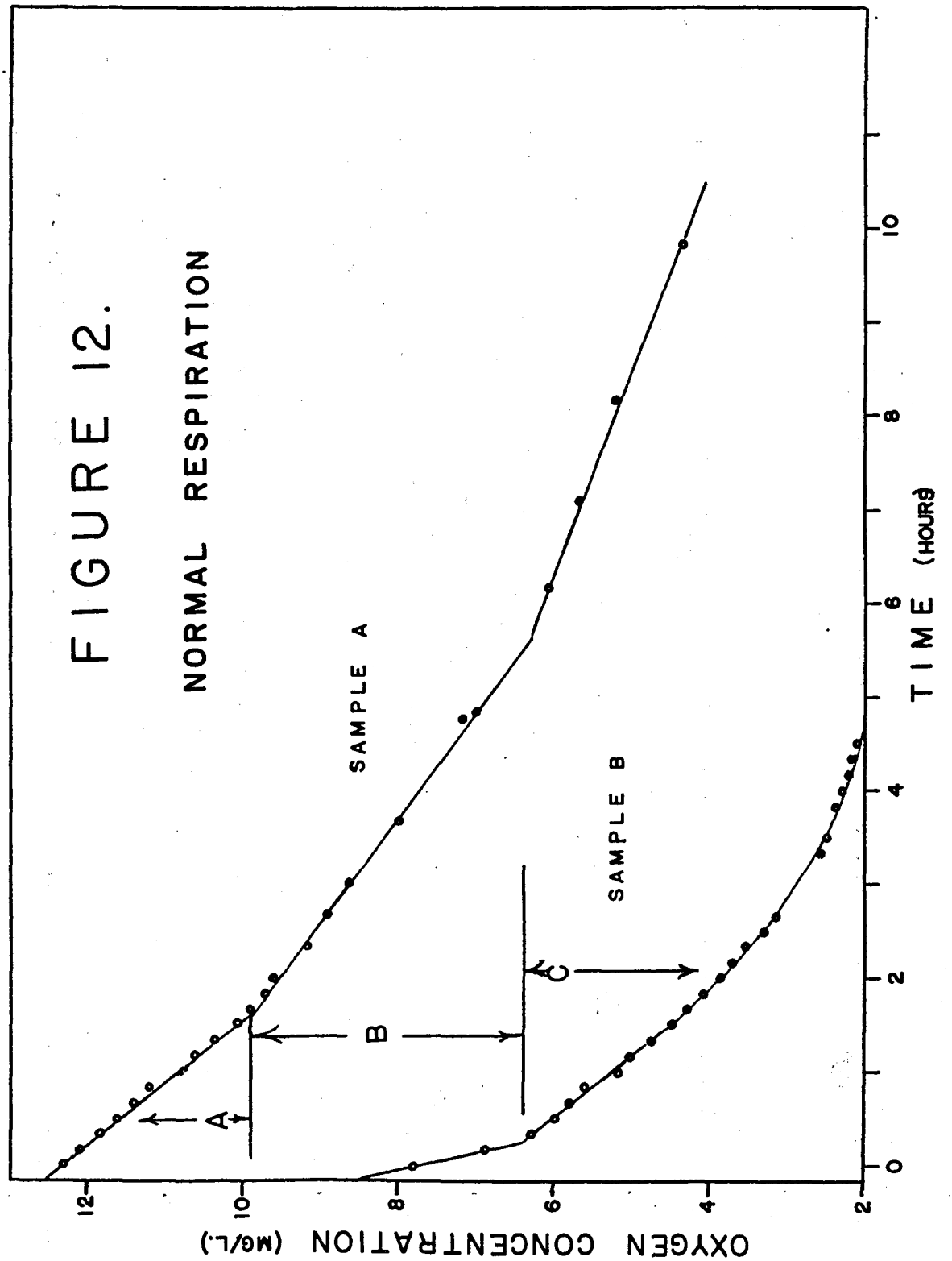


FIGURE 12.
NORMAL RESPIRATION
SAMPLE A
SAMPLE B
A
B
C
OXYGEN CONCENTRATION (MG/L.)
TIME (HOURS)
2
4
6
8
10
12
0
2
4
6
8
10

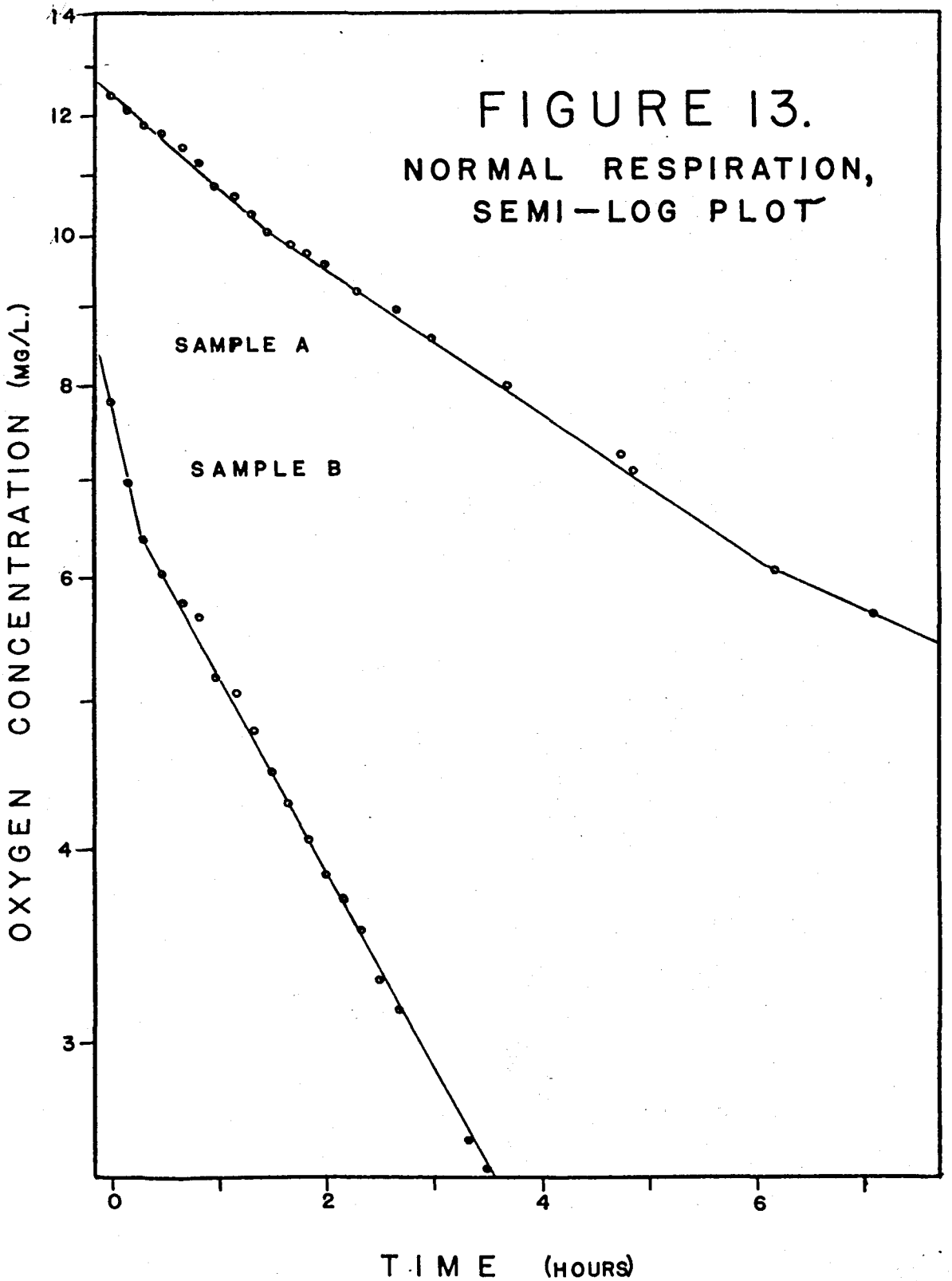


FIGURE 13.
NORMAL RESPIRATION,
SEMI-LOG PLOT
SAMPLE A
SAMPLE B
OXYGEN CONCENTRATION (MG/L.)
14
12
10
8
6
4
3
0
2
4
6
TIME (HOURS)

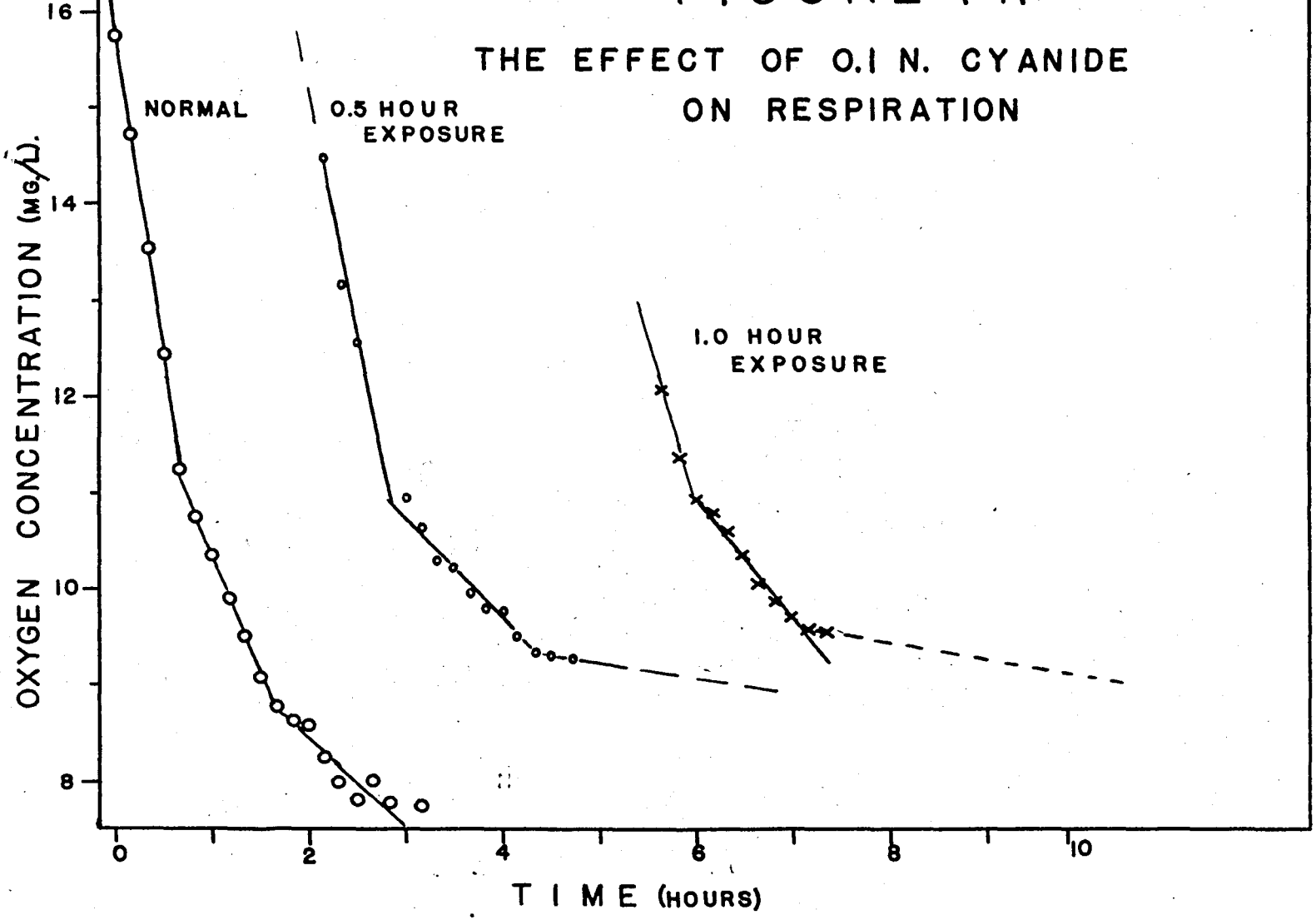
Table 5 - The effect of 0.1 N. Cyanide

Sample C

Normal - Raw Data (amp.)	Exposed 0.5 hour Raw Data (amp.)	Time	Exposed 1.0 hour	
			Raw Data (amp.)	Corrected Data(amp.)
15.75	16.47	0000	12.05	12.05
14.70	14.46	0010	11.35	11.35
13.55	13.16	0020	10.94	10.94
12.45	12.55	0030	10.79	10.79
11.25	-	0040	10.57	10.57
10.74	-	0050	10.34	10.34
10.34	10.96	0100	10.09	10.09
9.89	10.64	0110	9.89	9.89
9.52	10.29	0120	9.49	9.69
9.06	10.19	0130	9.39	9.59
8.80	9.96	0140	9.34	9.54
8.63	9.79	0150	-	-
8.58	9.74	0200	-	-
8.20	9.49	0210	-	-
7.98	9.37	0220	-	-
7.80	9.34	0230	-	-
8.00	-	0240	-	-
-	9.27	0245	-	-
7.73	-	0250	-	-
7.43	-	0300	-	-
7.68	-	0310	-	-
-	7.96	0500	-	-

FIGURE 14.

THE EFFECT OF 0.1 N. CYANIDE ON RESPIRATION



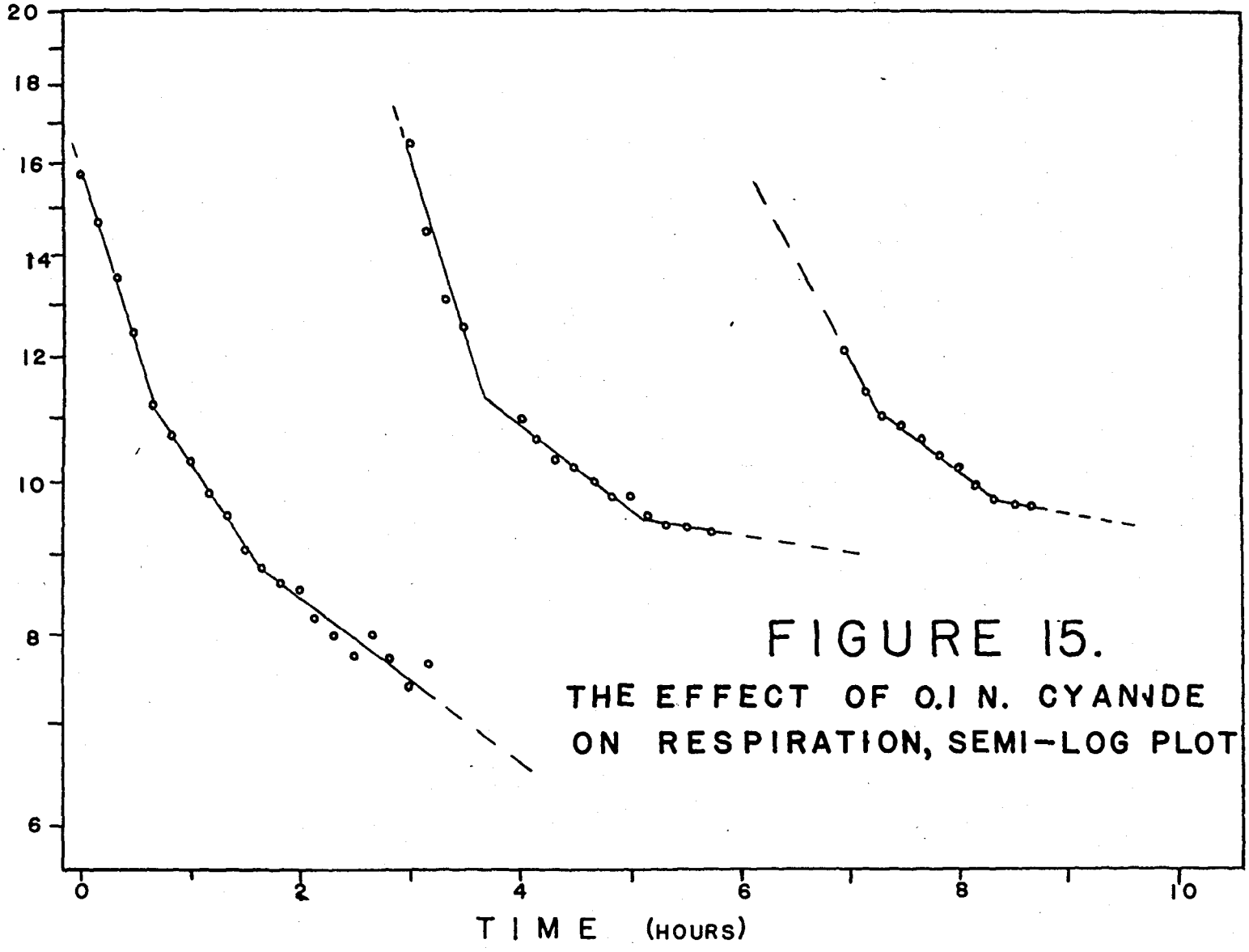


FIGURE 15.

THE EFFECT OF 0.1 N. CYANIDE ON RESPIRATION, SEMI-LOG PLOT

Table 6 - The effect of ultraviolet irradiation

Sample D - Irradiated with germicidal lamp for 15 hours while suspended in modified Beijerinck's solution.

Raw Data (amp.)	Corrected Data (amp.)	Time	Oxygen conc. (mg./l.
32.1	32.1	0000	8.71
30.7	30.7	0010	8.42
29.9	29.9	0020	8.20
29.3	29.3	0030	8.04
28.9	28.9	0040	7.93
28.6	28.6	0050	7.84
28.4	28.4	0100	7.79
28.1	28.1	0110	7.71
27.8	27.8	0120	7.63
27.6	27.6	0130	7.57
27.3	27.2	0150	7.46
27.0	26.7	0210	7.33
26.2	25.2	0310	6.91
25.8	24.8	0325	6.80
25.7	24.7	0335	6.78
25.6	24.6	0340	6.75
25.3	24.3	0350	6.67
25.1	24.1	0400	6.61
24.9	23.9	0410	6.56
24.7	23.7	0420	6.51
24.4	23.4	0430	6.42
24.1	23.1	0440	6.34
23.8	22.8	0450	6.25
23.5	22.5	0500	6.17
23.4	22.4	0510	6.14
23.1	22.1	0520	6.06
22.9	21.9	0530	6.01
22.8	21.8	0540	5.98
22.5	21.5	0550	5.90
22.3	21.3	0600	5.84
22.0	21.0	0610	5.76
21.8	20.8	0620	5.71
21.6	20.6	0630	5.65
21.4	20.4	0640	5.59
21.3	20.3	0650	5.57

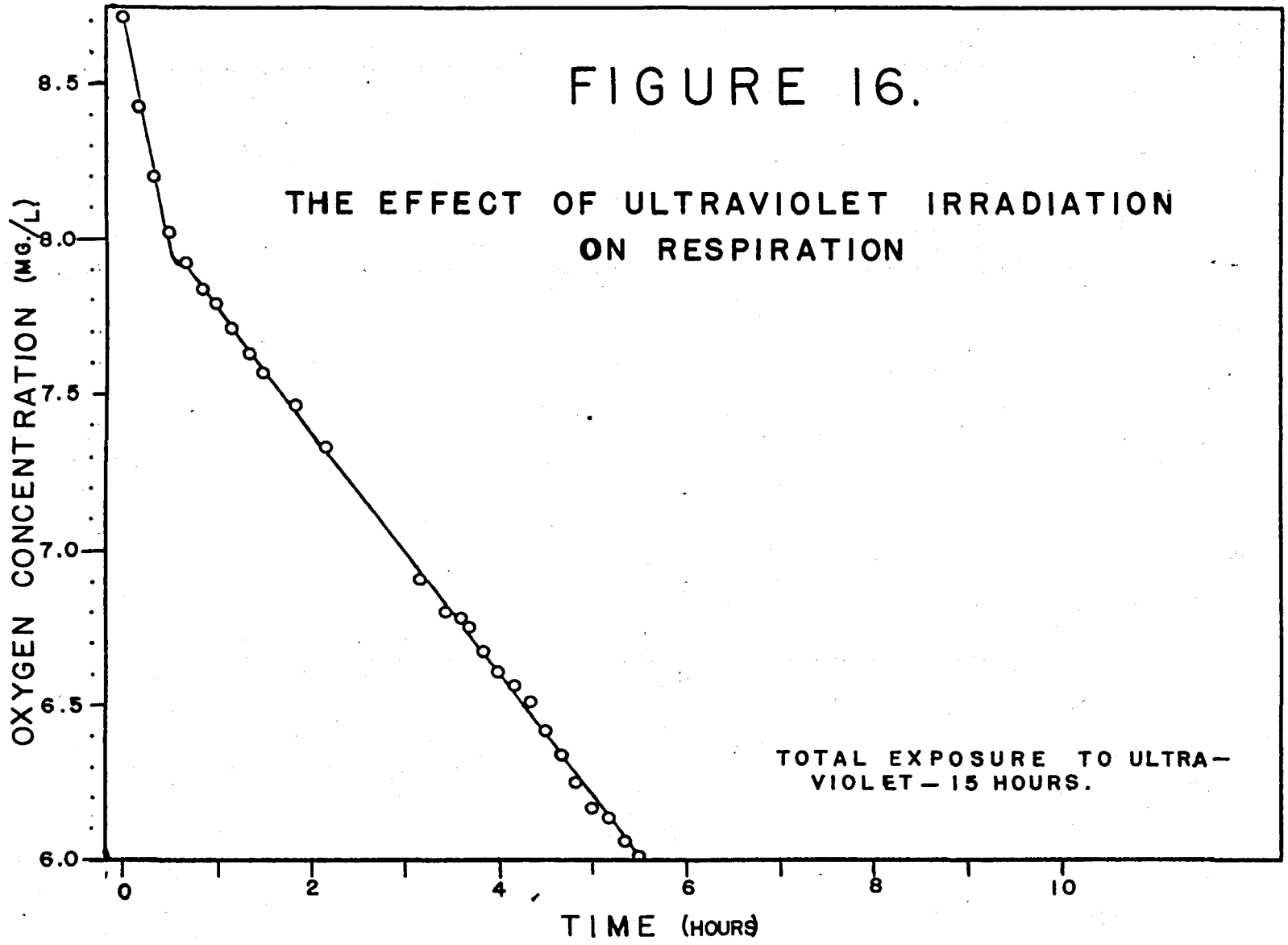


FIGURE 16.
THE EFFECT OF ULTRAVIOLET IRRADIATION ON RESPIRATION
OXYGEN CONCENTRATION (MG./L)
8.5
8.0
7.5
7.0
6.5
6.0
0
2
4
6
8
10
TIME (HOURS)
TOTAL EXPOSURE TO ULTRA-VIOLET - 15 HOURS.

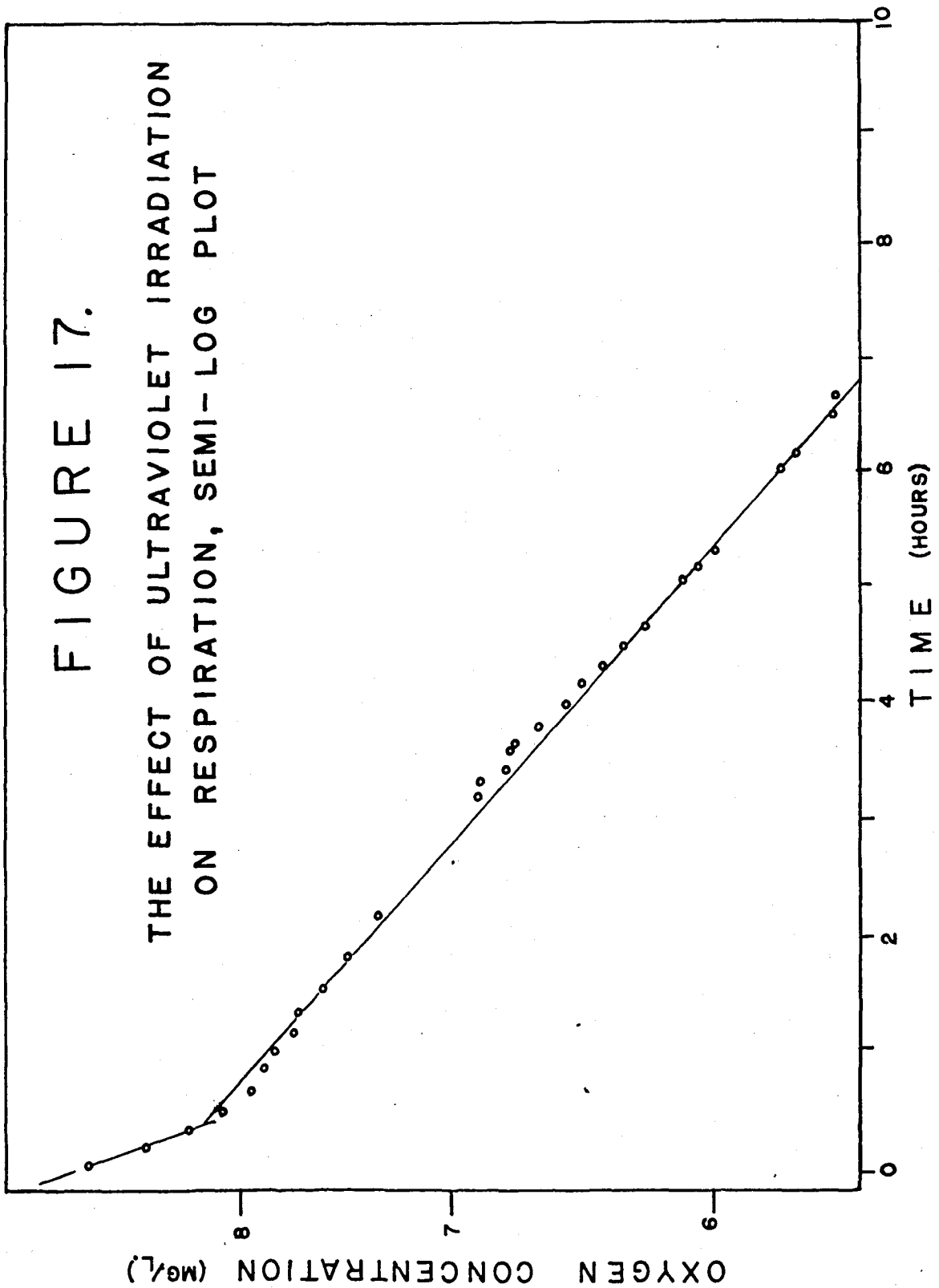


FIGURE 17.
THE EFFECT OF ULTRAVIOLET IRRADIATION ON RESPIRATION, SEMI-LOG PLOT
OXYGEN CONCENTRATION (mg/L)
6
7
8
TIME (HOURS)
0
2
4
6
8
10

Because cyanide forms insoluble products with Beijerinck's solution, it was necessary to use a solution of cyanide in deionized water to study the effects of this poison on respiration. It was therefore thought necessary to study the effects of suspending the algae in deionized water for a period of time. The data of Table 7 and Figure 18 demonstrate the effect on the respiration of the algae of 36 hours suspension in deionized water.

Table 7 - The effect of deionized water

Sample E - Placed in deionized water for 36 hours.

Raw Data (amp.)	Time	Oxygen conc. (mg./l.)
158.	0000	20.4
117.8	0035	15.2
87.4	0110	11.26
76.3	0130	9.83
60.0	0240	7.73
54.1	0300	6.97
50.9	0320	6.55
48.5	0330	6.25
45.6	0340	5.88
41.3	0350	5.32
39.6	0400	5.10
37.2	0410	4.79
35.4	0420	4.57
35.2	0430	4.41
31.0	0440	4.00
30.0	0450	3.86
27.1	0520	3.50
22.8	0550	2.94
22.0	0600	2.84
19.6	0610	2.53
18.8	0620	2.42
18.2	0630	2.35
16.9	0640	2.18
14.6	0650	1.89
12.0	0700	1.55

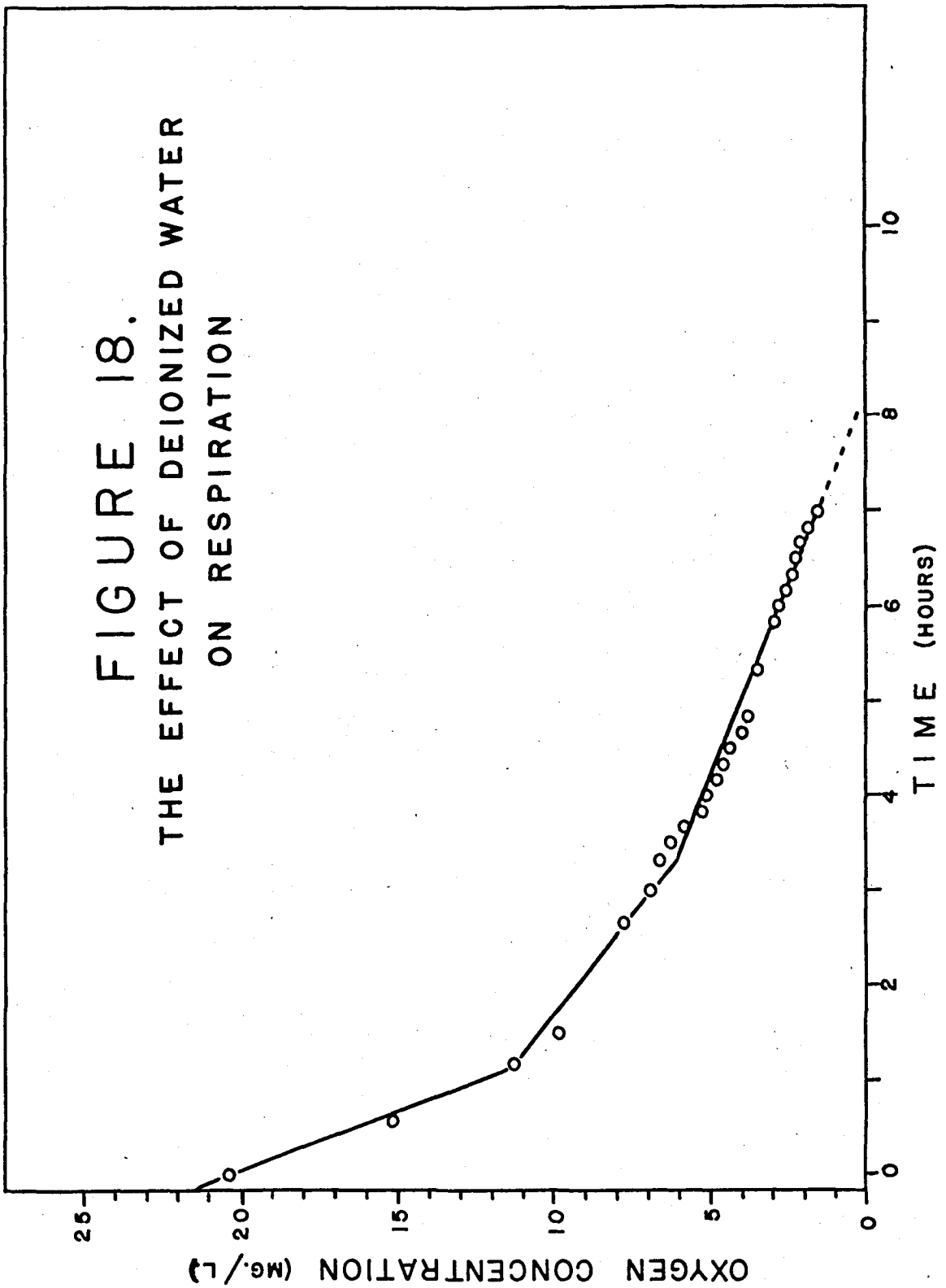


FIGURE 18.
THE EFFECT OF DEIONIZED WATER
ON RESPIRATION
OXYGEN CONCENTRATION (MG./L)
0
5
10
15
20
25
TIME (HOURS)
0
2
4
6
8
10

DISCUSSION

The data for the respiration of normal algae indicate that there are possibly three respiratory pathways. Each of these pathways can be distinguished by the critical oxygen tension at which the pathway becomes modified. Thus the range of oxygen concentrations indicated by the letter A in Figure 12 is high enough to permit all three pathways to function. However, at the critical oxygen tension of 10 mg./l. the pathway most sensitive to oxygen becomes modified and either ceases to function or becomes less active. In the following discussion, this pathway will be known as pathway I. The change in slope is thus caused by the loss or partial loss of this pathway while the other two pathways continue to function in the oxygen concentration range B (of Figure 12) in much the same manner as in region A. At the critical oxygen tension of 6.4 mg./l. a second pathway, pathway II, is evidently affected in a similar manner. Therefore at oxygen tensions in region C, i.e. below

6.4 mg./l., the final pathway, pathway III, maintains all or almost all of the respiration.

A similar interpretation of analagous data was made by duBuy and Olson.[1] The correlations which they observed between the respiration rate, protoplasmic streaming, and auxin transport at various oxygen tensions in Avena coleoptile strongly substantiate this line of reasoning. The one break which they found was at approximately 3.5 mg./l. Since they apparently did not conduct any studies at an oxygen concentration greater than 8 mg./l. it is therefore unknown if other respiratory pathways exist in Avena coleoptile.

The range of concentrations studied in this thesis work was from 25 mg. oxygen/l., approximately three times the normal atmospheric equilibrium value, down to less than 2 mg./l. It was thought that this range would be sufficient to permit a thorough study of the number of pathways involved.

The curves for normal respiration in Figures 12 and 13 serve to illustrate the fact that all pathways are apparently first order with respect to oxygen concentration. Some of the data for sample B which were tabulated in Table 4 were not graphed since they served only to show that diffusion from the atmosphere could take place when an oxygen depleted solution was in the respiration chamber as then used. Following this, the practice of sealing the neck of the respiration chamber to

1. duBuy, H. G., and Olson, R. A., Am. J. Bot., 27, 401 (1940).

the leg of the salt bridge with a drop of oil was initiated.

Although only two sets of data were presented, these breaks were found to be quite reproducible. The results of these other data were taken into consideration in the following discussion.

Because of the difficulties involved at the present time in measuring the amount of algae used, it was not possible to use the developed technique in a quantitative manner. It was thus found necessary to render the data from different studies comparable. This was accomplished by using the ratios of the slopes or some other similarly derived quantity rather than the absolute values of the slopes which would necessarily be dependent on the amount of algae used.

The simplest pseudo-separation of the three pathways can be effected by making a simplifying assumption. The assumption is that each of the pathways operates independently of the other pathways. Thus it is assumed that pathway III is as active in regions A and B as it is in region C where it is supposed to carry on all or almost all of the respiration. Likewise it is assumed that II operates in the same manner in region A as it does in region B. The major argument against this assumption stems from the fact that all three of these processes are related in that they are all competitive, i.e. they all remove oxygen. Thus pathway III might be more active in region C where it is operating alone (or almost so)

than in region B where it would be in competition with pathway II for the available oxygen.

Using this assumption, the contribution of pathway III to the respiration in region B is the same as in region C. The contribution of pathway II in region B can therefore be estimated by subtracting the contribution of pathway III from the slope at B. Likewise the relative contributions of the three pathways in region A, where all are operative, can be resolved. It is then possible to express these relative values in per cent and therefore make comparisons between different studies with different electrodes and chambers and with different amounts of algae.

Table 8 is a tabulation of such quantities for a number of studies in which the algae were subjected to the action of various agents.

According to table 8, pathway I is definitely the least sensitive of the three pathways with regard to the agents employed. This is shown by the fact that the portion of the total respiration maintained by this pathway was never less than the normal value. This indicates that the sensitivity of pathway I to these agents is either zero or very much less than the sensitivity of the other two pathways.

Table 8 shows pathway II to be completely poisoned by ultraviolet irradiation under the conditions employed. Pathway II also appears to have been influenced by cyanide under the condi-

Table 8 - Summary of derived data.

Sample and treatment	Data presented in Table:	Figure(s):	% respiration in region A maintained by pathway: I	II	III
Normal[1]	4[1]	12,13[1]	40	29	31
C- Normal[2]	5	14,15	55[3]	29	16
0.5 hour CN^2	5	14,15	80[3]	16	3.6
1.0 hour CN^2	5	14,15	67[3]	28	3.9
0.5hour CN exp.	not listed		70	19	11
Deionized water 36 hours exp.	7	18	72	13	15
Ultraviolet irradiation in deionized water	6	16,17	75	0	25
Senile culture-normal	not listed		45	32	23

1. Average of several studies.
2. Sample C was used for all of these data by rewashing and reexposing thus rendering the data more comparable.
3. Insufficient data would not permit a more accurate determination of the relative slope value thereby introducing an uncertainty in both of these values.

tions employed. This is clearly seen by the elevation of the height of the second break as the cyanide exposure was increased for sample C in Figure 14. Apparently the sensitivity of pathway II to oxygen is increased by the presence of cyanide. The inhibition of pathway II by cyanide does not appear to be significantly strong in Table 8.

Pathway III is observed in Table 8 to be strongly inhibited by cyanide and slightly inhibited by ultraviolet irradiation.

The information about the senile culture was included only for comparison with the normal. Insufficient data do not permit any conclusions to be drawn from this however.

These conclusions are not in complete accord with those of duBuy and Olson. This could be explained by the vast differences in the materials chosen for study. However, some comparisons can be quite pfofitably brought out.

The two pathways which they discovered were observed to contribute approximately equally to the total respiration in the upper oxygen concentration range. The position of the lower break in this thesis work and the fact that pathways II and III contribute approximately equally (Table 8) to respiration would indicate the regions investigated by them to correspond to the regions B and C studied herein. This would seem to lead the way to other similarities which however are not to be found. They observed their upper pathway to be

cyanide sensitive whereas this thesis work indicates both to be sensitive with the lower pathway being the more sensitive. Their conclusions as to the probable nature of the enzymes concerned would seem to be of little value with regard to the work with the blue-green algae.

It should be noted that no inhibition was apparent with cyanide concentrations less than 0.1 N., approximately five times the lethal concentration for most organisms. The lack of influence of cyanide could possibly be attributed to poor penetration of the membranes.

SUMMARY

In the course of a polarographic investigation of the respiration of a blue-green alga, the following observations were made:

1. There are three respiratory pathways operating in the normal algal cell investigated. These pathways can be distinguished by the critical oxygen tensions at which they become modified.

2. The upper pathway is least sensitive, the lower slightly sensitive, and the middle most sensitive to ultraviolet irradiation under the conditions employed.

3. The lower pathway is apparently inhibited the most by cyanide and the upper pathway the least. The sensitivity of the middle pathway to oxygen was increased by the presence of cyanide.

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In August, 1953, following a year at the Medical College of Virginia, I enrolled in the Graduate School of the University of Richmond to work for the M. S. degree in chemistry. This thesis is submitted in partial fulfillment of the requirements for that degree.