A CARBON DIOXIDE SENSITIVE PHOTOSYNTHETIC RATE SENSOR

by

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ABSTRACT

A glass electrode is described which is capable of detecting photosynthetic CO$_2$ uptake by monitoring the concentration of CO$_2$ dissolved in a small drop of algal suspension. Consideration of current theory suggests that transients should occur in the rate of CO$_2$ uptake which are similar to but distinct from those observed in O$_2$ evolution rate. These transients should be most readily observed when cells are illuminated alternately with wavelengths of 650 nm and 705 nm. The preliminary results show that CO$_2$ uptake by photosynthesis can be monitored by this apparatus. Although transients were not observed, this may be due to the rather low sensitivity. There is a discussion of shortcomings of the apparatus, and of improvements necessary before transients can be observed.
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CHAPTER 1
INTRODUCTION

1.1. The Purpose.

This thesis describes the design, construction, and testing of a CO₂ sensitive electrode, and the results of using it in some studies in photosynthesis.

When two different wavelengths of light are adjusted in intensity to give equal steady state rates of photosynthesis as measured by O₂ evolution, a rapid change from illumination by the one light to illumination by the other will in general result in a perturbation of the rate of O₂ evolution before it returns to the steady state. These perturbations are called chromatic transients.

In this thesis the conditions for similar transients in the rate of CO₂ uptake are discussed theoretically, and preliminary studies in photosynthesis are described. These define the response of the apparatus and show some of the further conditions which will need to be met if transients are to be observed in the rate of CO₂ uptake.

1.2. Advantage and Principle of the Method.

In the past, considerable study of photosynthesis has been done using the rate of O₂ evolution as a measure of photosynthesis. Some studies have been done using CO₂ uptake as a measure of photosynthesis, but convenient methods are not so readily available. Because it is expected that the two methods of measuring photosynthesis should not give entirely
equivalent results, we decided to develop a method of measuring CO₂ consumption.

A pH electrode is used to follow CO₂ concentration in a drop of cell suspension. Because in a small drop, the CO₂ consumed inside the drop causes diffusion of CO₂ into the drop from the surrounding atmosphere, the electrode measures a steady state CO₂ concentration. Disturbances in this steady state concentration are caused by changes in the net rate of CO₂ uptake.

1.3. The Apparatus.

The method used here for measuring CO₂ consumption was chosen because the action spectrum apparatus built and described earlier by Brooks (5) could be adapted for this purpose. The O₂ electrode and associated measuring equipment in that apparatus was replaced by the CO₂ detecting electrode. A few other modifications were made in the apparatus and these are also described in this thesis.
CHAPTER 2

INSTRUMENTATION


It is intended that the pH electrode in the apparatus shall monitor the rate of CO$_2$ consumption in a drop of algal suspension during photosynthesis. Because the algae in the drop consume CO$_2$ during photosynthesis, CO$_2$ diffuses into the drop from the surrounding gas to replace the CO$_2$ which was consumed. This establishes a steady state concentration of CO$_2$ within the drop. The steady state concentration of CO$_2$ is a function of the net rate of CO$_2$ uptake and the external CO$_2$ concentration. If the external CO$_2$ concentration is kept constant, a change in the rate of CO$_2$ uptake due to photosynthesis of the algae will establish a new steady state concentration. A higher CO$_2$ concentration will give a lower pH value and vice versa. Hence an increase in the rate of CO$_2$ consumption will result in a decrease in the CO$_2$ concentration which gives a higher pH. To permit a new steady state concentration to be attained rapidly after a change in uptake rate, the diameter of the drop must be small so that diffusion through the drop is rapid.

Carbon dioxide in aqueous solution goes to carbonic acid by the reaction: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{CO}_3^-$. Therefore a change in the steady state concentration of CO$_2$ will be reflected in a change of the pH of the drop. It is therefore possible to use a pH electrode to monitor changes in the steady state CO$_2$ concentration within the drop. To speed up the reaction of CO$_2$ to carbonic acid, the enzyme carbonic anhydrase can be used as a catalyst.
FIG. 2.1 THE pH ELECTRODE
Both the electrical potential difference across a semipermeable membrane and the pH are logarithmic functions of the $H^+$ ion concentration. The pH is therefore related linearly to the potential difference. This principle is employed in the glass pH electrode. Certain special glasses if sufficiently thin, act as membranes semipermeable to $H^+$ ions. The pH of solutions can then be compared in terms of millivolts (mv). At $20^\circ$ C a difference of one pH unit corresponds to 58 mv. An electrometer can therefore be used in conjunction with a glass pH electrode to monitor the steady state $CO_2$ concentration in the drop.

2.2. Electrode Design Considerations.

The semipermeable membrane of the electrode is Corning 0150 glass at the end of a stalk of Corning 0010 lead glass. To provide one constant reference solution of $H^+$ ions, 0.1 N HCl is put inside the glass electrode. Dipping into this solution is a reversible Ag-AgCl electrode made by plating silver wire with silver chloride. The silver wire is connected to the electrometer through a shielded cable. To measure potential difference a nonreversible reference electrode must be provided in the test solution. This consists of a calomel electrode connected to the solution through a salt bridge. The bridge uses a saturated solution of KCl.

The method to be used requires that a drop be held between a glass cover slip below and the electrode surface above. The surface of the electrode tip (the sensitive membrane) should therefore have a flat bottom. The action spectrum apparatus as previously used to monitor $O_2$ concentration (5) had an electrode of 2 mm. diameter. Hence it is
desirable that the bottom of the electrode be as flat as possible and have a diameter not greater than 2 mm. This combination of requirements—thin membrane of glass, flat bottom, and small diameter—proved difficult to obtain. The electrode which I used did not have an entirely flat bottom surface, but served the purpose adequately. A diagram of the electrode is given in fig. 2.1.

2.3. Electrode Measurements.

2.3.1. Measurement of Voltage.

To measure millivolt changes in the course of the pH measurement, I used an EIL Vibron Electrometer Model 33B. The electrometer has an input resistance of greater than $10^{13}$ ohms. It has ranges varying from 10 mv full scale deflection (f.s.d.) to 1000 mv f.s.d. A block diagram of the electrometer is given in fig. 2.2. Amplification of the signal is accomplished by an a.c. amplifier after the d.c. input signal has been modulated by a vibrator. The amplified signal is then converted back to d.c. by a synchronous detector and read out on an ammeter scale. An Esterline Angus graphic ammeter Model AW can be connected into the electrometer to make a continuous recording of the voltages. Voltages are normally read by connecting the electrodes to the High and Low positions on the electrometer.

In an actual voltage measurement, the voltage read on the meter is $V = E - iR$ where $E$ is the voltage output of the voltage source, $R$ is the resistive part of the circuit, and $i$ is the current through the circuit. It is therefore necessary to keep the resistance in the circuit
FIG. 2.2 BLOCK DIAGRAM OF THE ELECTROMETER
well below that of the meter so that iR is negligible compared to E. A factor of $10^2$ to $10^3$ greater resistance in the meter than in the rest of the circuit is ordinarily considered a minimum tolerance ($10^2$ gives V accurate to 1%). Because the resistance of pH electrodes is normally of the order of $10^8$ to $10^{10}$ ohms, it is necessary to select an electrometer with an input resistance $10^2$ to $10^3$ greater. The Vibron electrometer with an input resistance of at least $10^{13}$ ohms was therefore chosen.

Because the electrometer has such a high input resistance, it is necessary to use a shielded cable on the signal input lead to avoid induced voltages at the input.

To measure pH in the drop and hence monitor the CO$_2$ concentration the glass electrode was connected to High and the calomel reference electrode to Low.

2.3.2. Measurement of Resistance.

The electrical resistance of the glass electrode must not be too high. It must be less by a factor of $10^3$ than the input resistance of the electrometer, or less than $10^{10}$ ohms. Furthermore, the resistance must be small enough to permit rapid response of the circuit, which includes the capacitance of the input cable. In order to select electrodes of suitably low resistance, a method of measuring their resistance had to be devised.

To check the proposed method of measuring electrode resistance, the electrode was simulated by putting a voltage source in series with a variable high resistance. The voltage source was variable from
750 mv to +750 mv. A Keithley decade shunt Model 2008 with resistors increasing by factors of 10 from $10^3$ to $10^{12}$ ohms was used as the resistance portion of the electrode model. With this model, tests were made to determine the resistance range where the speed of response was best. With resistances greater than $10^{10}$ ohms, full response required several seconds. With resistance less than $10^8$ ohms considerable oscillation occurred in response to a voltage step. Acceptable electrodes should have $10^8 < R < 10^{10}$ ohms.

To measure resistance, the output voltage of the voltage source is first measured (set at 400 or 500 mv) by measuring its potential difference across the High and Low terminals of the electrometer. This is $V_1$. Then the model is connected to High and Guard and another variable resistor (another Keithley decade shunt) is put across High and Low. The electrometer reading with this arrangement is $V_2$. This arrangement is shown in fig. 2.3. The resistance $R$ is then determined to a first approximation by the equation $R = R_M(V_1/V_2)$, where $R_M$ is the variable resistance. The exact equation is $R = R_M(V_1/V_2 - 1/\beta)$ where $\beta$ is the degeneration factor as given in the instrumental manual.

To understand this measurement, consider the operation of the electrometer on the circuit. The amplifier gives a current output. In order to keep the potential difference between High and Guard as small as possible, the amplifier drives a current through the feedback resistor $R_f$ causing an IR drop that compensates for most of the voltage drop between High and Low in an ordinary measurement. In the arrangement of fig. 2.3, current is also driven through $R$ and $R_M$ to return back to the amplifier. Then $V_1 - IR - IR_M + I'R_f = 0$ where $I$ is the
FIG. 2.3  RESISTANCE MEASURING CIRCUIT
current through $R$ and $R_M$. $I'$ is the current through $R_f$. Because $R_f \ll R + R_M$, the potential difference across $R_f$ due to $I$ is negligible and therefore $V_2 = IR_M$ since $IR_M$ is the potential difference between High and Low, and $I$ through $R_f$ does not disturb the feedback. Let $V_{hg}$ be the voltage drop between High and Guard. Now $V_2 = V_{hg} + I'R_f$.

The equation $V_1 - IR - IR_M + I'R_f = 0$ then becomes

$$V_1 - IR - V_2 + V_2 - V_{hg} = 0 \text{ or } V_1 - IR - V_{hg} = 0.$$ 

Now divide this equation by the equation $V_2 = IR_M$ and get

$$V_1/V_2 = R/R_M + 1/\beta \text{ i.e. } R = R_M(V_1/V_2 - 1/\beta).$$

The instrument manual gives the value of $\beta$ as the degeneration factor. This factor represents the ratio of the total voltage drop from High to Low, to the voltage drop from High to Guard $\beta = V_2/V_{hg}$. This ratio depends on the size of the feedback resistor $R_f$ and therefore is different for each meter range. Its maximum value $\beta = 50$ occurs with the 1000 mv f.s.d. range and its minimum value $\beta = 5$ occurs with the 10 mv f.s.d. range. Hence on the larger ranges, the correction is smaller. For example, on the 1000 mv range $1/\beta = 1/50$, on the 10 mv range $1/\beta = 1/5$. However, if $V_1 > V_2$ on the largest range, and $V_1$ is kept at a set value, any range of the meter may be used to read the value of $V_2$ for an appropriate $R_M$.

For example, if $V_1 = 500$ mv, $R_M = a$ and $V_2 = 100$ mv on the 1000 mv range, $\beta = 50$. Then $R = a(500/100 - 1/50) = a(5 - 1/50)$. If with the same $R$, we use the 10 mv scale and set $R_M = a/100$, then

$$R = a/100(500/10 - 1/5) = a/100(50 - 1/5).$$

The correction is 0.4% in each case. Since it is important to have $V_1 > V_2$, $V_1$ is usually set at 400 or 500 mv.
The described method of measurement was tested by using a known large resistor for R and using the given arrangement to measure its resistance. For R, a 10 megohm resistor with 1% tolerance was used:

\[ R = 10^7 \pm 1\% . \quad V_1 = 500 \text{ mv} \pm 0.5\% . \]

With \( R_M + 10^6 \) ohms, \( V_2 = 49.5 \text{ mv} \pm 0.5\% \) on the 100 mv scale where \( \beta = 20 \). Then the calculated value of R is

\[ R = 10^6 \left( \frac{500 \pm 0.5\%}{49.5 \pm 0.5\%} \right)^{-1/20} = 1.005 \times 10^7 \pm 1\% \]

which is within the 1% tolerance of the resistor. For resistances on the Keithley shunt of greater than \( 10^8 \) ohms, the resistors are given to \( \pm 2\% \). If the meter can be read to \( \pm 0.5\% \) each time, the value of R can be calculated to within 3% if the exact equation is used.

The resistance of the electrode was measured as explained in the model study. \( V_1 \) was set at 500 mv. \( R_M \) was selected to give a value of \( V_2 < 500 \text{ mv} \). In this situation on any scale of the meter, the correction was less than 0.5% and hence the first approximation was considered sufficient. \( R = R_M(V_1/V_2) = 10^9 \) ohms \((500 \pm 3 \text{ mv})/(100 \pm 3 \text{ mv}) = 5 \times 10^9 \) ohms \( \pm 4\% \).

The electrode resistance is \( 5.0 \times 10^9 \) ohms \( \pm 4\% \).

The capacitance of the input cable was measured by taking a known resistance with a known length of cable, putting in a voltage step and observing on the recorder the time required for response to the step function. Now \( t_r = 2.2\tau \) where \( \tau \) is the time constant, and \( t_r \) is the time the circuit requires to respond from 10% to 90% of the value of the voltage step. Using this value of \( \tau \) and the known value of resistance, the capacitance is derived from the equation \( \tau = RC \). A 12.25 ft. cable with a \( 10^{10} \) ohm resistor had a response time \( t_r = 9.5 \) sec. Therefore \( C = t_r/2.2R = 9.5 \text{ sec}/(2.2)10^{10} \) ohms = 432 pf. The cable capacitance then is \( C = 432 \text{ pf}/12.25 \text{ ft.} = 35.2 \text{ pf/ft.} \), or approximately 35 pf/ft.
The response time of the circuit with the electrode can then be calculated. In the measurements with the electrode, an input cable 5 ft. long is required. The electrode resistance is $5 \times 10^9$ ohms. The response time then is

$$t_r = 2.2RC = (2.2)(5 \times 10^9 \text{ ohms})(5 \text{ ft.})(35 \times 10^{-12} \frac{\text{F}}{\text{ft}}) = 1.9 \text{ sec.}$$

The potential of the Guard terminal on the electrometer follows closely that of High. The shield of the input cable can be put either to the Low or to the Guard position. If it is put to the Guard position, the capacitor need charge only to a potential difference that is $1/\beta$ as great as the total potential difference across the input. In most work with photosynthesis the most sensitive range is used. Here $\beta = 5$ and hence if the shield is put to Guard rather than to Low the response time is reduced to $1/5$. Therefore when working with high resistance electrodes where rapid response is important, the input screen is put to Guard.

It is found that, especially with a long cable, when the Guard connection was used for the screen, considerable oscillation occurred in the response of the circuit to a step of greater than 1/5 of the full scale. Smaller deflections generally were characterized by a rapid response with little or no oscillation.

2.3.3. Measurement of Electrode Response to $H^+$ ions.

The response of the electrode to $H^+$ ion concentration was measured by successively immersing the electrode set into standard buffer solutions of known pH and measuring the millivolt output of the electrodes
FIG. 2.4
RESPONSE OF ELECTRODE
TO HYDROGEN ION CONCENTRATION
at each pH. The results are given in fig. 2.4, a graph of electrode potential difference against pH.

Between pH 2 and pH 10 the response was linear to within ± 3 mv. In the range pH 6 to pH 8 where most of the present work was done, linearity is good to within the limits of accuracy of the measurement, or to approximately ± 2 mv. The pH uncertainty of the buffer and the uncertainty of the meter reading are marked on the graph as the limits.

The response is 56 ± 1 mv/pH at 24.5°C. The theoretical response at this temperature is 59 millivolts.

2.4. Modification of the Action Spectrum Apparatus.

Brooks (5) has described in detail the construction and performance of an action spectrum apparatus. The same apparatus was modified for the present study. The modifications are described below.

As already indicated, CO₂ concentration rather than O₂ concentration in the drop is monitored, but the same steady state diffusion principle is employed as the basis of the measurement. The pH electrode was used for this measurement.

2.4.1. The Reference Beam.

The optical system was altered. The same light condensing cone was used to concentrate the light onto the sample. However, rather than producing the reference beam from the same entrance slit and separating the beams by a sliding mirror inside the monochromator box, the reference beam is taken from the opposite side of the lamp and directed around the
Fig. 2.5 Block Diagram of Apparatus
monochromator box. The desired beam is selected by moving a sliding mirror which permits the cone to be continuously filled with light while the beams are being changed. A block diagram of the optical system is given in fig. 2.5.

Because of the nature of the work with photosynthesis, it is necessary on most occasions to render the reference beam approximately monochromatic in order to study the effect of alternating two wave-lengths of light. Therefore a place is provided in the reference beam for a light filter.

2.4.2. Intensity Measurements.

For measuring intensity, we used the same principle as Brooks (5). Use of the slotted wheel meant that the cells received pulsed illumination. This has been avoided by using a small electro-mechanical chopper mounted close to the detector. The chopper is a magnetically activated tuning fork (Bulova light chopper). It is placed above the detector so that it chops the beam of light that is directed down onto the PbS detector by a small mirror placed just below the base of the cone. Because the copper operates at 400 Hz., the narrow band amplifier was modified to center on that frequency. A circuit diagram of the intensity measuring equipment is given in fig. 2.6. The Bias Voltage and Bucking Current are for the O₂ electrode, and hence were not used in this study.
Figure 2.6
Circuit Diagram of Phase Sensitive Detector

All cap. values in microfarads

ELECTRODE
OFF ↔ PSD
SWITCH
POSITIONS

BIAS VOLTAGE
BUCKING CURRENT
BUCKING VOLTAGE

Signal
In

GAIN CONTROL

+300V
-300V
-300V

12K

3.3K

3.2K

1.0

2.1M

3.1

100

33

470

2.1M

39K

22K

33K

1M

1M

33K

33K

21K

30

0.2

560

10K

20K

100K

10

0.47

1.5V

1.5V

25K

120K

10K

10K

100

100
1. Reference lead
2. Shielded lead
3. Lucite block
4. Threaded electrode holder
5. O-ring seal
6. Spring
7. Glass electrode
8. Air vent (one of two)
9. Chamber
10. Metal collar
11. Agar-Buffer contact
12. Syringe needle
13. Syringe
14. KCl-Agar
15. KCl solution
16. Calomel electrode
17. Cone channel condenser
18. Positioning screw

FIG. 2.7 ELECTRODE CHAMBER
2.4.3. The Measuring Chamber.

The chamber for performing the measurement is cut in a clear lucite block and is similar to the one used for \( \text{O}_2 \) concentration comparisons. The pH electrode holds the drop of cell suspension around it by surface tension. The calomel reference electrode is also held in the block and is linked to the drop through a KCl salt bridge. The arrangement is illustrated in fig. 2.7.
CHAPTER 3

THEORETICAL BACKGROUND

3.1. Light Reactions in Photosynthesis.

The present understanding of photosynthesis includes the hypothesis that photosynthesis in green plants and algae is driven by light absorbed in two different pigments, each having its absorption maximum in a separate region of the visible light spectrum. (6, 7, 11). Maximum efficiency of photosynthesis is therefore not attained with a single monochromatic beam of light. Addition of a second beam of such a wavelength that the other pigment absorbs it strongly, significantly increases the total efficiency of the process. This phenomenon is known as enhancement and was expressed by Eley and Myers (8) as:

\[ P_{12} = \Delta + P_1 + P_2 \]

where \( P_{12} \) is the rate of photosynthesis in beam 1 plus beam 2, \( P_1 \) is the rate of photosynthesis in beam 1, \( P_2 \) is the rate of photosynthesis in beam 2 and \( \Delta \) has a positive value.

For pigment system 1 (PS1) maximum absorption occurs in the red region of the spectrum, frequently at 705 nanometers (nm). The absorption maximum for pigment system 2 (PS2) occurs at shorter wavelengths. For Chlorella, Eley and Myers (8) found 650 nm and 700 nm to be convenient wavelengths for their study of the two pigment system. They found that with these wavelengths the greatest efficiency of photosynthesis was not obtained when the amount of light absorbed was equally divided between the two beams, but rather when more of the 650 nm light than of the 700 nm light was absorbed.
It is possible to select pairs of wavelengths such that if a beam of light of each of these wavelengths is adjusted in intensity to give equal steady state rates of photosynthesis, switching from one beam to the other will not result in a smooth transition. Rather a characteristic disturbance in the rate of photosynthesis occurs before the rate returns to its steady state value. These disturbances in the photosynthetic rate which result from changing the wavelength of illumination have been called chromatic transients. It is believed that they are a consequence of the two pigment system, and action spectra have shown them to be spectrally related to enhancement (2).

3.2. Kinetics of Photosynthesis by Oxygen Evolution and by Carbon Dioxide Uptake.

To study in detail the light requirements of photosynthesis many action spectra of enhancement and of transients were prepared in the past decade (1, 2, 10). Most of these studies were done by observing the rate of O₂ evolution and using this as a measure of the rate of photosynthesis. However in 1963 Blinks (1) reported measuring the rate of photosynthesis by determining the CO₂ uptake in a bulk solution with a pH electrode. Changes in the rate of CO₂ uptake were seen as changes in the slope of the curve of pH versus time. The pH increases as CO₂ is consumed from the carbonate buffer solution.

In his study of the kinetics of response to switching the light on and off, and in his study of enhancement, Blinks (1, 2) demonstrated that the changes in the rate of photosynthesis as observed by CO₂ uptake were not equivalent to those observed by O₂ evolution. For example, where
FIG. 3.1 A simplified scheme of photosynthesis showing the relations of the two pigment systems to each other, to the oxygen evolving mechanism, and to the carbon dioxide fixation cycle.
O₂ evolution showed a rapid rise in rate, CO₂ uptake showed a slower response. Where O₂ evolution showed a slow response, CO₂ uptake rate increased more rapidly. In addition, transients were observed both when measuring CO₂ uptake rate (1), and when measuring O₂ evolution rate (2), but the initial spikes occurred in opposite directions.

In order to gain an understanding of the significance of these results it is helpful to consider the simplified scheme of the photosynthetic mechanism given in fig. 3.1. Since PS2 is closely linked to O₂ evolution, stimulation of PS2 will evoke a rapid response in O₂ evolution, but because of the intermediates, the response in CO₂ consumption will be slower. Because CO₂ fixation is more closely linked to PS1, changes in the activity of PS1 would be more rapidly reflected in CO₂ uptake than in O₂ evolution, again because of the intermediates.

This rather simplified analysis suggests the importance of studying the response of photosynthesis to light by observing CO₂ consumption as well as by observing O₂ evolution, and of comparing the results. Such a study of enhancement and transients could increase our understanding of the kinetics of the two pigment systems and act as a check on present models.

Further insight into the kinetics of photosynthesis might be gained by studying the time course of events on switching from white light to monochromatic light of either long or short wavelength. Under white light illumination, both the systems are active, whereas the monochromatic light activates one system preferentially. A rapid change from white to monochromatic light might then show the effects of the "piling up" or the "draining off" of intermediates while the system readjusts.
3.3. Eley and Myers' Model of Photosynthesis.

In 1967 Eley and Myers (8) published a paper in which they compare the results of studies in photosynthesis done on Chlorella, and the results of an analog computer study of photosynthesis. The experiments with Chlorella were done by alternating light of the two required wavelengths and observing the rate of photosynthesis with an oxygen sensitive electrode. The analog computer study was based on a kinetic model of photosynthesis. The results of the analog study agreed remarkably well with those of their study of photosynthesis in Chlorella.

The model for analog computer study is given below.

Basic Equations:

1. Photoact 2: \[ E + \frac{1}{2}H_2O \xrightarrow{aI} EH + \frac{1}{2}O_2 \]

2. Dark Reaction: \[ EH + P \xrightarrow{k_d} E + PH \]

3. Photoact 1: \[ PH + X \xrightarrow{(1-a)I} P + XH \]

where:

- \( E \) represents the electron acceptors in their oxidized state
- \( EH \) represents the electron acceptors in their reduced state
- \( PH \) represents the electron donors in the reduced state
- \( P \) represents the electron donors in the oxidized state
- \( I \) represents the total absorbed intensity (quanta/sec) per \( E_0 \)
- \( a \) represents that fraction of \( I \) which is absorbed by PS2 at any wavelength
- \( (1-a) \) represents that fraction of \( I \) which is absorbed by PS1 at any wavelength.

\[ E_0 = E + EH \quad \text{and} \quad P_0 = P + PH \]
define:

\[ \varepsilon = \frac{E}{E_0} = \text{the fraction of the total electron acceptors in the oxidized state} \]

\[ \phi = \frac{PH}{P_0} = \text{the fraction of the total electron donors in the reduced state} \]

They obtained their E and P values by estimating pool sizes. In making these estimates, they used information from the work of Joliot (9). They estimated the K for the dark reaction from the redox potentials of E and of cytochrome f. The wavelength dependent a values were obtained from enhancement spectra.

The following assumptions are made in the model:

1. At the low intensities used, only the light reactions limit the rate.

2. The dark reaction is considered to be at equilibrium.

3. The photoacts are unidirectional with no back leakage.

4. The photoacts are first order reactions, i.e. the rate of photoact 2 depends only on the concentration of E and on the rate constant aI, and the rate of photoact 1 depends only on the concentration of PH and the rate constant (1-a)I. Rate equations are obtained as follows using the basic equations and the above assumptions.

The rate of change of E from equations 1 and 2 is given by

\[ \frac{d[E]}{dt} = -aI[E] + k_d[E_H][P] - k_r[E][PH]. \]

If now we divide equation 4. by \([E_0]\) it becomes

\[ \frac{d\varepsilon}{dt} = -aI\varepsilon + \frac{1}{[E_0]}\left( k_d[E_H][P] - k_r[E][PH]\right). \]

The rate of change of PH from equations 2 and 3 is given by

\[ \frac{d[PH]}{dt} = -(1-a)I[PH] + k_d[E_H][P] - k_r[E][PH]. \]
If now we divide equation 6 by $[P_0]$ it becomes

$$7. \frac{d\phi}{dt} = -(1-a)I\phi + \frac{1}{[P_0]} \left( k_d [EH] \cdot [P] - k_r [E] \cdot [PH] \right).$$

If now we set $[P_0]/[E_0] = m$, a constant, we can combine equations 5. and 7. to obtain

$$8.* \quad \frac{d\varepsilon}{dt} = -aI\varepsilon + m(1-a)I\phi + m \frac{d\phi}{dt}.$$

The dark reaction is considered to be at equilibrium and hence from equation 2.

$$9. \quad K = \frac{k_d}{k_r} = \frac{[E] \cdot [PH]}{[EH] \cdot [P]}.$$

Using the fact that $[P_0] = [PH] + [P]$ and that $[E_0] = [EH] + [E]$ we get from equation 9,

$$10. \quad [PH] \cdot [E] = K \cdot [P_0] \cdot ([E_0] - [E]) - K \cdot [PH] \cdot ([E_0] - [E])$$

whence

$$11. \quad \phi = \frac{1 - \varepsilon}{1 - \left( \frac{K-1}{K} \right) \varepsilon}.$$

Now set $K-1 = f$, a constant and equation 11. becomes

$$12. \quad \phi = \frac{1-\varepsilon}{1-f\varepsilon}.$$

Now differentiate 12. with respect to time to get

$$13. \quad \frac{d\phi}{dt} = -\frac{d\varepsilon}{dt} \left( \frac{1-f}{(1-f\varepsilon)^2} \right).$$

The rate equations then are equations 8, 12, and 13.

* In the reference (8) Eley and Myers give equation 8 as

$$\frac{d\varepsilon}{dt} = -aI\varepsilon + (1-a)I\phi + m \frac{d\phi}{dt}.$$

However since the mathematics gives the equations as in the text, it is assumed that the absence of $m$ in the second from the last term is a typographical error in the reference.
8. \[
\frac{d\varepsilon}{dt} = -aI\varepsilon + m(1-a)I\phi + m\frac{d\phi}{dt}
\]

12. \[
\phi = \frac{1-e}{1-f\varepsilon}
\]

13. \[
\frac{d\phi}{dt} = -\frac{d\varepsilon}{dt} \left(\frac{1-f}{(1-f\varepsilon)^2}\right).
\]

Using these three rate equations: 8, 12, 13, the computer gives outputs of \(\varepsilon\), \(\phi\), and \(aI\varepsilon\) which is the rate of \(O_2\) evolution.

3.3.1. Results of Particular Interest to this Study.

In their study of photosynthesis in Chlorella and in their analog study, Eley and Myers found that they could obtain:

1. transients between any wavelength of light 1 and any wavelength of light 2 (where light 1 has \(\lambda>690\) nm, and light 2 has \(\lambda<680\) nm);

2. transients between two wavelengths of light 2, provided that they were chosen so as to have different maximum enhancement values (the analog study showed that differing \(\varepsilon\) values were the essential requirement);

3. no transients between two wavelengths of light 1.

I decided that it would be interesting to compare these results with results we hoped to obtain from our study of transients in the rate of \(CO_2\) uptake, for the reasons given below.

1. As shown in fig. 3.1, PS2 is more directly related to \(O_2\) evolution than to \(CO_2\) uptake. PS1, however, is more closely related to \(CO_2\) uptake than to \(O_2\) evolution. Hence, one might suspect that the observation of a phenomenon such as chromatic transients which involves the interaction of the two pigment systems may depend on whether \(O_2\) evolution or \(CO_2\) uptake is being observed. The effect of illumination on the \(CO_2\) uptake
FIG. 3.2 Curves from Eley and Myers (8) showing $\epsilon$, $\phi$ and $a$ as a function of wavelength. A. gives values of $\epsilon$, $\phi$ and $a$ as estimated from experimental data. B. gives computer traces for $\epsilon$ and $\phi$. All data assumes a p.d. of 185 mv between E and P.
may not appear the same as its effect on O₂ evolution because of the separation of the two pigment systems by a chain of intermediates. This indeed is in agreement with the observations of Blinks (1, 2) that rise times in response to monochromatic illumination were faster for O₂ and slower for CO₂ if PS2 was activated, and vice versa if PS1 was activated.

Eley and Myers (8) found that O₂ evolution showed transients between appropriately chosen wavelength pairs of light 2, but none in light 1. In view of the preceding paragraph, one might suspect that by watching the CO₂ end, transients will be seen between some wavelength pairs of light 1, but not of light 2.

2. If we look again at Eley and Myers' model, we see that they took the O₂ evolution rate as equal to aIφ. The computer shows rate disturbances analogous to chromatic transients when the parameters are switched between those for 700 nm light and those for 650 nm light. This demonstrates that transients can be explained by assuming that on switching from λ = 700 nm to λ = 650 nm the a values change instantaneously, but the previous ε value holds and requires time to readjust to the new situation. This gives rise to the initial spike and the characteristic return to the steady state value.

If CO₂ fixation is related to the φ level in a similar manner as O₂ evolution is related to the ε level, then the CO₂ fixation rate is given by (1-a)Iφ. The CO₂ fixation rate would then follow the φ level even as the O₂ evolution rate follows the ε level. If now we look at Eley and Myers' graph (fig. 3.2) of the ε level as a function of wavelength, we see considerable variation of the ε level in the light 2 region—the condition for the occurrence of transients in O₂ evolution.
FIG. 3.3 Graphs from Eley and Myers (8).
The graphs show the rates of readjustment of $\varepsilon$ and $\phi$, and show how the transients in $O_2$ evolution depend on $a_1\varepsilon$. 
In the light 1 region, however, there is little variation, and in fact ε is approximately 1. In this region no transients are observed. Their graph of φ level as a function of wavelength (fig. 3.2) however shows the opposite trend. The φ level is high and fairly constant in the light 2 region, but dips sharply and rises in the light 1 region, i.e. it shows the irregularity necessary for transient behavior.

Both these considerations suggest that when observing CO₂ uptake, we can expect transients to occur between certain wavelength pairs of light 1, rather than of light 2.

3.3.2. A Caution.

Fig. 3.3 shows that the ε level readjusts much more slowly when the beam has been changed than does the φ level. Since O₂ transients depend on the time for readjustment of ε level we may expect CO₂ transients to be faster than O₂ transients, if our previous analysis holds. They may then occur too rapidly for our apparatus to respond to them.

3.4. The Proposed Experiment.

In view of the above discussion, we decided to focus this study on the observation of CO₂ transients with the pH electrode which we developed for this purpose. Therefore, we suggest looking for transients:

1. when changing from a beam of white to a beam of monochromatic light,
2. when changing between beams of λ = 700 nm and λ = 650 nm light,
3. when changing between wavelength pairs of light 1, and
4. when changing between wavelength pairs of light 2.
3.5. Measurement of Transients.

If transients do occur in any or all of the above mentioned situations more careful measurement can be made according to the equations derived below.

Assume that the rate of CO$_2$ uptake is given by $(1-a)I\phi$ by analogy with the assumption of Eley and Myers' model that O$_2$ evolution rate is given by $aI\phi$. There are two possible balance conditions:

A. If we adjust the intensities in order to obtain equal steady state rates of photosynthesis upon illumination by either beam, then the rates of CO$_2$ uptake are equal, or

$$(1-a_1)I_1\phi_1 = (1-a_2)I_2\phi_2$$

and since

$$\frac{\phi_1}{\phi_2} = \frac{PH_1/P_0}{PH_2/P_0} = \frac{(PH)_1}{(PH)_2}$$

$$\frac{I_{A1}}{I_{A2}} = \frac{(1-a_2)(PH)_2}{(1-a_1)(PH)_1}.$$ 

B. If now we adjust the intensity so that no initial change in rate occurs when we switch from beam 1 to beam 2, then since $\phi$ has not had time to change,

$$(1-a_1)I_1\phi_1 = (1-a_2)I_2\phi_1$$

when switching from beam 1 to beam 2, and

$$(1-a_2)I_2\phi_2 = (1-a_1)I_1\phi_2$$

when switching from beam 2 to beam 1. Therefore,

$$\frac{I_{B1}}{I_{B2}} = \frac{(1-a_2)}{(1-a_1)}.$$ 

Now combining equations from A. and B., we get

$$\frac{I_{B2}}{I_{B1}} \cdot \frac{I_{A1}}{I_{A2}} = \frac{(PH)_2}{(PH)_1}.$$
which leaves us with two equations defining the ratios of $PH_1/PH_2$ and of $(1-a_1)/(1-a_2)$ in terms of intensities which can be measured.

\[
\frac{I_{B1}}{I_{B2}} = \frac{1-a_2}{1-a_1} \quad \text{and} \quad \frac{I_{B2}}{I_{B1}} = \frac{I_{A1}}{I_{A2}} = \frac{(PH)_2}{(PH)_1}.
\]

These results provide us with several checks on the proposed model.

1. The ratio of $a_2/a_1$ and of $PH_2/PH_1$ can be calculated (assuming the model to be correct) and compared to values used by Eley and Myers.

2. The validity or consistency of the model can be checked. If transients occur and can be measured as described above, yet it is found that either $a_1/a_2 \neq 1$ or $PH_1/PH_2 = 1$ then the model is not a proper explanation of the observed transients because according to the model the occurrence of transients depends on $a_1$ and $a_2$ having different values, and on the $PH_1$ and $PH_2$ levels being different. According to the model if either $PH_1/PH_2 \neq 1$ or $a_1/a_2 \neq 1$, transients should not occur.
CHAPTER 4

EXPERIMENTAL RESULTS

This chapter reports some experimental results obtained with Chlorella, and defines the conditions under which these results were obtained.

4.1. The Sample.

Chlorella is easily grown, and since Eley and Myers' (8) work was based on results obtained with Chlorella, the same species was used in this study to permit comparison with their results.

The present work was done with Chlorella pyrenoidosa proteose grown under continuous illumination in culture medium 2-II (see appendix) at room temperature, from parent stock #252 obtained from the Indiana University culture collection 9/v/66.

After having been washed twice in buffer, the cells of Chlorella were taken up in a small amount of buffer containing carbonic anhydrase, making a very dense suspension. The buffer used was 0.01M Phosphate with pH near 8. Because pH 8 is near the top of the buffering curves of the buffers used, and a maximal response for a given change in CO₂ concentration is desired, the pH was adjusted to around this value.

The cells were harvested while the culture was still growing. The activity of the Chlorella suspension used in the experiment was characterized by observing the time required for oxygen, introduced by shaking the suspension well in air, to be consumed by the respiration
of the algae in the dark. The O\textsubscript{2} was depleted in 14 minutes in a sample which gave satisfactory photosynthesis.

4.2. The Illumination.

Illumination of the algae was provided by white light from the reference beam, 648 nm filtered light from the reference beam and 650 nm and 700 nm light from the grating monochromator. See fig. 2.5. The 648 nm light from the reference beam was produced by an interference filter with a half power band width of 18 nm. Because this filter also has a strong transmission band in the violet region, a sheet of red cellophane was added to isolate the 648 nm band. The monochromator has a linear dispersion of 6.6 nm/mm at the exit slit. With a 2 mm exit slit, the band width of these beams is 13.2 nm. The desired light beam was selected by sliding the moveable mirror. Since either beam could be blocked off, it was possible to switch the desired light beam on and off simply by sliding the mirror.

4.3. Introducing the Sample into the Chamber.

The cell suspension is drawn into a hypodermic syringe and introduced into the chamber through a syringe needle as shown in fig. 2.7. Because of the connection to the KCl - Agar plug, it is extremely difficult to introduce a drop of suspension into the chamber without air bubbles. The simplest adequate method of forming a drop of suspension was to remove the cover slip, push suspension from the syringe through the needle till all the air bubbles had gone and a drop of suitably
FIGS. 4.1 and 4.2 Photosynthetic response traces made on the Esterline-Angus recorder.

D — Dark

W — White Light on
POTENTIAL DIFFERENCE

FIG. 4.3

D — Dark
W — White Light on

FIG. 4.4

2. mv

8 sec.

D

W

2. mv

D

W

FIGS. 4.3 and 4.4 Response to switching white light on and off, Fig. 4.3 at 1 1/3 sec. (1 space) intervals and Fig. 4.4 at 2 2/3 sec. (2 space) intervals.
concentrated suspension appeared at the needle tip. The cover slip was put on again and the size of the drop adjusted. The chamber was then placed over the cone channel condenser and its position adjusted for optimal illumination of the drop.

4.4. Results.

4.4.1. Photosynthesis in Response to Light.

The traces given in figs. 4.1 to 4.4 were obtained with the Esterline Angus recorder. Fig. 4.1 shows that a 1 mv deflection could be obtained on switching white light on and off with algae in the buffer and under the conditions described. The traces show that a steady state (superimposed on some drift) is attained in each instance; i.e. changing the conditions of illumination changes the steady state pH value. Furthermore, the deflections are in the expected directions. Illumination should give rise to photosynthesis which consumes CO$_2$ and lowers the CO$_2$ concentration which in turn increases the pH value. In the dark the pH should again decrease due to an increase in the CO$_2$ concentration. This was indeed observed in the response—the steady state pH value was lower in the dark than in the light.

Fig. 4.2 shows a trace of the same response as fig. 4.1 but taken at a faster chart speed to determine the time required to achieve the new steady state. For a drop 2 mm in diameter, the time required for full response is approximately 10 sec. However, fig. 4.3 and fig. 4.4 show traces of the effect of switching white light on and off at short intervals—fig. 4.3 at 1.3 sec. intervals, and fig. 4.4 at 2.7 sec.
intervals. At 1.3 sec. intervals a change in slope is just perceptible and at 2.7 sec. intervals a change in slope can clearly be seen. Therefore it may be expected that a response will be noticed in two seconds.

These results show that the response time is mainly limited by the response time of the electrode, whose response time $t_r = 1.9$ sec.

To observe the response of photosynthesis to 650 nm and to 700 nm monochromatic light, these were switched on and off. However the intensity of the monochromatic beams was too small to produce photosynthesis. When slits on the monochromator which are normally set at 1 mm were opened to 2 mm, a slight change in slope could be observed on switching 650 nm light on and off, but no change could be observed when switching 700 nm light on and off.

Since the traces on the Esterline - Angus recorder show very little noise, it is possible to introduce some scale amplification between the electrometer and the recorder.

4.4.2. Scale Amplification.

The Esterline - Angus recorder is a 0 - 1 ma recording ammeter with a 1500 ohm resistance. Therefore to amplify the recorder scale, a 1500 ohm resistor was substituted for the recorder. Of the 0 - 1.5 volt potential drop across this resistor, a potential divider provided a variable fraction which was measured with a graphic voltmeter—a Heathkit Model EUW - 20A Servo-Recorder was used on its 100 mv scale. With this arrangement a suitable scale amplification was obtained. A convenient amplification was with 1/3 of f.s.d. on the electrometer corresponding to full scale on the recorder.
FIGS. 4.5 and 4.6 Noise traces using amplified scale on Heathkit Recorder.
FIG. 4.7 Photosynthetic response to white light on amplified scale.
D -- Dark
650 -- 650 nm monochromatic light on
700 -- 700 nm monochromatic light on

**FIG. 4.8** Response to 650 nm and to 700 nm monochromatic light.
The noise level with this arrangement is given in figs. 4.5 and 4.6. Fig. 4.5 shows the noise with a voltage fed into the electrometer on its 10 mv range. Fig. 4.6 gives noise with the electrode in a drop of 0.01 M Phosphate buffer and the electrometer on its 10 mv range.

Fig. 4.7 shows the response of cells to white light obtained with this arrangement. The deflection of 0.5 mv is about one-half of the response seen in fig. 4.1. The difference is due to differing concentration of algal samples.

4.4.3. Results Showing True Photosynthesis.

With the same arrangement and the same sample as described in the previous paragraph, traces were made of response to monochromatic light. A response of 0.15 mv to light of 650 nm can definitely be observed as seen in fig. 4.8. In response to switching 700 nm light on and off, a steady state offset of 0.04 mv was observed as seen in fig. 4.8. No change was observed when switching between 650 nm and 700 nm light. This may not be significant, as the same suspension of cells had been in use for 1.5 hrs. Upon one occasion erratic behavior occurred when switching between a low intensity white light and 650 nm monochromatic light. No transients could be recognized, but there was not time to study this further.

With a very dilute suspension of cells, a response was obtained when switching white light on and off as seen in fig. 4.9, but the deflections are in directions opposite to those seen in fig. 4.7, and slower. This same type of response can also be obtained with a drop of buffer as seen in fig. 4.10. It must therefore be due either to a
FIG. 4.9 Response of a very dilute algal suspension to white light. Note that deflections are in opposite directions to those in Fig. 4.7.
FIG. 4.10 Response of buffer to white light and to heat filtered light. The trace displays considerable drift but shows that in the light the pH decreases (decrease in p.d.). It also shows that when the heat filter is inserted the pH is similar to the pH in the dark rather than in the light.
D — Dark
W — White light on
f — White light with CuSO₄ heat filter inserted

**FIG. 4.11** Response of a dense suspension of algae to white light and to heat filtered light. Note that now the pH again increases (p.d. increases) in the light, and that inserting the heat filter reduces this response by only 1/3.
a heating effect or a photoelectric effect in the electrode. Further experiments were therefore designed to prove that the deflections shown in figs. 4.7 and 4.8 are indeed the result of photosynthesis.

To provide a check on heating effects, a heat filter was prepared. For this purpose a cuvette made with 1/16 inch thick lucite walls separated by a 1/4 inch spacer is filled with CuSO₄ solution of such a concentration that it looked very pale blue. This filter is placed in the white light beam to filter out infra-red radiation.

This filter eliminated much of the response of fig. 4.9 and 4.10 (deflection to the right reduced) as seen in fig. 4.10—when the heat filter is in the light beam the response is similar to switching the light off. However, when a heavy suspension of algae is used, the response is in the opposite direction (deflection to the left—in white light) and inserting the heat filter reduces this response only by about one third of the initial response as shown by fig. 4.11. This decrease in response occurs because the filter also decreases the total light intensity.

These results show that the response of fig. 4.9 and fig. 4.10 in buffer is due to a heating of the electrode. For the other response, as illustrated in figs. 4.7, 4.8 and 4.11 two explanations are still possible:

1. The response is due to true photosynthesis,

2. The response occurs because the dark green droplet is heated by the light causing expulsion of CO₂ with a resultant pH increase in the light. This response would be in the same direction as for photosynthesis, i.e. pH decreases in the light, and its rate would be increased by the
FIGS. 4.12 and 4.13 Response of a suspension of dead cells to white light and to heat filtered light. Both are on a great deal of drift. In Fig. 4.12 note the slope changes which are in the direction of pH decrease in the light. Fig. 4.13 shows that the previous (heat) response is eliminated by the heat filter.
presence of carbonic anhydrase in a way similar to that for photosynthesis. It is therefore necessary to demonstrate which effect is observed.

To differentiate between the two possibilities: 1. heating of the droplet with expulsion of CO₂ and 2. true photosynthesis, a culture of algae was sterilized for 15 minutes to kill the cells. These dead cells were prepared in the same way as live algae had been prepared for the previous experiments, and concentrated to a very dense green suspension. With this suspension of dead cells, the same effect as was seen in fig. 4.9 (i.e. deflection to the right, or pH decrease in the light) was observed, as seen in fig. 4.12. However, as seen in fig. 4.13, this deflection to the left was eliminated by inserting the CuSO₄ heat filter. Therefore, because heating the dead cells in the light gives a pH decrease, the responses observed in figs. 4.7, 4.8 and 4.11, a pH increase when under illumination, is due to photosynthesis.

4.4.4. Intensity Measurements.

To compare intensities at the top of the cone channel condenser, a PbS detector was masked off except for a round hole 0.04 inch in diameter. With the light chopper set above the exit slit, the PbS detector was placed 3/16 inch above the top of the cone and its millivolt output read. Since the millivolt output of the PbS detector is a linear function of the intensity (5) the intensity ratios can be determined simply by comparing millivolt outputs.

The response to different lights can be compared from the results given in figs. 4.7 and 4.8. The intensity ratios and response ratios
can then be compared to see whether the difference in response is simply due to different intensities of illumination.

The 650 nm light gave a response of 0.15 mv while the 700 nm light under the same conditions and with the same drop of cell suspension gave a response of 0.04 mv. The intensities of these lights are in the ratio of 650 nm/700 nm = 33 mv/28 mv = 1.2. The response ratio however is 650 nm/700 nm = 0.15 mv/0.04 mv = 3.7. So whereas the 650 nm light is only 1.2 times as intense as the 700 nm light, the cells respond by giving a change in pH 3.7 times as great. This illustrates the Emerson red drop effect, or that light 2 alone is much more efficient in photosynthesis than light 1 alone.

The very much more intense white light cannot be exactly compared because the intensity measuring signal was clipped due to overloading of the electronics. It can however be stated that both the intensity and the response were much greater than for either of the monochromatic beams.
5.1. Performance of the Electrode in Measuring Dissolved CO₂.

The response of the electrode to H⁺ ions is discussed in section 2.3.3. At 24.5°C the electrode gives a linear response of 56±1 mv per pH unit. Because CO₂ in solution goes to carbonic acid

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

a decrease of CO₂ concentration is observed as a pH increase (increase in potential difference) and vice versa. Measurements were not made of the sensitivity of the electrode system to changes in the CO₂ concentration.

5.2. Response of Chlorella Suspension to Illumination as Determined by the Electrode.

The response to light shown in fig. 4.7 is due to photosynthesis, because as was shown in the previous chapter, a heating response is slower and in the opposite direction. The CuSO₄ heat filter eliminates most of the heat response while reducing the photosynthesis response by only one third. This latter reduction in response is due to loss of light in the filter.

A test with the monochromator showed that the CuSO₄ filter cuts out light of wavelength greater than 685 nm, i.e. the infra red and all of light 1. To check whether the decreased response is due only to intensity decrease, or also to some loss of enhancement due to absence of light 1, I suggest that with the PbS detector, intensities could be adjusted so
that the white beam is set to give the same intensity as the CuSO₄ filtered beam. The magnitude of the two responses could then be meaningfully compared.

A change in the CO₂ concentration of the drop would result in a change in the pH. When CO₂ is consumed inside the drop, CO₂ from the atmosphere diffuses into the drop and if the drop is sufficiently small, a dynamic steady state can be attained both when the cells are consuming CO₂ by photosynthesis and when they are giving off CO₂ by respiration. The lower pH results from respiration in the dark and the higher pH from net CO₂ uptake by photosynthesis.

If in response to photosynthesis a pH change occurred for some reason other than a change in the CO₂ concentration, this change would not be balanced by diffusion from, or into the atmosphere. Hence it would show up as a change in slope which permanently changed the pH even if the conditions were reversed. For example, when the light is on, the pH might increase; then when the light is off, the pH would remain unchanged (unless there is also pH uptake in the dark). In either case no steady state would be attained.

It is therefore significant that the traces show that a steady state pH is attained both in the light and in the dark. These steady states are often superimposed on some drift, but even at worst, the slope changes show steady state characteristics. The steady state pH changes observed must therefore be attributed to a change in the CO₂ concentration due to photosynthesis. The magnitude of the pH changes measured ranged from $7.1 \times 10^{-4}$ to $1.8 \times 10^{-2}$ pH units.
When light 1 is eliminated by the CuSO₄ filter, CO₂ is still consumed in photosynthesis. Hence, the CO₂ end of the photosynthetic mechanism which is activated by pigment system 1 can function with only light 2 present. This result is consistent with results obtained by others (see discussion (4) by Blinks) showing that energy transfers can occur from pigment system 2 to pigment system 1 much more efficiently than in the reverse direction. This is the same phenomenon that gives rise to the Emerson's red drop.

According to Eley and Myers' theory the O₂ evolution rate follows the ε level and transients are due to the readjustment of this level. Readjustment requires 5 - 6 seconds. If according to the theory presented in this thesis, the CO₂ consumption rate depends similarly on the φ level, then the time-course of CO₂ transients should depend on the rate of readjustment of φ. Eley and Myers' graph suggests that φ readjusts more rapidly, and adjustment is complete well within a second. It was shown in chapter 4 that a response could be detected in 1 to 2 seconds with our apparatus: however, if Eley and Myers' graph is correct regarding rates of φ level readjustment, and if the time-course of CO₂ transients depends directly on the rate of readjustment of φ, then the response time of our equipment may not be fast enough to observe these transients.

5.3. Shortcomings and Suggested Improvements.

The system needs improvements in two areas: a) an increase in the signal to noise ratio, and b) a decrease in the time of response.
a) Increase in the signal to noise ratio.

The noise level is essentially the same with white as with 650 nm monochromatic light. This suggests that the noise will not increase if the monochromatic light intensity is further increased. This noise is in fact almost identical to the noise obtained when a voltage from a low resistance source is measured by the electrometer (see figs. 4.5 and 4.6). Therefore the observed noise is not due to the light nor to the electrode, and an increase in signal to noise ratio can be obtained with an increase of the monochromatic light intensity.

The monochromatic light intensity can be increased by increasing the width of the exit slit. This however also increases the bandwidth of the monochromatic light and so permissible bandwidths need to be considered in order to determine the maximum possible slit width. According to Eley and Myers' graphs (fig. 3.2) if the light centered on 700 nm is to produce a $\phi$ level different from that produced by the 650 nm light, it should have wavelengths longer than 695 nm and in no case shorter than 690 nm. The light could be made to center on 705 nm rather than on 700 nm. The shortest wavelengths could then be 15 nm shorter than the principle wavelength. Because the nature of the spectral transmission of the monochromator is such that the half-power bandwidth is one half the spread of the extreme wavelengths, a bandwidth of 15 nm would be permissible in this case. Since the dispersion of the grating monochromator is 6.6 nm/mm at the exit slit, a 2.3 mm exit slit will give a bandwidth of 6.6 nm/mm x 2.3 mm = 15.2 nm. If the light were centered on 705 nm, the exit slit should not be wider than 2.3 mm. In the
reported work a 2 mm exit slit was used. This is already near the bandwidth limit, and an increase of the slit width to 2.3 mm would raise the intensity only 17 per cent. For some applications the light could be centered at a longer wavelength permitting a still wider slit. Further increase in slit width may however render the light insufficiently monochromatic. If further intensity increases are necessary they will need to come by some other means.

The signal to noise ratio can also be increased by a reduction of the noise. The present noise signal as given in figs. 4.5 and 4.6 is 0.2 mv peak to peak. Since the noise is not introduced by the electrode system, but must come from the electrometer, noise can be reduced by narrowing the bandwidth of the recorder. In order to optimize the balance between response time and noise, the bandwidth of the recorder should be made equal to that of the electrode. The bandwidth is given by $\Delta f = \frac{1}{2\pi\tau}$ where $\tau$ is the time constant. The time constant for the recorder would then be equal to that of the electrode plus electrometer giving an overall time constant for the system of $2\tau$. This should significantly decrease the noise without reducing the signal or delaying the response unduly.

Also since it is the electrometer that introduces most of the noise, substituting an electrometer with a lower noise output will increase the signal to noise ratio of the system.

b) Decrease in response time of the system.

The fastest response observed is about the same as the time constant of the electrode-electrometer system. Presumably the overall response is
limited by this time constant. Since the time constant is determined by
the magnitude of the resistance and of the capacitance at the electric-
rometer input, \((\tau = RC)\) a decrease in either or in both of these would
decrease the response time. Thus an electrode with a lower electrical
resistance would respond more rapidly. The electrode used has a resist-
ance of \(5 \times 10^9\) ohms, whereas most larger pH electrodes have a resistance
of approximately \(5 \times 10^8\) ohms. It should be possible to produce a small
electrode with a lower resistance, making possible a shorter response
time.

The response time can also be shortened by decreasing the input
capacity by using different or shorter cables. By means of negative
capacity electrometer amplifiers the time constant at the input could
be reduced to tens of milliseconds.

Since time constant and bandwidth are inversely proportional, it
will be necessary to optimize conditions by the appropriate balance
between the noise voltage and the response time of the system.

Further experimenting with the buffer system using lower buffer
concentrations, different pK values, and different initial pH may
result in increased change in pH for a given change in CO\(_2\) concentra-
tion.

The effect of different concentrations of CO\(_2\) in the atmosphere
surrounding the drop should be investigated. It may be found that an
atmosphere slightly enriched in CO\(_2\) will give better results.
5.4. Summary.

1. A small pH electrode for monitoring the concentration of dissolved CO$_2$ has been developed and used to observe photosynthetic uptake of CO$_2$.

2. In both light and dark a steady state concentration of CO$_2$ was attained. As intended, the electrode in the apparatus does function as a monitor of the rate of CO$_2$ uptake.

3. The change in rate of CO$_2$ consumption upon illuminating the cells is due to photosynthetic uptake of CO$_2$.

4. In a theoretical study, a kinetic model of photosynthesis proposed by Eley and Myers has been used to consider the possibility of light induced transients in the CO$_2$ uptake rate of photosynthesis. It is suggested by analogy with the transients in O$_2$ evolution rate that these transients should occur in CO$_2$ uptake rate, but with different directions and rates than those which occur in O$_2$ evolution.

5. The light induced transients in photosynthetic CO$_2$ uptake were not clearly identified, but this may be because of insufficient sensitivity or intensity or not having discovered optimal physiological conditions for this effect.

6. Increasing the signal to noise ratio of the system through increasing the intensity of monochromatic light, and shortening the response time of the electrode is desirable and possible.

7. The conditions giving optimal pH change for a given change in CO$_2$ concentration should be sought.
BIBLIOGRAPHY


APPENDIX

Culture Medium 2 - II

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<tr>
<th>Ingredient</th>
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<tr>
<td>NaC\textsubscript{2}H\textsubscript{3}O\textsubscript{2} \cdot 3H\textsubscript{2}O</td>
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<tr>
<td>MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O</td>
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<td>CaCl\textsubscript{2}</td>
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<td>FeCl\textsubscript{3} \cdot 6H\textsubscript{2}O</td>
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*Trace metal solution* 

<table>
<thead>
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<td>H\textsubscript{3}BO\textsubscript{4}</td>
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*Trace metal solution: