ON THE DYNAMICS OF PHOTOSYNTHESIS.

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Although a great deal of attention has been paid to photosynthesis, nothing is known of the dynamics of the process. This aspect of the matter especially deserves investigation as furnishing a new point of attack upon this difficult problem.

We cannot analyze the dynamics of photosynthesis without first securing accurate data. A preliminary difficulty lies in the control of temperature; when leaves of land plants are exposed to sunlight, changes of temperature at once take place in the leaf and it is found that even under favorable conditions of control the temperature of the leaf may fluctuate as much as 10°C. in a half hour period. To avoid this difficulty, the writers have employed certain aquatic plants, which form thin layers or filaments, whose temperature can be regulated to a sufficient extent for the purposes of the investigation.

The fronds of the marine alga, *Ulva rigida* (sea lettuce), are so useful for this purpose that most of the experimental work was confined to them, although other material was used for comparison. These fronds consist of only two layers of cells and are so thin (about 0.078 mm.) that their temperature remains very close to that of the surrounding liquid. A further advantage of thin fronds is that gaseous exchange is extremely rapid.

The experiments on *Ulva* were carried on at the Marine Biological Laboratory at Woods Hole during the month of August when an abundant supply of excellent material was available.

To obtain data for the study of dynamics it is necessary to determine at frequent intervals how much photosynthesis has taken place. None of the available methods was satisfactory for our purpose. The method of counting bubbles is open to serious objections while the method of analyzing the gases in solution, as developed by Blackman and Smith,[1] is not sufficiently accurate and convenient. The difficulty was solved by developing a method which depends on the fact that as the plants abstract carbon dioxide from the solution it becomes more alkaline.

In collaboration with Loeb[2] one of the authors had observed that certain marine algæ when exposed to sunlight cause the sea water to become more alkaline. Similar observations had been previously made by various observers[3] upon fresh water plants in solutions containing bicarbonates. If bicarbonates are absent, little or no effect is observed. The greater degree of alkalinity produced in the presence of bicarbonates is due to the fact that the plants abstract CO_2 from bicarbonates. Thus in sea water (which normally contains carbonates and bicarbonates) the alkalinity produced in this way may amount to more than $pH = 9$.

In the case of marine plants it is not necessary to add bicarbonates, since the sea water contains a sufficient amount. Such plants can therefore be studied in their natural environment, which is a distinct advantage over the methods hitherto employed, in which concentrations of CO_2 greatly in excess of the normal were maintained during the experiments.

In this connection it may be mentioned that some authors state that the concentration of free CO_2 is about the same in solutions containing carbonates and bicarbonates as in the air above and that plants in such solutions have no more CO_2 at their disposal than land plants. Aside from the fact that the amount of free CO_2 in sea water is not known, they seem to overlook the fact that when free CO_2 is abstracted from a solution of carbonates and bicarbonates it is at once partially replaced as the result of the dissociation of the carbonates and bicarbonates, so that the plant receives at once what otherwise must be more slowly supplied by diffusion. The carbonates and bicarbonates constitute a reservoir of CO_2 which may be depleted by photosynthesis during the day and filled up during the night by

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[1] Blackman, F. F., and Smith, A. M., *Proc. Roy. Soc., Series B*, 1911, lxxxiii, 374.

[2] Loeb, J., The dynamics of living matter, New York, 1906, 98. Cf. Moore, B., Prideaux, E. B. R., and Herdman, G. A., *Proc. and Tr. Liverpool Biol. Soc.*, 1915, xxix, 233.

[3] Czapek, F., Biochemie der Pflanzen, Jena, 2te Aufl., 1913, i, 519.

diffusion from the air. The amount of CO_2 at the immediate disposal of the plant therefore depends largely on the amount of carbonates and bicarbonates present.

The usefulness of carbonates and bicarbonates in this connection is greatly increased by the fact that the plants are able to split them so as to extract from them much more CO_2 than can be removed by bubbling a stream of hydrogen through the solution.

In order to measure the degree of alkalinity produced by *Ulva*, a piece of the frond was placed in a tube of Pyrex glass[4] (about 12 mm. in diameter and about 5 cm. long) in such a manner that it completely covered the inside of the tube for the greater portion of its length. Fronds were chosen which were sufficiently stiff, so that their own elasticity caused them to remain pressed against the inner surface of the glass tube even when liquid was poured in and out or shaken back and forth in the tube.

The glass tube was sealed off at one end, while at the other it was furnished with a short piece of rubber tubing covered with paraffin.[5] The covering of paraffin was continuous and care was taken to renew it frequently.

After placing the frond in the tube, the latter was filled with sea water containing indicator[6] and the rubber tube was clamped shut. In some cases a small bubble of air was left in the tube to act as a stirrer; in other cases the tube was completely filled with sea water and the stirring was effected by a small piece of paraffin or by a glass bead covered with paraffin.

In order to determine the degree of alkalinity produced by photosynthesis two methods were used. In the first, the indicator was added to the sea water containing *Ulva* after a definite exposure to

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[4] This glass was chosen because it does not give off measurable quantities of alkali during the period of the experiment.

[5] It is necessary to use paraffin which will not give off measurable quantities of acid during the time of the experiment. For this purpose paraffin of a high melting point is usually advantageous. Rubber should be used which gives off the minimum amount of acid; the rubber used in these experiments was repeatedly boiled before using.

[6] Ten drops of saturated alcoholic phenolphthalein was added to 1 liter of sea water. This makes the concentration of alcohol 0.0067 M and that of phenolphthalein 0.0001 M.

sunlight; in the second, the indicator was added to the sea water before the exposure began. In the latter case there was a possibility that the presence of the indicator might affect the amount of photosynthesis but it was found by control experiments that this was not the case with the concentrations employed in these experiments.

There is an advantage in adding the indicator at the start since this permits us to compare the times required to produce a given amount of change under different conditions. A comparison of the values thus obtained is more valuable than a comparison of the amounts of CO_2 abstracted during equal times, for the former procedure compares the reaction velocities accurately while the latter may not. In case the indicator is added at the end, instead of at the beginning, curves may be constructed (plotting CO_2 against time) from which the time required for equal changes in alkalinity may be obtained by interpolation.

In case any substance is added to the solution which changes its buffer value, due allowance must be made for this fact. An apparatus for determining the buffer action of added reagents has recently been described by one of us.[7]

It was found by preliminary experiments that the amount of CO_2 abstracted by the plant was an approximately linear function of the pH value (in the range here employed, between pH 8.1 and pH 8.3).

It was necessary to ascertain whether the degree of alkalinity produced was a reliable measure of the amount of photosynthesis. This was done by making simultaneous determinations of the degree of alkalinity and the amount of oxygen evolved (by a modification of Winkler's method recently described by us[8]). The results show that the amount of photosynthesis, as indicated by the evolution of oxygen, is approximately a linear function (in this range) of the change in the pH value of the sea water. This being so we can measure the amount of photosynthesis by determining the change in pH value regardless of any possible complications, such as excretion of alkali by the plant.

Since the plants produce CO_2 by respiration this must be taken into consideration. Experiments conducted under precisely the same conditions, except that light was excluded, showed that the respiration was

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[7] Osterhout, W. J. V., *J. Biol. Chem.*, 1918, xxxv, 237.

[8] Osterhout, W. J. V., and Haas, A. R. C., *J. Biol. Chem.*, 1917, xxxii, 141.

practically constant. It is, therefore, easy to make a correction for it. It does not affect the form of the curve of photosynthesis found in the present investigation. That no acid other than carbonic is given off by the plant is shown by experiments in the dark in which the acidity produced was completely removed by a stream of hydrogen.

In order to ascertain how much photosynthesis had taken place after a definite time the pink color produced by the *Ulva* was matched against the colors of a series of Pyrex glass tubes[9] (of the same size) containing the same concentration of indicator in a series of buffer solutions of known alkalinity.[10] The matching was done under a "Daylight" lamp, which is invaluable for this purpose.

In this way the degree of alkalinity produced may be easily ascertained and since this corresponds to the amount of oxygen evolved, it gives us a direct measure of photosynthesis, provided we know the amount of CO_2 corresponding to the observed changes in alkalinity. This may be determined by the method referred to above.[7]

In order to carry out such investigations as the present one or to study the effects of temperature, light intensity, etc., it is not necessary to know the amount of CO_2 abstracted; it is sufficient to compare the time required to produce the same change in the color of the indicator under different conditions.

The experimental procedure was as follows: Young, vigorous plants of *Ulva* (deep green in color and not over 4 inches in diameter) were collected in the afternoon and placed in running sea water in the laboratory. In the evening they were covered with a dark screen so that the morning light could not reach them. On the following morning the plants were placed in a water bath at 27°C. and allowed to come to the temperature of the bath.

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[9] These tubes were prepared and clamped shut in precisely the same manner as the tubes containing *Ulva*.

[10] Cf. Sörensen, S. P. L., *Biochem.* Z., 1909, xxi, 131; *Ergebn. Physiol.*, 1912, xii, 393. Hoeber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 4th edition, 1914, 169. Bayliss, W. M., Principles of general physiology, New York, 1915, 203.

For the pH values needed in these investigations mixtures of 0.05 M borax and 0.2 M boric acid (to each liter of boric acid 2.925 gm. NaCl is added) are useful. The following table gives the pH values of a series of mixtures (Palitzsch, S., *Biochem. Z.*, 1915, lxx, 333; *Compt. rend. lab. Carlsberg*, 1916, xi, 199). Cf.

The experiment was started in the following manner. When everything (plants, sea water, and tubes) had come to the temperature of the bath, the plants were placed in the tubes, the rubber tubes were clamped shut, and the tubes were then placed in the bath so that they were about half an inch below the surface of the water, making an angle of about 20° with the surface, and exposed to direct sunlight.

Under these circumstances the alga receives abundant sunlight for photosynthesis. The amount of sunlight is affected by reflection from the surface of the water and the amount of reflection is influenced by the movement of the water due to stirring. But this is a fairly constant factor and of negligible importance. The effect of stirring can be obviated by placing a sheet of glass in contact with

McClendon, J. F., Gault, C. E., and Mulholland, S., *Carnegie Institution of Washington, Publication 251*, 1917, 21.

0.2 M boric acid. *cc.*	0.05 M borax. *cc.*	pH
0	10	9.24
1.0	9.0	9.11
2.0	8.0	8.98
3.0	7.0	8.84
4.0	6.0	8.69
4.5	5.5	8.60
5.0	5.0	8.51
5.5	4.5	8.41
6.0	4.0	8.31
6.5	3.5	8.20
7.0	3.0	8.08
7.5	2.5	7.94
7.7	2.3	7.88
8.0	2.0	7.78
8.5	1.5	7.60
9.0	1.0	7.36
9.4	0.6	7.09
9.7	0.3	6.77

By plotting the cc. of borax as ordinates and the pH values as abscissæ a curve is obtained from which intermediate values can be obtained by graphic interpolation. From the pH values found in sea water 0.21 must be subtracted on account of the "salt error." In the present investigation these values were carefully checked by means of the hydrogen electrode.

the surface so as to eliminate ripples, or by using in place of the tub a tank with a vertical glass wall against which the tube is held in such a way that the light falls upon the tube after passing through the glass wall.

The temperature of the bath was kept constant within 1°C. There was no need of more accurate control of temperature under the conditions of the experiment. A thermometer was inserted into the tube in many of the experiments.[11] On exposure to sunlight the temperature of the tube rose and remained slightly above that of the bath, but under the conditions of the experiment the difference was almost constant. Since the tissues used were so extremely thin, it is safe to assume that the temperature of the tissues was practically the same as that of the liquid in the tube.

The tube containing *Ulva* was allowed to remain in sunlight, with occasional shaking to stir its contents, until a standard shade of pink was produced which matched that of a selected buffer solution (for purposes of matching a clear space was left in the tube above or below the frond). When the color of the tube was seen to be closely approaching that of the selected buffer solution, the tube was removed for an instant from the bath, shaken, and placed beside the selected buffer solution under the "Daylight" lamp. If the tint was not as deep as that of the buffer, the tube was returned to the bath. As soon as the desired tint was reached the time was noted, the sea water was emptied from the tube, and a fresh sample of sea water (containing indicator) was poured in. The tube was again exposed to sunlight and the time required to produce the same tint was noted. This procedure was repeated as often as necessary.

The experiments were carried out on cloudless days during the month of August. In case clouds interfered with the sunlight at any time during the course of the experiment the whole experiment was rejected. At first it was feared that the increase in the intensity of the sunlight during the morning and its decline during the afternoon might affect the results. In order to ascertain whether this was the case the experiments were started at various times during the

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[11] The glass of the thermometer did not give off sufficient alkali to affect the results.

day. It was found that if the work was done between 9 a.m. and 4 p.m. the changes in the intensity of the sunlight might be neglected.

The results obtained are given in Table I, which represents the average of five experiments, and are illustrated in Fig. 1.

It is evident from Fig. 1 that the rate increases rapidly at first, then more and more slowly until it finally ceases to increase. From this point onward a steady rate is maintained.[12] The result is surprising, but in view of the fact that it has been confirmed by numerous experiments with *Ulva* as well as by experiments on *Enteromorpha*, *Spirogyra*, *Hydrodictyon*, *Potamogeton*, and other plants[13] it seems to be well established.

TABLE I.

Period.	Time required to produce standard alkalinity.	Total time exposed.	Amount of photosynthesis.	
			Observed.	Calculated.
	min.	*min.*		
1	35.7	35.7	1	0.92
2	25.9	61.6	2	2.07
3	23.3	84.9	3	3.18
4	21.7	106.6	4	4.23
5	20.4	127.0	5	5.23
6	20.3	147.3	6	6.22
7	20.5	167.8	7	7.22

Average of five experiments at 27°±0.5°C.

Two questions of great interest now present themselves. First, why does the rate increase at the start?[14] Second, why does it finally become stationary?

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[12] This steady rate is not the same for each piece of frond but each piece is fairly constant in its rate, so that if the material is kept over night it will be found the next morning that different pieces may start out at different rates, but the steady rate which each piece finally attains is practically the same as the steady rate which the same piece had the day before. In fact it is possible to recognize the various pieces by this means.

[13] In experiments on fresh water algæ a small amount of sodium bicarbonate was added to the water.

[14] This acceleration is not due to the increase in the intensity of light as the sun gets higher for it was also observed when the experiments were started at noon.

The suggestion which first offers itself is that photosynthesis belongs to the class of autocatalytic processes, in which the reaction is catalyzed by one of its own products. Such reactions begin slowly but as more of the catalyzing substance is produced the reaction goes on at an increasingly rapid rate until it begins to slow down as the reacting substances are used up. If these substances are constantly renewed, the reaction will not slow down but continue to go on more and more rapidly.

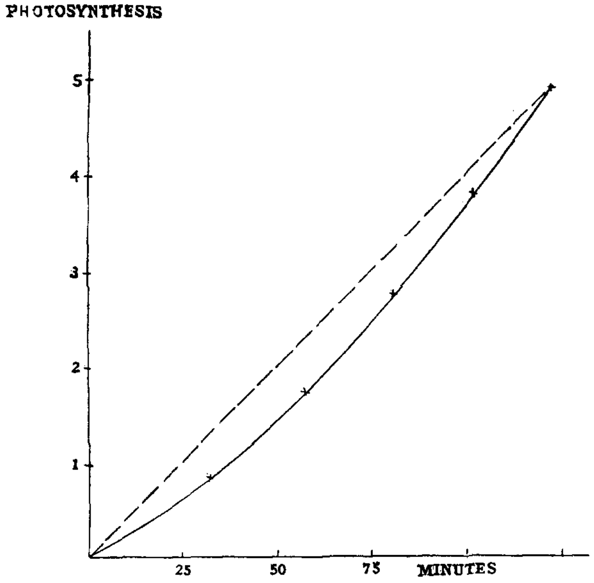

FIG. 1. Curve showing that where *Ulva* is exposed to light the speed of photosynthesis increases until a steady rate is attained. The dotted line expresses a uniform rate.

In our experiments on photosynthesis the reacting substances are constantly renewed.[15] The substances entering into the reaction are presumably carbon dioxide and water. The concentration of the water remains constant, while as soon as the concentration of the carbon dioxide has diminished by a very small amount it is brought back to the original point by the renewal of the sea water.

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[15] When the sea water is not changed during the experiment the curve rises more rapidly at first, then bends over to the right as the supply of CO_2 is used up.

If photosynthesis were an autocatalytic reaction, the amount of catalyzer should increase in the manner indicated in Fig. 2 and, under these conditions, the process should continue to increase in speed as time goes on. As a matter of fact it soon attains a steady rate. This might be accounted for by supposing that the concentration of the catalyst cannot exceed a certain amount, being limited by its own solubility. But in that case the rate would increase more and more rapidly up to a certain point and suddenly become stationary when the limit of solubility was reached, the curve of the catalyst being like that shown in Fig. 3.[16] This is not the case. The rate increases rapidly at first then more and more slowly until it finally becomes stationary.

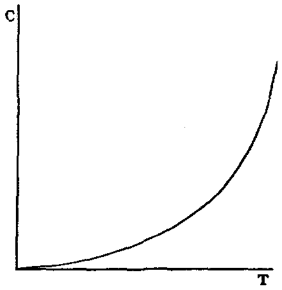

FIG. 2.

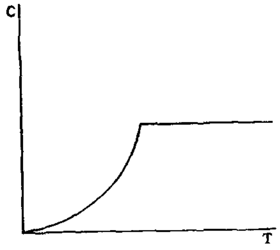

FIG. 3.

FIG. 2. Curve showing increase in the amount of the catalyst when the reaction is autocatalytic (*C*, catalyst; *T*, time).

FIG. 3. Curve showing behavior of the catalyst when its concentration is limited by such a factor as solubility (*C*, catalyst; *T*, time).

It might be supposed that the speed of the reaction is checked by the accumulation of the products of the reaction. In that case, however, the rate would not become constant but would gradually diminish to zero. Such influence of the products would be possible only in the case of a reversible reaction and we have no ground for believing that photosynthesis comes under this head.[17]

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[16] This is because the catalyst from the moment of its production is in solution. It is not analogous to a solid going into solution, which dissolves more slowly as the limit of solubility is approached.

[17] While respiration is in a sense the opposite of photosynthesis the steps in the process are apparently quite different from those found in photosynthesis.

It might also be suggested that the rate becomes constant through the operation of a "limiting factor" such as lack of light, carbon dioxide, or of temperature. But it is evident that the effect of such a factor would be fully felt at the very start of the reaction and that it could not cause a gradual falling off in the increase of speed.

This puts clearly before us a fundamental difficulty. The fact that the rate increases most rapidly at first and then more slowly shows that photosynthesis is not an autocatalytic reaction in the usual sense of the word, for in such a reaction[18] the rate would increase slowly at first, then more and more rapidly as time goes on. We must therefore conclude that photosynthesis belongs in a different category.

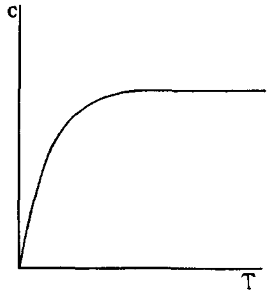

FIG. 4. Curve showing the behavior of the catalyst on the assumption that it is produced by the monomolecular reaction $A \rightarrow C$ (C, catalyst; T, time).

The key to the situation is furnished by the figures in the second column of Table I, which show that if the reaction is catalyzed by a substance, it must be produced more rapidly at first and then more and more slowly. It is also evident that this substance must be limited in amount and that when its production ceases the rate of photosynthesis becomes constant. We may assume that the rate of photosynthesis is proportional to the amount of the catalyst, which we will call C. The figures suggest that this substance may be produced in the manner characteristic of a monomolecular reaction as shown in Fig. 4. We may therefore assume that C is produced by a substance A, under the influence of sunlight, according to the monomolecular reaction: $A \rightarrow C$.

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[18] *I.e.*, under the conditions of the present experiment, where the reacting substances are kept approximately constant in composition.

We may now test this assumption by calculating the amount of photosynthesis which is to be expected after the lapse of a given time.

According to the ordinary equation for a monomolecular reaction,

$$C = A - Ae^{-KT}$$

in which T is time, e is the basis of natural logarithms, and K is the velocity constant of the reaction.

We may denote the amount of photosynthesis by P. If the rate of photosynthesis is directly proportional to the amount of C, we may, for convenience, put

$$\frac{dP}{dT} = C;$$

hence

$$\frac{dP}{dT} = A - Ae^{-KT}.$$

On integration this becomes

$$\frac{P}{A} = T - \frac{1}{K} + \frac{1}{K}e^{-KT}.$$

When the rate has become constant we find that a unit amount of photosynthesis is produced in 20.4 minutes (average of the last 3 periods in Table I), hence the rate of photosynthesis at that time is $1 \div 20.4 = 0.049$. This is by assumption equal to C when A is completely transformed into C and this is in turn equal to A at the beginning of the reaction. Hence A at the start $= 0.049$. We may substitute this value in the equation and find the value of K by trial. If we put $K = 0.049$ we get the values given in Table I. Better agreement with the observed values is obtained by taking lower values of K. This produces a gradual falling off in subsequent values, but it is possible that this might actually occur if the experiment could be continued for a sufficient length of time.

The agreement between the observed and the calculated values is very satisfactory except at the start. In this connection it may be pointed out that at the beginning of a reaction disturbances are to be expected.

It is therefore evident that the assumption justifies itself by giving an adequate quantitative explanation of the observed results. The question then arises whether it is a natural one. It would seem very probable that the light produces a substance which accelerates the reaction and unless this substance is produced in unlimited amount there must come a time when the rate will become steady (or fall off). The assumption therefore seems to be reasonable.

It is attractive to form a hypothesis as to the nature of the catalyst. One might be tempted to suppose that it is chlorophyll but for the fact that some plants which are deep green may not photosynthesize as rapidly as those which possess less chlorophyll.[19] It is of course possible that the less active plants are deficient in some essential factor other than chlorophyll. On the other hand it may be necessary for chlorophyll to be transformed by the light from an inactive into an active form,[20] so that the rate of photosynthesis depends on the amount of "active chlorophyll" present. This would be analogous to the well known activation of enzymes by various means.

An equally satisfactory quantitative explanation is obtained if we suppose the amount of photosynthesis to correspond to the amount of a substance P, produced (under the influence of light) by the monomolecular reactions

$$S \rightarrow M \rightarrow P,$$

in which S represents a constant source (*i.e.*, a substance which does not appreciably diminish during the experiment).

Let us suppose that in the morning, before the frond is exposed to the light, S alone is present. On exposure to light the formation of M and P begins. The amount of M will then increase until it reaches a constant value (when its rate of formation is equal to its rate of decomposition) but the value of P will continually increase, since it does not undergo decomposition. When M has reached a constant value we find (putting K as the velocity constant of the reaction $M \rightarrow P$) that the amount of M decomposed in 1 minute (unit time) is KM; this is also the amount of P which is formed in 1 minute, and since the reaction $S \rightarrow M$ produces just enough of M to balance the loss of M (by transformation into P) the amount of M produced each minute

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[19] Aquatic plants taken directly from ice-covered ponds in winter are found to possess but feeble photosynthetic power, though of a deep green color.

[20] Such activation of substances by light is well known in photochemistry.

is KM. Hence if we start in the morning with S alone there will be produced each minute KM and all of this will be transformed into P except what is present at any moment as M. Hence the amount of P produced in the time T is $KMT - M$.

When M has attained its constant value, we may, for convenience, put $M = 1$. The rate of increase of P is then constant and we find from the table that it takes 20.4 minutes to produce one unit of photosynthesis; hence $KMT = 1$. Substituting in this equation the values of M and T we have $20.4\ K = 1$, whence $K = 0.049$.

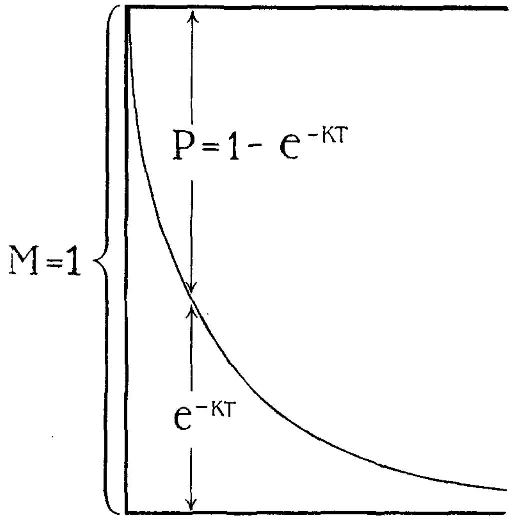

FIG. 5. Curve to illustrate that, as M decomposes in monomolecular fashion to form P, the amount of M left at any given time, T, is e^{-KT} and the amount of P is $1-e^{-KT}$. The abscissæ represent time; the ordinates the amount of M. At the start of the reaction $M=1$.

At the start of the reaction the value of M is 0; this gradually increases to 1 and remains constant. During this period of increase the value of M may be calculated as follows: When M has reached its constant value ($M = 1$) let us suppose that the reaction $S \rightarrow M$ suddenly stops while $M \rightarrow P$ continues; we shall find that if T minutes have elapsed after this occurrence, the amount of M which has disappeared is $1 - e^{-KT}$ (see Fig. 5). If the reaction $S \rightarrow M$ had not stopped it would have produced enough of M so that (in spite of the fact that M is constantly decomposing) the amount of M remaining at the time, T, would be just enough to balance the loss, or $1 - e^{-KT}$. Hence if we start with nothing but S (the values of M and of P being 0) the amount of M present after the lapse of any given time T will be $1 - e^{-KT}$ and the amount of P will be

$$P = KT - (1 - e^{-KT}).$$

This is the same as the equation

$$\frac{P}{A} = T - \frac{1}{K} + \frac{1}{K} e^{-KT},$$

when in the latter we put $K = A$ as was done in making the calculations given in Table I. Hence when we substitute the value $K = 0.049$ in the equation $P = KT - (1 - e^{-KT})$, we obtain the values already given in Table I.

If the chlorophyll takes part in the reaction by decomposing or by combining (as some recent evidence indicates), we might suppose that S represents inactive chlorophyll, M active chlorophyll, and P a derived substance which combines with CO_2. At present it does not seem profitable to attempt a more extended discussion of this question. But it may be pointed out that (as one of us has recently emphasized)[21] consecutive reactions of the type here discussed are to be looked upon as the rule, rather than as the exception, in living matter.

It is evident that either of the theories developed above gives a quantitative explanation of the results. Both seem to be based on reasonable assumptions. Future investigation must decide which is more useful.

In any event, it is clear that much is to be learned concerning the dynamics of photosynthesis, and it is hoped that the considerations here set forth may be of value in this connection.

SUMMARY.

Minute amounts of photosynthesis in marine plants can be accurately measured by adding a little phenolphthalein to the sea water,

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[21] Osterhout, *J. Biol. Chem.*, 1917, xxxi, 585; xxxii, 23.

and observing changes in the color of the indicator. In the case of fresh water aquatics bicarbonates are added.

By this method it is found that *Ulva* which has been kept in the dark begins photosynthesis as soon as it is exposed to sunlight and that the rate steadily increases until a constant speed is attained.

This may be explained by assuming that sunlight decomposes a substance whose products either catalyze photosynthesis or enter directly into the reaction.

Quantitative theories are developed in order to account for the facts.