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*DYNAMICAL ASPECTS OF PHOTOSYNTHESIS*¹

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Although a great amount 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, consisting of 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 suitable 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.

To obtain data for the study of dynamics, it is necessary to determine at frequent intervals how much photosynthesis has taken place. This was accomplished by a method elsewhere described.² This method enables us to measure quickly and accurately the amount of photosynthesis at definite intervals, without subjecting the plants to an abnormally high concentration of CO₂ as has heretofore been customary.³ It depends upon the fact that photosynthesis abstracts carbonic acid from the surrounding solution and renders it more alkaline. By the use of indicators the degree of alkalinity, and

consequently the amount of photosynthesis, can be determined with considerable precision.

The amount of photosynthesis is approximately a linear function of the change in PH value in the range here employed.

In order to measure the degree of alkalinity produced by *Ulva* under the influence of sunlight, a piece of the frond was placed in a tube of Pyrex glass filled with sea water and closed as described elsewhere.² The tube was then placed in a large water bath (the temperature of which fluctuated less than 1°C.) in direct sunlight. If clouds interfered with the sunlight at any time the experiment was rejected.

Since the plants produce CO₂ by respiration this must be taken into consideration. Experiments were conducted under precisely the same conditions, except that light was excluded. They agree in showing that the respiration was practically constant. It cannot, therefore, affect the form of the curve of photosynthesis.

TABLE 1

PERIOD	NUMBER OF MINUTES REQUIRED TO PRODUCE STANDARD ALKALINITY	TOTAL NUMBER OF MINUTES EXPOSED	AMOUNT OF PHOTOSYNTHESIS	
			Observed	Calculated
1st.....	35.7	35.7	1	0.92
2nd.....	25.9	61.6	2	2.07
3rd.....	23.3	84.9	3	3.18
4th.....	21.7	106.6	4	4.23
5th.....	20.4	127.0	5	5.23
6th.....	20.3	147.3	6	6.22
7th.....	20.5	167.8	7	7.22

Average of 5 experiments at 27°±0.5°C.

In each experiment the procedure was the same. Freshly collected *Ulva* (young, dark green fronds not more than 3 or 4 inches long) was placed overnight in running sea water and covered so that in the morning no light could reach it. In starting an experiment the *Ulva* was placed in a closed tube in sea water containing a trace of alcoholic phenolphthalein and exposed to light until a definite shade of pink was produced.⁴ This shade of pink matched that of a standard buffer solution (whose PH value was determined by the gas chain) containing the same concentration of indicator as the sea water (and observed in a Pyrex tube of the same size).

When the standard shade was attained, the time of exposure was noted. The sea water was then poured out of the tube containing *Ulva* (the last drop being removed by shaking), fresh sea water was added, and a new determination was made of the time required to attain the standard shade.

The results obtained are given in table 1 (average of five experiments). The table shows that the time required to reach the standard shade steadily diminishes until a constant value is reached.

This result is surprising, but it has been confirmed by numerous experiments on *Ulva*, as well as by experiments on *Enteromorpha*, *Spirogyra*, *Hydrodictyon*, *Potamogeton*, and other plants.⁵

It is therefore evident that photosynthesis is a process which shows a gradual acceleration until a steady rate is attained.⁶ A question of great interest now presents itself: What is the cause of the initial acceleration and why is a steady rate attained after a certain length of time?

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.

In our experiments on photosynthesis the reacting substances are constantly renewed.⁷ 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.

If photosynthesis were an autocatalytic reaction, it should, under these conditions, 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.⁸ This is not the case. The rate increases rapidly at first then more and more slowly until it finally becomes stationary.

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.⁹

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 reaction¹⁰ 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.

The key to the situation is furnished by the figures in the second column of table 1 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 stops increasing and 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. We may therefore assume that C is produced by a substance A , under the influence of sunlight, according to the monomolecular reaction: $A \rightarrow C$.

We may now proceed to 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.

If the rate of photosynthesis is directly proportional to the amount of C , we may, for convenience, put

$$\text{Rate of photosynthesis} = \frac{dP}{dT} = C;$$

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 1), hence the rate of photosyntheses 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 1. 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.¹¹ 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,¹² 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 reaction



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

We may 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 occurs. 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 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 $KMT - M$.

We may, for convenience, put $M = 1$ when it has attained its constant value; the rate of increase of P is then constant and we find that it takes 20.4 minutes to produce 1 unit of photosynthesis. Hence $KMT = 1$. Substi-

tuting in this equation the values of M and T we have $20.4 K = 1$ whence $K = 0.049$.

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 $1 - e^{-KT}$. 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 zero) 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 becomes 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 1. 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 1.

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)¹⁸ 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.—*Ulva* which has been kept in the dark begins photosynthesis as soon as it is exposed to sunlight. The rate of photosynthesis steadily increases until a constant speed is attained.

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

Quantitative theories are developed to account for the facts.

¹ Preliminary communication.

² The paper will appear shortly in *Science, New York*, N. S., 1918. All the precautions mentioned in this account were carefully observed in the present investigation.

³ The methods previously used in studying the photosynthesis of aquatic plants are not as accurate as the one here described, nor do previous experiments afford the kind of data needed for our purpose. Cf. Blackman, F. F., and Smith, A. M., *Proc. Roy. Soc., London*, (B) 83, 1911, (389).

⁴ All matching of shades was done under a 'Daylight' lamp, so that uniform conditions were assured throughout the experiments. Cf. *Science, New York*, N. S., 42, 1915, (764). A clear space was left in the tube below the *Ulva* to facilitate comparison of colors. In any single experiment the buffer may be dispensed with by using as a standard the pink solution produced by the first exposure. The first exposure should be as short as is consistent with obtaining a definite standard. Experiments showed that the trace of alcoholic phenolphthalein had no injurious effect.

⁵ In experiments on fresh water algae a small amount of sodium bicarbonate was added to the water.

⁶ 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.

⁷ 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 CO₂ is used up.

⁸ 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.

⁹ While respiration is in a sense the opposite of photosynthesis the steps in the process are apparently quite different from those found in photosynthesis.

¹⁰ I. e., under the conditions of the present experiment, where the reacting substances are kept approximately constant in composition.

¹¹ Aquatic plants taken directly from ice-covered ponds in winter are found to possess but feeble photosynthetic power, though of a deep green color.

¹² The activation of substances by light is well known in photochemistry.

¹³ *J. Biol. Chem., New York*, 21, 1917, (585); 22, 1917, (23).

MOBILITIES OF IONS IN AIR, HYDROGEN, AND NITROGEN

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In spite of the great number of investigations devoted to gases the question whether an ion is a molecule or an atom carrying an elementary charge, or whether it is a number of neutral molecules clustering about a charge is not as yet definitely settled. Both the 'cluster' hypothesis, according to which an ion is conceived of as a unit elementary charge surrounded by a satellite of neutral molecules, and the 'small-ion' hypothesis, according to which an ion is conceived of as a single molecule carrying an elementary charge, explain equally well the phenomenon which first necessitated the former, and also the older, hypothesis; this phenomenon being the fact that the mobilities and the diffusion coefficients of the ions in gases are relatively