The Temperature-coefficient of Photosynthesis : A Reply to Criticism.¹

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With two Figures in the Text.

INTRODUCTION.

THREE papers have recently appeared in the 'Philippine Journal of Science', two by Brown and Heise and one by Brown alone, adversely criticizing several current conceptions with regard to the rate of carbon assimilation in plants and the influence of the factors of temperature and light upon it. In the first (1917 *a*) Brown and Heise oppose the view that the temperature-relations of this process are such that it conforms to the van't Hoff rule. In the second (1917 *b*) they oppose the view that the magnitude of carbon assimilation is directly proportional to the intensity of illumination, holding that when the light is increased by equal increments the increments of photosynthesis form a rapidly declining series. In the third (1918), by Brown alone, some attack is made upon the evidence for the conception of limiting factors.

The first of these papers is the most considerable, and is directed against the conclusions reached in a series of papers by Blackman and his co-workers, especially against the work of Matthaei (1904). The view of Brown and Heise is that carbon assimilation in plants is governed by *low* temperature-coefficients, such as have been found to characterize photochemical reaction *in vitro* (1.04 to 1.42 for a rise of 10° C.), instead of by coefficients of the magnitude of about 2.0, which are the rule for 'dark' reactions *in vitro*.

Brown and Heise have not worked upon this subject themselves and provide no new data, but they endeavour to establish their position by selection, rejection, and correction of the published results of experimental workers in this field. The intensity of their conviction that these results must be wrong when they have no data of their own to start from would be rather puzzling were it not made evident that, having grasped the generalization that all photochemical reactions have low temperature-coefficients, they have thereupon decided, quite *a priori*, that photosynthesis must really

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have low coefficients also, however strong the evidence may appear to be against this. They therefore, without any misgivings, set out to explain away or discredit all measurements of carbon assimilation in plants which indicate high temperature-coefficients. There is nothing judicial or scientific in their method. Data are adopted or rejected quite apart from the amount of evidence supporting them. Sound progress can, however, only be made by shaping theories to fit facts and not *vice versa*. It has not occurred to them that though certain stages in the complex chain of reactions of carbon assimilation are certainly photochemical, yet other subsequent stages are 'dark reactions'. From this conception it follows that it would be a matter, not for *a priori* dogmatism, but for experimental investigation, whether the temperature relations of the total process were those of a 'light' or of a 'dark' reaction.

This view of the complex nature of carbon assimilation has been held by teachers and workers for many years now, and it has generally been accepted, on the available evidence, that the dark reactions govern the maximal possibilities of the system. The attack upon this view that Brown and Heise have made consists of a mass of detailed criticism of individual measurements of photosynthesis. As few readers are likely to weigh all these criticisms for themselves, it seems unfortunately necessary to point out in some detail how arbitrary and unsound they are.

ASSIMILATION AND TEMPERATURE.

Brown and Heise first discuss the work of van Amstel (1916) on *Elodea*.

Experiments of van Amstel on Elodea.

Miss van Amstel investigated the output of oxygen by *Elodea* in a current of water laden with CO_2 at a few temperatures, namely, 24° , 36.5° , and 40° .¹ She set out to measure the temperature-relations for these points, but admits that it is a difficult and complicated matter to get critical values for high temperatures. In one experiment only (Table V) did she compare the effect of the temperatures 24° and 36.5° on the same shoot of *Elodea*, and the observed increased rate indicated 1.26 as the value of K_{10} . This observation is the only low value for a temperature-coefficient of photosynthesis available in the literature, and it is of course eagerly adopted by Brown and Heise without any examination of credentials. It must first be pointed out in fairness to Miss van Amstel that she expressly disclaims that she has succeeded in establishing trustworthy ratios for these high temperatures. She says on p. 3: 'Thus the experiments, which are to be discussed here, will lead to the conclusion that in fact we did not measure the influence of temperature on the CO_2 assimilation itself, but that physical

¹ All temperature figures are in $^\circ\text{C}$.

processes have exerted their limiting influence.' 'In spite of this circumstance we are publishing our results now because they may indicate to others the probable way to obtain the desired results.'

In the results presented only five are at temperatures other than 24° : three at 36° , one at 40° , one at 45° . Of those at 36° only one can be compared with assimilation at 24° because the other two were done on smaller and undefined amounts of material. Throughout this work no adequate uniformity in the amount of material employed is attained, and the values for 'apparent assimilation', under as uniform conditions as possible, vary from experiment to experiment, 0.152, 0.166, 0.168, 0.186, 0.189.

Our present interest is therefore entirely concentrated on the case where, with constant light (2,482 Hefner candles), constant CO_2 supply (350 c.c. of water containing 0.152 mg. free CO_2 per litre every $4\frac{1}{2}$ to 5 minutes), the observed output of oxygen was 0.189 mg. per minute at 24° , while it rose to 0.258 mg. at 36.5° , thus indicating a ratio which would make $K_{10} = 1.26$.

For these values to be significant it is absolutely essential that each must represent the maximal value possible at that temperature. This can only be established by producing evidence that neither light nor CO_2 supply is limiting the assimilation to a value below the appropriate specific maximum. The value of 0.189 mg. O_2 we can accept as maximal for 24°C ., because raising the temperature alone considerably increased the value. But can we accept 0.258 mg. as maximal for 36.5° ? Van Amstel has given no proof in her paper that 0.258 is a maximal value. There is no experiment in which the temperature was raised from 36.5° to any higher value, using the same shoot. For information as to the possibilities of assimilation at 40° we must turn to an experiment on a different shoot from that which furnished the value 0.258 mg. This second shoot gave at 40° an average assimilation of 0.242 mg. Since, however, it had already at 24° given a lower value than the first shoot, van Amstel obtained, by allowing for its lower assimilatory activity, a figure for its assimilation at 40° which was 5 per cent. higher than that of the first shoot at 36.5° . There are no data from which to calculate the probable error of van Amstel's experiments. In their absence it is safe to say that there is no proof that this rise of 5 per cent., obtained in such an indirect way, represents a real rise in assimilatory activity due to the higher temperature. Consequently it is unproven that 0.258 is the maximal value possible at a temperature of 36.5° .

Van Amstel was of opinion that she had given proof that both CO_2 supply and light were more than sufficient for the assimilation possible at any of the temperatures used in her experiments. Closer inquiry, however, may lead to a different conclusion. With regard to CO_2 supply, she trusted only to alteration of rate of flow; the concentration of CO_2 in the water-supply was the same in the whole of her experiments. The shoot

occupied the axis of the cylindrical vessel through which the water flowed and most of the flow would be along the unimpeded track at the periphery of the vessel. Now the important thing is the concentration in the more stagnant layers at the surface of the leaves and the diffusion gradient maintained from them into the leaf tissue. So when she found in one experiment that halving the total flow-rate through the cylinder did not appreciably lower the rate of photosynthesis, we cannot conclude that the concentration at the surface of the plant was seriously diminished by the reduced flow out beyond it. Experiments made at Cambridge by Mr. F. Summers on the bubbling of water-plants in a stream of tap-water supersaturated with air showed that doubling the rate of flow only increased the bubble-rate by 15 per cent. Indeed, if the flow is very fast, a further increase of 20 per cent. in rate may not increase the bubble-rate at all. Concentration of CO_2 is the only significant thing, especially in a water medium where diffusion is so sluggish, and it is a pity that the whole of van Amstel's work was done with but one concentration.

As proof that the light, 2,482 Hefner candles, was sufficient for the full assimilation at 36.5° van Amstel furnishes one experiment in which at this temperature an increase of light to 3,377 candles did not increase the assimilation (van Amstel, Table II). The value of the assimilation in this experiment (0.148 mg.) is, however, so widely different from that obtained in the later experiment at 36.5° (0.258 mg.) that, unless some explanation of the discrepancy is forthcoming, the result cannot be considered significant. For all these reasons, therefore, the figure 0.258 mg. O_2 per min. cannot be regarded as having been proved to be maximal for a temperature of 36.5° .

Further, there is the important point that no account is taken of the respiratory gas-exchange going on at the same time as photosynthesis. At low temperatures this will be small, but at high temperatures it cannot be neglected for exact work. Therefore at 36.5° the real photosynthesis value must be appreciably higher than the apparent value actually observed by just the amount of oxygen that is circulating round and round in the respiration of the moment.

It appears, therefore, that the temperature-coefficient of 1.26 is not sufficiently substantiated.

The Work of Blackman and Smith.

Brown and Heise quote experiments made by Blackman and Smith (1911) and calculate therefrom three temperature-coefficients for assimilation in *Elodea*. The first, 2.05, between 7° and 13° , agrees with the calculations of that paper. The second, 1.75, between 7° and 21° , and the third, 1.35, between 13° and 21° , were not calculated in the paper, because there was no proof that the figure obtained at 21° was the real measure of the

full temperature effect. In all probability it was the measure of a light effect. As will be seen from Fig. 7 of the paper in question, a temperature of 21° should allow of considerably more assimilation than was obtained at that temperature in either of the Expts. D or E. It was stated implicitly concerning Expt. D, and specifically of Expt. E, that the values obtained at 21° were light-limited values. Until Brown and Heise can bring forward proof that this was not the case, they are not entitled to use the results at 21° in order to calculate therefrom a coefficient for *temperature*.

It appears, therefore, from a reconsideration of the work of van Amstel and of Blackman and Smith that of the five coefficients given in Brown and Heise's Table III, four are worthless because they are calculated from results in regard to which there is no proof that temperature was exerting its maximum effect. On the contrary, there is every probability that some other factor was limiting the intensity of the assimilation. The remaining coefficient, 2.05, is perfectly sound and is of a magnitude which agrees with the van 't Hoff rule.

BROWN AND HEISE'S TABLE III.

Range of Temperature.	Coefficient.	Calculated from data of
7-13°	2.05	Blackman and Smith, p. 402
7-21°	1.75	„ pp. 400, 401
13-21°	1.35	„
24-36.5°	1.28	van Amstel
36.5-40°	1.25	„

The Work of Kreusler.

Kreusler (1887) worked with one shoot of *Rubus*, kept in his chamber for three weeks and exposed to a different temperature every day. Matthaei (1904) showed that the activity of the shoot is declining day after day and that the material soon becomes quite unsound, so that it is impossible to analyse out pure temperature effects from it. At the end the shoot makes little response to change of temperature, while at the beginning it may make a large response. Brown and Heise, though aware of this criticism, welcome the results of the abnormal material as being nearer to their ideal of 1.04 for the value of the temperature-coefficient. It hardly seems necessary to go further into this line of evidence.

The Work of Matthaei on Cherry-laurel.

The work of Matthaei (1904) on Cherry-laurel has been the chief experimental groundwork for the calculation of temperature-coefficients for carbon assimilation and has formed the chief support of the statement that such temperature-coefficients are in agreement with the van 't Hoff rule for ordinary chemical processes *in vitro*. Brown and Heise make a detailed attack on Matthaei's work, and it will therefore be necessary to consider somewhat fully their criticisms.

The method of Matthaei was to adopt only those numbers as measures of the influence of the temperature-factor which were obtained under conditions in which the temperature was shown to be limiting and the other factors in considerable excess. Working with one fixed light and a series of increasing temperatures, she found for that series a curve of the well-known inflected form with a rising limb for the lower temperatures, followed by a horizontal limb for the higher temperatures. Starting all over again with a more intense light, this finding repeated itself with the inflexion point

FIG. 1. This figure is redrawn from Matthaei's Fig. 3, rectifying a mistake and adding, from her tables, two omissions.

at a higher temperature, and similarly with still more intense lights, the collection of curves being shown in Fig. 1.

Her interpretation is that the rising limb is a true measure of temperature effect, while the horizontal limb is limited by light and merely expresses the light intensity, uninfluenced by temperature. Now, since the object of Brown and Heise is to show that assimilation is very little affected by temperature, they proceed on the general plan of casting doubts upon the soundness of the numbers in the rising limb of the curve (and upon these only) by appealing to seasonal changes, experimental errors, or other unsupported hypotheses. They are then left with merely a set of horizontal

limbs, one for each intensity of light used by Matthaei. As each horizontal limb does cover assimilation over a fair range of temperature, they thus reach the desired conclusion that temperature has practically no effect upon assimilation.

We must now consider in some detail the criticisms levelled at the rising limbs of each of Matthaei's four curves, which were carried out with 1, 2, 4, and 8 arbitrary units of light respectively. The first of these curves sets forth the results of twenty-one experiments ranging from -6° to $+33^{\circ}$, all in unit intensity of light. The curve is of very definite form, and it is clear from it that the results obtained at temperatures below 3° increase in proportion to increasing temperature, while the remainder of the results show the limiting effect of unit light. The eight experiments on the rising part of the curves are in direct opposition to Brown and Heise's contention, and they therefore simply rule them out of the discussion by announcing their intention of confining their consideration to values obtained between $+3^{\circ}$ and 33° . This method of arbitrarily choosing a lower limit (3°) which will be convenient for their theory is felt by them to be too summary a procedure to pass unsupported by any argument. They therefore say, 'The part of the curve below 3° shows very much higher temperature-coefficients than would be called for according to the van't Hoff principle. At these temperatures many plant processes are just coming into activity. Under such conditions it would not be surprising to find that a general ratio would not hold for any particular function.'

To this it may be replied that results below 3° with weak light are perfectly consistent with results above 3° in somewhat stronger light when treated from the point of view of Matthaei, Blackman (1905), Kanitz (1915), and many others who accept the application of the van't Hoff rule. This is, therefore, in favour of their outlook and against the view of Brown and Heise, who have to assume a sudden discontinuity of principles at this temperature, passing from big temperature-coefficients below it to very small ones above it.

Brown and Heise then pass to Matthaei's second curve, expressing the results at different temperatures with light of twice unit intensity. It will be seen that the general form of the curve is the same as that for unit light just discussed. The important point is that though double light may produce increased assimilation, as pointed out by Brown and Heise, *yet it only does so when the temperature is raised. So long as the temperature remains low, no amount of light increase causes increased assimilation.* This fact, unchallenged, would be fatal to Brown and Heise's whole contention. They deal first of all with Matthaei's proof that at temp. 0.4° doubling the light does not increase the assimilation, while at temperatures 9° , 11° , and 25° the assimilation is thereby almost doubled. Their comment is as follows:

'The temperature is below that which we are considering. It may be

said, however, that with unit intensity of light the result at 0.4° is very similar to those obtained at all higher temperatures, and so is greater than would be expected from the more complete results recorded in Table I and plotted in Fig. 1. This figure shows an increase in assimilation of considerably more than 50 per cent. between 0.4° and 3.6° . Doubling the intensity of light did, however, increase this apparently too high result in Table II for unit intensity of light at 0.4° .

This passage is obscure, and has been rendered more so by carelessness in the citation of tables. Table I should evidently be Table V and Table II should be Table VI. In Matthaei's paper these results are incorporated in Figs. 2 and 3 respectively.

The first sentence of their comment is a repetition of their evasion of data which do not suit them. The statement that the result in Matthaei's Fig. 3 (Fig. 1 of this paper) for 0.4° is greater than would be expected from the more complete results in Matthaei's Fig. 2 is very curious, for comparison will show that the values from the two curves are exactly the same. Apparently Brown and Heise suppose that the figure for 0.4° should in every series of experiments be the same proportion of the result for 3.6° as it is in Matthaei's Fig. 2. A moment's consideration will show the improbability of such a supposition. The value for the higher temperatures shown in Matthaei's Fig. 3 (Fig. 1 of this paper) for unit light is depressed because of a lowered seasonal activity of the leaves. This phenomenon is perfectly familiar to any one who has worked with either land or water plants, and, though its causes are obscure, it is best expressed in Matthaei's words: 'It appears as if in the sluggish condition of the leaves more light were necessary to do the same amount of work.' The assimilation for unit light is therefore lower in Matthaei's Fig. 3 than in her Fig. 2. But at 0.4° , where, according to all the evidence, light is not the limiting factor, but temperature, there is no reason why the assimilation should be depressed from its usual temperature value. The result at 0.4° and light intensity one unit is therefore not too high, but, as has already been remarked, exactly the same and confirmatory of the result in Matthaei's Fig. 2. The result at the same temperature and light intensity *two* units is the same within the probable error of the experiment, and this constitutes a proof that a temperature of 0.4° is the limiting factor, for results of another order altogether are obtained when temperature is not limiting, as is shown by the three concordant experiments at 9° , 11° , and 25° .

It is well established, therefore, that with light intensity of two units a temperature of 0.4° is limiting and *a fortiori* that lower temperatures are limiting also. This is important, for it cannot be expected that in every fresh series of experiments with more intense light the proof that these lower temperatures are limiting should again be repeated. When, therefore, Matthaei comes to the experiments with light intensity of four units she is

perfectly justified in adopting these already well-established values at which temperature is limiting as part of her curve. This must be said, because Brown and Heise deal with the five experiments with light intensity of four units as an isolated set of experiments with no relation to previous values or results. These five experiments are part of the whole body of results and must be viewed in the light of them. They yield four separate temperature values, those for 9.2° , 11.4° , 24.8° , and 25.2° . These values, as plotted by Brown and Heise, seem to exhibit inconsistency, but let us look at them in the light of previous results. The curve for four units of light must be allowed to have associated with it the temperature-values well established in the previous series, e.g. those at -6° and at 0.4° . When these are added to the four values of the four-unit series itself there are obtained a series of six results which can only be consistently explained in the way adopted by Matthaei, namely, by the omission of the result for 24.8° , given by a leaf believed to be abnormal. We are not confined to the two alternatives offered by Brown and Heise, who say: 'If the figures for 11.4° and 24.8° are correct, there is very little or no rise in the rate of assimilation; if the results for 9.2° and 25.2° are reliable, there is a considerable rise.' They conclude that 'the results are such that no reliable conclusions can be drawn from them'. Even if only the four values obtained at four units light are considered there is a third explanation, the one adopted by Matthaei, viz. that the results at 9.2° , 11.4° , and 25.2° are reliable and the one at 24.8° irregular. Reinforced by the values at -6° and 0.4° her interpretation gives a consistent explanation of five out of six results, whereas the only conclusion from Brown and Heise's theory is that the whole series is valueless, a very unlikely hypothesis. Matthaei's treatment of the data is substantiated by her pointing out that the one value which is too low (at 24.8°) is given by a leaf that in a previous experiment had given an unusually low result for a less intense illumination. It is worth while to calculate the ratio for this particular leaf and compare it with the ratio for normal leaves. The ratio $\frac{\text{assimilation at 4 units light}}{\text{assimilation at unit light}}$ for the leaf in question = $\frac{485}{180} = 2.70$. The same ratio for the normal leaf is $\frac{610}{220} = 2.77$. The closeness of these two ratios supports Matthaei's view that the experimental result at 24.8° is due to the lower assimilatory power of the leaf in question. Nor is this view upset by the fact that at unit light the results from the two types of leaves are close enough to be within the limits of experimental error.

It is necessary here to consider the peculiar views held by Brown and Heise as to experimental error. They say 'the percentage of increase between the figure for 9.2° and the one for 11.4° , and four units of light, is, moreover, within the possible limits of experimental error, indicated in our Table V, Expts. 13 and 14'.

Now Expts. 13 and 14 of Brown and Heise's Table V (Matthaei's Fig. 2) differ by 0.00088. It is therefore not true to say that 0.00100, the difference between the experiments at 9.2° and 11.4° with four units of light, is within the previous extreme limits of error. It is outside those limits. Moreover, that error in itself was an extreme error and one which by the laws of chance would occur extremely rarely. This is made perfectly clear when one sets out precisely the probable error of the long series of fourteen experiments with light of unit intensity, ranging from -6° to +33.1°.

The set of fourteen experiments gives a mean value of 0.0029 and a standard deviation of 0.000235, so that the probable error of a single experiment works out at ± 0.000158 .

That the small deviations throughout the series are devoid of any significance is shown by considering them in three groups: (a) the five coldest observations, (b) the four medium observations, and (c) the five hottest observations. The probable error between the mean of a group of four and the mean of a group of five is, according to the formula kindly given me by Mr. G. Udny Yule, $\pm 0.000158 \times \sqrt{\frac{1}{4} + \frac{1}{5}} = \pm 0.00011$. As the actual means of the three groups respectively are (a) 0.00286, (b) 0.00292, (c) 0.00288, the differences between these means are found to be actually only about half the probable error.

Brown and Heise go against all the probabilities in saying that such a large difference between two values as 0.0010 is not significant and is to be attributed to experimental error, for the unlikely deviation of ± 0.00044 only occurred once.

Having claimed 0.00100, i. e. ± 0.00050 , as within the limits of experimental error, Brown and Heise immediately go on to claim a much smaller difference, i. e. ± 0.00020 (the difference between the results for light intensities of four and six units at 11.4°), as a real light effect, not due to experimental error, indicating that further increase of light would increase still further the assimilation. This is surely merely juggling with figures in the interests of a theory. As a matter of fact, Matthaei is interpreting the results according to the probabilities, considering the general form of all her previous curves and considering how little wider this variation is than the probable error of her previous experiments, in claiming that these two results are the same within the limits of experimental error.

Brown and Heise next turn their attention to the results embodied in Matthaei's Fig. 4. These are the results of eleven experiments with eight units of light at temperatures of 11° (one expt.), 25.4° (2 expts.), 32.1° (2 expts.), 38.3° (2 expts.), 40.9° (2 expts.), and 42.9° (2 expts.). With the exception of the two highest temperatures, over 40°, these results, like those of previous sets with less light, present an initial rising curve, followed by a flat top. The difference between the results at 11° and 25.4° here is too great to be attributed by Brown and Heise to experimental error, yet, as it is necessary

for their theory to throw doubt upon the result at 11° , they have recourse to another suggestion, which I hope to show is equally improbable. 'The difference (between the result at 11° and those at 25.4° and 32.1°) can readily be explained as due to differences in the seasonal activity of the leaves,' say Brown and Heise. Now the experiment at 11° was done on Mar. 4, the experiments at 25.4° on Jan. 30 and 31, and the experiments at 32.1° on Feb. 4 and 5. In discussing the change in the seasonal activity of the leaves, Matthaei, who along with Blackman and independently had worked with Cherry-laurel leaves for some years, states that the change in the seasonal activity of the leaves occurred comparatively suddenly at the beginning of April, with the onset of warmer weather, and thus long after Mar. 4. She found in 1901 and 1902 a high assimilatory activity through March, and from her experience she felt justified in regarding this experiment of early March as belonging to the same category, as regards assimilatory activity, as the experiments at the beginning of February. Against this testimony it will need more than the unsupported assertion of Brown and Heise, who are observers in a tropical climate and have never carried out a single experiment on a Cherry-laurel leaf, that they can readily explain the difference between the result at 11° (Mar. 4) and the result at 25.4° (Jan. 31) as due to difference in the seasonal activity of the leaves. We may conclude that these experiments agree with all the previous ones and establish that with sufficient light the values put forward as characteristic of -6° , 0.4° , 9.2° , and 11° are limited by the temperature and that the true effect of increase of temperature is a rapid rise in assimilation.

Finally, Brown and Heise consider the experiments at high temperatures with very intense light and with thermo-electric records of the internal temperature of the leaf. From these experiments Matthaei obtained a rapidly rising curve. As, however, in the first three experiments both light and temperature were increased, it was necessary for Matthaei's hypothesis that she should furnish evidence that the temperature and not the light was the controlling factor. This she did by comparing these experiments with those of a former series in which in each case a larger amount of assimilation was obtained with a less intense light. The inference was drawn that the lower values in the present series were due to the limiting effect of temperature. Brown and Heise's criticism is that Matthaei's comparison was made between two sets of leaves plucked at two different seasons and therefore differing in assimilatory activity. They say that the results in the second series are lower than would be expected from the temperature of these experiments, judging from the results of the first series, and that the only possible conclusion that can be drawn from a comparison of these experiments is that the leaves used in April (the second series) were less active than those used in March (the first series). Now I propose to show that while there is indeed an error in Matthaei's reasoning, yet we

are by no means confined to the barren conclusion suggested by Brown and Heise. It is obvious that the leaves in the second series were less active than those in the first series, and Matthaei pointed this out and was fully aware of it. This lessened activity implies that the less active leaf requires more light to perform the same assimilation as the more active leaf. Now, since more light is needed for the same assimilation, comparisons cannot safely be made between the more active and the less active leaves. Thus, when Matthaei says, as a result of the comparison between the less active leaf of Expt. 56 and the more active leaf of Expt. 37, that the former must be exposed to nearly twice the light necessary for the same assimilation in a normal leaf, we cannot accept her conclusion without further inquiry. For while the more active leaf performed 0.0072 assimilation with 8 units of light, the less active leaf would certainly require more light than this for the same assimilation, and we do not know exactly how much more. It is, indeed, probable that by increasing the light to 13 units (i. e. by 65 per cent.) a sufficient margin was provided, but it is not certain. It is a practical certainty that the margin was insufficient in Expt. 57, for the light used was more than three times that used in Expt. 38, and it could scarcely therefore have been limiting even for the more sluggish leaf. But even in the case of Expt. 56 it is possible to calculate approximately whether the light provided was in excess. If the experiments of Matthaei's Fig. 3, made in April with unit light, are compared with those of her Fig. 2, made in February with unit light, it is seen that on the average the activity of the more sluggish leaf is about 75 per cent. of that of the more active one, $\frac{0.0028}{0.0022} = 0.77$. It follows that if the more active leaves perform 0.0072 assimilation with 8 units of light the less active ones will require $\frac{4}{3} \times \frac{8}{1} = 10.7$ units for the same assimilation. Therefore 13 units of light is in excess and cannot be the factor limiting the assimilation of Expt. 56 to 0.00705. Thus Matthaei's assumption is justified.

A similar calculation will show that the margin in Expt. 57 compared with Expt. 38 is so large that full allowance can be made for the lessened activity of the leaves and yet the light must be in excess and therefore the temperature limiting. Though no exact calculation can be made for Expt. 58 compared with Expt. 50, yet the probability again is that the light is in excess. This is confirmed by the result of Expt. 59, in which, though the light is not increased, the assimilation (both the initial and the average values) is greater.

This increase is therefore obviously due to the rise in temperature, showing that at 30.5° C. the temperature was the limiting factor. In this case the proof does not depend upon the comparison of leaves of differing activities at all. This fact has been dealt with by Brown and Heise as follows:

'Expt. 59, at a temperature of 37.5° and light intensity of 45 units,

shows a marked increase in the rate of assimilation over that shown in Expt. 58 with the same light and a temperature of 30.5° . The rate of assimilation at 37.5° fell off very rapidly with successive readings. This shows that at this temperature there are complicating side-reactions of considerable magnitude, so that it is not to be expected that a photo-chemical ratio would hold. The importance of side-reactions will be shown in another connexion. Moreover, one experiment under extreme conditions cannot be regarded as reliable when we consider the magnitude of the experimental error with medium temperatures. For the above reasons we have thought it best not to attempt to draw any conclusion from the experiment at the temperature of 37.5° . It is interesting to note that Expts. 42 and 43 (Matthaei, Table VII), with light intensity of eight units and temperature of 38.3° , do not show the decrease in the rate of assimilation that is seen in Expt. 59.

Their first sentence recognizes a fact which they are quite unable to explain, as they will not admit the obvious solution that temperature, the only factor altered, was limiting in Expt. 58. The fact that assimilation fell with time in Expt. 59 makes their difficulty greater, for obviously it is the higher values which most nearly represent the true assimilatory activity. Hypothetical side-reactions, therefore, do not help them. Their statement about experimental error is incorrect. The difference of the first readings in Expts. 58 and 59 is 0.00080, i. e. five times the probable error, which has been shown to be ± 0.00016 , so that it cannot be held to be insignificant. Nor is this experiment an isolated one. It is part of a concordant series. Nor, indeed, is the temperature condition of 37.5 an 'extreme' condition. After casting up this cloud of unjustified innuendo the authors can only say that they find it best to leave the experiment out of account altogether.

The Work of Blackman and Matthaei.

Blackman and Matthaei (1905) have shown that the temperature-coefficient of *Helianthus* is even greater than that for Cherry-laurel and is probably about 2.5. They base their temperature-curve upon the results of four experiments, in each of which definite proof is given that the temperature was the controlling factor. Brown and Heise's comment on these four experiments is as follows: 'It is not evident why the changes in the rate of assimilation cannot be explained as due to variation in light intensity, especially since it is evident from their individual experiments that fluctuations in light intensity are accompanied by marked changes in assimilation.' It will scarcely be believed, after this comment, that in each of the four experiments quoted Blackman and Matthaei gave specific proof that increase of light did not increase the assimilation at that temperature. Since, however, this does not seem to have been made clear to Brown and Heise, it will be necessary to study each of the experiments mentioned.

Take first Blackman and Matthaei's Expt. X. The experimenters' statement is as follows :

' For the first four readings the temperature was kept down to about 18° , and again in the last two the temperature was the same. In all these readings except the first, which was low, due to the extremely overcast leaden sky, the assimilation numbers are remarkably uniform, 0.0089, 0.0090, 0.0089, 0.0089, 0.0092 ; while the light varied up and down, being especially brighter in the last two readings. This can only be interpreted as being due to the fact that the assimilation is limited by the temperature, which has been kept steady throughout. Striking confirmation of this is obtained by raising the temperature for the fifth reading. *The sky was no lighter than before*, but yet, on the temperature being brought up to 30.5° , the assimilation at once doubled, becoming 0.0163.'

In Expt. XI it is shown that the same assimilation value is obtained at the sixth reading as at the first, although the light was much brighter at the sixth reading. This can only be due to the controlling influence of temperature, as claimed by Blackman and Matthaei.

In Expt. XVI also, two readings were obtained at a temperature of 30° , in the second of which a considerable proportion of the illumination was cut off by a wooden tube, yet the assimilation did not fall, proving that temperature and not light was the limiting factor.

When there are provided these definite proofs in each experiment that the temperature was the controlling factor, of what scientific avail is Brown and Heise's statement that they would prefer to attribute all the changes to variations in illumination ?

In their final discussion of the temperature-coefficient of assimilation Brown and Heise have attempted to show that its value is 1.04 ± 0.03 , i. e. that assimilation is practically unaffected by temperature. They claim that this value is supported by the results of the investigators named in their Table I.

<i>Name of Investigator.</i>	<i>Name of Plant used.</i>	<i>Coefficient calculated by Brown and Heise.</i>
Matthaei	Cherry-laurel	1.0 +
Prjanischnikow	<i>Typha</i>	1.0 +
Kreusler	<i>Rubus</i>	1.16
Blackman and Smith	<i>Elodea</i>	1.35
van Amstel	<i>Elodea</i>	1.26

The results of all these workers have now been reviewed in this paper with the exception of those of Prjanischnikow (1876), which are not sufficiently accurate in method to merit discussion in this connexion, and it has been shown that the only accurate and trustworthy values for the coefficient are those of Matthaei on Cherry-laurel, which is 2.1 ; of Blackman and Smith on *Elodea*, which is 2.05 ; and of Blackman and Matthaei on *Helianthus*, which is not definitely given, but is probably about 2.5. All

these values are in agreement with the van't Hoff rule for 'dark' reactions and are much higher than photochemical coefficients. Recently Osterhout and Haas (1919) have obtained a coefficient of 1.81 for *Ulva*, which is nearer to the values characteristic of 'dark' reactions than to those usual in 'light' reactions. Even this figure, however, is probably not high enough, for in the very brief details given of their experiments in the low CO₂ tension of sea-water they provide no evidence against the natural view that low CO₂ tension, based on slow dissociation, and diffusion were limiting their observed value at 37° to something lower than would have been obtained had temperature been exerting its maximum effect.

It was stated in the introduction to this paper that carbon assimilation is a complex process involving both 'light' and 'dark' reactions, and that the 'dark' reactions govern the maximal possibilities of the system. The survey since made of the work done on this problem has shown that the reliable experimental evidence is all in favour of the idea that the velocity of the whole process is determined by some reaction having a high temperature-coefficient, and not a low coefficient such as is characteristic of pure photochemical reactions.

ASSIMILATION AND LIGHT INTENSITY.

In their second paper Brown and Heise (1917 *b*) maintain that the relation between light and assimilation is not one of direct proportionality, as has been held broadly by Blackman and his co-workers. They state that the relation is such that from each increase in light intensity there results a progressively smaller increase in the velocity of assimilation. This contention is based upon the experiments of Matthaei (1904), Pantanelli (1903), Reinke (1883), and Timiriazeff (1889). Brown and Heise themselves state that Timiriazeff's results are based upon faulty experimentation. They do not, therefore, put these results forward as independent evidence for their thesis. There is thus no need for any discussion of Timiriazeff's results here. The results of the other workers as interpreted by Brown and Heise need, however, some consideration.

The Work of Matthaei on Cherry-laurel.

In Fig. 3 of their first paper Brown and Heise have drawn a curve supposed to represent the relation of light intensity and carbon dioxide assimilation in Cherry-laurel based on Matthaei's data. It is clear that such a curve ought to consist of the results of experiments in which light has been definitely proved to be the limiting factor. Now of the ten values which make up this curve it can be asserted of only three that there is proof that they are light-limited values obtained from normal leaves. The value 0.0022 with unit light is light-limited, as is proved by increasing the light to two units, resulting in a large increase of assimilation at the same temperatures. The value 0.0038 with two units of light is light-limited, as is shown

by the fact that an increase of light to four units increases the assimilation at the same temperatures. The value 0.0061 at 25° and L. In. = 4 is light-limited, as it is much lower than is obtained at the same temperature with greatly increased light (cf. assimilation 0.0101 at 23.7° and L. In. = 26, Expt. LVII) from leaves at the same season of the year. On the other hand, 0.00365 at 9.2° and L. In. = 4 is quite clearly limited by the temperature, since all the remaining values for the same light at higher temperatures are considerably greater. The value 0.00485 is obtained from an abnormal leaf (see previous argument, p. 525). The value 0.00465 at 11.4° is limited by temperature, since an increase to temp. 25.2° is followed by a considerable increase of assimilation at the same illumination. The value 0.00505 with L. In. = 6 and temp. 11.4° is limited by the temperature, as it lies, within the limits of probable error, upon the temperature-curve. The value 0.0063 at 25.2° does not exist. It is an erroneous addition on the part of Matthaei to her summary table and does not appear in her full tables or in her curve.

The remaining values, 0.0070 at 15° and L. In. = 13; 0.0101 at 23.7° and L. In. = 26; and 0.00136 at 30.5° and L. In. = 45, are all values limited by the temperature for reasons already fully set forth. Thus Brown and Heise's curve is partly a light-curve, but principally a temperature-curve, and therefore does not show the relation between light intensity and assimilation as asserted by them.

The Work of Pantanelli.

In the paper by Blackman and Smith (1911) it was shown that Pantanelli's curve was of the usual compound nature, only the first part showing a true light effect, the later part showing probably the effect of limiting CO_2 supply or possibly of temperature. Brown and Heise reject this explanation of Pantanelli's curve and draw the curve again in such a way that they can interpret it as giving support to their thesis. Now in their exposition, in addition to minor matters of questionable soundness which are here omitted for lack of space, Brown and Heise have arbitrarily omitted from their curves all values above unit light. Yet Pantanelli conducted experiments at light intensities of 4, 9, 16, 25, 36, 49, and 64 units. It has been shown in our paper that while the values obtained in the highest intensities of light are justly omitted from the curve, giving smaller values, attributed to 'fatigue' of the chloroplast, yet the values at 4 and 9 units form an important part of the whole evidence. If it be asked why Brown and Heise have omitted, without any explanation or reference to the fact, all the results in light intensities greater than unity, the only answer at all possible is that these results are entirely fatal to their theory. Whether the stationary results at medium intensities of light or the depressed results at higher intensities are considered, there is here a long range of experiments in which the assimilation *does not increase at all* and the values cannot be

represented by a rising curve of any kind, logarithmic or other, but simply by a straight horizontal line or indeed later a falling curve. Just as Brown and Heise, in the interests of their *a priori* theory, ignored the low temperature results of Matthaei, so here, and for the same reason, they ignore the results of Pantanelli at the higher intensities of light.

The Work of Reinke.

Brown and Heise have used Expt. VIII of Reinke as the chief basis of the argument in which they attempt to obtain support for their theory from Reinke's work. The curve of Expt. VIII, however, can be interpreted, with greater probability, as one which shows the action of two limiting

FIG. 2. Reinke's results. Curve A. Average of all results in Tables I to VIII. B. Average of all results except those obtained after the plant had been once exposed to 16 units of light. Reading 60? with unit light in Expt. VI omitted from both curves.

factors, first the light and later the CO_2 supply. But Expt. VIII does not show this so conclusively as do some of the other experiments, notably Expts. I, III, and VIII. In order to avoid all theoretical bias in the interpretation of Reinke's experiments, Fig. 2, Curve A, has been drawn showing the average of *all* the experiments made by Reinke with his first arrangement of lighting, i.e. all the results shown in his Tables I to VIII. There can be no doubt that the curve obtained is one of the familiar type showing the action first of light as the limiting factor and later of CO_2 supply. Already at unit light the assimilation reaches its maximum and remains sensibly at that figure up to an intensity of 16 times sunlight.

Brown and Heise's Table II gives the results for all Reinke's experiments, and they say, 'These results are in agreement with the more detailed calculations given in Table I for Reinke's Table VIII'. As a matter of fact, the results in their Table II do *not* support their hypothesis. In Expts. I, III, and VII no increase is shown between unit sunlight and 16

units. In fact the only figures which give their hypothesis a semblance of support are those in Expts. IV, V, and the later portions of VIII. It will be noticed that in these cases the plant has been exposed for some time to high intensities of light, and that on being taken back into lower intensities its curve is slightly different from that of a fresh plant. It seems clear that the plant has been to some extent injuriously affected by the intense light. This supposition is supported by the fact that in Expt. VIII, where the plant is twice exposed to a light intensity of 16 units, the bubble emission gradually falls off through the experiment, so that the last row of figures is throughout the smallest of the whole series. In order to avoid this source of error Curve B, Fig. 2, has been drawn, representing the readings after ignoring all those taken after the plant has been once exposed to 16 units of light. This curve is a typical limiting factor curve and cannot possibly be interpreted as supporting the hypothesis of Brown and Heise. It is significant of the small amount of support which Brown and Heise can obtain from any of the experiments, that Curve B is scarcely more conclusively in favour of the interpretation of Blackman and Smith than is Curve A, obtained from all Reinke's readings whatsoever. In fact Brown and Heise practically admit this when they say, 'This progressive falling off in assimilation per unit of increase of light is very rapid, and it is, therefore, not surprising that with high light intensities increasing the intensity does not greatly augment the rate of assimilation', and later, 'if a direct proportionality is found in such a case (i.e. in low intensities of light) this is due to the selection of a particular range of light intensities'. In the first of these sentences they admit the fact that at higher intensities increase of light does not increase assimilation, and in the second that at the lower intensities the assimilation is proportional to the light. It only remains for it to be noted that the latter is true up to half sunlight and the former from unit sunlight to 16 units in order to make it clear that the hypothesis of two limiting factors is correct, on Brown and Heise's own admission, through the whole range of intensities used in Reinke's experiments.

Recently Boyson-Jensen (1918) has given curves showing the effect of light upon assimilation. As he points out, these curves are of a dual nature, showing first the limiting effect of light and later that of CO₂ supply. Owing, however, to the fact that individual variations were not eliminated, the results are not sufficiently exact to form a decisive test between the interpretation of Brown and Heise and that of Blackman.

ASSIMILATION AND LIMITING FACTORS.

In a third paper W. H. Brown (1918) has made an attempt to prove that the results of Blackman and Smith (1911) on *Elodea* and *Fontinalis* can be more accurately represented by a so-called optimum curve than by the

limiting factor curve drawn in that paper. A critical glance at Brown's Fig. 1 will show that the so-called improvement in presentation put forward by Brown is merely a matter of drawing a line arbitrarily between the various experimental values, so as to produce an illusory effect of convex curvature, rather than horizontality. His proposed correction of the latter part of the curve by allowing for the temperature-coefficient is quite illegitimate, for convincing evidence was given that both the values to which Brown has applied this correction were already much below the maximal values for those temperatures. The values were, in fact, kept low by the controlling influence of some other factor, presumably light. As, therefore, they are not temperature-values at all, they cannot be subjected to a correction for temperature-coefficient. Apart from this proposed change, the difference between Brown's curve and that put forward by Blackman and Smith is insignificant. The attempt made by Brown to separate the earliest part of the curve into two individual parts is seen, when closely examined, to involve him in inconsistencies. It is not proposed, however, here to follow him into these details of interpretation, for it is not upon such details as these that the validity of the theory of limiting factors as applied to carbon assimilation depends. This theory does not depend upon any single curve, nor can it be overthrown by suggesting, as Brown has done, minor differences in the interpretation of any single curve. Thus Matthaei's supposition that in her earliest curve light was the limiting factor at all temperatures higher than 3° , did not become established from a consideration of the form of that particular curve, but from the fact that by subsequent increases of the intensity of the light she obtained *a whole series* of similar inflected curves, each one higher than the last in correspondence with the increased light. In a similar way, though not so completely, Blackman and Smith, in the paper under discussion, showed that a light of 6.0 units was limiting the assimilation in their general curve, because raising the light from 4.2 to about 6.0 units caused an increase in the rate of assimilation.

The revival by Brown of the hypothesis that there exists an 'optimum concentration of carbon dioxide', which is indicated by his drawing of Blackman and Smith's curve, has surely very little value in the light of the experimental proof that the assimilation corresponding to that optimum can be varied as desired by changing the intensity of the light used in the experiments. When such a series of curves is obtained, as was obtained by Matthaei for Cherry-laurel in increasing intensities of light, any one of these curves may perhaps be of the form preferred by Brown, i.e. such as he would call an optimum curve. But how can all the curves be optimum curves, and what is the value of the idea of an optimum which changes with every change of one of the factors conditioning the experiment?

It is not claimed that a limiting factor curve always adheres rigidly to a typical form with a sharp angle at the point of change of the limiting factor.

It is conceivable, and is indeed probable, that when, so to speak, two factors are close to the limiting value a change in the one not limiting may have some appreciable effect on assimilation. This will show itself about the inflexion of the curve where the limiting factor is changing. For example, when CO_2 is limiting, increase of temperature may cause a small increase of assimilation by increasing the rate of diffusion of the CO_2 . But all minor details like these apart, the hypothesis of limiting factors rests broadly on the possibility by its means of interpreting simply and logically the greatest number of the known facts about the rate of carbon assimilation.

Finally, Brown reveals his singular lack of appreciation of the complexity of the system involved in assimilation when he asks how such a principle as that of limiting factors can hold in the phenomena of assimilation when he can see no sign of this principle at work in the action of hydrochloric acid upon marble or the solution of a gas in water.

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