P^{32} labeled compounds of the xylem exudate. Only radioactive inorganic sulfate was present on chromatograms of the exudate collected over 24 hours. The S^{35} inorganic sulfate in the exudate fluid was isolated chromatographically as easily as the phosphate, but no detailed study of time versus amount of S^{35} in the exudate has been run.

Summary

The xylem exudate of several species of plants contained three P^{32} labeled compounds—inorganic phosphate, and two unknown phosphorus containing compounds. As much as 20 % of the total radioactive phosphorus has been observed in the unknown compounds from the exudate on decapitated tomato stems. Sulfur was present only as inorganic sulfate.

The two unknown phosphorus-containing compounds are normal constituents of the xylem sap, but they have not been detected in the exudate from squash peduncle. The unknown present in largest amounts in the exudate from decapitated plants was also found in barley guttate. The two unknowns are normal constituents of the xylem exudate rather than wound compounds on the cut surface of the stem or leaf base or products formed by radiation injury from the P^{32}.

The percentages of P^{32} in the unknowns reached a maximum value about 4 hours after the addition of P^{32} to the nutrient solution bathing the roots. This time lag was independent of when the plant was decapitated to collect exudate. It is interpreted as indicative of the rate of incorporation of phosphate into these compounds.

Both the seeds of seedlings and the roots of older plants contributed to the formation of the two unknown phosphorus-containing substances. Removal of the seed from young seedlings severely reduced the amount of these compounds present. The amount of the unknowns in the xylem exudate decreased rapidly with age of the barley seedling, but remained present in considerable quantity in older plants.

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TRANSIENT CHANGES IN CELLULAR GAS EXCHANGE AND THE PROBLEM OF MAXIMUM EFFICIENCY OF PHOTOSYNTHESIS[1,2]

ROBERT EMERSON and RUTH CHALMERS

Botany Department, University of Illinois, Urbana, Illinois

I. Formulation of Issues

Since 1938 different investigators have reached very divergent conclusions regarding the maximum efficiency of photosynthesis, in spite of the fact that most of the calculations of efficiency have been made from measurements of the rate of photosynthesis of the green alga, Chlorella. For example, Rieke (25), Emerson and Lewis (11), and Daniels with various co-workers (15) found that the efficiency of photosynthesis did not exceed a value of about 30 %, while Warburg (30) with various co-workers (6, 7, 31, 34) has published a series of papers in which he reports efficiencies of 70 % and upwards to 100 %. We refer here to measurements in the red region of the spectrum where the carotenoid pigments do not contribute to light absorption. This region, near the long-wave limit of light absorption by chlorophyll, should favor the attainment of maximum possible efficiency, if the quantum yield is independent of wave length.

From published discussions of the subject, it seems clear that the great differences in calculated efficiency are due primarily to differences in the estimation of the rate of photosynthesis, rather than to disagreement as to the absorbed light energy. Questions have arisen concerning the application of certain specialized techniques for measuring the rate of photosynthesis. Most of the rates from which efficiencies of 70 % and higher have been calculated, are derived from manometric measurements of pressure changes in the gas space above aqueous suspensions of cells. In general these measurements have been made with cell suspensions dense enough to absorb practically all the energy of the incident beam of measured light.

There are some reports (Warburg, 30; Damaschke et al, 8) of confirmatory results from measurements with thin suspensions of cells, by both manometric and electrometric methods. We shall not discuss these

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measurements in the present communication, because they raise issues different from those connected with the measurements with dense suspensions and short periods of exposure to measured radiation. Since these latter measurements constitute the principal foundation for the claims of very high efficiencies, it seems appropriate to give them primary consideration.

Disagreement concerning the significance of manometric measurements with dense suspensions of cells stems from disagreement as to the proper allowance for time delay or lag between changes in rate of metabolic gas exchange inside the cells, and the attainment of corresponding rate changes in the observable motion of the manometric fluid, from which the rate of photosynthesis is calculated. Changes in rate are necessarily involved, because the rate of pressure change attributable to photosynthesis is not directly observable but must be derived from the difference between two observed rates, one when the cells are exposed to a light beam of measured intensity, and the other when the cells are darkened. In principle it is of no consequence whether the cells are darkened between exposures to the measured light, or whether the measured beam is superimposed from time to time upon a background of continuous illumination from some other source (a practice often followed by Warburg and co-workers in their measurements of efficiency). In either case the rate of pressure change attributable to the beam of measured light must be derived from the difference between two observed rates, so there must be a change from one rate to another between the two observations.

If rates of photosynthesis are calculated from rates of pressure change observed in close succession after changes in illumination, neglect of the effects of time lag can lead to error in the calculated rates of photosynthesis, and hence to error in the estimation of efficiency.

Warburg and co-workers have expressed the opinion that in their application of the manometric technique, time lag between changes in rate was of negligible importance. Their published values for the efficiency of photosynthesis are derived from rates of pressure change in close succession after changes in illumination, at intervals sometimes as short as one minute (34), without any allowance whatever for time lag.

Others who use the manometric technique for studying photosynthesis have observed time lags of several minutes between changes in illumination and the approximate attainment of new steady rates of pressure change (19). The apparent absence of time lag reported by Warburg and co-workers therefore calls for explanation. One possibility is that the frequency and amplitude of shaking which they specify are sufficient to make the approach to equilibration between gas and fluid space so rapid that time lag is, as they suppose, of negligible importance. An alternative explanation is that the time lag in their manometer system is much the same as is commonly observed in other laboratories, but that under the conditions of their experiments, compensatory processes obscure the effects of time lag. Our first objective is to present experimental evidence showing that under conditions closely approximating those specified by Warburg and co-workers, time lag is appreciable, and follows a course which would be expected if it were due primarily to diffusion between liquid and gas space. Absence of apparent evidence of time lag is therefore to be regarded as evidence of compensatory processes, and should not be interpreted as indicating that the manometric pressure changes respond without time lag to changes in metabolic rates.

This makes it necessary to consider the nature of the errors which would be introduced by neglecting the effects of time lag due to diffusion. In the case of measurements of rate of exchange of a single gas, calculated from pressure changes in a single manometer vessel, the neglect of diffusion lag would ordinarily result in underestimation of the magnitude of any change in rate, and hence in underestimation of the rate of photosynthesis. Roughton (26) has shown that by application of a simple correction for the effect of diffusion, it is possible to calculate the actual rate of gas production, even during the lag period. But Warburg and co-workers have made their measurements by the so-called two-vessel method, in order to follow the rates of production or consumption of two gases, oxygen and carbon dioxide. In the case of two-vessel measurements, the effects of diffusion lag are more complex. The technique calls for the measurement of two rates of pressure change, in two reaction vessels containing equal samples of cell suspension. It remains true that the neglect of time lag will generally result in underestimation of the magnitude of changes in rates of pressure change in each reaction vessel, but in the two-vessel measurements the rates of oxygen and carbon dioxide production are functions of the ratio of the rates of pressure change in the two vessels, so the effect of diffusion lag upon calculated rates of gas production will depend upon its effect on this ratio. (We shall use the term "production" to cover both production and consumption, since consumption may be regarded as negative production.) The application of the two-vessel method presupposes not only that the metabolic rates of gas production remain equal in the two vessels at all times, but also that during periods of time lag the rates of diffusion of gas between liquid and gas phase remain equal in the two vessels. These requirements must be fulfilled with respect to both oxygen and carbon dioxide. If diffusion lag is not equal in the two vessels, then neglect of its effects could result in either under- or overestimation of the rates of gas production.

The problem will be further complicated if at the times of change in illumination the rate of metabolic gas production does not come immediately to its new value, but undergoes some transient changes before it reaches a steady value. This will lengthen the period during which errors may arise from differences in diffusion lag in the two vessels.

We have mentioned above that we shall present evidence (Part III) which we interpret as indicating

that there are compensatory processes which can obscure the effects of diffusion lag. These compensatory processes bring about transient changes in rates of gas production. We shall also present evidence (Part IV) that in vessel pairs like those used by Warburg and co-workers there are sometimes substantial differences in diffusion lag. Therefore the contingencies discussed in the preceding paragraphs may actually be encountered, and are not mere hypothetical possibilities.

In their publications, Warburg and co-workers have not dealt with these contingencies. Doubtless they thought it unnecessary because in their experiments they noticed no evidence of time lag. But our evidence that time lag is nevertheless a significant factor under the conditions of their measurements leads inevitably to the conclusion that the next step toward assessing the significance of their measurements of efficiency of photosynthesis is the comparison of diffusion lag in vessel pairs such as they describe. We shall present measurements which indicate that equality of diffusion lag cannot be assumed for such vessel pairs, but that by modifying the shape of one of the members of the pair it is possible to obtain pairs which show equality of diffusion lag with respect to one gas (oxygen) within the limits of sensitivity of the method. The establishment of equality of diffusion lag with respect to one gas is an improvement over the uncertainty which prevails in the case of the measurements of Warburg and co-workers, where it is probable that diffusion lag was unequal with respect to both gases. However, equality of diffusion lag with respect to one gas implies, as we shall explain in Part IV, inequality with respect to the other. This also must be taken into consideration in the application of the method.

It is appropriate to mention that Warburg et al (35) have recently noticed the effects of time lag in manometric measurements of photosynthesis, and have reported making allowances for these effects. They report no change in their conclusions concerning the efficiency of photosynthesis. The reader might infer from this that our worries about possible errors from time lag in earlier experiments are groundless, and that since Warburg has now begun to make allowance for the effects of time lag, the work which we report here might now be regarded as superfluous. To any reader who may be inclined to adopt this viewpoint, we would reply that the experiments in which no allowance was made for time lag remain the principal foundation of Warburg's fundamental claims regarding the efficiency of photosynthesis. Warburg and co-workers have yet to publish any measurements of time lag which would provide a basis for deciding whether the allowance they now propose to make for it is adequate. They have yet to recognize the tendency of transient metabolic processes to prolong the period of diffusion lag; and their two-vessel measurements are still made with vessel pairs for which there are no published data on equality of diffusion lag. Under these circumstances, discussion of the issues we have raised in this communication is not superfluous.

II. Experimental Methods

Since our ultimate purpose is to assess the significance of Warburg's conclusions derived from two-vessel measurements of the rate of photosynthesis, we have followed his specifications in many respects. However, in order to obtain greater precision of measurements of pressure change, it has also been necessary to introduce some changes.

We have followed the recent practice of Warburg and co-workers, and used rectangular reaction vessels shaken with a horizontal linear motion, instead of the larger circular vessels shaken with rotary motion which had been used by Warburg and Negelein (36) and also by Emerson and Lewis (10, 11). The combination of rectangular vessel shape and horizontal shaking leads to less foaming of the cell suspension than the rotary shaking in circular vessels, and also makes it easier to shake two vessels simultaneously with identical motion.

Warburg's two-vessel measurements of photosynthesis have been made with the open, constant-volume type of manometer. Three are required for an experiment—two for the two samples of cell suspension, and a third to correct for changes in barometric pressure. Two visual estimations of fluid level enter into the reading of each manometer; one for the pinch-cock adjustment to constant volume, and one for the pressure reading itself. Since the barometric pressure correction contributes to each measurement of metabolic pressure change, four visual estimations of fluid level enter into each measurement. All these estimations are made while the manometers are in rapid motion. Even with the aid of a hand lens, a precision of $\pm$ 0.5 mm is the utmost that can be expected. If the pressure changes are 5 mm or less, the random errors of $\pm$ 0.5 mm are important. In the experiments of Warburg and co-workers, many rates of photosynthesis are derived from pressure changes of about 3 mm. A change read as 3.0 mm might be anything from 2.5 to 3.5 mm. Here the range of uncertainty is 30 %. Nishimura et al (20) have given examples of the errors that can arise in the computation of rates from two-vessel measurements, through reading errors which would be of only minor importance in single-vessel measurements. Pirson et al (24) have given a more general and extensive discussion of random errors in the two-vessel method, from which it may be seen that an error of 30 % in one of the two rates of pressure change can lead to an error of several fold in the rate of gas production.

Greater precision is attainable by reading the manometers with a cathetometer (horizontal telemicroscope with cross-hairs and screw adjustment for height, and scale divided into hundredths of a mm), but the constant-volume type of manometer does not lend itself to reading by cathetometer because a barometric pressure correction must be read in addition to the pressure changes for the two cell samples, and because of the pinchcock adjustment for constant volume. In order to avail ourselves of the greater precision of the

cathetometer, we have substituted differential manometers for the constant-volume type used by Warburg. The differential manometer is a closed system, and requires no control for barometric pressure changes nor adjustment to constant volume. For two-vessel measurements only two manometers are required, each with a compensation vessel and a vessel with cell suspension. One manometer with its two vessels is illustrated in figure 1.

The manometers were of uniform-bore pyrex capillary with an area of cross section of 0.426 mm^2.

One might suppose that a double cathetometer unit (two telemicroscopes) would suffice for reading two differential manometers. In the case of each manometer, if the fluid level rises on one side it should fall an equal amount on the other, so a single reading should suffice to establish the change in pressure. However,

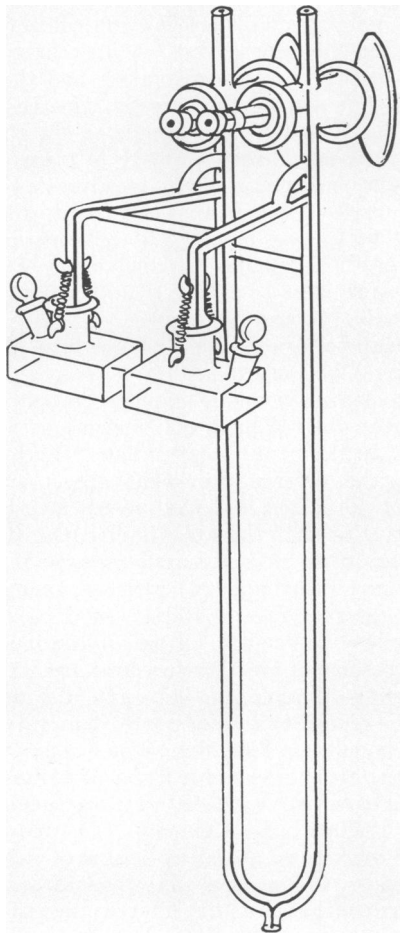


FIG. 1. Differential manometer, with vessels of the shape used for measuring H. One vessel is used as a compensating vessel, with suspending fluid but no cells. The other vessel is for the experimental material. For two-vessel measurements, a second manometer is required, with a pair of vessels larger than the pair shown here. The shapes of the two types of larger vessels used are shown in figure 2 (shapes B and C).

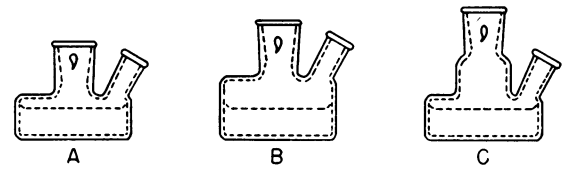


FIG. 2. Vessel shapes used for two-vessel measurements. Vessels of shape A were used for measuring H. For measuring h, Warburg and co-workers used vessels of B shape (like A except deeper). We have used vessels of C shape for measuring h. The depth is the same as for the A vessels, except for the central tower.

the ascending meniscus does not always travel the same distance as the descending one, because on the descending side manometer fluid is left on the wall of the capillary tube, while on the ascending side the meniscus may be moving up into dry tubing, or may be adding to its level by picking up fluid left on the wall during a previous descent. The simultaneous reading of both meniscus levels minimizes (but does not eliminate) errors arising from the wetting of the capillary walls by the manometer fluid. A quadruple unit is therefore necessary for reading two differential manometers. The simultaneous setting of all four columns by four observers presented too many difficulties, and we found that with practice, two observers could set all four columns within four seconds, and repeat the process at intervals of one minute, for many minutes. The four-second interval between settings introduces no error when the rates of pressure change are constant. There is, of course, a small error when the rates change between one set of readings and the next. We have not attempted to make a correction for this error, which is small compared with random errors. The four cathetometer settings within four seconds are more nearly simultaneous than naked-eye readings of three constant-volume type manometers, an operation which often takes 20 seconds.

Although the cathetometers are read to the nearest hundredth of a mm, the precision is not as great as this. Besides the errors introduced by wetting of the capillary walls, there are errors due to back-lash in the cathetometer screws, and the presence of the observers causes noticeable effects on the apparent meniscus levels, probably through warming of the metal parts of the cathetometer. Systematic attention to minor details does not seem to improve the precision beyond about $\pm$ 0.03 mm. However, this represents an improvement of more than ten-fold in the precision attainable with the constant-volume manometers.

We have compared pairs of reaction vessels similar to the ones described by Warburg and co-workers and also other patterns. Figure 2 shows the three shapes with which we have done most of our work. Shapes A and B represent the pair described by Warburg and Burk. These two vessels are supposed to differ in depth only. We have found that all hand-formed rectangular vessels of this type differed in other dimensions as well as depth. In agreement with Nishimura

et al (20, pp. 200–201, table 5) we always found significant differences in diffusion lag in such vessel pairs, but, because of the differences in other dimensions as well as depth, it was not possible to be sure whether the observed differences in lag were characteristic of differences in depth. Recently we have obtained (from Fischer and Porter Co.) vessels made to within 0.1 mm of specified dimensions, from pre-formed rectangular pyrex tubing. With such vessels we have been able to compare diffusion lag in pairs which differed in depth alone, and these tests, described in Part IV, have been the basis of our conclusions regarding the comparability of diffusion lag in vessel pairs of shapes A and B.

The shape C shown in figure 2 represents the kind of vessel suggested by Nishimura et al (20, pp. 189, 206), as a substitute for Warburg's deep vessel. The space in which the cell suspension circulates is supposed to be the same as in the A shape, the required difference in volume being obtained by adding the central tower, instead of increasing the depth over the entire area. Nishimura et al (20) thought that with an A-C pair differences in diffusion lag would be less likely than with an A-B pair.

We are now able to obtain C-shape vessels made to accurate dimensions from rectangular tubing. However, all experiments with C-shape vessels reported in this paper were done with hand-formed vessels, matched with hand-formed vessels of the A shape, since precision-formed vessels of the C pattern did not become available until the work was nearly completed.

Since our purpose was to study the effects of diffusion lag under conditions approximating those prevailing in the measurements of Warburg and coworkers, we adjusted conditions to be at least as favorable to diffusion of gas between liquid and gas phase as in their experiments. Diffusion rate is a function of the area of liquid surface, the liquid volume, the irregularities in the internal shape of the vessels, and the amplitude and frequency of the shaking motion. Warburg et al (33, pp. 337–338) describe their vessels as about 22×38 mm in internal width and length, and having volumes of either 13 to 14 ml or 18 to 19 ml, depending upon depth. In most of the experiments which they report with such vessels, they used 7 ml of cell suspension. Our hand-formed vessels of shapes A and C were of comparable length and width. Their volumes were 14.9 or 18.4 ml, depending on whether the central tower was present. In such vessels we also used 7 ml of suspension.

For our tests of equality of diffusion lag in vessel pairs like the A-B combination, we used the vessels made from precision-formed rectangular tubing, accurate to 0.1 mm in all internal dimensions, in order to exclude the effects of all differences except depth. These vessels were 26×40 mm in internal width and length, thus providing a considerably larger surface area than the vessels described by Warburg and coworkers. The volumes were 14.7 ml or 22.1 ml, depending on whether the internal depth was 13 or 20 mm. In such vessels we used either 7 or 8 ml of suspension. The 8 ml filling still gives a more favorable surface-volume ratio than the 7 ml filling in a vessel 22×38 mm (dimensions specified by Warburg), so that with the 8 ml filling our vessels should still provide more favorable diffusion conditions than his.

In different publications Warburg and co-workers have specified shaking amplitudes of from 1.7 to 2 cm and frequencies of from 140 to 200 per min (for example, Warburg et al, 33, p. 337, 140–180 per min; Burk and Warburg, 7, p. 14, 200 per min). Throughout our work we have used amplitudes of 1.8 cm and a frequency of 200. Higher speeds or larger amplitudes result in occasional splashing of the liquid into the entrance of the capillary tube connecting manometer with reaction vessel, with consequent interruption in the sequence of readings.

From 200 to 300 μl of cells were used in each vessel. This amount of cells gives total absorption of a beam of red light ($\lambda = 644$ mμ) in all three vessel shapes when they are at rest. A little light is transmitted when the vessels are shaken, and the amount transmitted is appreciably greater for the B shape than for the A or C, which appear to absorb equally. However, the eye is very sensitive to traces of transmitted light, and comparison of steady rates of photosynthesis in the different vessels led us to the conclusion that with these densities of cell suspension, the fraction of the light absorbed in vessels of all three shapes was so close to 100 % that the differences detected by the eye were negligible.

Although under conditions of near-total absorption the difference between the A and B vessel shapes with respect to light absorption may be negligible, this is not the case with thinner cell suspensions which absorb 50 % or less of the incident light. With thin suspensions, the difference in light absorption during shaking of the A and B shaped vessels may be significant, and as we shall show in a later paper, this difference is probably a contributory source of error in experiments of Warburg (30) with suspensions so thin that the rates of photosynthesis could be computed from steady-state pressure changes in continuous light. These experiments are therefore free from the errors arising from diffusion lag, but again because of the sensitivity of the two-vessel method to small errors, a small inequality in light absorption can lead to a relatively large error in computed rate of photosynthesis.

The light source was a 450 watt mercury-cadmium arc from Phillips Lamp Company. The red cadmium line (644 mμ) was isolated by means of a red-transmitting glass and a heat absorbing glass. Of the energy transmitted by this filter combination, 83 % was found to be in the 644 mμ cadmium line while 17 % was of longer wave lengths, including the mercury line at 691 mμ, with some continuous background radiation from the faint glow of the quartz envelop of the arc, and from the hot electrodes. Most of the 17 % beyond 644 mμ was of wave lengths longer than 700 mμ, chiefly from cadmium and mercury lines between 700 and 1000 mμ. By using a glass which absorbed the 644 mμ line and transmitted the lines of

longer wave length, we were able to show that all the combined energy of wave lengths longer than 644 $m\mu$ produced no detectable photosynthesis. Therefore in calculating efficiencies we considered only the fraction of the energy from the 644 $m\mu$ line (83 %) was available for photosynthesis. All energy measurements were made bolometrically, with a large-surface platinum bolometer, calibrated against a radiation standard from the U. S. Bureau of Standards. For calibration, a fluorite window was used. This window was tested by the Bureau of Standards and found to transmit 92 % of the energy emitted by the radiation standard (reflection losses only).

The beams of light incident upon the vessels containing the cell suspensions provided about 1.5 μ einsteins of energy per minute at the 644 $m\mu$ wave length, for each vessel. A thermocouple was used to monitor the lamp output at this wave length during the photosynthesis measurements.

Twin beams were taken from opposite sides of the same lamp, by the optical system shown in Nishimura et al (20, p. 191, fig 4). Duplicate systems of prisms and lenses projected rectangular light spots on the bottoms of the two reaction vessels containing the cell suspensions. The size of the light spots and the length of the shaking path of the vessels were adjusted so that the light spots were always completely intercepted by the suspensions. The illuminated areas were 20×20 mm. Vessels 40 mm long could be shaken over an 18 mm path, and still intercept the entire light beams during shaking.

The light beams were adjusted to give equal absorption of energy for photosynthesis in the pair of vessels to be used for the two-vessel measurements. Hand-formed reaction vessels scatter, reflect, and refract different fractions of the incident beams, so equality of absorbed energy could not be assured simply by making the incident beams equal. It was necessary to adjust the intensities for each vessel pair used. Equality of photosynthesis was the criterion for equality of energy absorption. A suspension of cells was prepared in carbonate-bicarbonate buffer #9, and 7 or 8 ml were pipetted into each vessel. The two vessels were then shaken in a thermostat, and pressure changes were observed. The two light beams thus adjusted to give equal photosynthesis in the two vessels, differed according to bolometric measurements by about 2 %, for some of the pairs of hand-formed vessels.

Since the two equal samples of cells may be assumed to respire at equal rates in darkness, one should expect that in darkness the pressure changes in the two vessels would be inversely proportional to the vessel constants for oxygen (the pressure changes in carbonate buffer experiments being attributed to oxygen exchange alone). Therefore, in order to assure equality of photosynthesis in the two vessels, it should only be necessary to adjust the intensity of the two light beams so that in light also, pairs of pressure increments were inversely proportional to the vessel constants. If we designate the pressure increments in the two vessels by H and h, and use the capital letter for the *larger* of the two pressure changes (which will refer to the vessel with the *smaller* gas space), we may use capitals to designate all quantities for the vessel with smaller gas space, and small letters for the other vessel. The relationships, expressed algebraically, should be as follows: Since in darkness it is assumed that $X_{O_2} = x_{O_2}$, and since $HK_{O_2} = X_{O_2}$ and $hk_{O_2} = x_{O_2}$,

$$\frac{h}{H} = \frac{K_{O_2}}{k_{O_2}}$$

We always observed, however, that for steady-rates of respiration in darkness, h/H proved to be a little smaller than K_{O_2}/k_{O_2}. Whatever the reason for this difference, if the cell samples in the two vessels are equal, and respire at equal rates, then the ratio h/H (which we may call ρ), determined experimentally from steady-rate measurements in darkness, is a better criterion for equality of photosynthesis during illumination, than the calculated ratio K_{O_2}/k_{O_2}. Our procedure, therefore, was to determine ρ experimentally from steady-rate measurements of respiration in darkness, and then to adjust the light beams so that during steady-rate photosynthesis the ratio of the observed increments H and h was also equal to ρ.

We considered several possible reasons why the values of ρ determined experimentally from pressure increments observed in darkness during steady-rate conditions failed to match the calculated value of K_{O_2}/k_{O_2}, and we made experiments to test several hypotheses. The difference is small (of the order of 2 or 3 %) and, if several factors contribute to it, then it is not surprising that no single factor makes a contribution large enough to be clearly identifiable. With the simple constant-volume manometers we never had encountered difficulty in confirming the ratio K_{O_2}/k_{O_2} through measurements of h/H in darkness, but a difference of 2 %, which is easily detectable in cathetometer measurements with differential manometers, might escape notice in the less precise readings of the constant-volume manometers. On the other hand, the calculation of K_{O_2} and k_{O_2} for the differential manometers involves some approximations because of the changing volume due to motion of the manometer fluid. No such approximations enter into the calculation of K_{O_2} and k_{O_2} for the constant volume manometers. But in the case of the differential manometers, when the area of cross-section of the capillaries is small the approximations seem to be too small to account for observed differences between ρ and K_{O_2}/k_{O_2}.

Another possibility is that the carbonate-bicarbonate buffer fails to suppress carbon dioxide exchange as completely as has always been assumed on the basis of the dissociation constants of carbonic acid, so that the pressure increments H and h represent not only oxygen exchange but some appreciable exchange of carbon dioxide as well. Mass spectrometer measurements made for us through the courtesy of Professor Allan Brown at the University of Minnesota, showed that under conditions approximating those in our experiments, quite appreciable changes in carbon dioxide

concentration above carbonate buffer mixture #9 could be demonstrated. The buffer appears to suppress only about 90 to 95 % of the carbon dioxide exchange, instead of about 99 %, as has been generally assumed. This indicated fraction of carbon dioxide exchange could account for a portion of the difference between ρ and K_{O_2}/k_{O_2}, but probably not for all of it.

Another factor possibly contributing to the disagreement between ρ and K_{O_2}/k_{O_2} is the hanging of manometer fluid on the capillary walls, which makes the downward motion of the meniscus on one side unequal to the upward motion on the opposite side. Any effect due to this factor should vary with the rate of travel of the meniscus, but the difference between ρ and K_{O_2}/k_{O_2} seemed to be independent of rate of travel.

Regardless of what may be the source of the discrepancy, there can be no doubt that ρ_{dark}, measured experimentally, is a better basis for adjusting the light beams for equality of photosynthesis in the two vessels, than the calculated value of K_{O_2}/k_{O_2}. The criterion for equality of photosynthesis is:

$$\rho_{dark} = \rho_{light}$$

Except for the measurements shown in figure 5, all experiments were made at 20° C. Warburg has reported that this temperature was favorable for demonstrating maximal efficiency (Warburg and Burk, 31, p. 429).

The basic equations for calculating gas exchange from two-vessel measurements are the same whether the pressure measurements are made with differential or constant volume manometers. When large numbers of calculations of rates are to be made from pressure measurements, the function ρ serves a useful purpose. In two-vessel experiments, where the pressure increments represent both oxygen and carbon dioxide, the conditions: $X_{O_2} = x_{O_2}$ and $X_{CO_2} = x_{CO_2}$ must both be fulfilled for each pair of values of H and h. For each pair meeting this requirement there is a pair of factors, F_{O_2} and F_{CO_2}, by which H can be multiplied to give X_{O_2} and X_{CO_2}:

$$X_{O_2} = F_{O_2}H$$
$$X_{CO_2} = F_{CO_2}H$$

F_{O_2} and F_{CO_2} are linear functions of the ratio ρ:

$$F_{O_2} = \frac{K_{CO_2}}{\frac{K_{CO_2}}{K_{O_2}} - \frac{k_{CO_2}}{k_{O_2}}} - \rho \frac{k_{CO_2}}{\frac{K_{CO_2}}{K_{O_2}} - \frac{k_{CO_2}}{k_{O_2}}},$$

$$F_{CO_2} = \frac{K_{O_2}}{\frac{K_{O_2}}{K_{CO_2}} - \frac{k_{O_2}}{k_{CO_2}}} - \rho \frac{k_{O_2}}{\frac{K_{O_2}}{K_{CO_2}} - \frac{k_{O_2}}{k_{CO_2}}}$$

The lines can be conveniently plotted from their intercepts.

For F_{O_2} the intercepts are:

$$\text{on } \rho \text{ axis: } \frac{K_{CO_2}}{k_{CO_2}},$$

$$\text{on } F_{O_2} \text{ axis: } \frac{K_{CO_2}}{\frac{K_{CO_2}}{K_{O_2}} - \frac{k_{CO_2}}{k_{O_2}}},$$

For F_{CO_2} the intercepts are:

$$\text{on } \rho \text{ axis: } \frac{K_{O_2}}{k_{O_2}},$$

$$\text{on } F_{CO_2} \text{ axis: } \frac{K_{O_2}}{\frac{K_{O_2}}{K_{CO_2}} - \frac{k_{O_2}}{k_{CO_2}}}$$

Figure 3 shows a plot of these two lines, for a vessel pair found to be equal in diffusion lag for oxygen (the pair tested in fig 8). All the two-vessel experiments reported in this communication were made with this vessel pair. The volumes of the compensating and experimental vessels in ml were as follows:

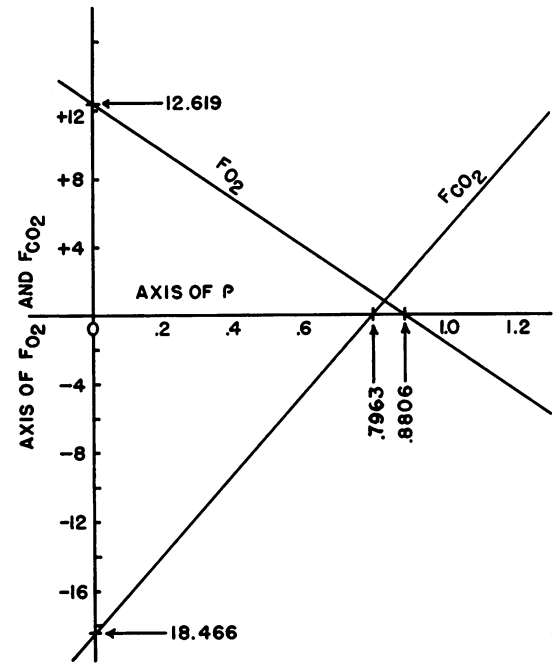


FIG. 3. The lines for F_{O_2} and F_{CO_2} as functions of ρ. These lines represent the equations:

$$F_{O_2} = 14.33\ (0.8806 - \rho)$$
and
$$F_{CO_2} = 23.15\ (\rho - 0.7963).$$

The data from which these equations were derived are:

Manometer for H		Manometer for h	
Experimental vessel	Compensating vessel	Experimental vessel	Compensating vessel
14.94 ml	14.92 ml	18.38 ml	18.46 ml

7 ml fluid in each vessel, 5 % carbon dioxide in air, temperature 20° C, manometer capillary volume 0.00426 ml /cm length.

Shape A vessels	V_F	V_G	Shape C vessels	v_f	v_g
Compensating vessel	7.00	7.92	Compensating vessel	7.00	11.46
Experimental vessel	7.00	7.94	Experimental vessel	7.00	11.38

With a capillary cross-section of 0.426 mm², and with 5 % carbon dioxide in air in both the compensating and experimental vessels, the values of the constants for these vessel combinations are:

$$K_{O_2} = 1.208 \qquad k_{O_2} = 1.517$$
$$K_{CO_2} = 1.955 \qquad k_{CO_2} = 2.220$$

The intercepts plotted in figure 3 were derived from these constants. When the plot is made on a large scale, it is a simple matter to read off for any value of ρ the corresponding values of F_{O_2} and F_{CO_2}.

It is to be noted that the ρ used here is the reciprocal of the one used by Nishimura et al (20). This change was made because their notation leads to equations in which F_{O_2} and F_{CO_2} are not linear functions of ρ, and are less convenient to plot than our linear functions. Our F_{O_2} and F_{CO_2} correspond to their K'_{O_2} and K'_{CO_2}, which we have dropped because they seem too easily confused with K_{O_2} and K_{CO_2}.

Figure 3 illustrates several features of the two-vessel method. It shows that for a certain value of ρ (ca 0.65 for this combination of manometers, vessels, and temperature), F_{O_2} and F_{CO_2} are equal in numerical value and opposite in sign (+3.3 and −3.3). In other words, a value of −1 for γ is characterized by a ρ of 0.65.

The curve for γ as a function of ρ is a rectangular hyperbola (cf. Pirson et al 24, pp. 18–19, figs 6–7). All values of ρ to the left of 0.7963 and to the right of 0.8806 give negative values of γ, and as ρ becomes infinitely negative or infinitely positive, γ approaches an assymptote of about −1.62. At a ρ value of 0.7963, γ is zero. It is positive for ρ values between 0.7963 and 0.8806, becoming infinitely positive as ρ approaches 0.8806, and infinitely negative as ρ passes above 0.8806.

The value of ρ where the line for F_{CO_2} crosses the zero line (0.7963) coincides with a value of $F_{O_2} = 1.208$. This is close to the value of K_{O_2} for carbonate buffer experiments (1.218), and the two would be identical except for the fact that the constants for the differential manometer depend slightly upon the gas mixture. The value of K_{O_2} is figured for air in the case of carbonate buffer experiments, and for 5 % carbon dioxide in air for the two-vessel measurements. Apart from these small differences due to the dependence of constants on the gas mixture, the values of F_{O_2} and F_{CO_2} which coincide with a ρ of 0.7963 (1.208 and zero respectively) are the values which should apply to the measurements in carbonate buffer.

We have explained that when we used carbonate buffer as a medium for experiments to adjust the light beams for equality of photosynthesis in a pair of vessels, we expected to find, for equality of oxygen exchange,

$$\rho = \frac{K_{O_2}}{k_{O_2}}$$

Steady rates of respiration in darkness gave a ρ of about 0.77, while K_{O_2}/k_{O_2} was about 0.80. Among possible explanations for the difference between ρ and K_{O_2}/k_{O_2}, we mentioned that the buffer might permit appreciable carbon dioxide exchange, or, stated in another way, F_{CO_2}, instead of being zero, would have a finite value. Figure 3 shows the finite value that would have to be attributed to F_{CO_2} to account for a ρ of 0.77 instead of 0.80. It indicates that F_{CO_2} would have to be about 1/5 the value of F_{O_2}. It seems unlikely that the buffer should permit such large changes in carbon dioxide pressure, so we concluded that this factor alone could not explain the difference between ρ and K_{O_2}/k_{O_2}.

In two-vessel measurements, the values of F_{O_2} and F_{CO_2} depend upon the ratio of the two pressure changes. Small errors in the measurement of either one can therefore lead to disproportionately large errors in F_{O_2} and F_{CO_2}, and to correspondingly large errors in X_{O_2} and X_{CO_2}. The effects of random errors have been discussed by Nishimura et al (20), and more extensively by Pirson et al (24).

Our experiments on diffusion lag with single vessels (fig 5), and our tests of the comparability of diffusion lag in pairs of vessels (figs 7 and 8) were made with cells suspended in carbonate-bicarbonate buffer #9 (15 parts 0.1 M K_2CO_3, 85 parts 0.1 M $NaHCO_3$). Our two-vessel measurements (figs 9–12) were made with cells suspended in acid culture medium (KH_2PO_4) saturated with 5 % carbon dioxide in air (pH about 4.8). Whatever was used as suspending medium for the cells, the same was used in the compensating vessel of each manometer, and the same gas mixtures were used in experimental and compensating vessels.

The cells were prepared for experiments by centrifuging them out of the culture medium, washing them twice in freshly prepared medium, making them up in fresh medium to the desired suspension density, and pipetting 7 or 8 ml into each manometer vessel. For all experiments with acid culture medium, the cell suspensions were saturated with a gas mixture of about 5 % carbon dioxide in air, and the gas spaces and manometer tubes were swept out with the same mixture. Analyses were made of the concentration of carbon dioxide in the mixtures used for the experiments.

Ordinarily the manometers were shaken in darkness for a few minutes, and the rate of pressure change was followed until it was observed to be steady in both vessels, before starting the sequence of light and dark periods. We made experiments with light periods of many different lengths, from one minute up to thirty minutes, usually alternated with dark periods equal to the light periods. For the most part, we used sequences of 10 minutes light–10 minutes dark, repeated several times. One of our objectives was to compare rates of photosynthesis calculated from transient and steady rates of pressure change, and since the transient changes usually lasted five minutes

or longer after each light–dark or dark–light transition, 10-minute periods were about the shortest that could be used to provide a basis for calculation from steady rates. The 10-minute periods make the experiments directly comparable with the tests of the technique shown in figures 5, 7, and 8.

Minor modifications in the culture medium were made from time to time in the course of the work. In general the medium was prepared according to the specifications of Emerson and Lewis (10, p. 818), but in some cases urea was substituted for nitrate as the nitrogen source. We departed from their specifications by omitting $CaCO_3$. Some cultures were grown over incandescent lamps, some over fluorescent lamps. Ofter a reduction in light intensity was made after one or two days' initial growth of the cultures. In general from 10 to 20 μl of cells were inoculated, and cultures were harvested after 5 to 20 fold increase in cell material. Such increases were obtained in from 24 to 72 hours.

Composition of culture medium and conditions during culture growth exert a marked influence upon the transient pressure changes which follow darkening and illumination when the cells are suspended in acid medium saturated with 5 % carbon dioxide (figs 9–12). Emerson and Lewis (11, p. 796) mentioned similar correlations.

In this communication we make no attempt to correlate transient metabolic behavior with culture conditions, because we have been concerned primarily with the demonstration of diffusion lag and its effects in two-vessel measurements. The examples of transient metabolic gas exchange (figs 9 to 12) are chosen to illustrate the range of results obtainable, rather than to demonstrate any special dependence upon culture conditions.

III. The Character of Transitional Pressure Changes, and the Extent to Which Diffusion Lag Is a Contributory Factor

We explained in Part I that time lag due to diffusion may be an important factor in the application of the two-vessel method to measurements of changing metabolic rates. There is no doubt about the presence of a diffusion barrier between the interior of the cells where gas production takes place, and the gas space where the measureable changes in pressure take place. The question is only whether diffusion lag makes a significant contribution to the rates of pressure change during the minutes immediately following changes in illumination.

Disagreement prevails regarding the importance of diffusion lag because under different conditions the transition from one rate of pressure change to another may follow very different courses.

Figure 4 is a diagram showing different courses of transition, which may be observed under different conditions. It shows a steady rate of pressure change of – 3 mm per min in darkness. At time t = 0 light is turned on, and photosynthesis, superimposed upon the continuing respiration, brings about a change in the

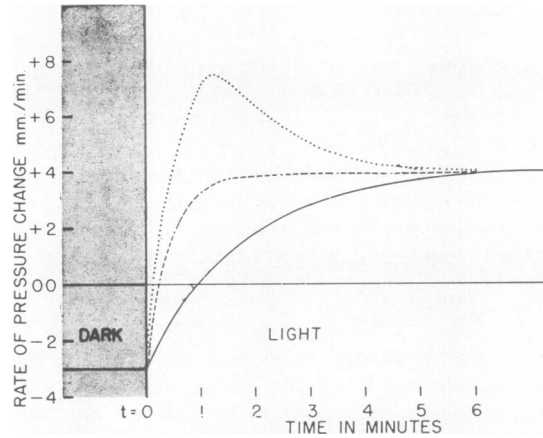


Fig. 4. Diagram showing different courses of transition from a steady dark rate of – 3 to a steady light rate of + 4 mm per min. The solid line represents the kind of transition observed by Emerson and Lewis for cells suspended in carbonate buffer. The dotted line shows the path of transition they observed for cells in acid phosphate medium. The dashed line is representative of a course of transition corresponding to the behavior described by Warburg and co-workers, for cells suspended in acid culture medium.

metabolic gas exchange, leading to a new steady rate of pressure change, of + 4 mm per min. The solid line shows the kind of transition from one rate to another which we would expect if diffusion lag were a factor of appreciable importance. According to the way the curve is drawn, a period of about 5 minutes elapses from the termination of the steady dark rate at time t = 0 until there is a close approach to the new steady rate in light. Pressure changes observed at intervals of 5 or 10 minutes after t = 0 would show noticeable effects of diffusion lag of this magnitude.

The dashed line shows a sharper change in rate. A close approach to the steady rate in light is attained within about one minute from t = 0. If this represented the course of diffusion lag, it would be scarcely noticeable in pressure readings at intervals of 5 or 10 minutes. The effect of such a lag as this would be noticeable if readings were made at intervals of one minute.

The dotted line shows a course of transition which indicates that some process other than diffusion is determining the rate of pressure change. The rate rises to a maximum within the first two minutes after t = 0, and then descends gradually, approaching close to the level of the final steady rate after five minutes. Such a course suggests that besides the steady rate of gas production which leads to the final steady rate of pressure change, there may also be temporary changes in the rate of gas production, to account for the transient maximum. From the dotted curve alone, it would be impossible to judge what might be the contribution of diffusion lag, but it is easy to see that a combination of the diffusion lag represented by the solid line, and some process tending to produce such

a maximum as is shown by the dotted line, would lead to an intermediate course for the rate of pressure change which might approximate the dashed line.

All the three types of transition illustrated in figure 4 have been described in publications reporting measurements of rate of photosynthesis for the purpose of calculating efficiency. The solid curve is representative of the course of transition observable with cells suspended in carbonate buffer. Emerson and Lewis (11, p. 792, fig 5) showed a similar course of transition. Warburg and co-workers have published no data in their recent papers to show the course of transition in carbonate buffer, but there is no doubt that their technique of measurement leads to the appearance of similar behavior. For example, according to Warburg, Burk, and Schade (32, p. 309), "with cells in carbonate . . . transition periods of at least 5 min have to be considered, ..."

Again without producing numerical data, Warburg and co-workers have described transitions similar to the dashed curve in figure 4, for experiments with cells suspended in acid culture medium saturated with 5 % carbon dioxide in air. In describing experiments in which rates of pressure change were observed at intervals of 5 minutes, Burk et al say (6, p. 226) " it was a further improvement owing to this motion that physical transition effects were not observed upon change from dark to light and vice versa, that is, the gas equilibration was virtually perfect for our purposes." A more specific statement, implying transitions which showed even less evidence of diffusion lag than appears in the dashed line of figure 4 reads: " . . . transition effects of equilibration were not ordinarily observed when dark cells were illuminated and vice versa, even when readings were taken every 1 or 2 instead of 5 to 10 min " (Warburg and Burk 31, p. 427).

Rate transitions similar to the dotted line in figure 4 were reported by Emerson and Lewis (10, p. 815; 11, p. 792, fig 2). They attributed the pressure maxima to transient maxima in the metabolic rate of carbon dioxide production (" carbon dioxide burst ").

Warburg has referred to the pressure maxima as something peculiar to the experiments of Emerson, but not identifiable as a normal feature of the metabolic activity of the Chlorella cells used in his experiments. He reported (29, p. 202) that he had encountered them at rare intervals, and suggested that they were connected with foaming of the cell suspension (an interpretation which cannot be sustained by either his observations or ours). He has published no discussion of the possibility that the same processes responsible for pressure maxima in our experiments, might have compensated for diffusion lag in his experiments, and made the rate transitions appear to be practically instantaneous. However, after claiming for several years that in his experiments diffusion lag was not appreciable, and that the pressure changes came to steady rates immediately following changes in illumination, he and his co-workers reported sequences of pressure change after changes in illumination which, if they had been plotted as successions of rates (as Emerson and Lewis had plotted their observations), would have looked very similar to the dotted curve in figure 4, and to the pressure maxima which Emerson and Lewis had interpreted as resulting from transient maxima in carbon dioxide production (cf. Warburg, Geleick and Briese 34, pp. 418–419).

Clearly there is a large measure of agreement regarding the transitional rates of pressure change observable under different experimental conditions. In our laboratory, as well as in the laboratories of Warburg and co-workers, it has been observed that when cells are suspended in carbonate buffer a time lag of the order of minutes is observable, while with cells suspended in acid culture medium saturated with about 5 % carbon dioxide in air, instead of a time lag there may be a transient maximum in either positive or negative rate of pressure change.

Disagreement arises over the interpretation of such observations. Warburg and co-workers leave no room for doubt as to their opinion regarding the transient rates. They (Warburg et al, 32, p. 309) write: " . . . it is important to know that in carbonate there is an induction period of minutes until the light action is fully developed manometrically. The cause of this induction is chemical; it cannot be mere physical equilibration, because for the same shaking rates and same conditions there is no corresponding induction if the cells are suspended in acid culture medium."

This statement was published earlier than the papers in which they describe pressure maxima (Warburg, Geleick and Briese, 34; Warburg et al, 35), but there is no evidence that their tardy recognition of the existence of pressure maxima has caused them to modify their belief that diffusion lag is negligible in their experiments, for up to 1954 they continued to treat the sequences of observed pressure changes as if they were quantitatively representative of the rates of gas production in the cells, according to the usual equations of the manometric technique, which presuppose a condition of close approach to equilibrium between gas and liquid phases.

In the publication quoted (32), Warburg refers to the lag in carbonate buffer experiments as " chemical," thus avoiding a direct statement as to whether he regards the lag as physiological. There is no basis for supposing that the establishment of steady states close to equilibria among CO_2, H_2CO_3, and carbonate and bicarbonate ions could lead to time lags of the order of minutes, and other " chemical " causes which would not apply equally to the measurements in acid culture medium seem remote. The only likely alternatives are physiological lag or diffusion lag.

Many years ago Warburg himself observed a time lag in experiments with Chlorella suspended in carbonate buffer (28, p. 192). He investigated this time lag at both high and low intensities. He was able to distinguish between lag due to diffusion, and lag due to physiological factors (so-called " induction "). He concluded that the effects of physiological induction were identifiable at high light intensities, but not at

low intensities. The lag which he observed at both high and low intensities he attributed (correctly, in our opinion) to diffusion. The absence of evidence of physiological induction at low light intensities has been confirmed by many other investigators (recently, for example, by Hill and Whittingham, 14). The increments of light used for measuring efficiency are "low", in the sense that in the dense cell suspensions they bring about average rates of photosynthesis far below the saturation rate, so under the conditions of the efficiency measurements no large effects from physiological induction would be anticipated.

Our new combination of differential manometers and rectangular vessels, and cathetometer for reading pressure changes, makes it possible to follow transitions from one rate to another with a degree of precision which justifies the study of transitional rates under conditions of shaking which approximate those specified by Warburg and co-workers, to see if there is evidence as to the nature of the lag.

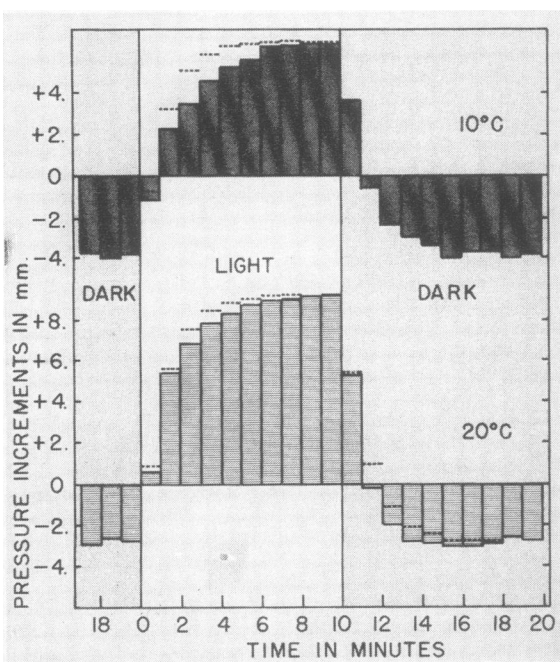


FIG. 5. Pressure increments, observed at one-minute intervals, for dark–light and light–dark transitions, with suspensions of Chlorella in carbonate buffer #9. *Above:* experiment at 10° C, with 260 μl cells in 7 ml of medium, $K_{O_2} = 1.265$. *Below:* experiment at 20° C, with 250 μl cells in 7 ml of medium, $K_{O_2} = 1.208$. Each set of measurements represents the sum of five repeated cycles of 10 min light–10 min dark.

The data for the light-to-dark transition at 10° C were used to derive the value of k for the theoretical curves plotted in figure 6. The fit of the observations for this transition is shown by the open circles plotted in figure 6.

The other three transitions would fit the curves in figure 6 if the succession of increments followed the course indicated by the dotted marks.

Figure 5 shows a record of two experiments, one at 10° C, the other at 20° C. The columns represent increments of pressure change, read at intervals of one minute, for five successive sequences of 10 minutes light–10 minutes dark. The time scale of the figure shows only one sequence of 20 minutes. Each plotted column represents the sum of the five increments observed during the five repetitions of the indicated minute in the sequence. This has the effect of averaging the results of the five cycles. The time scale starts at zero at the beginning of the light period. The increments of the last three dark minutes are re-plotted to the left of time zero, to show the starting point of the transition from dark rate to light rate.

The four sets of transitions appear similar, but they are not identical. If we suppose there is no physiological induction, and that a steady metabolic rate of gas production in either light or darkness is established in cells at the moment of each light–dark or dark–light transition, we may consider what course should be followed by the observable pressure increments if the observed gradual transitions from one rate to another were due to diffusion lag. For this purpose we may treat all rates as positive, measured from a zero line drawn through the average steady rate of respiration, and thus avoid the necessity of formulating our expressions to include both negative and positive rates. If at time $t = 0$ the metabolic rate changes from zero to a steady rate R_∞, and a simple diffusion barrier is all that delays the registration on the manometer of a rate of pressure change commensurate with R_x, then the rate R_t indicated by the manometer at time t will be related to R_x according to the equation

$$R_t = R_\infty(1 - e^{-kt}), \qquad (1)$$

where e is the base of natural logarithms, and k is a constant that includes the various physical characteristics of the system which determine the rate of diffusion of gas from the cells to the gas space. We may call it the apparent diffusion factor of the system. Apart from possible effects of temperature on viscosity of components of the system, we should expect a Q_{10} of some 1.2 for diffusion, so k will be temperature-dependent.

The observations plotted in figure 5 are *increments* from minute to minute, and do not tell us the *rates*, R_t, at the times when the increments were observed, until R_t has become essentially equal to R_x. To make a direct test of the fit of our data to equation *1* we may let A represent the total accumulated pressure change from time zero to t (time zero representing the start of either a light or a dark period). It can then be shown that

$$R_x t - A = (R_x/k)(1 - e^{-kt}). \qquad (2)$$

The values of A for successive minutes are derivable from the data plotted in figure 5. R_∞ is the difference between the steady rates of pressure change in darkness and light.

By choosing different values for k, it is possible

to obtain a close fit of equation 2 to each of the four transitions shown in figure 5. The light-to-dark transition at 20° C shows definite evidence of a small maximum in respiration, suggesting that a small accelerating effect of light on respiration might have influenced this sequence of increments. The dark-to-light transition at 10° C is definitely slower than the corresponding one at 20° C, suggesting a small effect of temperature, perhaps no larger than should be anticipated for a diffusion process. However, we recall that although the *average* rate of photosynthesis in the entire dense suspension is well below saturation, the few cells exposed to the full intensity of the incident beam may be exposed to a level of illumination not far from saturation, so that some effect of physiological induction could be anticipated. According to van der Paauw (23, pp. 595–598, fig 16) and also to Hill & Whittingham (14) induction is greater at lower temperatures, and this supports our opinion that induction probably modifies the course of both the dark-to-light transitions. No induction effects are to be anticipated for the light-to-dark transition, and the one at 10° shows no evidence of acceleration of respiration due to previous illumination. Of the four transitions plotted, this one alone shows no clear evidence of complex origin, so we have used it to derive a value of k for the purpose of testing the fit of equation 2 to the observed data.

Figure 6 shows $(R_\infty/k)(1-e^{-kt})$ plotted on a scale of 100, against time in minutes, for a value of 0.78 for k. The solid lines represent the rise and fall of the expression in light and dark, and the open circles represent the values of $R_\infty t - A$ for the light-to-dark transition at 10° C.

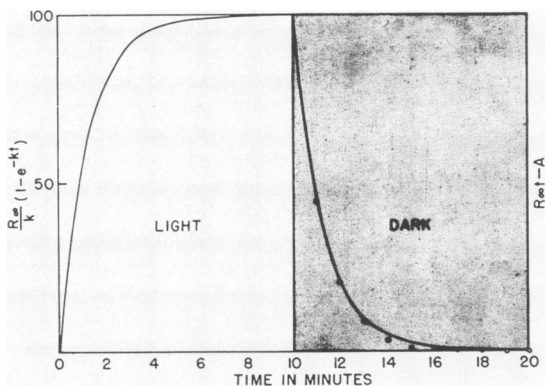


FIG. 6. Comparison of the transitions shown in figure 5 with the expression $(R_\infty/k)(1-e^{-kt})$. The value of k was derived from the observations of the light-to-dark transition at 10° C, shown in figure 5. The open circles show the values of $R_\infty t - A$ derived from these observations. The scale of $(R_\infty/k)(1-e^{-kt})$ is arbitrary. The curves pass the half-way point of upward and downward transitions at about 0.9 min. The transitions shown in figure 5 would fit the course of these curves if the increments followed the sequence of the dotted marks, instead of the observed values.

The amounts by which the other three transitions depart from the course of rise and fall predicted by the curves in figure 6 may be judged from the dotted marks above and below the increments plotted in figure 5. Had the increments come to the levels indicated by the dotted marks, the fit of the experimental values of $R_\infty t - A$ to the curves $(R_\infty/k)(1-e^{-kt})$ would have been exact for all four transitions.

The departures of the increments plotted in figure 5 from the values which would fit the lines plotted in figure 6 for a diffusion process are small, and are easily accounted for in terms of minor effects of light on respiration, a limited effect of physiological induction, and a small effect of temperature on diffusion (for which no allowance was made in calculating the levels of the dotted marks). It could well be that the same diffusion process is the major rate-limiting factor in all four cases.

On the other hand quantitative interpretation of the transitions shown in figure 5 primarily in terms of physiological factors would be inconsistent with certain well established facts regarding photosynthesis. It would require the assumption that cessation of photosynthesis upon darkening follows a slow course nearly symmetrical with the rise after a dark period, and that the induction effects in light were large at low intensities. It would also be necessary to suppose that induction was nearly independent of temperature. If interpretation in terms of physiological induction were rejected, then it would be necessary to assume some rate-limiting chemical process which would have a symmetrical effect for both dark–light and light–dark transitions, and which was furthermore characterized by a temperature coefficient unusually low for chemical processes. Apart from such improbable alternatives, it appears necessary to accept the evidence that under the conditions of these experiments, transitions from one rate of pressure change to another involve a diffusion lag of the order of minutes.

The value of k used for plotting the curves in figure 6 implies a half-time for the change from one rate to another of about 0.9 minute. We have also made direct measurements of diffusion under the same conditions of shaking as were used for the experiment in figure 5, but with water instead of cell suspension in the reaction vessels. Such measurements lead to half-times less than 0.3 min. However in these measurements we have not been able to achieve a degree of precision comparable with that which is attainable in experiments with suspensions of algal cells. The stopping and starting of photosynthesis by repeated alternation of light and dark periods provides a method for repeated reversal of diffusion gradient without interruption of shaking, while direct measurements without cells involve starting each measurement with the manometers and reaction vessels at rest. If there is a concentration gradient between gas and liquid, then when the reaction vessel is at rest the concentration of gas in the liquid phase will not be uniform. There are also other factors which tend to diminish the precision of direct measurements. In

any case, since we are interested in the time lag of the system during the measurements of photosynthesis, cell suspensions are to be preferred, since the presence of the cells increases the viscosity of the liquid. Also, the diffusion barrier between the interior of the cells and the suspending fluid is included, and this may contribute appreciably to the total time lag.

Warburg's argument that there is no diffusion lag in acid culture medium is not conclusive because of the evidence that in acid medium there can be compensatory processes. As we have explained, the transient pressure maxima which are evidence of compensatory processes appear in Warburg's experiments as well as in our own. We find it understandable that the evidence of pressure maxima appears only in the later publications of Warburg and co-workers, because in their earlier experiments they used slower speeds of shaking (for example Burk et al, 6, p. 226, 150/min), while later publications mention the use of higher speeds. (200 per min, Burk and Warburg, 7, p. 14.) Less efficient mixing of the cell suspensions would tend to smooth out the pressure maxima over longer periods and make them less conspicuous. Emerson and Lewis used shaking speeds up to 700 per min in their efforts to make the maxima as clear and sharp as possible.

It is also easy to understand why the maxima appear in acid culture medium saturated with 5 % carbon dioxide, but not in alkaline carbonate buffers. In the case of carbonate buffers, carbon dioxide pressure is maintained at a nearly constant and relatively low level by the buffering action of the carbonate and bicarbonate ions. Changes in pressure are attributable almost entirely to oxygen production. Emerson and Lewis attributed the pressure maxima to transient changes in carbon dioxide production, which would not be observable in carbonate buffer experiments. In the case of acid culture medium, the pressures of both oxygen and carbon dioxide are free to change in response to metabolic production of gases, and the high concentration of carbon dioxide serves to increase the transient maxima. According to the results of Emerson and Lewis, at the low carbon dioxide partial pressures of the carbonate buffers, no prominent maxima in carbon dioxide exchange would be expected, even in acid culture medium.

This review of the evidence shows that there is no difficulty in accounting for the behavior of transitional pressure changes observed in our laboratory and in Warburg's, without recourse to the improbable assumption that diffusion lag was negligible. Among users of the manometric technique, diffusion lag of the order of minutes is so universally recognized and so well supported by experimental evidence (cf Myers and Matsen, 19), that any discussion of it would have been superfluous, were it not for the fact that the repeated statements by Warburg and co-workers that they could find no evidence of diffusion lag led many people to believe that Warburg had devised shaking conditions which practically eliminated diffusion lag.

Having found strong evidence that diffusion lag is a factor of significant magnitude under the conditions of Warburg's measurements of efficiency of photosynthesis, we must next consider what effects it might have upon his application of the two-vessel method, because as we mentioned in Part I, the application of the two-vessel method to periods of transient rates presupposes equality of diffusion lag in the two vessels.

IV. The Testing of Vessel Pairs for Equality of Diffusion Lag

Emerson and Lewis (11), applied the two-vessel method to the study of oxygen and carbon dioxide production during the periods of pressure maxima, and recognized that inequality of diffusion lag in the two vessels would lead to errors. They tried to maintain equality by designing the vessels so that liquid circulation during shaking would be as near alike as possible in the two vessels. But they made no tests of equality of diffusion lag, and emphasized that their results for periods of changing rates should be regarded as approximations, because of the uncertainty about equality.

Warburg and co-workers have recognized the requirement for equality of lag in the two vessels (for example Warburg, Burk and Schade, 32, pp. 307–308), and have stated that in the case of their experiments the requirement was fulfilled, but they have published no evidence from which such a conclusion could be drawn, and it may be doubted whether their method of reading the meniscus levels of manometers with a magnifying glass during shaking at a frequency of 200 per min would permit the comparison of diffusion lag in two different vessels with a degree of precision which would be significant for their application of the two-vessel method.

Nishimura et al (20, p. 200, table 5) tested vessel pairs similar in dimensions to those of Warburg and co-workers, and found clear evidence of inequality of diffusion lag. They also tested pairs of vessels like the A-C combination (fig 2), but came to the conclusion that more precise measurements than naked-eye or hand lens readings of meniscus levels would be required to demonstrate equality of lag.

As far as we are aware, no techniques have been developed for testing the equality of diffusion lag in pairs of manometer vessels destined to be used for two-vessel measurements during periods of transition from one metabolic rate to another. There seems to be no published analysis of the applicability of the method to transitional rates. Pirson's thorough and up-to-date discussion (24) covers steady rates only. There has been no need for a method to test equality of lag, because apart from the work of Warburg and his collaborators, there appear to be no instances where conclusions of wide interest and substantial theoretical importance have been based upon an application of the two-vessel method to the measurement of transitional rates.

Our comparison of diffusion lag presupposes equality of steady-rate respiration and photosynthesis in the two vessels. We have explained (Part II) how we

adjusted the twin light beams to give equal rates of photosynthesis in the two vessels, by first determining the value of h/H, or ρ, during steady rates of respiration in darkness, and then adjusting the intensity of the light beams to give the same value of ρ for steady rates of pressure change in the light. These adjustments were made with dense suspensions of cells, to absorb all the incident light.

These steps to establish equality of steady rates of gas production in the two vessels in both light and darkness depend upon having the cells suspended in carbonate buffer, so that the pressure changes are attributable to oxygen production alone. If the production of two gases contributed to the rates of pressure change, it would be impossible to establish the value of ρ representing equality of gas production in the two vessels, unless γ, the ratio of production of the two gases were known. The need for application of the two-vessel method arises when the value of γ is in doubt.

Similarly, tests of equality of diffusion lag must be made under conditions when the pressure changes represent the production of only one gas, so that there can be no ambiguity as to the significance of the two pressure changes, H and h. If, with cells suspended in carbonate buffer, the light beams have been adjusted to give equality of ρ in both light and darkness, then constancy of ρ as the rate of pressure change changes from one level to another, is a criterion of equality of diffusion lag.

We have made this test by exposing the cells to successive 10-min periods of light and darkness, and observing pressure increments at one-minute intervals. For each pair of increments H and h, we calculated a value of ρ. We show first (fig 7) our application of this test to a vessel pair analogous to the pair described by Warburg and co-workers for their two-vessel measurements (shapes A and B in fig 2).

Pressure changes were read for six successive cycles of 10 min light–10 min dark. The readings of the first cycle are not included in the results. To minimize random fluctuations, the pressure changes for corresponding minutes of the remaining 5 cycles have been summed, as was explained in connection with figure 5.

The top row of columns shows the pressure increments, in mm per min, which represent the values of H. The next row shows the corresponding increments representing h. In each case they are plotted upward and downward from zero.

The last 3 dark minutes are re-plotted at the beginning of the light period, just as was done in figure 5, to show the starting point for the transition to the light rate.

The third row of columns in the figure shows the value of ρ for each minute in the sequence. ρ is defined as h/H, but in the first minutes after light–dark and dark–light changes, it often happens that the increments of h and H are too close to zero to give significant values of ρ. In these cases, instead of calculating from the *increments* of H and h, we have used the *differences* between successive increments.

$$\frac{h_2 - h_1}{H_2 - H_1}$$

When this difference is large compared to the increments themselves, it gives a more significant comparison of diffusion lag than does the pair of increments.

For the vessel pair in figure 7 the steady-rate value of ρ in darkness was 0.53. The light beams had been adjusted so that the same value of ρ was maintained during steady-rate photosynthesis.

Figure 7 shows that after each change in illumination, the values of h approach their new level more promptly than the values of H. This evidence of a difference in diffusion lag is confirmed by the plotted values of ρ, which show systematic departures from the base line of 0.53 during the times of transition from one rate to the other. This indicates that the increments of pressure change in the gas spaces of the two vessels represent unequal amounts of gas during the transition periods. We have explained that for correct application of the two-vessel method, it is essential that the pressure increments always represent equal increments of gas in the two gas spaces. Since this combination of vessel shapes fails to meet the requirement, we must conclude that it is not suitable for two-vessel measurements during transition periods, at least under the conditions of this test.

Whether conditions could be found under which vessel pairs of the A and B shapes would maintain the steady-rate value of ρ during periods of changing rate is a question that should be dealt with by anyone wishing to use such vessel pairs for two-vessel measurements during transition periods. The inequality of diffusion lag shown in figure 7 does not prove that under all conditions such a vessel pair would necessarily show inequality. The evidence shows only that equality should not be assumed, without objective test.

If instead of pairing a vessel of shape B with the A vessel, we use a vessel of shape C for the one with larger gas volume, we shall have more reason to expect equality of diffusion lag, because circulation of the liquid takes place in space of the same dimensions in both vessels. Figure 8 shows a test of an A-C vessel pair, analogous to the test of the A-B pair shown in figure 7. In contrast to figure 7, we see in figure 8 that the values of H and h follow very similar courses of transition from one rate to the other. The values of ρ are plotted upward and downward from a base line of 0.77, the value found for this vessel pair during steady rates of respiration. There is scarcely any evidence of systematic deviations of ρ greater than the random fluctuations. It would hardly be possible to tell from the sequence of ρ values where the light period began and ended.

This test shows that diffusion lag for oxygen may be considered equal in this pair of vessels. Our efforts to devise a correspondingly simple and direct test for

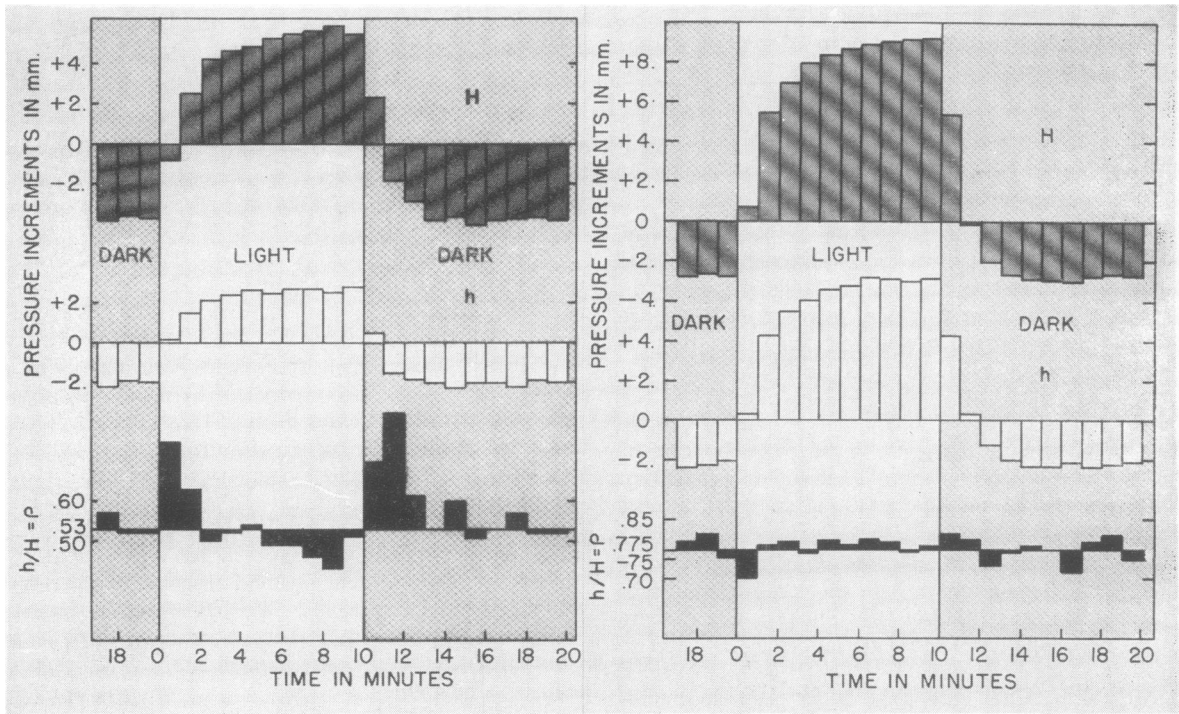


FIG. 7 (*left*). Experiment with carbonate buffer #9, to test equality of diffusion lag for oxygen in a vessel pair similar to the pair described by Warburg and co-workers (shapes A and B in fig 2). The volumes of the vessels containing the cell suspensions were 14.72 and 22.14 ml. Eight ml of suspension and 200 μl of cells were used in each vessel, and K_{O_2} was 1.076, $k_{O_2} = 1.824$. The base line of 0.53 from which the values of ρ are plotted represents the ratio h/H for steady-rate respiration in darkness. The plotted increments of H and h represent the sums of four successive cycles of 10 min light–10 min dark.

FIG. 8 (*right*). An experiment corresponding to the one shown in figure 7, but with a shape C vessel substituted for the shape B vessel, to test equality of diffusion lag for oxygen. Cells were suspended in carbonate mixture #9 again. The volumes of the vessels containing the cell suspensions were 14.94 and 18.38 ml, $K_{O_2} = 1.208$, $k_{O_2} = 1.517$. Seven ml fluid and 250 μl of cells were used in each vessel. The base line of 0.775 from which the values of ρ are plotted represents the value of h/H for steady-rate respiration in darkness. The plotted increments of H and h represent the sums of five successive cycles of 10 min light–10 min dark.

comparison of diffusion lag with respect to carbon dioxide were unsuccessful, and we thought it an open question whether diffusion lag with respect to carbon dioxide would be equal in a vessel pair shown to have equal diffusion lag with respect to oxygen.

After our experimental work was completed, Professor G. E. Briggs showed us that the solubility of the gas enters into the general expression for diffusion lag in such a way that if diffusion lag in a given vessel pair is equal with respect to one gas, it is necessarily unequal with respect to another gas of different solubility, unless the two vessels have equal gas and liquid volumes, a condition which would make the pair useless for two-vessel measurements. The difference in solubility of oxygen and carbon dioxide is so great that the effect of this factor upon diffusion lag cannot be overlooked.

In principle it is therefore impossible to make two vessels equal in diffusion lag with respect to both carbon dioxide and oxygen, and at the same time provide the difference in proportions of gas and liquid volume upon which the application of the two-vessel method depends. This does not diminish the value of the method for the measurement of steady rates, but its application to transient rates is subject to limitation. However, if diffusion lag in the two vessels has been shown to be equal with respect to oxygen, this also establishes the degree of inequality for carbon dioxide. If equality prevails with respect to oxygen, the inequality with respect to carbon dioxide will depend upon the ratio of the constants of the two vessels,

$$\frac{K_{CO_2} k_{O_2}}{k_{CO_2} K_{O_2}}.$$

With the vessel pair listed in figure 8, the predicted difference in diffusion lag for carbon dioxide is only about 10 %. While such a difference may not be negligible, it is nevertheless small enough so that useful information can be obtained from two-vessel measurements with a vessel pair of these characteristics. As we shall show, the method is capable of demonstrating important qualitative differences between the transient rate of gas production in different species

of algae, and in samples of a given species cultured in different ways (figs 9 to 12). It will also be possible to show how rates of photosynthesis leading to very high estimates of efficiency might be derived from two-vessel measurements of transient rates, and to assess the probable significance of the transient rates of gas production and the high estimates of efficiency.

V. Two-vessel Experiments with a Vessel Pair Chosen for Equality of Diffusion Lag for Oxygen

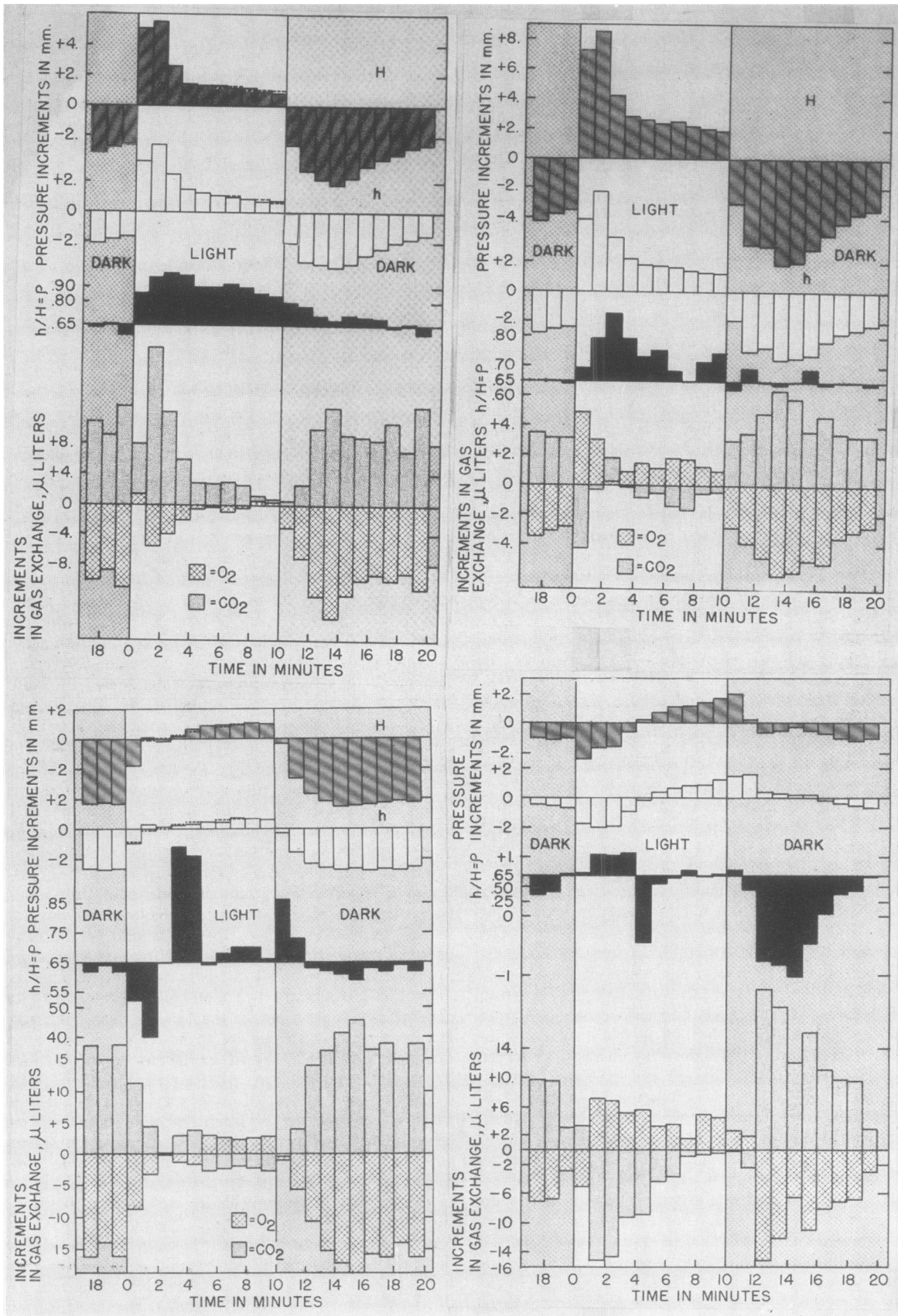
The experiments described in this section were made with the vessel pair used for the test shown in figure 8. It is the same pair for which the values of F_{O_2} and F_{CO_2} are plotted as functions of ρ in figure 3. From time to time in the course of the work, the equality of photosynthesis in the twin light beams was tested according to the method described in Part II. With cells suspended in carbonate buffer, it was verified that the value of ρ for steady rates of pressure change in the light remained equal to 0.77, the value for steady rates of pressure change in darkness. Apart from these tests, the measurements were made with cells suspended in acid culture medium saturated with 5 % carbon dioxide in air. As explained in Part II, under these conditions ρ has a significance different from that for measurements in carbonate buffer. In the case of acid culture medium, changes in ρ no longer imply inequalities in rate of gas production, but instead indicate changes in F_{O_2} and F_{CO_2}, the values by which H is to be multiplied to obtain the indicated rates of oxygen and carbon dioxide production. The base line from which ρ was plotted in the carbonate buffer experiments is of no significance for the experiments in acid culture medium. In the case of the two-vessel experiments it is useful to plot ρ upwards and downwards from the value at which $\gamma = -1$. For the vessel pair used, a ρ of about 0.65 corresponds to a value of -1 for γ, and we have plotted ρ from this base line in the figures 9 to 12.

If no adjustment is made for the great sensitivity of two-vessel measurements to random errors, the fluctuations in increments of calculated gas exchange from minute to minute may sometimes be so great that it is difficult to discern the course of the gas production indicated by the pressure changes. Emerson and Lewis (11) calculated gas exchange from smoothed curves drawn through their observations, instead of from the observations themselves. For the most part we have calculated gas exchange directly from our pairs of observed increments of H and h, but in some cases, especially where H and h are small, there has been an obvious need to smooth out random fluctuations. Where this has been done, dotted marks indicate the adjustments made in the original observations before calculating the values of ρ and the increments of gas exchange. For example in figure 9 it was assumed that in the last five minutes of light the values of both H and h should follow a smooth downward course, instead of the slightly irregular downward course of the observations. Smoothing was done arithmetically, so the total net change was kept identical with the total of the original observations. The dotted lines in the plots of both H and h show the very small extent of the adjustments made. Similar small adjustments may be seen in subsequent figures. No alteration in conclusions nor in calculated rates of photosynthesis results from these adjustments. The totals of the increments of oxygen and carbon dioxide exchange remain the same. The only effect is the smoothing out of random fluctuations in the increments of oxygen and carbon dioxide production, which would be far larger than the apparently trivial fluctuations in H and h.

Figure 9 illustrates the kind of pressure maxima which we have already mentioned as being typical of experiments with acid culture medium. The plotted increments of H and h represent the sums of three successive cycles of 10 minutes light–10 minutes dark. Both sets of increments show a sharp maximum in the first two minutes of light, and a less pronounced maximum in the third, fourth, and fifth minutes of darkness. These maxima are less extreme than those illustrated by Emerson and Lewis, but they are similar in character, and constitute evidence of transient changes in metabolic gas exchange.

Below the increments of H and h are plotted in solid black the values of ρ derived from corresponding pairs of H and h. The last three dark minutes which precede the beginning of the light period show ρ values close to 0.65, indicating approximately equal increments in oxygen and carbon dioxide of opposite sign. In the first three minutes of light ρ rises sharply, then declines gradually toward the end of the light period. In darkness it drops quickly back to 0.65.

The apparent increments in production of the two gases indicated by the pairs of pressure increments are plotted below the values of ρ. After steady rates of gas production have been attained in the cells, and after diffusion between gas and liquid phases has produced a steady state approximating equilibrium between the two phases, then the calculated increments in gas production do actually correspond to the concurrent metabolic increments. During the time of approach to this condition, the calculated increments do not correspond to the metabolic increments. In general, if diffusion lag in the two vessels is not too far from equal with respect to either gas, it may be assumed that the apparent increments will fall short of correctly representing the concurrent changes in metabolic increments. Under special circumstances the apparent increments might for a brief period exceed the actual changes in metabolic increments. But any such errors resulting from inequalities in diffusion lag must be promptly followed by equal errors in the opposite direction, before steady rates of pressure change are re-established. No matter what the errors from minute to minute, as long as the metabolic rates of gas production remain equal in the two vessels, the sum of the calculated apparent increments during the transition period (from the termination of


PRESSURE INCREMENTS IN mm.
H
h
h/H=P
DARK
LIGHT
DARK
INCREMENTS IN GAS EXCHANGE, μ LITERS
=O2
=CO2
TIME IN MINUTES

one steady rate until close approach to the new steady rate), cannot fail to include, for both oxygen and carbon dioxide, the actual metabolic increments accumulated during the transition period. Also included between the initial and terminal increments of such a period, are the amounts of carbon dioxide and oxygen by which the gas phase fell short of being in equilibrium with the liquid phase at the moment of termination of the preceding steady rate. Missing from the total will be the amounts of the two gases by which gas phase falls short of equilibrium with liquid phase at the time of close approach to establishment of the new steady rate. Allowance can be made for these two effects of diffusion lag, and thus the total increments of oxygen and carbon dioxide during the transition period can be correctly estimated within the limits of accuracy of the method. Departures from a constant ratio of oxygen and carbon dioxide production, or from a steady rate of production of either gas, can therefore be identified with reasonable assurance.

For example in figure 9, we see that the sum total of increments of carbon dioxide between the beginning and end of the light period greatly exceeds the corresponding total of increments for oxygen. The beginning of the light period was preceded by several dark minutes during which the pressure increments in the two vessels were nearly constant, and the corresponding oxygen and carbon dioxide increments were also nearly constant. While a similar constancy has not quite been attained by the end of the light period, the increments are apparently close to attaining steady values. If we assume that the cell samples were equal and maintained equal rates of respiration, and that the twin light beams maintained equal photosynthesis in the two vessels, then there is no escape from the conclusion that at some time during the light period there was a large excess of carbon dioxide production. There remains uncertainty as to the exact course by which this excess was built up, and one can certainly question the significance, for example, of the small maximum in oxygen production shown in figure 9 for the first minute of light, an increment which is clearly out of line with the rest of the sequence.

The same reasoning may be applied to the dark period in figure 9. From the end of the close approach to steady rates which had been attained in the last minutes of the light period, up to the approach to new steady rates at the end of the dark period, there were certainly appreciable maxima in the rates of both carbon dioxide production and oxygen consumption. No doubt their distribution in time was different from that shown by the apparent increments, but in spite of all uncertainties the sums of the apparent increments must match the total metabolic increments, subject only to allowance for diffusion lag at beginning and end of the transition period.

The quantum requirement of photosynthesis that can be calculated from this experiment may be compared with the requirement indicated by the experiments in carbonate buffer, in figure 8, for example. If in both cases we base the calculation upon the more or less steady rates observed in the last few minutes of both light and darkness, we find for the carbonate experiment a requirement of 11.6 quanta per molecule of oxygen, and for the experiment in figure 9 a requirement of 9.6 quanta. In the case of the experi-

FIG. 9 (*upper, left*). Experiment with *Chlorella pyrenoidosa* cells suspended in acid culture medium with 5 % carbon dioxide in air, for measurement of transitional changes in oxygen and carbon dioxide exchange. Seven ml of suspension and 295 μl cells were used in each vessel. The plotted increments of pressure and of apparent gas production or consumption represent the sums of three successive cycles of 10 min light–10 min dark. The vessel pair is the one for which the constants are plotted in figure 3.

The random fluctuations in the last five light minutes for H and the last 4 light minutes for h have been smoothed before calculating the plotted values of ρ and the increments of oxygen and carbon dioxide. The smoothing is indicated by the dotted marks. It does not alter the total exchange of either oxygen or carbon dioxide, but leads to a smoother course for the calculated gas exchange during these minutes.

FIG. 10 (*upper, right*). Experiment with *Chlorella pyrenoidosa* cells suspended in acid culture medium with 5 % carbon dioxide in air, for measurement of transitional changes in oxygen and carbon dioxide exchange. Seven ml of suspension and 255 μl cells were used in each vessel. The plotted pressure increments represent the sums of 4 successive cycles of 10 min light–10 min dark. The increments of apparent gas production or consumption have been divided by 4, so they represent the average values for the 4 cycles. The constants for this vessel pair are the ones plotted in figure 3.

FIG. 11 (*lower, left*). Experiment with Scenedesmus suspended in acid culture medium with 5 % carbon dioxide in air, for comparison of the transitions with those shown for *Chlorella pyrenoidosa* in figures 9 and 10. Some smoothing has been done in the light readings, indicated by the dotted marks. The values of ρ and oxygen and carbon dioxide exchange are based on the smoothed values of H and h. No change is made in the total exchange of oxygen or carbon dioxide.

Seven ml of suspension and 265 μl of cells were used in each vessel. The plotted increments represent the sums of four successive cycles of 10 min light–10 min dark. The constants for this vessel pair are plotted in figure 3.

FIG. 12 (*lower, right*). Experiment with Chlorella ("strain 1") suspended in acid culture medium with 5 % carbon dioxide in air, for comparison with *Chlorella pyrenoidosa* and Scenedesmus experiments shown in figures 9, 10, and 11.

Seven ml of suspension and 320 μl cells were used in each vessel. The plotted increments represent the sums of 4 successive cycles of 10 min light–10 min dark. The constants for this vessel pair are plotted in figure 3.

ment in figure 9 we can arrive at a lower quantum requirement by estimating the rate of photosynthesis from the highest positive increment (first minute of light) and he highest negative increment (fourth minute of dark). This leads to a quantum requirement of 6.0. But there is no evidence that the maximum rate of oxygen consumption calculated for the fourth dark minute was prevailing during the minute of light which gave the maximum positive increment, and neither of these increments can be assumed to be correctly representative of the concurrent metabolic rate. The calculated quantum requirement of 6.0 is therefore not identifiable with any metabolic rates that can be shown to have taken place. On the other hand the quantum requirement of 9.6 is derived from nearly steady rates of pressure change, which must therefore be close approximations to the values which would correspond to the steady metabolic rates of gas production.

As for the carbon dioxide "burst," it appears to be much smaller than the bursts described by Emerson and Lewis. The difference is attributable to differences in cultural and experimental conditions. Emerson and Lewis had observed that the carbon dioxide burst was maximal at about 10°, and that it was larger after a long dark period. Our present interest is in the magnitude of the burst under the conditions of Warburg's measurements of efficiency of photosynthesis. The 20° C temperature and the 10-minute dark periods between light exposures would be expected to give a much diminished carbon dioxide burst. The significant conclusion to be drawn from the experiment shown in figure 9 is that the apparent carbon dioxide burst is still identifiable at 20° C, and that it can persist for cycle after cycle of 10 minutes light–10 minutes dark. At a temperature of 10° C, and with long periods of light and darkness, and cells grown according to the specifications of Emerson and Lewis, our present technique gave results in good agreement with theirs.

Without giving supporting figures, Emerson and Lewis mentioned indirect evidence that the magnitude of the carbon dioxide burst depended upon the amount of cell material, while the steady rate of photosynthesis depended on the absorption of light. To test the effect of cell quantity on carbon dioxide burst, we have repeated the experiment shown in figure 9, with 195 μl of cells in each vessel instead of 295. The cells were from the same stock suspension in each case. The smaller quantity of cells still gave practically total absorption of the incident light, and in accordance with expectation we calculated nearly the same efficiency of utilization of light for photosynthesis, on the basis of the last few minutes of light and darkness. However, the extra carbon dioxide produced was nearly proportional to the quantity of cells used. Table I shows a comparison of these figures. The near-proportionality between the amount of extra carbon dioxide and the quantity of cells is in accord with the suggestion put forward by Emerson and Lewis that the extra carbon dioxide comes from

TABLE I

COMPARISON OF EXTRA CARBON DIOXIDE AND QUANTUM REQUIREMENT (ϕ^{-1}, QUANTA ABSORBED PER MOLECULE OF OXYGEN PRODUCED) IN PHOTOSYNTHESIS FOR TWO DIFFERENT QUANTITIES OF CELLS

AMT OF CELLS IN EACH VESSEL	ABSORBED LIGHT ENERGY	PHOTOSYNTHETIC OXYGEN PRODUCTION	ϕ^{-1}	EXTRA CO_2* IN 10 MIN
μl	*micromole-quanta/min*	*micromoles per min*	*quanta/O_2 produced*	μl
195	1.5	0.17	8.8	12.9
295 **	1.5	0.15	10.0	17.5

Each experiment represents the average of 3 successive cycles of 10 min light–10 min dark, and the same stock suspension of cells was used in each case.

* Extra carbon dioxide means the amount in excess of the quantity equal and opposite to the oxygen exchange. The figures represent the average of the three cycles in each experiment.

** Represented in figure 9.

metabolic intermediates. The greater the quantity of cells, the larger the pool of metabolic intermediates available for extra carbon dioxide production. On the other hand since the larger quantity of cells resulted in no appreciable increase in absorbed light energy (the absorption being practically 100 % in each case), the photosynthetic oxygen production should be about the same for both the smaller and the larger concentration of cells. The tabulated figures show that this was the case.

The pressure increments for the light period in figure 9 are in agreement with the results of Emerson and Lewis, to the extent that in both cases the pressure maximum appears to result from a maximum in carbon dioxide exchange. For the dark period, the evidence is in conflict with the results of Emerson and Lewis. Both their results (11, p. 792, fig 2) and ours show a pressure maximum in darkness, but in their case the maximum indicated a deficit of carbon dioxide production (roughly equal to the carbon dioxide burst in the light), while in our figure 9 the dark maximum is associated with maxima in both oxygen consumption and carbon dioxide production.

There may also be apparent maxima in oxygen production during the light period. As we have said, the small maximum indicated for the first minute of light in figure 9 is too small to be significant, but figure 10 illustrates a case in which there is a larger oxygen maximum, lasting for two minutes. This experiment represents four successive cycles of ten minutes light–10 minutes dark, instead of the three cycles represented in figure 9.

Just as in the case of figure 9 we considered the *sum-total* of oxygen and carbon dioxide production between periods of approximately steady rates, so we may look at the corresponding totals in figure 10. In the case of the light period we see that although the excess of oxygen production over carbon dioxide is not as great as was the excess of carbon dioxide in the

light period of figure 9, it is nevertheless large enough to be regarded as evidence that at some time during the light period there was an appreciable excess in oxygen production. Again we must keep in mind that its time course may be different from the time course indicated by the plotted increments.

The apparent carbon dioxide maximum in figure 9 and the apparent oxygen maximum in figure 10 are both the result of roughly similar maxima in the observed pressure increments. But in the case of figure 10 the successive pairs of increments lead to a slower increase in ρ, which does not attain as high a maximum as in figure 9. (Note that the scale for ρ is larger in figure 10 than in figure 9.) This leads to different values for F_{O_2} and F_{CO_2}, and accounts for the difference in increments of gas production calculated from patterns of pressure increments which look roughly similar.

The differences to be seen in figures 9 and 10, with respect to patterns of pressure increments, and with respect to apparent maxima in oxygen and carbon dioxide production, are also evident if the results are calculated from individual cycles of light and darkness, instead of from the summed increments of three or four cycles which are represented in the figures. In each experiment, the individual cycles lead to the same pattern of results as the summed cycles. The differences must therefore be regarded as systematic, and cannot be attributed to the accidental combination of different extremes of random errors. The conditions under which the cells for these two experiments were grown were different. For figure 9, cultures had been grown a short time from a heavy inoculum. About 50 μl cells had been inoculated into each flask, and they had been grown about 18 hours at high intensity, followed by 20 hours at reduced intensity. The increase in cell material had been about five fold. For figure 10, the inoculum had been about 12 μl per flask, the growth for 48 hours at high intensity and 48 hours at reduced intensity, resulting in 24 fold increase. We attribute the differences to be seen in figures 9 and 10 to differences in culture conditions, but we cannot say whether any of these conditions are more important than others in bringing about the difference in results.

The results for the dark period in figure 10 are in good agreement with those for the dark period of figure 9. The value of ρ remains close to 0.65 throughout the dark period, indicating near-equal and opposite increments in oxygen and carbon dioxide. There are apparent maxima for both gases, about four minutes after the beginning of the dark period. As in figure 9, the 10-minute periods of light and darkness appear to be barely long enough to give a close approach to steady rates, in the last two minutes or so of each period.

Because of the greater extremes in oxygen exchange, the experiment shown in figure 10 offers a greater range of possibilities for calculating rates of photosynthetic oxygen production and quantum requirements, than does the experiment in figure 9. First, taking the values of oxygen exchange from the nearest approach to steady rates, during the last two minutes of light and darkness, we find a quantum requirement of 8.3, in reasonable agreement with the value calculated from steady rates in figure 9. The opposite extreme is to calculate from the maximum oxygen production (first light minute) and the maximum consumption (fourth dark minute). From these extremes we can calculate a rate of photosynthetic oxygen production equivalent to one molecule for only 3 absorbed quanta. Warburg and co-workers have reported a number of similar values from calculations based similarly, upon the periods which gave maximum difference in increments of oxygen production between light and darkness. By basing their calculations upon the estimated slopes of rate curves, instead of upon actually observed increments, they arrived at quantum requirements of unity (Warburg et al, 34, pp. 418–419). We remind the reader that during the periods of transition from one rate to another, the calculated increments of gas production cannot be assumed to be equal to the concurrent metabolic rates. It is easily possible that for short periods the latter could attain much higher levels.

Since both figures 9 and 10 illustrate similar pressure bursts in the first minutes of illumination, it might be suggested that these bursts are due to purely physical causes, and have no real counterpart in the metabolic behavior of the cells. The fact that the positive and negative maxima in light and darkness are not symmetrical in pattern is not conclusive evidence against this suggestion, although symmetry of reversible physical effects might be expected on general grounds. Emerson and Lewis (11) mentioned several items of evidence that the pressure maxima were representative of physiological behavior of the cells rather than of physical behavior of the measuring system. In particular they observed that cells grown under different culture conditions showed significant differences in capacity to produce pressure bursts, under identical conditions of light absorption, etc. We show in figures 11 and 12 that cells of different species show even larger and more striking differences. These two figures show experiments identical with the ones represented in figures 9 and 10, except for the substitution of different species of algae. Figures 9 and 10 show experiments with a strain of *Chlorella pyrenoidosa* widely used for efficiency measurements and other experiments on photosynthesis. It is the strain used by Emerson and Lewis, and has been designated as the "Emerson strain" by Whittingham and by Myers. The cells of this strain are small (about 20 to 30×10^6 cells/μl packed volume). Figure 11 shows an experiment with Scenedesmus D^3 (Gaffron's strain), and figure 12 shows a corresponding one with a strain of Chlorella of undetermined species, which we designate simply as "strain 1." Its cells are larger than *C. pyrenoidosa,* and run about 2 to 3×10^6 cells per μl packed volume. According to Dr. Paul Silva, it corresponds closely to the taxonomic description of *Chlorella ellipsoidea.*

The measurements with Scenedesmus (fig 11) show no clear evidence of pressure maxima, in either light or darkness, but it is an open question whether periods longer than 10 minutes would show a decline from the apparently steady light and dark rates indicated for the 10-minute periods. The transitions are definitely sharper than those shown in figure 2 for carbonate buffer experiments, and this constitutes evidence of metabolic maxima, though of much smaller magnitude than the maxima indicated for *C. pyrenoidosa* in figures 9 and 10. Perhaps in Scenedesmus the maxima last longer and do not attain such extremes. The values of ρ show large deviations from the base line of 0.65, but these are limited to periods when the pressure increments are near zero, so that the sensitivity of ρ to random errors is very great, and consequently these fluctuations (which appear to be random rather than systematic) are hardly significant.

The absence of evidence of carbon dioxide maxima in figure 11 is in disagreement with the results of Brown and Whittingham (5), who, presumably using the same strain of Scenedesmus, found appreciable bursts of carbon dioxide in response to illumination. This difference is probably due to differences in culture conditions. We have not attempted to find ways of culturing Scenedesmus which would lead to carbon dioxide bursts.

Figure 12, on the other hand, shows marked pressure maxima and consequent transitional fluctuations in oxygen and carbon dioxide, but they are opposite to the effects observed with *C. pyrenoidosa*. Illumination brings about a maximum in *negative* pressure change, associated apparently with a transient maximum of carbon dioxide consumption, instead of a burst of production. The first minutes of darkness show an increase in positive pressure changes, which indicate a transient peak in carbon dioxide production. There is also evidence of transient changes in oxygen exchange in both light and darkness.

Figures 9, 10, 11, and 12 show such diverse patterns of transitional pressure changes that their physiological origin is hardly to be doubted. Errors due to difference in diffusion lag in the two vessels might obscure the actual course of transient metabolic gas production, but could hardly lead, under one set of physical conditions, to such a range of transient pressure changes as is illustrated in these figures.

Table II shows a comparison of the quantum requirements (ϕ^{-1}, number of quanta required per molecule of photosynthetic oxygen production) that can be calculated from the data of the experiments shown in figures 5, 7, 9, 10, 11, and 12, from different choices of time intervals. The purpose of this table is to compare the values of ϕ^{-1} from apparent maximal and from steady or near-steady rates of gas exchange. For each value of ϕ^{-1}, the table shows the minutes of the light and dark periods chosen as a basis for calculation of photosynthetic oxygen production. In the case of the experiments in carbonate buffer (figs 5 and 7), the *maximal* rates are also the nearest approach to *steady* rates, since the effects of diffusion lag overshadow all other transient effects in this medium. Photosynthetic oxygen production is calculated on a single-vessel basis, pressure changes being multiplied by K_{O_2}. In the case of the experiments in acid culture medium with 5 % carbon dioxide in air (figs 9, 10, 11, 12), photosynthetic oxygen production was calculated on the basis of two-vessel measurements. Reference to the figures will show in each case the character of the pressure increments and apparent gas exchanges during the minutes chosen as a basis for estimation of ϕ^{-1}. The figures show that steady rates of gas exchange were scarcely attained within the ten-minute periods. The nearest

TABLE II

QUANTUM REQUIREMENT (ϕ^{-1}, NUMBER OF QUANTA ABSORBED PER MOLECULE OF OXYGEN PRODUCED) MEASURED AND CALCULATED IN DIFFERENT WAYS

MEDIUM	BASIS OF CALCULATION	FIG NO.	MIN CHOSEN FOR CALCULATION OF RATE		CALCULATED PHOTOSYNTHETIC O_2 PRODUCTION	ABSORBED LIGHT ENERGY	ϕ^{-1}
			LIGHT	DARK			
					micromoles per min	*μ einsteins per min*	*μ einsteins per micromole*
Carbonate buffer #9*	Final or steady rates of pressure change	5, 10°	8, 9, 10	8, 9, 10	0.12	1.45	12.0
		5, 20°	8, 9, 10	4, 5, 6	0.13	1.5	11.5
		7	8, 9, 10	6, 7, 8	0.11	1.45	13.0
Acid culture medium,** 5 % CO_2 in air	Final or steady rates of calculated gas exchange	9	6, 7, 8, 9, 10	6, 7, 8, 9, 10	0.15	1.5	10.0
		10	9, 10	9, 10	0.18	1.5	8.3
		11	9, 10	9, 10	0.22	1.3	6.0
		12	6, 7, 8, 9, 10	8, 9, 10	0.11	1.3	11.8
	Maximum calculated increments of gas exchange	9	1	4	0.25	1.5	6.0
		10	1	4	0.50	1.5	3.0
		11	6	6	0.24	1.2	5.0
		12	2, 3	3, 4	0.24	1.3	5.4

* Measurements calculated by single-vessel method.
** Measurements calculated using two-vessel method.

approach to steady rate was used as a basis, usually the final minute or two of the light or dark periods. Maximal rates of photosynthetic oxygen production were calculated from intervals showing maximum difference between oxygen production in light and oxygen consumption in dark. As we have explained, these maxima are only apparent rates, and the true metabolic rates in light and darkness may very likely pass through brief maxima much larger than anything indicated by the measurements. From such rates much smaller values of ϕ^{-1} would be derived. On the other hand in the case of the steady-rate values there is a tendency to overestimate the rates, because the ten-minute periods are not quite long enough for attainment of steady rates, so the values of ϕ^{-1} are more likely to be too low.

For the carbonate buffer experiments, the values of ϕ^{-1} range from about 11 to 13. If allowance were made for some contribution of carbon dioxide to the pressure changes these values might be reduced by as much as 10 percent.

Of the measurements in acid medium, those calculated on a steady-rate basis show values of ϕ^{-1} ranging from about 8 to 12, except for the value of 6 for Scenedesmus. As we have explained periods somewhat longer than 10 min may sometimes be required for approach to steady rates, so perhaps the value of 6 should be regarded as more representative of apparent maximum than of steady rate of oxygen exchange.

VI. Discussion and Conclusions

The results of the experiments described in Parts III and IV hardly call for further comment. In our opinion they leave no doubt that in manometric experiments, even under conditions of rapid shaking, the effects of diffusion lag are easily identifiable. We have shown that for vessel pairs of certain shapes the difference in diffusion lag with respect to oxygen may be large enough so that the use of such pairs for two-vessel measurements involves risk of serious errors in the calculation of rates of gas production from rates of pressure change during periods when the distribution of gases between gas and liquid phases is undergoing change from one steady state to another.

The application of the two vessel method to periods of transient changes in rate can lead to errors of unpredictable magnitude in the rates of both oxygen and carbon dioxide production. Although we have no quantitative information on diffusion lag in the vessel pairs used by Warburg and co-workers, the results of the test shown in figure 7 suggest that with the combination of shapes which they used, differences in lag may have been so great that the calculated rates of oxygen and carbon dioxide production and the derived values of γ could be far from correct. Certainly they do not correspond to the actual rates of metabolic gas production during the time intervals over which the rates of pressure change were measured, although up to the publication of Warburg et al (35), they have been treated as if they did so correspond. Conclusions upon which Warburg and co-workers place especial emphasis (the quantum yield of unity for oxygen production during illumination (7), the capacity of the cell to make up the energy required for photosynthesis by supplementing the energy of the light quantum with energy from oxidative metabolism (34), the equivalence of the oxygen capacity of Chlorella cells and red blood corpuscles (35), etc) are supported primarily by two-vessel measurements which are clearly subject to the limitations we discussed above. These conclusions are, therefore, of doubtful significance.

We have shown that in vessel pairs so shaped that liquid circulation tends to be equal, diffusion lag for oxygen may be equal within the limits of measurement, and we have used such a vessel pair for the two-vessel experiments shown in figures 9 to 12. We explained that after our experimental work was completed, our attention was drawn to the fact that in vessels of unequal volume diffusion lag with respect to one gas implies inequality with respect to a second gas of different solubility. Therefore, no vessel pair can meet the requirements for the two vessel method for oxygen and carbon dioxide during and immediately following changes in metabolic rate of gas production or consumption. However, by establishing equality of diffusion lag with respect to one gas, we have also established the degree of inequality with respect to the other gas, a substantial improvement over inequality of unknown magnitude.

As far as we know, there is no published analysis of the consequences of diffusion lag for two-vessel measurements, such as Roughton (26), for example, has given for single-vessel measurements of the rate of production of a single gas. In collaboration with Professor G. E. Briggs, we plan to prepare such an analysis for publication. Preliminary study shows, however, that calculation of metabolic rates of gas production from changing rates of pressure change (as was done by Roughton for single-vessel experiments) will not be possible in the case of the two-vessel experiments unless the course of transition in rate of production of one of the two gases can be mathematically specified.

Present evidence indicates that there may be transient fluctuations in the metabolic rate of production of both oxygen and carbon dioxide, so there is little prospect that we shall soon be able to specify the transient course for one gas with sufficient accuracy to calculate the course for the other.

The application of the two-vessel method to the study of transient metabolic rates is therefore an approximation. All experimental methods are to some extent approximations, and although this one may be fairly rough we should not dismiss it on that account without first considering whether alternative methods promise to be closer approximations. The transient maxima in rates of pressure change are observable beyond all doubt, and we think there is also no room for doubt that they are of physiological origin. If better and more direct methods can tell us more about these maxima than we can learn through the

application of two-vessel manometry, then there may be no need to make further application of the manometry, except, perhaps, for the measurement of rates under steady-state conditions.

The trouble is that for the most part, the direct methods which are applicable to the special conditions under which the pressure maxima appear, give little or no evidence of transient changes which could account for the pressure maxima.

We recall that considerable evidence of transient maxima in gas exchange has been produced by certain direct methods (for example, in the work of Blinks and Skow, 2; Aufdemgarten, 1; Gaffron, 13 and McAlister, 16). We attempt no complete listing of such observations nor comparison of the methods because for the most part the organisms used have been so different from the unicellular alga Chlorella (higher plants or multicellular algae in many cases), and the conditions of the experiments have been so different from those which we recognize as necessary to produce pressure maxima, that we could hardly claim the effects have a common origin. On the other hand direct methods applied to *Chlorella pyrenoidosa*, under conditions more or less approximating those during which pressure maxima have been observed, have resulted in surprisingly little evidence of transient maxima. Transient changes in the carbon dioxide production of Chlorella were observed by McAlister and Myers (17) and also by van der Veen (27). In the work of McAlister and Myers, the light intensity and carbon dioxide concentration were rather different from the range of these factors in our experiments, but there is a possibility that the effects have something in common. In the case of van der Veen's experiments the conditions were quite similar to ours, and one might regard the results as confirmatory. But experiments of Manning, Daniels, Moore, and others, with the dropping mercury electrode (cf. references listed by Moore and Duggar, 18) showed no indication of maxima in oxygen exchange. Yuan et al (38) used a specially constructed combination of magnetic oxygen analyzer and infrared carbon dioxide analyzer, and searched unsuccessfully for evidence of transient maxima in the exchange of either gas. Brackett et al (3) reported some rather small fluctuations in oxygen exchange, revealed by measurements with a platinum electrode method described by Olson et al (22). Whittingham (37) followed oxygen exchange by a modification of Hill's hemoglobin method, and was unable to confirm the fluctuations reported by Brackett et al (3), or to find any evidence of transient fluctuations in oxygen exchange. Van Norman and Brown (21) used a recording mass spectrometer to follow both oxygen and carbon dioxide exchange during alternations of light and darkness, and reported no evidence of transient maxima. Brown (4) made a more detailed study of oxygen exchange, using isotopes of oxygen to identify the photosynthetic and respiratory exchanges, and reported that respiratory oxygen consumption by *Chlorella pyrenoidosa* followed an essentially constant rate through alternations of light and darkness. The rate of photosynthetic oxygen production during illumination was also steady.

In one case only has a direct method revealed gas exchanges which would clearly lead to pressure maxima such as can be observed manometrically, and this was achieved after several years of unsuccessful effort. Brown and Whittingham (5), after improving the mass spectrometer technique to make it applicable to carbon dioxide assay up to concentrations of 5 %, were able to demonstrate bursts of carbon dioxide production quite analogous to those described by Emerson and Lewis on the basis of their two-vessel manometric measurements. They confirmed the findings of Emerson and Lewis in considerable detail, and also added new and interesting information concerning the carbon dioxide maxima.

There is no corresponding confirmation by direct methods, for such maxima in rate of oxygen production as are indicated in figure 10 for the light period, and in both figures 9 and 10 for the dark period. It is possible that these evidences of transient changes in rates of oxygen production are spurious, and that the pressure maxima are due entirely to carbon dioxide. Emerson and Lewis concluded from their experiments that the rate of oxygen exchange probably followed a steady course, and made smooth transitions between light and dark rates. The equations for effects of diffusion lag in the two-vessel method show that under certain conditions a pressure maximum due to one gas could appear to be representative of the other. In our work we were initially inclined to ascribe the pressure maxima to carbon dioxide, and to regard the evidence of oxygen maxima as doubtful. We cannot specify with certainty the conditions which lead to sequences of pressure increments in the two vessels, from which oxygen maxima can be calculated for the light periods. But although the positive oxygen maximum in the light is elusive, and appears in only a few of our experiments, the negative oxygen maximum in darkness is common to nearly all our experiments, and for the reasons set forth in our discussion of figure 9, we think the manometric evidence is probably significant.

The fact that confirmation by direct methods for the oxygen maxima calculated from manometric measurements has not yet been found, need not be regarded as evidence that these maxima are spurious. In many cases there is doubt as to whether the direct methods could reveal the transient fluctuations in rate indicated by manometry. For example in the method of Yuan et al (38) rapid fluctuations in rate might become lost in the volume of circulatory system required to bring the gas mixture to the analyzing elements of the system. It is perhaps more difficult to understand why Brown, and van Norman and Brown, were unable to find maxima in oxygen with the mass spectrometer, although Brown and Whittingham, using essentially the same method, were able to find full confirmation of the carbon dioxide maxima. We note, however, that the oxygen maxima calculated from our

experiments are considerably smaller than the large carbon dioxide maxima obtainable in light. It was by following the specifications of Emerson and Lewis for producing large carbon dioxide bursts that Brown and Whittingham were just able to demonstrate carbon dioxide bursts with the mass spectrometer. Effects of one tenth this magnitude might well be lost in the random scatter of points in the mass spectrometer readings, whereas they remain large and easily observable in the cathetometer readings of pressure changes for the two-vessel method. Through exchange of information between our laboratory and Brown's, we are led to believe that cells grown in the same manner in both laboratories may show small but easily identifiable pressure maxima although with the mass spectrometer there may be no clear evidence of rate maxima.

The fact that Hill and Whittingham observed no oxygen maxima with the very sensitive and rapid hemoglobin method is also inconclusive, because this technique requires the use of oxygen partial pressures far lower than those prevailing in our manometric experiments, and it might well be that, just as high partial pressures of carbon dioxide are required to demonstrate maxima in rates of carbon dioxide production, oxygen maxima would not appear except at higher partial pressures of oxygen. Also Hill and Whittingham used quantities of cells much smaller than the amounts used in our experiments. We know that carbon dioxide maxima are proportional to quantity of cells, and although we have not demonstrated the same relationship for oxygen maxima we think it likely that this will prove to be the case.

This discussion of the evidence from our own experiments and from those of other investigators in regard to transient maxima makes it clear that as far as carbon dioxide is concerned, there is no doubt that large fluctuations in rate do actually occur. In the case of oxygen the evidence is less convincing, because the manometric results have not yet been confirmed by direct methods. But we think that in all probability the rates of both oxygen and carbon dioxide production undergo large fluctuations immediately following changes in illumination. The evidence suggests that during these changes the rates of gas exchange may not be representative of photosynthesis and respiration in the usual sense. The transient maxima probably represent changes in the size of pools of metabolic intermediates which are maintained at steady levels during steady-rate photosynthesis or respiration, but which must be quickly changed to new levels by any change in the balance between these two processes. This is essentially the interpretation which Franck (12) has proposed for the high efficiencies of photosynthesis reported by Warburg and coworkers. It may be true (as we showed in the discussion of fig 10) that very high yields of oxygen production per quantum of absorbed light may be calculated from the maximum observable difference between transient rates. Reasonable allowance for diffusion lag suggests that rates even higher than those estimated by Warburg could be calculated, leading to yields larger than one oxygen molecule per quantum of light absorbed. But if these high rates represent changes in the size of pools of intermediates, it should be clear that the maximum difference between rates in darkness and light cannot be assumed to represent the rate of oxygen production attributable to the light, because there is no basis for assuming that the processes represented by the negative maximum in darkness were continuing at the same rate during the time of the positive maximum in light. On the contrary, there is every reason for believing that they were not. Apart from this, even if the rate of oxygen production could be shown to attain a temporary maximum indicative of a quantum yield of unity or better, this would not indicate an equivalent efficiency for photosynthesis. Oxygen production from pools of intermediates represents energy changes of quite unknown magnitude, but in all probability far smaller than the energy required for production of carbohydrate and oxygen from carbon dioxide and water, the chemical change which we associate with the term "photosynthesis."

Our experiments offer no clues as to the nature of the intermediates which might constitute the pools from which we suppose the transient maxima are produced. Emerson and Lewis reported (11) that the total extra carbon dioxide production, over and above a carbon dioxide exchange equal and opposite to the oxygen, was sometimes as much as one micromole per 100 μl of cells. Assuming that 100 μl of cells contain about 1 mg of chlorophyll, or roughly one micromole, we see that the extra carbon dioxide may be about the molar equivalent of the chlorophyll. The extra carbon dioxide shown in our table I for the 10-minute light periods of the experiment in figure 9 is only about 6 μl per 100 μl cells in ten minutes, or about 0.25 micromole of carbon dioxide, from a quantity of cells containing roughly one micromole of chlorophyll. Brown and Whittingham (5) reported finding about 0.5 micromoles extra carbon dioxide per μl cells.

These results indicate that the pools of intermediates giving rise to the carbon dioxide maxima attain levels comparable with the concentration of chlorophyll in the cells.

The oxygen maxima generally represent smaller fractions of the volume of cells. In the case of the oxygen maximum in darkness in figure 9, the total excess, over and above the average of the last two minutes, is about 0.04 micromole per 100 μl of cells. The oxygen maximum in darkness in figure 10, calculated on the same basis, represents about 0.2 micromole. The oxygen maximum in the first two minutes of light in figure 10 represents about 0.1 micromole per 100 μl cells. These figures are only approximations, which serve to establish orders of magnitude.

One could consider the possibility that the extra oxygen might represent changes in equilibria between components of the cytochrome system. Davenport and Hill (9, p. 332) have estimated that the amount of cytochrome f present in chloroplasts is about one four-hundredth the amount of chlorophyll. This is so

much smaller than the amounts of extra oxygen estimated from our experiments that the hypothesis is not promising. However, there may be other cytochrome components involved besides cytochrome f, and perhaps oxidation-reduction equilibria outside the chloroplasts contribute something to the maxima.

Finally, this discussion of the interrelations of diffusion lag and transitional rates of gas exchange leads inevitably to the conclusion that measurements of the efficiency of photosynthesis are significant only when they are based upon steady metabolic rates. The results we have reported here support the conclusion reached earlier by a number of other investigators, that a quantum requirement of about 8 per molecule of oxygen produced represents the highest efficiency that can be sustained by the evidence (equivalent to about 30 % in red light). The claims put forward by Warburg and co-workers that from one to four quanta suffice per molecule of oxygen produced, appear to be founded upon experimental methods which cannot be counted upon to give results which are numerically correct, and the results, whether correct or not, cannot be regarded as an appropriate basis for calculating the efficiency of photosynthesis.

Summary

The two-vessel manometric method has sometimes been applied to measurement of photosynthesis during periods of transient rates without proper regard for its limitations. Tests show that diffusion lag is an important factor, and that vessel pairs equal in diffusion lag with respect to oxygen can be selected. Theoretical considerations show, however, that equality with respect to two gases of different solubility is not possible. When the method is applied with vessels matched for oxygen, the results indicate transient maxima in both oxygen and carbon dioxide exchange. If the evidence for oxygen maxima is accepted, then values of ϕ^{-1} (the number of quanta required per molecule of oxygen produced) as low as 3 can be calculated, and actual metabolic rates must represent still lower values. However, evidence is presented which suggests that the transient gas exchanges are not representative of respiration and photosynthesis. Steady-rate measurements, which may be regarded as representative of respiration and photosynthesis, lead to values of ϕ^{-1} from about 8 to 12.

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PASSIVE PERMEATION AND ACTIVE TRANSPORT OF IONS IN PLANT ROOTS[1,2]

EMANUEL EPSTEIN

AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

Active ion transport in plant roots, according to the most widely held view, involves the operation of carriers. The hypothesis is as follows: The ions combine with the carrier molecules and the resulting ion-carrier complexes traverse membranes of limited permeability to the free ions. At the inner surface of the membranes, the ions are released from the carriers. This active process of ion transport depends on metabolism, and is characterized by a high degree of selectivity. Ions taken up by this mechanism are largely nonexchangeable with ambient ions of the same or other species.

The process briefly outlined above is not the only one whereby ions may penetrate plant roots. Cations may be non-metabolically adsorbed on negatively charged surfaces within the root, in stoichiometric exchange for other cations residing on these exchange surfaces. Epstein and Leggett (6) exposed excised barley roots to solutions of radioactive $SrCl_2$ (Sr^*Cl_2). When, after 60 minutes, the roots were briefly rinsed with water and then exposed to a solution of non-radioactive $SrCl_2$ of the same concentration, they lost a large part of their Sr* by exchange. Other cations also displaced exchangeable Sr* from the roots. However, the amount lost to water was only a fraction of the amount lost to salts.

When similar experiments were performed on the absorption of sulfate, from solutions of $K_2S^*O_4$, a large labile fraction was again observed. However, in this instance, the amounts of S^*O_4 lost to water were identical with the amounts lost to non-radioactive sulfate, indicating that no exchanging or displacing ions were needed to effect this removal. It appeared, rather, that a fraction of the S^*O_4 ions in the tissue freely diffused out upon transfer of the tissue to water or salt solutions. Another fraction, however, neither diffused out into water, nor exchanged with ambient non-radioactive sulfate. It will be shown that the former process is a manifestation of an "outer region," "water space," or "apparent free space," to which ions have free and reversible access by diffusion. The second irreversible process is active transport of sulfate which will be discussed in greater detail elsewhere (9). The relation between these two processes will be examined. The findings will be shown to apply also to ions other than sulfate.

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