THE COURSE OF RESPIRATION DURING THE LIFE CYCLE OF CHLORELLA CELLS*

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INTRODUCTION

The hereditary, environmental, and developmental sources of variability among organisms are of constant concern to students of cell physiology. Though heredity and environment are usually considered, far less attention has been devoted to eliminating the variability caused by the growth and development of the cells. In this paper the importance of cell age, or of the developmental stage of a cell as it influences its metabolic characteristics, is emphasized, as studied on synchronized algal suspensions.

Material and Methods

The work was done with the high-temperature strain, 7-11-05, of *Chlorella pyrenoidosa* (Sorokin and Myers, 1953 and 1954). To induce synchronization in cell development algal suspensions were grown at 39°C. under diurnally intermittent illumination: 9 hours' light and 15 hours' dark. After 9 hours of continuous illumination the wall of the mother cells (at the beginning in only a few cells) began to rupture. This rupturing, with the liberation of autospores, is referred to in later discussion as cell division. If after 9 hours of illumination a cell suspension is darkened, growth in mass is interrupted in all cells, but the divisions continue. In the absence of organic carbon the growth of liberated autospores is postponed until after illumination is started. After 3 to 4 cycles of this light: dark regimen an algal population contains at the end of a dark period up to 99 per cent of the cells as small autospores. When illuminated, cells of such an algal suspension start growing simultaneously. To maintain synchronization in cell development the described regimen of light:dark was kept throughout the entire period during which a certain algal culture was the source of cells for the studies reported in this paper. The developmental stages of the cells are denoted by the lapse of time in hours from the start of growth (start of illumination) to the moment when growth was interrupted by harvesting cells from the culture chamber.

Cells were grown in a continuous culture apparatus (Myers and Clark, 1944) under

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conditions permitting the maintenance of constant temperature, illumination, and nutrient supply throughout the investigation. Carbon dioxide was supplied by bubbling a 3.5 per cent CO_2—in air mixture through the algal suspension during both the light and dark periods; temperature was maintained at 39°C.; light intensity from tungsten bulbs as measured on the surface of the culture chamber was near 4,000 lux. Density of the algal suspension was kept at about 1 mm.^{3} packed volume of cells per 1 ml. of suspension. In grams per liter the culture medium contained: KNO_3—1.25, KH_2PO_4—1.25, $MgSO_4 \cdot 7H_2O$—1.00, $CaCl_2$—0.0835, H_3BO_3—0.1142, $FeSO_4 \cdot 7H_2O$—0.0498, $ZnSO_4 \cdot 7H_2O$—0.0882, $MnCl_2 \cdot 4H_2O$—0.0144, MoO_3—0.0071, $CuSO_4 \cdot 5H_2O$—0.0157, $Co(NO_3)_2 \cdot 6H_2O$—0.0049, ethylenediaminetetraacetic acid (as a chelating agent)—0.5. The pH of the medium was 6.8. Sterile conditions were maintained in the growth chamber.

Respiration studies were done by means of the direct Warburg method. An algal suspension was harvested from the continuous culture apparatus. An aliquot of this suspension was centrifuged and the deposit resuspended in a three salt suspending fluid containing in grams per liter: KNO_3—1.25, $MgSO_4 \cdot 7H_2O$—1.00, KH_2PO_4—2.50. In studies of glucose respiration 10 gm. of glucose were added to the above solution. After this preparation the cell suspension was stirred by bubbling air through it. A pair of vessels was used to determine both oxygen and carbon dioxide exchange. Into each vessel were measured 2.5 ml. of algal suspension containing about 20 mm.^{3} of algal cells. The central well of one vessel contained 0.25 ml. of 15 per cent KOH. Data from at least two pairs of vessels were averaged to obtain a single point for the figures. When only oxygen consumption was determined a single vessel method was used. KOH was placed in the central well and at least two vessels were used to obtain the average values presented in figures and tables. Vessels were shaken in the bath at a speed of 120 oscillations per minute. Temperature in the bath was 39°C.

The time factor was of considerable importance and was recorded as routine. It took about 50 minutes from the moment of harvesting cells until the respiration vessels were placed into the bath. During this time algal cells were at room temperature of about 26°C. The first readings were taken after an equilibration period of 10 minutes. Readings at zero time (the moment of placing vessels into the bath) were obtained by graphic extrapolation.

Endogenous respiration was studied at two levels of pH, 4.5 and 6.8. In measuring glucose respiration at pH 6.8 two techniques were employed. In one case glucose was added to the suspending fluid before cells were transferred into it. In this way the time was cut to a minimum during which cells were deprived of organic carbon after having been harvested from the growth chamber. In the second case cells after centrifugation were taken into a suspending fluid from which glucose was absent and allowed to undergo endogenous respiration. After readings on endogenous respiration had been taken for 60 minutes, glucose was added from the side arm, and readings on glucose respiration were started. Rates of endogenous respiration, and of glucose respiration of cells introduced into the glucose medium after centrifugation, are given for the intervals of time 0 to 30 minutes after placing flasks into the bath. When glucose was added after 60 minutes of endogenous respiration, rates of glucose respiration are for 0 to 30 minute intervals after the addition of glucose.

RESULTS AND DISCUSSION

In Table I are listed respiratory quotients (CO_2/O_2) for cells of seven successive developmental stages. In each case measurements are given for two intervals of time of the same respiration experiment: 0 to 30 and 50 to 80 minutes after placing manometric vessels into the bath.

In general, the respiratory quotient is rather stable in the course of cell development, an observation recorded also by Nihei *et al.* (1954). Some increase in the respiratory quotient after exposure of cells to light was due to the reduction of nitrates (Cramer and Myers, 1949). The relative stability of the respiratory quotient both in the course of cell development and during a respiration experiment allows us to base the following discussions largely upon the rates of oxygen evolution for which more extensive data are available.

TABLE I

Respiratory Quotients (CO_2/O_2) for Cells of Successive Developmental Stages of C. pyrenoidosa, Strain 7-11-05

Developmental stage of the cells Duration of illumination or darkness in growth chamber in hours		R.Q. for intervals of time during one respiratory experiment	
Dark	Light	0 to 30 min.	50 to 80 min.
(1)	(2)	(3)	(4)
15	0	1.3	1.2
15	1	1.5	1.2
15	3	1.5	1.2
15	4	1.3	1.2
15	6	1.5	1.4
15	9	1.5	1.5
5	0	1.4	1.3

As seen in Fig. 1 there is practically no difference in the rates of endogenous respiration measured at two different pH values. Rates for glucose respiration, as is usually the case, are considerably higher than those for endogenous respiration but in general they follow the same pattern in the course of cell development. Other conditions being equal, cells after being harvested from the growth chamber continue to respire at a rate depending on the supply of respiratory material available to the cell. In presenting data on endogenous respiration the time interval at which they have been recorded is of utmost importance. This change in the rate of endogenous respiration with the progress of the experiment, and the importance of the time factor in recording manometric readings acquire even more significance in work with cells of different developmental stages, subjected to illumination for different lengths of time.

In Fig. 2 are presented time course curves for the rates of endogenous respira-

tion of cells of six successive developmental stages. Centrifugation, transferring cells into suspending fluid, aerating the algal suspension, and filling the respiratory flasks took as usual about 50 minutes. After flasks were placed into the bath they were shaken for 10 minutes. Readings were then started and continued for 3 hours.

As seen from Fig. 2 the course of endogenous respiration after darkening the algal cells depends greatly on the developmental stage of the cells. Small cells

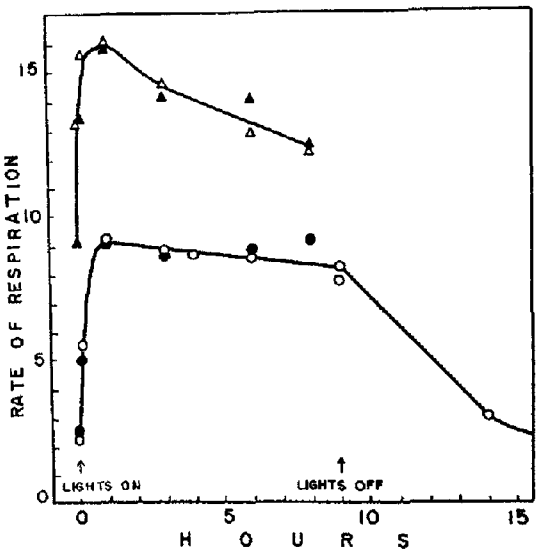

FIG. 1. Rates of endogenous and glucose respiration in cubic millimeters O_2/cubic millimeter packed cells/hour for cells of successive developmental stages of *C. pyrenoidosa*, strain 7-11-05. Open circles, endogenous respiration at pH 4.5; filled circles, endogenous respiration at pH 6.8; open triangles, glucose respiration at pH 6.8, glucose added to the suspending fluid before cells were transferred into it; filled triangles, glucose respiration at pH 6.8, glucose added after 60 minutes of endogenous respiration; all measured by the one vessel method.

from the dark (0 hour cells) have a low respiration rate which decreases only slowly with time. Cells of intermediate developmental stages (1 and 3 hour cells) have the highest starting respiration rate which undergoes a rapid decline as soon as cells are darkened. In cells well advanced in their development (6 and 8 hour cells) the starting respiration rate is lower but this rate remains stable for almost 3 hours of keeping cells in darkness—a surprisingly long time for endogenous respiration, particularly at 39°C. The importance of the time factor in recording respiration rate after harvesting cells is shown in Fig. 3 where the course of respiration during cell development is depicted as based on manometric readings during different intervals of a respiratory experiment.

The longer the preparatory period, and the later the time during which rates of respiration are recorded, the greater is the probability that the curve describing respiration in the cell development will be of the slowly ascending type. In Fig. 3 this would fall somewhere between the curve depicting the rates for 0 to 30 minute interval and that for the 90 to 120 minute interval. It is evident that the course of respiration during cell development, as this process takes place in the growth chamber at the moment of harvesting the cells, is described by neither of these curves but by one more or less approaching that obtained

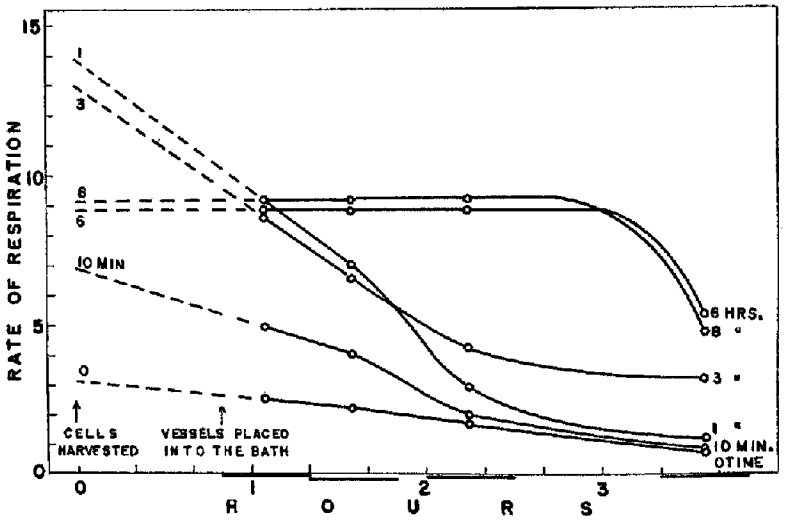

FIG. 2. Time course of the rates of endogenous respiration during respiratory experiment in cubic millimeters O_2/cubic millimeter packed cells/hour for cells of successive developmental stages of *C. pyrenoidosa*, strain 7-11-05. Rates of oxygen consumption calculated for 4 intervals of time, 30 minutes each, indicated on the time scale by the solid lines. The portions of the curves corresponding to the span of time between harvesting the algal cells and placing the manometric vessels into the bath extrapolated graphically. All measured by one vessel method at pH 6.8. Developmental stages are indicated on the curves.

by extrapolation of the data to the moment of harvesting (depicted by the dotted line connecting crosses in Fig. 3). It is interesting that the shape of this extrapolated curve closely approaches that for glucose respiration (Figs. 1 and 4).

Contrary to the picture for endogenous respiration (Fig. 3) the shape of the curve for glucose respiration (Fig. 4) in the course of cell development is far less dependent on the time in which it is recorded during an experiment. The curves in Fig. 4 describing the rate of glucose respiration for two different intervals run almost parallel to each other; the small differences in the shape of these curves will be discussed later.

These studies suggest that the rate of both endogenous and glucose respiration rises immediately after the start of the development of a cell, soon reaches a maximum, undergoes a slow decline, and eventually, by the time the mother

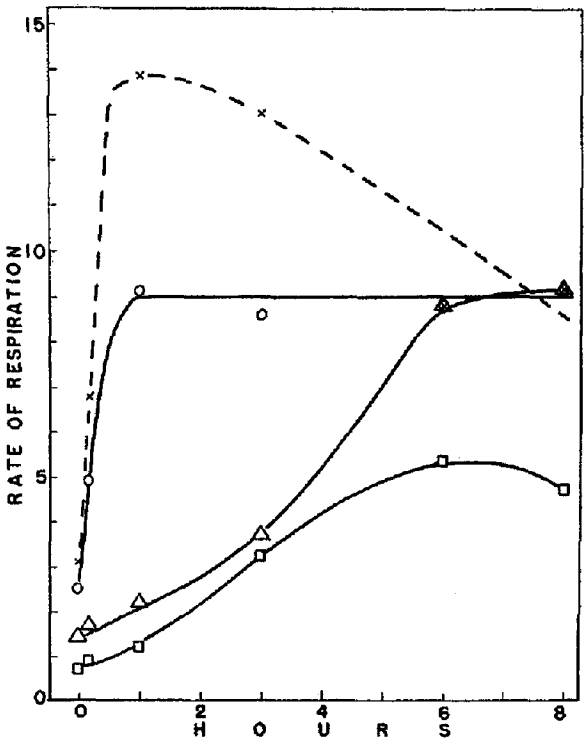

FIG. 3. Rates of endogenous respiration in cubic millimeters O_2/cubic millimeter packed cells/hour for cells of successive developmental stages of *C. pyrenoidosa*, strain 7-11-05. Circles, rates based on readings during the 50 to 80 minute interval after harvesting cells (0 to 30 minute interval after placing vessels into the bath); triangles, rates based on readings during the 140 to 170 minute interval after harvesting cells (90 to 120 minute interval after placing vessels into the bath); squares, rates based on readings during the 200 to 230 minute interval after harvesting cells (150 to 180 minute interval after placing vessels into the bath); crosses, rates extrapolated to the moment of harvesting cells from the growth chamber; measurements by the one vessel method at pH 6.8.

cell ruptures into a number of daughter cells, reaches the lowest level characteristic of autospores in the dark. The dry weight and specific gravity of cells generally increase during development. For this reason the downward slopes of the curves for endogenous and glucose respiration, if calculated on a dry weight basis, become even steeper than in Fig. 1.

Support for this picture of changes in the respiratory rate during the cell development is given by Neeb's data on *Hydrodictyon* (Neeb, 1952), which

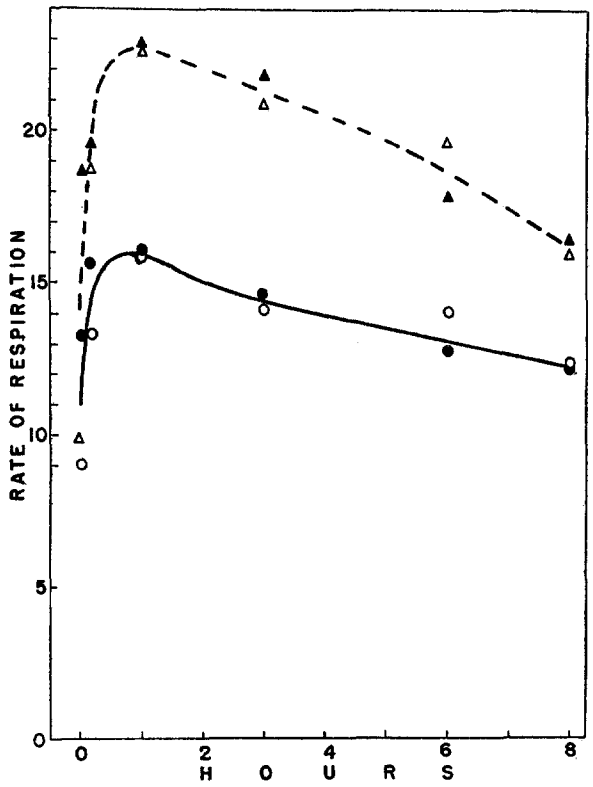

FIG. 4. Rates of glucose respiration in cubic millimeters O_2/cubic millimeter packed cells/hour for cells of successive developmental stages of *C. pyrenoidosa*, strain 7-11-05. Circles, rates based on readings during the 0 to 30 minute interval after placing vessels into the bath or, for the technique in which glucose was added after 60 minutes of endogenous respiration, during the 0 to 30 minute interval after adding glucose; triangles, rates based on readings during the 90 to 120 minute interval after placing vessels into the bath or after adding glucose; filled symbols, glucose added to the suspending fluid before cells were transferred into it; open symbols, glucose added after 60 minutes of endogenous respiration; measurements by the one vessel method at pH 6.8.

show a clearly descending respiration rate during the life cycle of this alga. Neeb did not observe a rise in the respiration rate at the beginning, but he started his respiration measurements 2 days after the start of algal development and possibly missed the phenomenon.

Contrary to these findings Nihei *et al.* (1954) describe the course of the rate of endogenous respiration in *Chlorella ellipsoidea* as gradually rising during the whole period of cell development with the highest value of respiration rate observed at the final stage of what they call light cells. Only after the cell suspension is transferred into darkness does the respiration rate start to decline. Contradictions between these results, and Neeb's and ours may be due to a variety of factors: the use of different organisms, differences in conditions of growing cells, in methods of inducing synchronization, in technique of preparing algal suspensions for manometric studies and in interpretation of the observations. One of the most obvious differences lies in the way of reporting the observed data. What Nihei *et al.* indicate in their discussions as endogenous respiration rate is by no means a rate characteristic of the cells as they grow in the growth chamber. What they measured was the rate after a considerable lapse of time while the cell suspension was prepared for manometric measurements following harvest from the growth chamber. As was shown in Figs. 2 and 3 cells of certain intermediate developmental stages (in our experiments 1 hour, and to a less extent 3 hour cells) possess the highest rate of endogenous and glucose respiration. After being harvested and placed in the dark in the absence of organic carbon these cells use their supply of respiratory material faster than cells of later developmental stages. If manometric readings are delayed long enough after harvesting, the curve describing the course of respiration rate in cell development has a shape close to that reported by Nihei *et al.* (1954) or to that given in Fig. 3 for 90 to 120 minute readings. It can even be concave as in Neeb's experiments (1952) when he measured respiration rate at certain intervals during one light period following a dark period. All these curves are brought together for comparison in Fig. 5.

The time scale in Fig. 5 necessarily differs for different curves. In our experiments it embraces 8 hours, in the observations by Nihei *et al.* it is 28 hours, in Neeb's data for the life cycle of *Hydrodictyon* it is 34 days, and in those depicting the course of respiration during one light period it covers 12 hours. The developmental status of cells to which the starting points of the curves in Fig. 5 refer is also different. In our work the starting point on a curve refers to the zero cells from the dark. In Neeb's experiments, covering the events during one light period, the starting point also indicates cells from the dark. In his experiments, covering the development of the organism for 34 days, measurements were started only after 2 days of growth and therefore the initial period of 2 days has no place on the curve. Nihei *et al.* used a technique of cell synchronization in which a steady state algal suspension grown at *ca.* 6000 lux was transferred to 200 to 400 lux and kept at this light intensity for 7 days. After this time the algal suspension was considered to be rich in what they call "dark" cells and was used as a starting point in their observations on the events taking place during the life history of algal cells. Actually the "dark" cells of

Nihei *et al.* are not comparable in developmental stage to our cells from the dark. After emerging from cell division these cells were immediately influenced by light, and at the start of respiratory measurements they were already in some advanced stage of their development. The absence of the actual zero point in cell

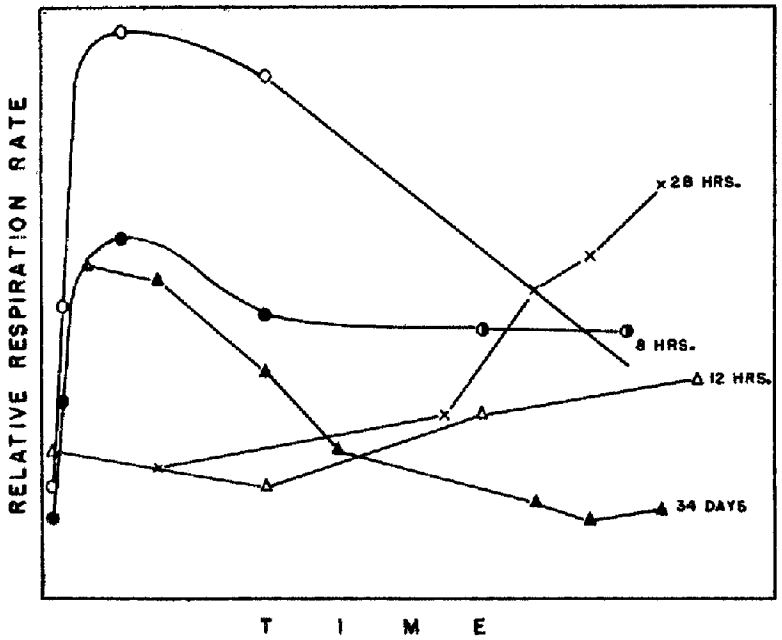

FIG. 5. Rates of endogenous respiration—O_2 consumed—on dry weight basis. Filled circles, data for *C. pyrenoidosa*, strain 7-11-05, for the 8 hour period and based on readings during the 0 to 30 minute interval after placing vessels into the bath; open circles, data for *C. pyrenoidosa* for the 8 hour period extrapolated to the moment of harvesting cells from the growth chamber; filled triangles, Neeb's data for *Hydrodictyon* for the period embracing the course of the development of a colony for 34 days; open triangles, Neeb's data on *Hydrodictyon* for one light period of 12 hours; crosses, Nihei *et al.* data on *C. ellipsoidea* covering the development of a synchronized batch of cells for 28 hours. Time and respiration rate scales are different for different strains.

development from the curve presented by Nihei *et al.* can also help explain the differences in the shape of this curve and ours.

In discussion, it is convenient to distinguish two phenomena: a rapid rise of respiration rate during the first period of cell development and a decline of rate over the second much longer part of the life history of the cell. This decline is slow as long as the algal suspension is kept in the light, but is considerably accelerated by darkening cells at an advanced stage of development.

The slow decline of respiratory rate during development in the light seems

to have no direct connection with the level of carbohydrates in a cell: an increase in the amount of carbohydrates has not been shown to impede respiration. One could think that this slow decline of the rate might be caused by the proportional increase of storage material and dead substance (cell wall) if these processes took place in our experiments during the first 8 hours of cell development. However, this possible explanation proves to be inadequate or insufficient. Microscopic examinations show that, if cells are darkened after 9 hours of illumination, cell divisions take place at a high rate. One might expect that the liberation of daughter cells from mother cell wall and the decrease in proportion of dead material would raise the rate of respiration. This expectation does not materialize. With continued cell divisions the rate of respiration of an algal suspension still declines. It reaches the lowest level at the time when cell divisions are completed and remains at this low level as long as cells are prevented from growth by keeping them dark in the absence of organic carbon.

Probably the decrease in respiratory material plays a part in the rapid decline of respiration rate in the dark. The transition from light to darkness may also directly influence the activity of respiratory enzymes. *But the fact that the decline in the respiration rate starts early in cell development, and extends over the whole period during which the cell is in the light, speaks against the assumption that only these two factors operate to reduce respiration after the cells are darkened.* Evidently bursting of the mother cell wall is preceded by preparatory processes, somehow connected with the decrease in respiration.

The decline of respiration rate after darkening has been studied on steady state suspensions and explained by the decrease in amount of the respiratory substrate (French, Kohn, and Tang, 1934; Cramer and Myers, 1949). The rate of respiration declines very rapidly at first, but after some time (about 25 hours in the French, Kohn, and Tang experiments) comes to an almost steady value, decreasing only slowly thereafter. It was assumed that two substances, A and B, were used in respiration during the first period and the transition to the second period was caused by the exhaustion of A after which only B was used for a long time at a low rate (French *et al.*, 1934).

There is no doubt that a decrease in the amount of respirable material is partially responsible for the decline in endogenous respiration rate with time. But the work with synchronized algal suspensions suggests that the changes in the speed of this decline (the transition from the above mentioned first period to the second) coincide with the accomplishment of cell divisions. The processes bringing about the decline in the respiration rate start early in cell development and culminate at cell division. After that, if cells are prevented from growth by being kept in the dark, the respiration rate decreases very slowly. This course of events with non-synchronized suspensions underlines observations on so called starvation. Darkening a steady state suspension interrupts growth, causes most cells to divide, and this, together with a decrease

in the supply of respirable material, brings the respiration rate rapidly to a low level. After most of the cells are brought to the stage of small autospores their respiration rate changes slowly with time.

The coincidence of cell division with the rapid decline in respiration rate after darkening, can also be demonstrated in another way. It was shown in Fig. 3 that 6 and 8 hour cells have a similar rate of respiration and, if darkened, respire at a constant rate for about 3 hours. One would expect that 6 hour cells which had received less light and therefore accumulated less respirable material would be the first to show signs of a decline in the respiration rate. Actually the opposite is true. The 8 hour cells are advanced further in their development than 6 hour cells and after being darkened, start cell divisions first. The result is that the rate of respiration shows the first signs of a decline in 8 hour cells after 160 minutes in the dark, and at the end of the experiment declines to a lower level than in 6 hour cells in which the respiration rate starts to decline only after 180 minutes in the dark.

To close the cycle of changes in the respiration rate during its life cycle, a cell has to come back to the initial level at the beginning of development. It is difficult to separate the initial rise of rate from the influence of accumulation of carbohydrates in light. The level of carbohydrates in a cell is known to influence the rate of respiration. With the technique of synchronization employed in these investigations the start of cell development and a concomitant rapid rise in respiration rate coincide with the increase in respiratory material in the cell transferred from dark into light. The question is whether this rise in rate is due to anything else than the increase in carbohydrates. An attempt was made to prove that the decline in respiration rate is a normal process taking place during cell development in light. A logical extension of this would be a reverse process bringing the rate of respiration back to a high level to make a new cycle possible.

Another line of reasoning in favor of cyclic changes in respiration rate, induced by one method or another, was provided by the work of Pirson *et al.* (1954) with *Hydrodictyon*. Colonies of this alga were maintained at different regimens of light:dark; *i.e.*, 12:12 hours, 6:6 hours, and 10½ hours:7 hours. Then the conditions were changed to continuous darkness. The rhythmic changes in respiration rate established at each light:dark periodicity continued for some time in the dark with maxima in respiration rate corresponding to the time expected if the particular light:dark regimen had not been changed to continuous darkness. Evidently there was no increase in respiratory substrate after the alga was transferred into darkness; fluctuations in respiration rate occurred with no connection to changes in the level of substrate.

The rate of glucose respiration provides final evidence in favor of the view that increase in respiration rate at the beginning of cell development is influenced by other factors than the level of carbohydrates. As shown in Fig. 4, not

only are the initial rates of glucose respiration different in cells of different developmental stages, but these differences remain after about 2 hours of manometric observations. As a matter of fact these differences become even more pronounced with time.

In the fourth column of Table II the increase in the rates of glucose respiration for 90 to 120 minute intervals of a manometric experiment is expressed in per cent of the rates for 0 to 30 minute intervals. Zero hour cells low in their respiration rate at the start of readings increase the rate by only 9 per cent. Cells in more advanced developmental stages not only have a higher initial respiration rate, but their glucose respiration rate increases more with time, reaching 40 to 47 per cent. In 8 hour cells the rate of glucose respiration is not only comparatively low at the start of an experiment but also increases

TABLE II

Rate of Glucose Respiration in Cubic Millimeters O_2/Cubic Millimeter Packed Cells/Hour for Cells of Successive Developmental Stages of C. pyrenoidosa, Strain 7-11-05

Developmental stage of the cells	Rate of respiration for intervals of time during one respiratory experiment		Per cent increase with time
	0 to 30 min.	90 to 120 min.	
(1)	(2)	(3)	(4)
0	9.0	9.8	9
10 min.	13.3	18.7	41
1 hr.	15.8	22.6	43
3 hrs.	14.1	20.8	47
6 hrs.	14.0	19.6	40
8 hrs.	12.4	15.9	28

more slowly with time (28 per cent) than in cells of earlier developmental stages. Evidently cells of different developmental stages differ in their capacity to respire exogenous carbon sources.

It seems that changes in respiratory activity during cell development are to some extent independent of light. This, however, does not exclude the possibility that a direct light activation of respiratory enzymes takes place parallel to the processes related to cell development. An indirect influence of light through an effect on the respiratory substrate level is also clearly indicated. Studies on the influence of light on respiration have been generally done on steady state suspensions. They have been confused by the fact that changes from light to darkness and *vice versa* have an indirect influence on the rate of respiration by affecting the developmental status of an algal suspension. Large cells which accumulated enough carbohydrates to respire for a considerable length of time at a more or less constant rate, but which did not advance in their development far enough to be ready for cell division, could be given

alternate light and dark periods for some time without influencing their respiration rate. Small daughter cells kept in the dark or under weak illumination can be expected to increase their respiration rate if brought into light or if light intensity is increased. Large cells old enough to divide if darkened may undergo cell division in the course of the experiment and cause the respiration rate to go down. Depending on the previous culture conditions and also on the intensity of illumination and the duration of light and dark periods, the average developmental status of an algal suspension can be changed and a certain degree of synchronization can be induced unknowingly. This will bring about a change in the rate of respiration. Thus the influence of illumination on the rate of respiration of non-synchronized algal suspensions may result from an interplay of a complex array of processes proceeding in different cells at different speed and in different directions. The intricacy of the situation makes it understandable why studies on the influence of light on the respiration of non-synchronized algal suspensions have not produced concordant results.

SUMMARY

Endogenous and glucose respiration were studied during the life history of *Chlorella pyrenoidosa*. A generalized picture of the course of respiration during the life cycle is suggested. At the liberation of daughter cells from the wall of the mother cell, or soon after, the respiration rate reaches its lowest level. If the daughter cells are placed in light the respiration rate rapidly increases with time, soon reaches a maximum, and then declines slowly. Two factors are important in the initial increase—the early developmental stage of the cells and the influence of light. In autotrophically developing algae the parts played by developmental processes and by light have not been separated. Direct activation of respiratory enzymes by light, in addition to the level of respiratory substrate, cannot be excluded.

The decline of respiration rate over most of the cell history seems to have no connection with light and is probably bound to the developmental processes *per se*. Darkening the suspension interrupts growth and induces liberation of daughter cells, with concomitant faster decrease in respiration rate. The rate of respiration of small daughter cells decreases in darkness only slowly with time. Illumination seems necessary to bring these cells back to a high level of respiratory activity.

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