*INTEGRATION OF ENERGY CONVERSION AND BIOSYNTHESIS IN THE PHOTOSYNTHETIC BACTERIUM RHODOPSEUDOMONAS CAPSULATA**

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Recent reports from this laboratory described the use of intermittent illumination as a technique for exploring the intimacy of coupling between photophosphorylation activity and biosynthesis in *Rhodopseudomonas capsulata*.[1, 2] *In vivo* pulsing of adenosine triphosphate (ATP) production at certain frequencies, by programmed intermittent illumination, was found to result in marked inhibition of growth rate. Significant alterations in macromolecular composition of the cells are associated with this inhibition, particularly in respect to total ribonucleic acid (RNA) content. This communication describes in greater detail the effects of intermittent illumination on *Rps. capsulata* and their implications in regard to the mechanisms which regulate the integrated formation of major cell constituents, including the energy-converting membrane system.

Materials and Methods.—Organism and culture conditions: The strain of *Rps. capsulata* employed was isolated in 1963 and was recently deposited in the American Type Culture Collection (no. 23782; strain "St. Louis"). The medium used (initial pH, 6.8) contained 0.4% DL-malic acid, 0.1% $(NH_4)_2SO_4$, 0.0001% thiamine hydrochloride, and additional inorganic salts as specified by Sojka *et al.*[1]

Stock liquid cultures, the inoculum source for experimental cultures, were transferred every 24 hr and, unless otherwise noted, incubated in continuous saturating light (550 foot-candles (ft-c)) provided by Lumiline lamps (see below). Screw-cap test tubes of 17-ml capacity, selected to fit a Klett-Summerson photometer, were routinely used; each tube contained several glass beads to facilitate mixing prior to turbidity measurements. Subsequent to inoculation, the tubes were completely filled with sterile medium and then capped; initial culture densities were always lower than 30 photometer units, and frequently much less. The test-tube cultures were incubated at approximately 34°, and air in their vicinity was vigorously circulated by fans. Ambient temperature in the immediate region of the tubes ranged from about 32° to 36°; the growth rate of *Rps. capsulata*, however, is little affected by temperature over the span 26–38° (ref. 3).

Growth kinetics: Growth rates were estimated from serial turbidity measurements of the culture tubes in a Klett-Summerson photometer equipped with a no. 66 filter. Under the various conditions used, the turbidity value up to at least 220 photometer units is proportional to bacterial mass; 200 units is equivalent to 440 μg dry wt/ml.

Illumination: In all experiments with intermittent illumination, light and dark intervals were always equal; the total time elapsed for one light interval and a succeeding dark interval is designated as "cycle length." Light intensity during the dark intervals was less than 5 ft-c. To obtain intermittency with cycle lengths between 10 sec and 60 min, the light source was alternately turned on and off by automatic timing devices. For cycle lengths between 0.1 and 2.4 sec, the light beam was chopped by a rotating semicircular disc, driven by an appropriate synchronous motor, interposed between the light source and the cultures. In "L arrangement," the cultures, placed on a bench top, were illuminated by a bank of two Lumiline lamps (60 w, except as noted) mounted in juxtaposition in a "light box," the front of which consisted of a thin clear plastic sheet. In "PF arrangement," cultures were placed in a "dark box" constructed with a fan and wire screening at opposite ends. The front of the box was fitted with a plastic sheet backed with a piece of black cardboard, in which a vertical window of 11.5 × 4.5 cm was

cut. Two duplicate cultures were placed just behind the transparent window and were illuminated by an external 30-w reflector flood lamp.

Light intensity measurements were made with a Weston illumination meter model 756. It should be noted that for apparently equal values of light intensity, the actual intensity in the L arrangement is doubtless significantly higher than in the PF arrangement because of oblique illumination from the long lamps. With continuous "saturating" illumination (550 ft-c), the growth rate of *Rps. capsulata* in the L arrangement is distinctly faster than in the PF arrangement. Possibly, this effect may be related to a difference in color temperature of the two types of lamps employed.

Chemical determinations: Protein and RNA determinations were made on cultures in logarithmic growth, at turbidities between 165 and 200 photometer units. For protein estimation, cells were harvested and the pellet was treated with 1 *N* NaOH (at boiling water-bath temperature); protein in suitable aliquots of the resulting solution was determined by the method of Lowry *et al.*,[4] with the use of crystalline bovine serum albumin as standards.

For RNA determination, 10 ml of culture were rapidly mixed with an equal volume of 10% trichloroacetic acid (TCA), previously cooled to −3°. After 45 min at −3°, the suspension was centrifuged. The precipitate was washed twice with cold 5% TCA, and RNA was then extracted by two successive 15-min incubations with 5% TCA at 70°. The extracts were combined and aliquots analyzed by the orcinol method, as described by Schneider.[5] D-ribose was used as the standard, and ribose equivalents were multiplied by a factor of 4.9 (see ref. 6) to give the RNA values shown in Table 2.

Bacteriochlorophyll (BChl) estimations also were made on cultures in logarithmic growth, at turbidities between 100 and 200 photometer units. A suitable volume of culture was centrifuged and the pellet extracted with acetone-methanol according to the procedure of Cohen-Bazire *et al.*[7] Absorbancy of the extract at 775 mμ was measured and an extinction coefficient of 75 mM^{-1} cm^{-1} (see ref. 8) used for calculation of the results recorded in Table 3.

Results.—Growth kinetics in intermittent light: If pools of photoproducts can accumulate during a given period of illumination of growing cells, sufficient to maintain optimal biosynthesis for an equal time period in darkness, the growth rate in an intermittent light:dark regimen with this periodicity should approximate that observed in continuous light. Our initial experiments[1] aimed at finding a cycle length of this kind disclosed that the growth rate of *Rps. capsulata* was markedly inhibited in intermittent light, even with cycles as short as 10 seconds. With relatively long cycles, in which the limited photoproduct pools presumably would be rapidly consumed during the early part of each dark interval, an over-all growth rate of about half the control (continuous light) value was expected, but observed only when the cycles were 30 minutes or longer. Figure 1 illustrates typical growth kinetics obtained in experiments with continuous and intermittent light, and Table 1 summarizes the effects of intermittency on growth rate, with cycles ranging from 0.1 second to 60 minutes. In all instances the incident light intensity was 550 ft-c, which is saturating for growth in continuous light (the growth rate vs. light intensity curve for *Rps. capsulata* is similar to that obtained by Sistrom[9] for *Rps. spheroides;* see Fig. 2).

The data of Table 1 indicate that as the cycle length with intermittent light increases beyond one minute, the over-all growth rate gradually approaches the theoretical value, i.e., one half the rate in continuous light. With a 60-minute cycle it would appear that the growth rate is maximal during the light intervals and virtually zero in the dark intervals. Frequent turbidity measurements of

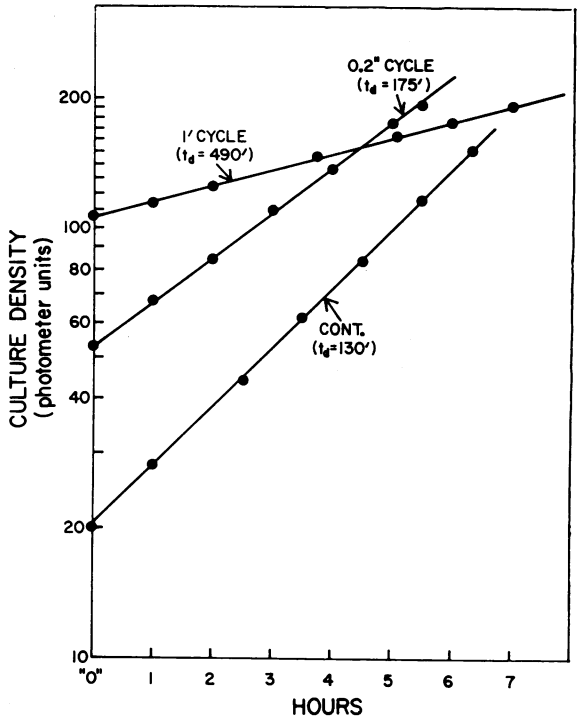

FIG. 1.—Growth kinetics of *Rps. capsulata* in continuous light and in two intermittent light regimens (0.2-sec and 1-min cycles). The cultures were grown in the PF arrangement. History of cultures before "zero" time: *Cont.:* cells were kept in logarithmic growth in continuous light for more than 24 hr by repeated subculturing, very small inoculum was used for the last subculture, and turbidity measurements were initiated when the photometer reading was 20; *0.2-sec cycle:* cells grown for 15 hr (*ca.* five mass doublings) in this regimen and subcultured to fresh medium at "zero time"; *1-min cycle:* culture grown overnight (*ca.* two doublings) in this regimen, at "zero time" the turbidity was as shown. The ordinate is on a logarithmic scale; t_d = mass doubling time (in minutes).

cultures in a 60-minute cycle, in fact, show stepwise growth increases consistent with this conclusion. Maximal inhibition of growth rate is seen with cycles between about ten seconds and one minute. With a 2.4-second cycle, the theoretical rate is again observed, and further shortening of the cycle leads to substantially increased growth rates. These seem to plateau with cycles between 0.1 and 0.5 second at a rate still distinctly slower than the growth rate in continuous light. In all the intermittent light regimens used, the cultures received the same quantity of light per hour, and it is consequently evident that cells growing in very short cycles (<2.4 seconds) utilize the incident energy for biosynthesis more efficiently than those subjected to longer cycles. The reduction in efficiency with intermittent cycles of the order of 30 seconds to one minute is such that the cells, though receiving saturating light (550 ft-c), behave as if they were growing in *continuous dim* light of only about 60 ft-c intensity (see Table 2).

The decrease in growth rate observed with intermittent cycles of 30 seconds to one minute is much greater than would be expected if only the quantity of incident light were the decisive factor. With continuous light in the L arrangement, decrease of intensity from 550 to 275 ft-c leads to a relatively small increase in mass doubling time; typical values are 105 and 135 minutes, respectively. Similar experiments in the light-limiting region also showed the same effect, i.e., the mass doubling time in a one-minute cycle with 120 ft-c intensity (L arrangement) was 600 minutes, while in continuous light at 120 and 60 ft-c intensities the doubling times were 175 and 275 minutes, respectively. Another way of demonstrating the severe temporal energy stress caused by intermittency

TABLE 1. *Effect of intermittent illumination on growth rate of Rps. capsulata.*

Length of light:dark cycle	No. of expts.	Mass doubling time* (min)	Intermittency value†
0 (Continuous light)	6	132	
Seconds:			
0.1	2	180	0.73
0.2	2	185	0.71
0.5	2	180	0.73
1.0	3	212	0.62
1.2	3	205	0.64
1.8	2	210	0.63
2.4	7	255	0.52
10.0	4	465	0.28
30.0	2	508	0.26
Minutes:			
1	4	501	0.26
3	3	400	0.33
5	3	398	0.33
10	5	337	0.39
20	2	325	0.41
60	3	246	0.54

* Average from the number of experiments indicated in column 2; duplicate tubes in each experiment.

† Doubling time in continuous light/doubling time in intermittent light. PF illumination arrangement (550 ft-c). Several mass doublings were permitted to occur in each regimen before turbidity measurements were initiated for determining the growth rate; rates were calculated from serial photometer readings (220 units maximum).

is to superimpose a continuous beam of low-intensity light, e.g., 30 ft-c, on cultures growing in saturating intermittent (one-minute cycle) light. This causes a growth rate increase equivalent to that resulting from a similar increment in intensity with cultures growing in continuous illumination in the lower portion of the light-limiting region.

RNA and protein composition of cells growing in continuous and intermittent light: It has been established that the total RNA content of heterotrophic bacteria, growing with continuous energy supply at a given temperature, is proportional, within limits, to the growth rate.[6] Changes in protein content are less pronounced and, accordingly, as the growth rate decreases, the protein/RNA ratio increases.[10] Since intermittency leads to striking effects on the growth rate of *Rps. capsulata*, it seemed likely that the protein/RNA ratio would be affected, and possible that this index might provide some insight into the molecular bases of the changes in growth kinetics caused by pulsing energy flow. Relevant data on the RNA and protein composition of cells grown in continuous light and several intermittent regimens are shown in Table 2. Attention is drawn to the fact that the protein/RNA ratios differ somewhat from the preliminary results given in an earlier report,[1] due to the use of different analytical procedures.

Cells grown in continuous saturating light, i.e., at maximal growth rate, showed the lowest protein/RNA ratio (4.1), mainly because of the high RNA content. The values for cells growing at about the "theoretical" over-all rate in a 60-minute intermittent cycle were similar. Intermittency in a one-minute cycle, however, leads to a definite diminution in protein content and a substantial

TABLE 2. *RNA and protein composition of Rps. capsulata cells grown in continuous and intermittent light.*

Culture incubation conditions	No. of expts.	Mass doubling time* (min)	Protein (μg/ml)	RNA (μg/ml)	Protein (% of dry wt)	RNA (% of dry wt)	Protein/RNA
Continuous light	6	132	314	75.9	71.3	17.2	4.1
60-Min cycle	6	246	330	69.1	75.0	15.7	4.8
1-Min cycle	4	501	267	49.2	60.7	11.2	5.4
Continuous light—low intensity (60 ft-c)	4	470	260	41.3	59.1	9.4	6.3
2.4-Sec cycle	4	255	305	47.1	69.4	10.7	6.5

PF illumination arrangement.

The protein and RNA contents of cultures with turbidities ranging from 165 to 200 photometer units were measured and all data normalized to 200 units (440 μg dry wt/ml); values given are averages from the numbers of experiments indicated. A minimum of three mass doublings was permitted to occur in each regimen before samples were taken for analysis; in the 60-min cycle experiments, cells were always harvested in the middle of a light period.

* Except for the 60 ft-c continuous light expts., these values are from Table 1; growth rates of the cultures used for analysis were also determined and were within the usual experimental error.

decrease in the RNA level. Separate experiments have shown that the ribosome content/unit dry weight of "one-minute cycle cells" is only about one half that of cells cultivated in continuous saturating light.[3] The similarity between cells in a one-minute intermittent cycle and in continuous dim light (60 ft-c) in respect to growth rate was noted above, and this is seen also in the protein and RNA composition.

Comparison of the values for the one-minute and 2.4-second cycles is of special interest. Cells growing in the 2.4-second cycle clearly utilize the incident light energy for biosynthesis with considerably greater efficiency. Moreover, with energy flow pulsed at these frequencies the usual relationship between growth rate and RNA content seen with continuous energy supply is obviously violated. Assuming the same ribosome content in both types of cells, the results suggest, not surprisingly, that growth rate is a function of both ribosome content and the "average" chemical energy flux.

Effects of intermittency on BChl synthesis: The BChl content of *Rhodopseudomonas* and other purple bacteria is inversely related to the incident light intensity during growth with continuous illumination.[9, 11] Below the saturation point, light intensity determines the mass doubling time and it has been suggested[12] that the synthesis of BChl is somehow regulated in concert with (or by) the growth rate, rather than by intensity *per se*. It was consequently of interest to determine the effect of intermittency on BChl formation. Results of a number of experiments with both the L and PF illumination arrangements are summarized in Table 3.

In comparison with cells growing rapidly in continuous light (550 ft-c) in the L arrangement, cells multiplying more slowly in a one-minute intermittent cycle have a greatly elevated BChl content. Approximately the same BChl value was found in cells growing at comparably slow rates in continuous dim light of 50 ft-c intensity. Thus, the relative BChl contents also indicate that in a one-minute intermittent cycle the cells "perceive" a disproportionately small fraction of the

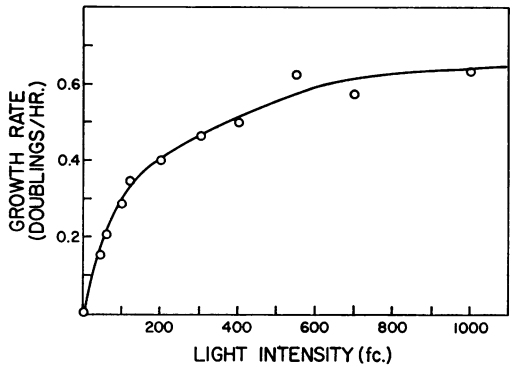

FIG. 2.—Relationship of growth rate of *Rps. capsulata* to incident light intensity. Rates are expressed in terms of mass doublings/hour (1/mass doubling time) during logarithmic growth in the L illumination arrangement. To obtain different intensities, the tubes were placed at appropriate distances from the light source (30-w lamps for intensities up to 120 ft-c).

incident energy, and the results are consistent with the general correlation between growth rate and BChl synthesis.

In the PF arrangement, the growth rate in continuous light is somewhat slower and this is reflected in a higher BChl level. Intermittency in a 60-minute cycle does not influence the BChl content appreciably. The increase seen in the one-minute cycle is considered significant; in fact, the value observed was identical with that found in the one-minute cycle with the L arrangement. Since the doubling times were substantially different in the one-minute cycles in the two arrangements, it appears that factors other than growth rate as such are involved in regulation of BChl formation. Dissociation of BChl synthesis from growth rate is clearly seen by comparing the results with the one-minute and 2.4-second cycle regimens in the PF arrangement. More rapid pulsing of the energy supply increases the growth rate markedly and also leads to an *increase* in BChl content. These results imply that pulsing of the energy supply at certain frequencies can cause disturbances in the chemical signal(s) that governs "balanced" BChl synthesis.

Since the BChl content of cells growing in continuous light increases with decrease of light intensity, the validity of the results in Table 3 depend, in part, on the absence of appreciable "self-shading" effects as the culture density increases. It was found that under the experimental conditions used with high light in-

TABLE 3. *Bacteriochlorophyll content of Rps. capsulata cells grown in continuous and intermittent light.*

Illumination arrangement	Regimen	No. of expts.	Mass doubling time (min)	BChl content* (% of dry wt)
L	Continuous light	30	113	0.66
"	1-Min cycle	16	367	1.20
PF	Continuous light	10	132	0.97
"	60-Min cycle	3	246	0.88
"	1-Min cycle	6	501	1.19
"	2.4-Sec cycle	10	255	1.67

Incident light intensity, 550 ft-c.

Cells in logarithmic growth were harvested at culture turbidities between 100 and 200 photometer units and analyzed for BChl as described in *Materials and Methods*. The mass doubling times for cultures in the PF arrangement are from Table 1; those for the L arrangement cultures are averages of a number of determinations.

* Average from the number of experiments indicated.

tensity, the BChl content (% of dry wt) did not change significantly during increase of bacterial density from 100 to 200 photometer units. With low-intensity incident light (50 ft-c), a definite gradual increase in BChl content does occur over this range; cells growing with 50 ft-c illumination have a relatively high BChl content, and with such low incident intensities self-shading effects are to be expected.

Discussion.—Intermittent and flashing illumination have been used in numerous photosynthesis researches, primarily in studies designed to determine the time constants of dark reactions closely associated with early photochemical events in nongrowing cells or pigmented subcellular fractions. Inhibition of growth of both higher plants[13] and unicellular algae[14, 15] in intermittent light has been reported, however, and usually is ascribed to "induction" (lag) periods in operation of the "photosynthetic system," associated with dark to light transitions (e.g., see discussion in ref. 16).

There is good reason to believe that, as far as biosynthesis is concerned, the primary result of intermittent illumination of *Rps. capsulata* is pulsing of ATP formation.[17] As a working hypothesis, we suggest that ATP and its congeners (adenosine diphosphate (ADP) and adenosine monophosphate (AMP)) function in a signal system which participates in regulation of the integrated biosynthesis of key macromolecular cell constituents, including the BChl-containing energy-converting membrane system. Direct determinations of the "ATP pool" in photosynthetic bacteria indicate that this is rapidly filled upon illumination.[18, 19] With intermittent light, the composition of the total adenylate nucleotide pool presumably oscillates between limiting states which can be described in terms of a concentration ratio parameter, "energy charge," defined[20] as (ATP + 0.5 ADP)/(ATP + ADP + AMP). Atkinson and his colleagues[20, 21] have shown that, with a given total adenylate nucleotide concentration, the activity of certain enzymes which utilize ATP is strongly affected by the energy charge. It may be that repeated interruption of ATP formation at certain frequencies in *Rps. capsulata*, by intermittent illumination, affects the average energy charge so as to accelerate abnormally the activity of certain biosynthetic sequences and to inhibit others, leading to unbalanced slow growth. With cycles in the range of ten seconds to several minutes, lags in any energy-requiring process (including, for example, restoration of optimal intracellular concentrations of nutrients or repair of cell components degraded during dark intervals) which occupy an appreciable fraction of each light period could contribute to the disturbed physiological state.

We assume that increase of (continuous) light intensity up to the saturation point increases the rate of ATP generation and the energy charge, and that these are controlling factors which dictate stimulation of RNA (and thereby, protein) synthesis and inhibition of BChl formation; in connection with the latter, it is noteworthy that studies[11, 22] with two species of photosynthetic bacteria indicate that changes in BChl level reflect comparable changes in quantity of the energy-converting membrane system. The foregoing interpretation implies that, with the use of saturating intermittent illumination, cycles in the strongly inhibitory range cause reduction of the average ATP production rate and energy charge to values characteristic in cells growing slowly in continuous dim light, i.e., cells

which show increased BChl and decreased RNA synthesis. Common chemical signals with opposing effects on BChl (membrane) and RNA formation would rationalize, in part, the reciprocal relationship invariably found between the quantities of these components. A preferential utilization of available ATP for synthesis of "excess" BChl (membrane) in cells growing under conditions of energy stress could then dispose to inhibition of the growth rate. Extension of the present line of investigation hopefully will provide insight into the relationship of ATP production rate and energy charge and their control functions during normal growth.

Summary.—Growth kinetics and cell composition (RNA, protein, and bacteriochlorophyll) of the photosynthetic bacterium *Rhodopseudomonas capsulata* during anaerobic multiplication with continuous illumination and in various intermittent light regimens have been examined as an approach to elucidation of regulatory mechanisms involved in the integration of energy conversion and biosynthetic processes. The results are interpreted in terms of a general scheme through which ATP generation rate and steady-state microconcentrations of adenylate nucleotides may control balanced synthesis of major macromolecular cell components.

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