METABOLIC CONDITIONS IN CHLORELLA*

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(Received for publication, May 10, 1948)

The variability of metabolism observed quite generally in microorganisms has never been systematically studied in the green algae. The frequent use of forms such as *Chlorella pyrenoidosa* in the study of photosynthesis has led to common use of purposely standardized culture conditions. This practice has been pushed toward its limit in the development of a continuous culture apparatus (Myers and Clark, 1944) which provides uniform experimental material day after day. At the same time the continuous culture method also affords a basis for the study of variability in metabolism imposed by various conditions.

The quotient of gas exchange has long been recognized as a practical tool in metabolic studies. Its usefulness in *Chlorella* has been extended by the work of the preceding paper (Cramer and Myers, 1948 *b*) which showed that the effect of nitrate reduction on the gas exchange quotient provides an index of the rate of nitrogenous synthesis. This finding is now applied to cells subjected to such conditions as might be expected to affect their metabolic activities.

The present study began in an attempt to explain the effect of light intensity on the CO_2/O_2 quotient noted in the preceding paper. It has been extended to include consideration of the changes in over-all metabolism induced by starvation, high light intensity, and nitrogen deficiency in comparison with the metabolism of growing cells. The general experimental methods and the over-all carbon and nitrogen metabolism in growing cells have been described in the preceding paper. It should be emphasized that the term *growing cells*, as used herein, specifically describes a standard preparation; *i.e.*, cells cultured photosynthetically under an illumination light-limiting for both growth and photosynthesis. The merit of this particular choice of reference will be justified in the subsequent discussion.

Starvation

Growing cells may be starved aerobically by shaking a suspension in the dark. Starvation results in a gradual decrease in capacity for photosynthesis, a disappearance of starch, and a marked decrease in endogenous respiration which approaches a constant rate with an R.Q. of 1.0 (Cramer and Myers, 1948 *a*). Table I describes the time course of the R.Q. after addition of glucose to growing and starved cells.

* Supported by a grant from the University of Texas Research Institute.

Initial R.Q.'s of starved cells in nitrate are similar to those of growing cells in ammonia, indicating complete absence of nitrate assimilation in starved cells, although it is known that carbohydrate assimilation is taking place during this time (Myers, 1947).[1] Only after a considerable period of assimilation do starved cells exhibit the high R.Q. resulting from nitrate reduction that is characteristic of growing cells. This is borne out also by the final pH values of the cell suspensions. Nitrate uptake is accompanied by an increase in pH and the higher final pH values are related to the longer periods of high R.Q. and nitrate assimilation. The later R.Q. values for growing cells are probably somewhat low due to the high final pH and retention of carbon dioxide as bicarbonate.

TABLE I

Variation of R.Q. *and pH during Glucose Assimilation in Starved Cells*

In Knop's solution with nitrate as the nitrogen source. Original pH = 4.45.

Time interval	R.Q.		
	Growing	Starved 1 day	Starved 3 days
min.			
0–50	1.46	1.10	1.22
50–100	1.55	1.18	1.15
100–150	1.57	1.53	1.26
150–200	1.52	1.58	1.35
200–250	1.57	1.65	1.47
250–300	—	—	1.65
Final pH	5.9	5.6	4.5

Effects of the nitrogen source and light intensity on the CO_2/O_2 quotient in growing and starved cells are presented in Table II. The low light intensity of 40 foot-candles was chosen as being light-limiting for both growth and photosynthesis and approximately the same as the effective light intensity of culture; the high light intensity of 600 f.-c. is light-saturating for both growth and photosynthesis. Each of the data describes the CO_2/O_2 quotient during the period of 30 to 90 minutes after the beginning of illumination. At both light intensities of measurement the CO_2/O_2 quotients of starved cells on nitrate approach a value of 1.0 and approach still more closely the quotients found in growing cells on ammonia where no nitrate reduction is taking place. Evidently the metabolism of starved cells requires a high C/N assimilation ratio.

[1] Attention is called to an author's error in Table III of the paper cited where the R.Q. values for glucose and acetic acid were reversed. Observed values of the R.Q. on acetic acid were 0.99 to 1.04; on glucose, 1.09 to 1.16. The latter values are in agreement with the initial R.Q.'s observed here.

High Light Intensity

Table II also contains data bearing on the metabolism at high light intensity. Growing cells studied at high light intensity in nitrate media show a CO_2/O_2 quotient of 0.88 in agreement with the often cited value of 0.9. The differential in the quotient between nitrate and ammonia (0.88 *vs.* 0.95) is smaller than the differential observed at low light intensity (0.68 *vs.* 0.94).

TABLE II

Variation of the CO_2/O_2 Quotient with Nitrogen Source and Light Intensity

Knop's at pH 4.5 with nitrogen source as indicated; ~ 12 c.mm. cells/flask; 4 per cent CO_2.

Nitrogen source	Measured at 40 f.-c.			Measured at 600 f.-c.		
	Growing	Starved 1 day	Starved 3 days	Growing	Starved 1 day	Starved 3 days
NO_3^-	0.76	0.82	0.89	0.86	0.90	0.95
	0.68	0.81	0.88	0.87	0.93	0.95
	0.67	0.87	0.96	0.88	0.90	0.95
	0.71	0.86	0.91	0.86	0.91	0.98
	0.70	0.85	0.92	0.87		
	0.68	0.89		0.88		
	0.65			0.90		
	0.66			0.90		
	0.60					
	0.64					
Average.....	0.68	0.85	0.91	0.88	0.91	0.96
NH_4^+	0.91			0.94		
	0.96			0.96		
	0.91			0.94		
	0.95			0.97		
	0.96			0.92		
	0.97			0.95		
Average.....	0.94			0.95		

At high intensities there must occur proportionately less nitrogen synthesis and proportionately greater carbohydrate synthesis.

After 3 hours' exposure to an intensity of 600 f.-c. and transfer to fresh Knop's solution (with nitrate) the CO_2/O_2 quotients observed at low light intensity were 0.37 and 0.44. Such experiments led to a study of the CO_2/O_2 quotient in cells which had been grown at high light intensity (300 f.-c.) in the continuous culture apparatus. Comparison of cells grown at high and low light intensities is presented in Table III. Cells cultured at high light intensity show a greater rate of respiration and a higher R.Q. indicative of considerable

accompanying nitrate reduction. Under low light intensity of measurement the same cells show a very low CO_2/O_2 quotient again indicating rapid nitrate reduction. All the data indicate that at high light intensities there occurs an increase in ratio of C/N assimilation. On return to low light intensities the

TABLE III

Effect of Light Intensity of Culture on the Subsequent Gas Exchange

Medium Knop's solution (with nitrate) at pH 4.5. Rates of gas exchange in c.mm./hr./c.mm. cells.

	Cells grown at high light intensity (300 f.-c.)			Cells grown at low light intensity (45 f.-c.)		
	O_2	CO_2	CO_2/O_2	O_2	CO_2	CO_2/O_2
Low light (45 f.-c.)	3.08	−0.97	0.32	5.35	−3.19	0.60
Darkness	−2.05	3.23	1.58	−1.47	2.07	1.41

TABLE IV

Gas Exchange of Normal and Nitrogen-Deficient Cells

Measurements made before and after exposure to high light intensity in Knop's solution minus nitrate. Rates of gas exchange in c.mm./hr./c.mm. cells (referred to the cell volume before exposure).

Experiment	Studied at	Before exposure			Time of exposure	After exposure		
		O_2	CO_2	CO_2/O_2		O_2	CO_2	CO_2/O_2
		Growing cells in Knop's ($+NO_3^-$)			*hrs.*	Nitrogen-deficient cells in Knop's ($+NO_3^-$)		
1	Low light (40 f.-c.)	4.57	−3.17	0.69	4.5	3.92	−0.65	0.17
	Darkness	−1.68	2.23	1.33		−3.09	4.85	1.57
2	High light (600 f.-c.)	42.5	−36.5	0.86	3.0	39.0	−28.7	0.74
	Darkness	−1.37	1.79	1.31		−2.04	3.24	1.59
						Nitrogen-deficient cells in Knop's minus NO_3^-		
3	High light (600 f.-c.)				4.0	31.6	−31.4	0.99
	Darkness					−1.38	1.38	1.0

C/N assimilation ratio decreases to a value still lower than that characteristic of cells always grown under low light intensity.

Nitrogen Deficiency

Effects of the C/N assimilation ratio may also be expected as a result of limited nitrogen supply. Nitrogen-deficient cell preparations could be ob-

tained by transferring growing cells to a Knop's solution with the usual KNO_3 replaced by K_2SO_4 and illuminating at a high intensity (~300 f.-c.) under 4 percent carbon dioxide. Three typical experiments are detailed in Table IV. When the nitrogen-deficient cells are taken up in fresh nitrogen deficient media (Experiment 3) the CO_2/O_2 quotients of 1.0 indicate a com-

TABLE V

Summary of Four Metabolic Conditions in Chlorella

Metabolic condition	CO_2/O_2 quotient with NO_3^-		C/N ratio of assimilates	C/N ratio of cells (inferred)
	In low light	In high light		
Growing (at low light intensity)	0.7	0.9	Normal	Normal
Starved	0.9	1.0	>Normal	<Normal
High light exposed	~0.4	—	<Normal	>Normal
Nitrogen-deficient	~0.2	0.7	<Normal	>Normal

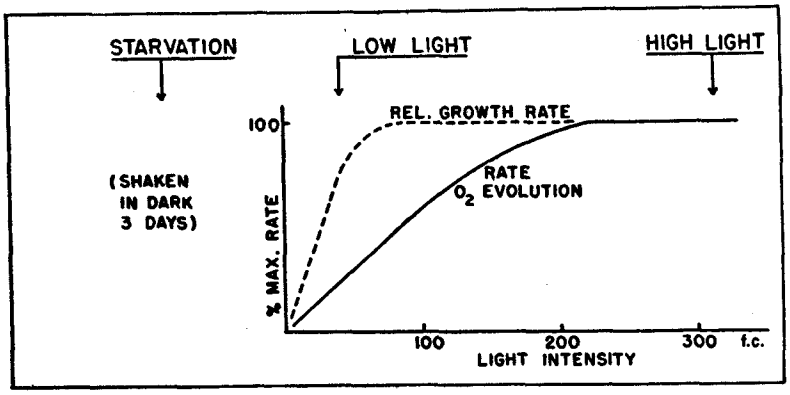

FIG. 1. Graphical description of conditions which induce three different types o metabolism in *Chlorella*. Curves for relative growth rate and rate of oxygen evolution are idealized from experimental curves (Myers, 1946).

pletely carbohydrate metabolism.[2] On return to nitrate-containing media (Experiments 1 and 2) the nitrogen-deficient cells exhibit a CO_2/O_2 quotient depressed markedly at low light intensities and significantly even at high intensities. The return of a nitrogen supply results in a C/N assimilation ratio far below the normal ratio of growing cells.

[2] Note, however, that these are short time experiments. It is likely that on longer exposure in nitrogen-deficient media considerable lipid materials may be produced (*cf.* Spoehr and coworkers, 1946).

DISCUSSION

Characteristics of the four types of cells studied are summarized in Table V and may be considered with reference to Fig. 1. One is forced to select as a *normal* or *reference* condition that which occurs during growth at low light intensity. Here the rate of carbon assimilation limits the rate of growth and metabolism is not "growth bound." Assimilated carbon distributes itself freely between the nitrogenous, carbohydrate, and lipid cell constituents. As the rate of carbon assimilation increases under increasing light intensities a point is reached at which some factor, very likely the rate of nitrogen assimilation, becomes growth-limiting. Further increase in light intensity to a saturating intensity for photosynthesis now results in a greater and greater proportion of carbohydrate synthesis. On return to low light intensity, however, the cells which are glutted with carbohydrate now show a rapid nitrate assimilation with remarkably low CO_2/O_2 quotient.

Nitrogen deficiency, as high light intensity, leads to a predominantly carbohydrate metabolism. On return to a nitrate supply, nitrogen-deficient cells show evidence of very rapid nitrate assimilation in their very low CO_2/O_2 quotients. Starvation, on the other hand, decreases the proportionate amount of carbohydrate so that starved cells at either high or low light intensity or in the dark show quotients approaching those of carbohydrate synthesis alone. Starved cells can again become growing cells only after restoring the C/N balance characteristic of growing cells.

The variable metabolic pattern in *Chlorella* has practical importance with regard to studies on photosynthesis. The quantum yield must be measured at low intensities where the CO_2/O_2 quotient is particularly sensitive to metabolic conditions. In reviewing work on this problem it is difficult to decide whether the algae used were growing or starved in the sense used here. Attention may be called to the wide variation in the CO_2/O_2 quotient observed by Manning, Stauffer, Duggar, and Daniels (1938) in their quantum efficiency studies on *Chlorella* in nitrate-containing media. The CO_2/O_2 quotient varied so widely that the average value of 1.02 cited by Rabinowitch (1945) is of doubtful significance. It now appears possible that these variations are explainable in terms of varying rates of nitrate reduction in response to the metabolic conditions of the cells used.

The present observations also have bearing on studies of *Chlorella* at high light intensities. In the usual short time manometric experiments at photosynthesis-saturating light intensity a constant rate of gas exchange may continue for an hour or more. Strictly steady state conditions do not obtain, however, since the cellular composition is shifting in the direction of increasing carbon content and the shift cannot continue indefinitely at a constant rate. Additional information on the effects of high light intensity has been sought in mass culture experiments and will be reported elsewhere.

It is instructive to apply to *Chlorella* the same considerations of nutritional economy which Foster (1947) has proposed for the interpretation of mold metabolism. Under frugal nutritional conditions, as experienced in their natural habitat, the molds are highly efficient in converting substrates to cell materials. When provided with high concentrations of carbohydrate or when limited in nitrogen supply their metabolism becomes deranged to an *overflow* metabolism with the conversion of large amounts of substrate to storage or excretory products. Nutritional efficiency in *Chlorella pyrenoidosa* is likewise geared to a marginal economy; it will grow at a light intensity of less than 10 f.-c. and reaches a maximum growth rate at less than 100 f.-c. (Myers, 1946). High light intensity must provide here the same conditions of overabundant metabolic substrate (reduced products of carbon dioxide) that high carbohydrate concentrations provide for molds, but the lack of extensive excretion limits the overflow products to storage carbohydrate and lipid. From such considerations the metabolism of *Chlorella* in high light intensity may be interpreted as an abnormal or overflow metabolism.

SUMMARY

1. The effect of nitrate reduction and assimilation on the CO_2/O_2 quotient of gas exchange has been used as an index of the relative rates of carbon and nitrogen assimilation in *Chlorella pyrenoidosa*. Changes in over-all metabolism induced by starvation, high light intensity, and nitrogen deficiency have been studied in comparison with the metabolism of cells growing at light-limiting intensities.

2. Starvation, which results in depletion of carbohydrate reserves, gives rise to a high CO_2/O_2 quotient ($\sim$0.9) during photosynthesis and, therefore, a high C/N assimilation ratio. Starved cells apparently restore their normal C/N ratio before becoming growing cells.

3. Under photosynthesis-saturating light intensities cells show the high CO_2/O_2 quotient (0.9) indicative of a high C/N assimilation ratio. Return to low light intensities is followed by the abnormally low CO_2/O_2 quotient ($\sim$0.4) of a low C/N assimilation ratio. High light intensity apparently gives rise to a condition of a limiting rate of nitrogen assimilation and to an overflow metabolism analogous to that found in other microorganisms.

4. Nitrogen deficiency leads to a completely carbohydrate metabolism in short time experiments and makes still more pronounced the effects characteristic of high light intensity alone.

5. Considerations of nutritional economy sustain the experimental evidence in establishing the metabolism of cells growing under light-limiting intensities as the normal or reference metabolic condition in *Chlorella*.

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