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Dark and Photometabolism of Sugars by a Blue Green Alga: Tolypothrix Tenuis[1]

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Summary. The carbohydrate metabolism of the autotrophically grown blue-green alga, *Tolypothrix tenuis*, was studied. The alga respires glucose, fructose, galactose, and ribose.

About 60% of the glucose consumed is converted by starved cells into a glucose polysaccharide. Glucose uptake and O_2 consumption are not inhibited by 0.01 M arsenite or by 0.005 M iodoacetamide.

The distribution of ^{14}C in the polysaccharide glucose was established after feeding of glucose-1-^{14}C, -2-^{14}C, -6-^{14}C, ribose-1-^{14}C, and fructose-6-^{14}C. Randomization of isotope between the 2 halves of the glucose from polysaccharide is limited when the experiments are carried out in the dark. After an extended incubation glucose-2-^{14}C yields a glucose molecule with isotope labeled approximately equal in C-1, C-2 and C-3.

When the labeled glucoses were fed at a light intensity of compensation point, and in the presence of carbon dioxide, a greater degree of randomization of isotope occurred. The enhanced randomization of isotope is thought to result from an additional supply of triose phosphates as a result of photosynthesis which creates an environment favorable to the reversal of the glycolytic reactions.

To account for the labeling patterns and the resistance of respiration to the inhibitors, it is proposed that the oxidative pentose phosphate cycle is the major pathway of carbohydrate breakdown in this alga.

Most of the studies of algal carbohydrate metabolism have been done with the green algae (6). Based on studies with inhibitors, enzymic assays and ^{14}C distribution in the glucose moiety of polysaccharide, the Embden-Meyerhof pathway appears to be the principal route of glucose respiration in the *Chlorellaceae* (6). However, little is known about the carbohydrate metabolism of the blue green algae. The resistance of glucose respiration of *Tolypothrix tenuis* to respiratory inhibitors such as arsenite and iodoacetamide was interpreted to mean that a pathway or pathways other than classical glycolysis was of importance to cellular respiration (2). Examination of the distribution of ^{14}C in the polysaccharide glucose from short-term photosynthesis with both *Anacystis nidulans* and *Chlorella pyrenoidosa* (12) revealed a similar pattern of asymmetry, as described by Gibbs and Kandler (7).

Blue green algae are usually considered as obligate autotrophs. The ability of *T. tenuis* to grow heterotrophically with glucose as the main source of carbon (13) makes this organism particularly useful in a study dealing with carbohydrate breakdown in the *Cyanophyta*. This report is an attempt to evaluate the pathways involved in the metabolism of sugars deduced from ^{14}C distribution in the polysaccharide. In addition, the effects of light and glycolytic inhibitors on the glucose metabolism are also examined.

Materials and Methods

Organism. T. tenuis, provided by Dr. A. Watanabe, was grown batchwise at 33° to 35° in 5-liter flasks containing 3 liters of culture medium. The composition of 1 liter of medium was: KNO_3, 3 g; $Na_2HPO_4 \cdot 12\ H_2O$, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.02 g; Arnon's A 5 solution, 1 ml; 1 % EDTA-Fe solution, 1 ml. Air enriched with 3 % CO_2 was continuously bubbled through the cultures. A bar magnet kept the medium agitated and the gas bubbles dispersed. Vigorous stirring was found essential for good growth.

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The cultures were illuminated continuously by fluorescent lights (Sylvania white) supplemented with 100-w incandescent bulbs. The light intensity at the surface of the flask was roughly 300 ft-c. Starved cells were obtained by aerobic incubation of 3-day cultures in darkness for 24 hours at the same temperature used for growth. Cells were harvested by filtration onto S and S No. 588 paper. The cell mat was removed from the paper with distilled water and then centrifuged at 6000 × *g*. The cells were suspended in distilled water to a final concentration of about 10 mg dry weight per ml. The yield was about 100 mg dry weight of cells from 1 liter of medium. To check for bacterial contamination, 2 ml of cell culture were introduced into 10 ml of growth medium which had been fortified with casamino acids and glucose. If no turbidity or bacterial growth resulted after 2 to 3 days at 30° the culture was taken as free from contamination.

^{14}C-Labeled Substrates. Glucoses labeled at positions 1, 2 or 6 were purchased from New England Nuclear Corporation and ribose-1-^{14}C from Nuclear Chicago Corporation. Fructose-6-^{14}C was prepared from glucose-6-^{14}C by utilizing crystalline yeast hexokinase, and highly purified preparations of rabbit muscle phosphohexose isomerase and potato phosphatase. Purity of the fructose was established by paper chromatography.

Dark Incubation with Labeled Substrates. Incubation was carried out at 37° either in 150 ml or 50 ml Warburg vessels using a total reaction volume of 12 ml, or 5.5 ml, respectively. In some experiments, the center well contained a filter paper wick wetted with 0.5 ml of 20 % KOH. For incubation under air enriched with 1 % CO_2 ,the center well contained 0.5 ml of Pardee buffer; an additional 0.7 ml was placed in 1 of the 2 side arms. The flasks were flushed with a 1 % CO_2-air mixture and then equilibrated for 30 minutes.

Incubation at Compensation Point. The same incubation system as described under dark incubation was used. Warburg flasks were illuminated from below with 150-w Sylvania Flood lamps. The compensation point was reached at about 30 ft-c as measured at the water surface of the Warburg bath with a Weston light meter. A pair of monitor flasks was used for each feeding experiment to check for the proper light intensity by taking readings of the gas exchange.

Isolation of Polysaccharide Glucose. At the end of incubation, 12 ml of absolute ethanol were pipetted into the main compartment of the Warburg vessel to stop the reaction. After centrifugation, the cell material was extracted exhaustively with 80 % (v/v) ethanol at 80° until the washings were free of radioactivity. The cell debris was then hydrolyzed in 5 ml of 1 N HCl for 2 hours at 100°. The unhydrolyzed fraction was centrifuged and the hydrolysate was evaporated to dryness under vacuum over P_2O_5 and NaOH pellets. Carbohydrate content of the hydrolysate was determined with anthrone. Polysaccharide glucose was isolated and identified by paper chromatography. In every experiment, all radioactivity in the polysaccharide fraction was accounted for as glucose. After elution from the paper and addition of carrier, the glucose was degraded by the *Leuconostoc mesenteroides* technique (5).

Time Course of Glucose-1-^{14}C and Glucose-6-^{14}C Respiration. Twenty-four 15 ml Warburg flasks were equally divided into 6 sets. Each set was subdivided into 2 subsets, one to be run in dark, the other at compensation point. One of the flasks in the subset contained glucose-1-^{14}C and the other glucose-6-^{14}C. In the main compartments were K-phosphate buffer, pH 6.0, 50 μmoles; $MgCl_2$, 5 μmoles; substrate 8.8 μmoles containing 5.6 μc (tipped in at time zero from sidearm); cells, 9.4 mg of dry weight; the final volume was 3.0 ml and 0.2 ml of Pardee buffer was placed in the center well. One of the side arms was occupied by 0.2 ml of substrate, the other by 0.5 ml of 18 N H_2SO_4. The 2 sets of experiments were performed concurrently. After the system has been flushed with a mixture of 1 % CO_2 in air and equilibrated for 30 minutes, substrate was tipped in. At intervals of 10 minutes up to 1 hour, incubation of each of 2 flasks was stopped by tipping in the H_2SO_4. After the reaction has been stopped, 0.4 ml of 20 % KOH was injected into a side arm. The Pardee buffer and the KOH were rinsed out with the aid of a syringe. Three water washings, amounting to about 0.4 ml from each center well and a similar amount from the KOH side-arm, were pooled. $^{14}CO_2$ equivalent to 350 μmoles from the KOH-Pardee buffer solution was converted to $BaCO_3$. Radioactivity was measured at infinite thickness with a window type GM counter, Model 186, Nuclear-Chicago.

Results and Discussion

Respiratory Properties. Our data provide evidence confirming the studies of Watanabe and his associates (14) that *T. tenuis*, in contrast to most other *Cyanophyta* which have been studied (15), exhibit a strong response in O_2 uptake following the addition of sugars. Among the carbohydrates examined, glucose gave the best response, followed by fructose and galactose (fig 1). Ribose supported an O_2 uptake roughly equal to that of galactose. Similar to the studies of Kratz and Myers (15) with *Anabaena variabilis*, *Anacystis nidulans* and *Nostoc muscorum*, *T. tenuis* did not exhibit noticeable response to added organic acids, including acetate, pyruvate, and D-gluconate.

The rate of glucose uptake was found to be about 0.5 to 1 μmole per mg dry weight of cells per hour. The Q_{O_2} values (μl O_2 uptake per hour per mg dry wt) were 4.3 and 16.6 for the endogenous and glucose stimulated respirations, respectively. Assuming that the endogenous respiration was not disturbed by substrate, about 0.6 μmole of O_2 was consumed per μmole of glucose taken up. Of the glucose assimilated,

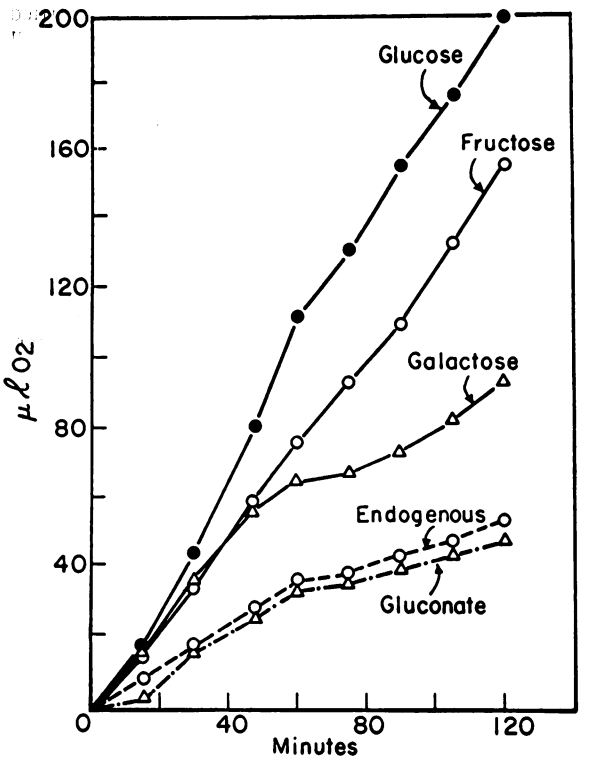

FIG. 1. Time course of O_2 consumption of hexoses by *T. tenuis*. Each 15-ml Warburg flask contained substrate, 30 μmoles; K-phosphate, pH 6.0, 30μmoles; $MgCl_2$, 5 μmoles and 6 mg dry weight of 24 hour starved cells in a final volume of 3.0 ml. The center well carried 0.2 ml of 20 % KOH. The substrate was tipped in at time zero from the side arm after an equilibration period of 30 minutes. The atmosphere was air and the temperature was 37°. At the end of incubation for 120 minutes, the content of each flask was pipetted into a centrifuge tube. The cell material was spun down and an aliquot of the supernatant fraction was analyzed for residual carbohylrate with anthrone. There were consumed 11.8 μmoles of glucose, 7.0 μmoles of fructose and 4.5 μmoles of galactose, respectively.

60 % or more was converted into polysaccharide.

Another characteristic common to both *Chlorella* (17) and *Tolypothrix* was noted when the effect of light on the uptake of glucose was determined. In our experiments, the total amount of glucose consumed or converted to polysaccharide differed only slightly under conditions of either darkness or compensation light intensity, in the presence or absence of CO_2 (table I, see also ref. 14).

Unlike *Chlorella* (11) and *Scenedesmus* (4), the respiration of *T. tenuis* was resistant to high concentrations of arsenite and iodoacetamide. Arsenite at 0.01 M markedly stimulated glucose uptake and its conversion to polysaccharide (table I) and O_2 consumption (table II). In contrast, inhibition of both O_2 evolution and subsequent uptake of O_2 after addition of 0.01 M arsenite to cells photosynthesizing at a light intensity of approximately compensation established entry of arsenite into the cells and its blocking of photosynthesis (table II). Not shown, 0.005 M iodoacetamide gave similar results. These findings with arsenite and iodoacetamide suggest that the conversion of glucose to pyruvate by the usual glycolytic pathway (Embden-Meyerhof) and the subsequent oxidation of the α-ketoacid serves in a limited capacity in the breakdown of glucose by *T. tenuis*.

Metabolism of ^{14}C-Labeled Sugars in the Dark. Polysaccharide glucose isolated after dark feeding of glucose-^{14}C or ribose-1-^{14}C revealed that the bulk of the isotope remained in the position equivalent to that in the added sugar (table III). When glucose-1-^{14}C was substrate, some isotope moved to C-3 of the polysaccharide glucose. When glucose-2-^{14}C was respired, isotope was also found in C-1 and C-3 of the polysaccharide glucose. Even when the glucose-1-^{14}C, glucose-2-^{14}C and ribose-1-^{14}C was completely consumed, tracer was not found to any substantial amount in the lower half of the isolated polysaccharide glucose. Contrary to these findings with glucose-1 and -2-^{14}C, where little randomization of isotope occurred, isotope located in C-6 of the fed glucose was detected consistently in C-1 of the polysaccharide glucose. Not shown, but similar in labeling

Table I. *Glucose Uptake and Conversion to Polysaccharide in the Absence or Presence of* 0.01 M *Arsenite in the Dark and at Compensation Point*

Each flask contained K-phosphate buffer, pH 6.0, 50 μmoles; $MgCl_2$, 5 μmoles; glucose, 20 μmoles; cells, 9 mg dry weight; final volume 3 ml; in center well, 0.2 ml of 20 % KOH or Pardee buffer. The temperature was 37°. The gas phase was CO_2-free air or 1 % CO_2. After 3 hours the cells were centrifuged. The supernatant solution was assayed for glucose. The cells were hydrolyzed in 5 ml of 1 N HCl for 2 hours at 100°. Carbohydrate content of the hydrolysate was determined with anthrone and is expressed as μmoles of hexose after hydrolysis.

	Dark		Compensation	
Gas	Air-CO_2	1 % CO_2	Air-CO_2	1 % CO_2
		μmoles of glucose consumed		
Control	8.2	7.6	10.6	7.5
0.01 M Arsenite	14.4 (1.76)*	13.2 (174)	12.6 (119)	10.7 (143)
		μmoles of hexose after hydrolysis		
Control	5.7	4.7	...	5.4
0.01 M Arsenite	11.2 (197)	9.0 (192)	9.2	8.3 (154)

* Percent over control.

Table II. *Consumption or Production of O_2 in the Absence or Presence of Arsenite and in the Dark or at Compensation Point*

Conditions identical to table I. A minus (−) indicates uptake and a plus (+) indicates evolution of O_2.

Conditions	Dark μmoles	Compensation μmoles
Endogenous	−4.1	+5.3
Glucose	−12.2	−3.7
Endogenous + 0.01 M arsenite	−6.5	−1.0
Glucose + 0.01 M arsenite	−17.0	−5.3

pattern to glucose-6-^{14}C was the ^{14}C distribution obtained with fructose-6-^{14}C Interestingly, isotope was detected in C-5 of polysaccharide glucose when glucose-6-^{14}C was substrate.

The isotope labeling patterns in the polysaccharide glucoses will be considered in relation to the scheme depicted in figure 2. The ^{14}C of ribose is considered to enter the scheme by way of intermediates of the pentose-P pathway. It has been demonstrated that some of the enzymes of the glycolytic and pentose-P pathways (4, 18) are present in the *Cyanophyceae*. By means of these reactions, glucose can be converted into polysaccharide in 3 ways: A) direct conversion involving reactions 1 and 2 with no rearrangement of isotope; B) breakdown of hexose phosphate by Embden-Meyerhof reactions (reactions 1, 2, 3, 4, 5, and 6) down to triose phosphates and back again which permits randomization of isotope between upper and lower half of the hexose chain; and C) reaction 7, the pentose-P pathway together with reactions 3 and 2.

The reactions of the pentose-P cycle (reaction 7 together with 3,2) are of particular interest because they provide a mechanism for introduction of ^{14}C into C-1 and C-2 from C-3 and into C-1 and C-3 from C-2. On each turn of the cycle, 3 moles of hexose-P are converted into 2.5 moles of newly reformed hexose-P with the concomitant loss of 3 moles of CO_2. The half mole of hexose-P is derived from C-4, 5, and 6 of hexose and in this scheme these carbons have no influence upon the spread of tracer within the upper half. The effect of repeated cycling on the ^{14}C distribution of a 2-labeled hexose is illus-

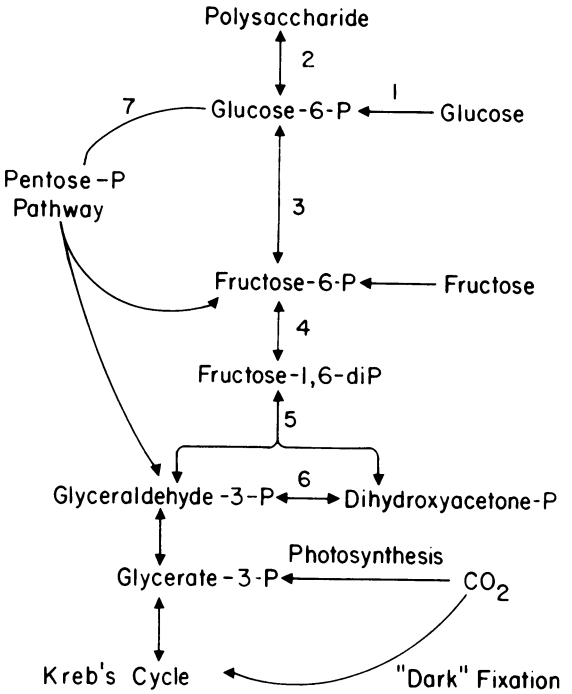

FIG. 2. Outline of reactions which may effect ^{14}C distribution in polysaccharide glucose.

Table III. *Distribution of ^{14}C in Polysaccharide Glucoses after Dark Feeding of Labeled Glucoses or Ribose-1-^{14}C*

Two sets of experiments, A and B of duration 60 minutes and 140 minutes, respectively, are described here. In experiment A, the incubation mixtures of 12 ml contained: substrate, 60 μmoles; K-phosphate, pH 6.0, 330 μmoles; $MgCl_2$, 50 μmoles; cells, 54 mg dry weight. The amount of isotope was: glucose-1-^{14}C, 56 μc; glucose-2-^{14}C, 40 μc, and glucose-6-^{14}C, 50 μc. In experiment B, the incubation system of glucose-1-^{14}C and -2-^{14}C of 5.5 ml contained substrate, 38 μmoles each of glucose-1-^{14}C (5.2 μc) or glucose-2-^{14}C (4.7 μc); K-phosphate pH 6.0, 100 μmoles; cells, 66 mg dry weight. In the last 2 columns, the incubation mixture of 11 ml contained: substrate, 30 μmoles of glucose-6-^{14}C (15 μc) or 33 μmoles of ribose-1-^{14}C (15 μc); K-phosphate, pH 6.0, 200 μmoles; cells, 130 mg dry weight. The center well of each flask contained 20 % KOH. The gas phase was air and the temperature was 37°. The figures represent the percentages of ^{14}C in the various carbon atoms. The value in parentheses is the specific activity expressed in mμc/mg carbon.

Carbon atom	Glucose-1-^{14}C A	Glucose-1-^{14}C B	Glucose-2-^{14}C A	Glucose-2-^{14}C B	Glucose-6-^{14}C A	Glucose-6-^{14}C B	Ribose-1-^{14}C B
C-1	82.5(5.3)	71.0(2.3)	19.6	32.5	7.4	8.4	44.6(0.68)
C-2	2.5	...*	61.4(3.7)	32.6(2.3)	1.5	1.2	17.1
C-3	7.7	16.7	13.9	23.6	1.9	1.6	30.8
C-4	4.4	3.5	1.4	5.2	0.8	0.7	2.7
C-5	0.0	...*	1.9	1.7	3.3	7.8	2.5
C-6	2.9	...*	1.9	4.5	85.0(5.4)	80.4(5.2)	2.2

* Carbon lost.

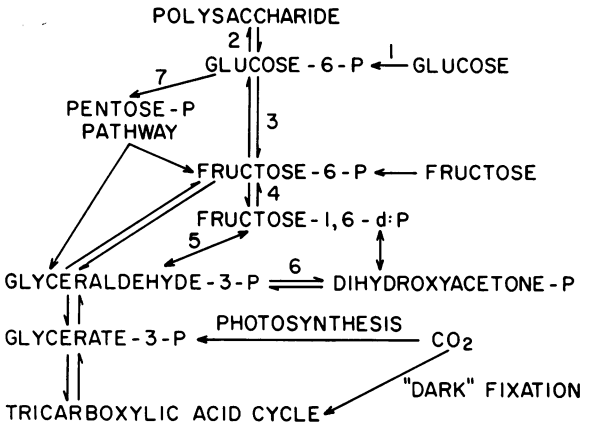

FIG. 3. Effect of the oxidative pentose-P cycle on the ^{14}C distribution of a C-2-labeled hexose with repeated cycling (see ref. 20 for comparison with glucose-3-,4-^{14}C).

trated in figure 3. On the first turn of the cycle ^{14}C is introduced into C-1 and C-3 in a proportion of 2:1 (9). On further turns of the cycle, a hexose unit is formed with isotope essentially equally divided among C-1, 2, and 3. It is noted that randomization of ^{14}C between the 2 halves of the hexose chain is not permissible by way of the pentose-P cycle.

The spread of isotope (table III) into C-1 and C-3 of glucose from C-2 of glucose and C-1 of ribose indicated active participation of the pentose-P cycle reactions (1). It is interesting to compare the calculated values in the hexoses (fig 3) with the observed values from the feeding experiments. For feeding of ribose-1-^{14}C with C-1 taken as 100, the values are C-2 = 38, C-3 = 69, and the calculated values for 3 turns of the cycle are C-1 = 100, C-2 = 50, C-3 = 77. Very convincing evidence of a complete recycling of the pentose-P cycle is the approximately uniform distribution of isotope in C-1, 2, and 3 of polysaccharide glucose after a 140 minute feeding of glucose-2-^{14}C (Column b). In this extended experiment, essentially all the fed glucose was consumed.

Further evidence for the participation of part of the reactions of this oxidative pathway is derived from the glucose-1-^{14}C data. Pentose units derived from a 1-labeled hexose through the decarboxylation of 6-P-gluconate are unlabeled. Obviously, another explanation is required to account for the appearance of isotope in C-3 of polysaccharide glucose following consumption of glucose-1-^{14}C. An exchange reaction catalyzed by transketolase involving C-1 and C-2 of fructose-6-P-1-^{14}C and xylulose-5-P, an intermediate of reaction 7, would place isotope at C-1 of the ketopentose. Resynthesis of a hexose unit yields a labeling pattern similar to that obtained with ribose-1-^{14}C (table III).

While the bulk of the isotope was still conserved at C-6 of the polysaccharide glucose (table V) when glucose-6-^{14}C was substrate, limited randomization to C-1 had occurred. Recycling through the pentose-P pathway results in a relocation of ^{14}C only in the upper half of the hexose. Through this series of reactions, glucose labeled at C-6 would not lose its isotope and would be retained at C-6 of the newly resynthesized hexose. The symmetry of a hexose with respect to isotope labeling is dependent upon triose-P isomerase (reaction 6) which brings the dihydroxyacetone-P and glyceraldehyde-3-P into equilibrium. The fact that randomization of isotope from C-1 and C-2 of the hexose to their homologous partners, C-6 and C-5 was considerably smaller when compared to the reverse process rules out the importance of a mechanism involving solely the glycolytic reactions as a means of achieving a symmetrically labeled hexose. It would appear that randomization is the result of a mechanism whereby glyceraldehyde-3-P labeled in the beta carbon and derived from C-3,4, and 5 of a pentose-5-^{14}C unit (reaction 7) is brought into isotopic equilibrium with dihydroxyacetone-P. The reversal of the glycolytic reactions would introduce isotope into C-1 of polysaccharide glucose. It is clear that by such a series of reactions, the movement of isotope is unidirectional, proceeding only from the lower half of the consumed hexose to the upper half of the newly synthesized hexose.

A related point of discussion is the small but significant increase in radioactivity at C-5 of the polysaccharide glucose obtained from the cells fed with glucose-6-^{14}C. (table III). A scheme involving the glycolytic pathway and the isotope shuttle of the tricarboxylic acid cycle may be considered (19). In this series of reactions, glyceraldehyde-P-3-^{14}C would be converted to acetyl CoA-2-^{14}C. Movement of the latter compound through the intermediates of the tricarboxylic acid cycle and the relevant reactions of the glycolytic pathway would result in the formation of glyceraldehyde-3-P-2,3-^{14}C. Isotope could enter C-5 of the hexose unit either through the aldolase system or by the participation of an exchange reaction catalyzed by transaldolase (16). The fact that the isotopic content of C-2 of the polysaccharide glucose remains constant during the experiment suggests participation of transaldolase rather than of aldolase.

We interpret the labeling patterns of the polysaccharide glucoses, the inhibitor data, and the C_1/C_6 ratios (see below) as proof that the oxidative pentose-P pathway and not the Embden-Meyerhof pathway plays a dominant role in the respiration of glucose by *T. tenuis*. The inhibitor data can be considered as supporting evidence for this view since iodoacetamide and arsenite are known to inhibit D-glyceraldehyde-3-P dehydrogenase and pyruvic acid oxidase, respectively, but do not appear to affect any enzyme of the oxidative pentose-P cycle (3, 8). Pertinent here is our finding that arsenite does not alter the isotope distribution patterns in the polysaccharide glucoses after the feeding of glucose-1-^{14}C, glucose-2-^{14}C and glucose-6-^{14}C under the conditions listed in table I. Enzymic studies (4, 10) have led to a similar conclusion.

Effect of Light. When the incubations were carried out at the compensation point, the bulk of the isotope in the polysaccharide glucose was again retained in the labeled position of the added glucose (tables IV, V). This finding was most evident in the presence of light but in the absence of CO_2. Light in the presence of CO_2 caused a striking increase in the randomization of isotope from C-2 to C-5 and from C-6 to C-1 (tables IV, V).

Table IV. *Distribution of ^{14}C in the Polysaccharide Glucose after Feeding of Glucose-2-^{14}C in Dark or at Compensation Point*

The incubation mixtures of 12 ml contained glucose 60 μmoles (21 μc); K-phosphate buffer, pH 6.0, 330 μmoles; $MgCl_2$, 50 μmoles; cells, 38 mg dry weight. Gas phase, CO_2-free air or 1 % CO_2 in air; 60 minutes, 37°. The figures are percentages of ^{14}C in various carbon atoms. The values in parentheses are the specific activities expressed in mμc/mg carbon.

Carbon atom	Air-CO_2	1 % CO_2	1 % CO_2
C-1	2.2	2.7	23.5
C-2	86.6(15.6)	73.4(8.1)	54.5(8.1)
C-3	5.3	6.9	17.4
C-4	0.5	0	0.3
C-5	5.0	15.0	2.6
C-6	0.5	1.5	2.4

Table V. *Distribution of ^{14}C in Polysaccharide Glucose Insolated after Feeding of Glucose-6-^{14}C in Dark or at Compensation Point*

The incubation mixtures of 12 ml contained glucose, 60 μmoles (31 μc) and cells, 62 mg dry weight; other components and experimental conditions identical to table IV. The figures are percentages of ^{14}C in various carbon atoms. The values in parentheses are the specific activities expressed in mμc/mg carbon.

	Compensation Point		Dark	
Carbon atom	Air-CO_2	1 % CO_2	Air-CO_2	1 % CO_2
C-1	6.6	14.5	7.6	8.0
C-2	1.5	1.5	1.7	1.1
C-3	1.2	1.3	1.4	0.6
C-4	1.0	0.5	0.6	0.3
C-5	3.7	2.8	1.3	1.9
C-6	86.1(1.44)	79.5(1.76)	87.5(1.54)	88.0(1.04)

The investigation of Kandler and Gibbs (10) with *Chlorella* showed that photosynthesis had only a small effect on the randomization of ^{14}C between the 2 halves of the glucose molecule following assimilation of specifically labeled glucoses. In contrast to *Chlorella*, the effect of light together with CO_2 on this kind of randomization of isotope was very pronounced in *Tolypothrix* (table IV, V). The increase of isotopic crossing over may be the result of A) decrease of the pentose-P pathway with a concomitant increase in the traffic entering the glycolytic pathway or B) conditions favorable for the reversal of the glycolytic pathway including an increase in the triose-P isomerase reaction with respect to other reactions depicted in figure 2.

To determine the validity of postulate (A), the C_6/C_1 ratio was determined in the dark as well as at compensation point. Six samples of CO_2 were collected at 10-minute intervals for a period of 1 hour. The ratios (dark) obtained, starting with the initial 10-minute sampling period were: 0.16, 0.34, 0.23, 0.34, 0.28, 0.38; the corresponding values at compensation point were: 0.15, 0.18, 0.25, 0.21, 0.28, 0.33. The C_6/C_1 ratios were again found to be essentially identical when the experiment was carried out in the presence of 5×10^{-6} M 3-(*p*-chlorophenyl)-1, 1-dimethyl urea. This concentration of herbicide inhibited O_2 evolution more than 90 % and had no effect of glucose uptake either in the dark or at compensation. These findings argue against postulate (A).

The other possibility, postulate (B), will now be considered. In the context of this investigation, the action of photosynthesis is thought to provide a situation favorable for the reversal of the Embden-Meyerhof pathway by increasing the flow of traffic from triose to hexose and polysaccharide. In this respect, it is of interest to compare the distribution of ^{14}C in the glucose formed during the assimilation of $^{14}CO_2$ under the conditions of photosynthesis or of darkness. By means of glycolysis and the tricarboxylic acid cycle (fig 2), a sugar labeled equally in positions 3 and 4 would be expected to be formed during dark assimilation of $^{14}CO_2$ (dark fixation).

After exposure of *Tolypothrix* to $^{14}CO_2$ in the dark for 1 hour, we found the distribution pattern in the polysaccharide glucose to be C-1 = 7.5, C-2 = 23.4, C-3 = 18, C-4 = 100, C-5 and C-6 = 0. It is quite clear that the 6-carbon chain formed in the dark has an extremely unsymmetrical ^{14}C distribution pattern. While the incomplete isotopic equilibration by triose isomerase can partially account for the asymmetric pattern, it does not account, however, for the higher content of ^{14}C in C-1 and C-2 as compared to C-5 and C-6 or for the fact that C-2 and C-3 have the same specific activities. The reactions of the pentose-P pathway are pertinent because the effect of this cycle upon the ^{14}C distribution of a 3,4-labeled hexose is the introduction of isotope into C-2 and subsequently into C-1 coupled to a dilution of ^{14}C in C-3 (20). The specific activity of C-4 is unaffected. Thus C-4 would have a higher activity than C-3 and C-3 would become roughly equal to C-2 if the rate of the triose isomerase and aldolase reactions were relatively slow compared to the conversion of glucose-6-P to fructose-6-P and polysaccharide by way of the pentose-P pathway. In agreement with this notion, randomization of isotope from C-1 and C-2 to their equivalents, C-5 and C-6, in the lower half of the 6-carbon chain is limited when the experiment is carried out in the absence of light. Kindel and Gibbs (12) reported the following pattern in polysaccharide glucose formed during photo-

synthesis at saturating light intensity using the blue-green alga, *Anacystis nidulans*: C-1 = 26, C-2 = 21, C-3 = 73, C-4 = 100, C-5 = 11, C-6 = 19. The labeling pattern observed in the glucose synthesized during photosynthesis tends toward symmetry when compared to that synthesized in the dark. During photosynthesis the photosynthetic carbon cycle is active and it has been established that this cycle leads to complete randomization of isotope within the hexose molecule (7). The fact that both light and CO_2 were necessary to obtain the randomization of isotope when $^{14}CO_2$ or a sugar was substrate establishes the important role of the photosynthetic carbon cycle in explaining our results recorded in tables IV and V. Thus, when glyceraldehyde-3-P is formed by the reactions of the oxidative pentose-P cycle, it can enter into the chloroplast and be metabolized by the series of reactions comprising the photosynthetic carbon cycle. While the photosynthetic conditions used in this investigation seemed to effect the respiratory mechanism at least with respect to carbon metabolism, it should be emphasized that dim light and CO_2 had little influence on the uptake of glucose nor did it result in an increased polysaccharide formation.

We suggest that 1 effect of photosynthesis upon glucose metabolism in *Tolypothrix* is the creation of an additional supply of glycolytic intermediates from the photosynthetic carbon cycle, giving rise to a situation favorable for the formation of hexose from triose through the aldolase and triose isomerase reactions.

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