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Although the functions of the essential major elements in plant metabolism have been studied for many years, little work has been done concerning the effect of these elements during short-term incorporation of radioactive carbon dioxide. This may be of some importance as it has been the general custom during photosynthesis studies in this laboratory to suspend algae in various dilute buffer solutions (Bassham, Shibata, Steenberg, Bourdon, and Calvin, 1956; Barker, Bassham, Calvin and Quarck, 1956) or in distilled water alone (Lynch and Calvin,1953; Norris, Norris and Calvin, 1953), assuming that the salts remaining within the cells from the time of growth in nutrient solution are sufficient in quantity for the cells not to become deficient in one or more of the essential elements during the course of the experiment. There are some indications, however, that the addition of salts to algae suspended in distilled water may have a rapid, pronounced effect on some metabolic system within the plant. Thus, Clendenning, Brown and Eyster(1956) have reported that Nostoc muscorum, if rinsed and resuspended in distilled water, loses most of its photosynthetic capacity, which can, however, be completely restored by the addition of potassium ion in concentrations no greater than a few parts per million. Also, K. Baalsrud (personal communication) found that the photosynthetic rate of a marine diatom, when suspended in synthetic magnesium-free water, can be

greatly increased by the addition of magnesium salts. In view of these observations it appeared worthwhile to investigate the effects of the addition of the essential elements in those photosynthesis experiments in which the cells are kept in distilled water for varying periods of time.

In much of the work dealing with the path of carbon during photosynthesis (Benson, Kawaguchi, Hayes and Calvin, 1952; Moses and Calvin, 1958a; Bassham, Benson, Kay, Harris, Wilson and Calvin, 1953), the cells were suspended in distilled water and kept under constant conditions of temperature, lighting and aeration for periods of up to 30 minutes or more before the radioactive carbon dioxide was introduced in order to achieve a "steady internal metabolic state." In experiments in which the adaptation time and the exposure time to ^{14}C are short, the possibility of a deficiency of one or more mineral elements affecting the pattern of ^{14}C incorporation is slight, but in longer term experiments extending for an hour or more, the probability increases of such a deficiency occuring when cells are suspended solely in distilled water. This possibility of such a mineral deficiency would be of particular interest in those studies which purport to show some of the metabolic pathways of carbon not involved in the carbon cycle itself, such as the incorporation of carbon into pigment systems (Blass, 1957), or the interaction between the respiratory and photosynthetic pathways (Benson and Calvin, 1950; Calvin and Massini, 1952). This paper reports the effects of the effects of the major mineral elements, both singly and in combination, on the incorporation of radiocarbon in both light and dark in Chlorella. The effect of the ammonium ion, though not a constituent of the nutrient solution, has also been studied and its effects described.

METHODS

The alga Chlorella pyrenoidosa was grown in bacteria-free culture in steady-growth tubes under conditions previously described (Holm-Hansen, Hayes and Smith, 1956). The composition of the nutrient medium was as follows: KNO_3, 1.2×10^{-2}M.;

$MgSO_4$, 1.0 x 10^{-2} M.; KH_2PO_4, 8 x 10^{-3}M.; $Ca(NO_3)_2$, 1 x 10^{-4} M.; Fe (as the ferric-EDTA complex), 5 p.p.m.; B (as H_3BO_3) and Mn (as $MnCl_2$), 0.5 p.p.m. each; Zn (as $ZnSO_4$), 0.05 p.p.m.; Cu (as $CuSO_4$), 0.02 p.p.m.; Mo (as MoO_3) and Co (as $CoCl_2$), 0.01 p.p.m. each. Samples were drained from the tubes and either pipetted into the small vessels used in the actual photosynthesis test, or centrifuged, rinsed, and resuspended in a variety of solutions prior to being pipetted into the small vessels. The construction and functioning of the apparatus which was used for the exposure of the algal cells to radiocarbon has been described elsewhere (Moses, 1957; Moses and Calvin, 1958b). The algal suspension (1.0 ml.) was contained in a cylindrical vessel which had a flat, optical-glass bottom and which was equipped with a glass top containing aeration and venting tubes; these flasks, which were partially submerged in a water bath, were mechanically shaken over a bank of fluorescent lights which illuminated the algal suspension with an intensity of approximately 2,000 f.c. The shaker held 12 such flasks, which enabled many parts of any one experiment to be performed simultaneously. After a certain period of adaptation of the shaker, during which time 1 per cent (v/v)CO_2 in air was flushed through the vessels, any desired additions were pipetted directly into the algal suspension, following which 50 µl. of 0.026 N.-$NaH^{14}CO_3$ (400 µc/ml.) were added. After varying periods of photosynthesis with the labelled carbon, 4 ml. of boiling absolute ethanol were added to kill the cells. After extraction with 80 percent and 20 per cent ethanol, the total insoluble cell material was centrifuged and discarded. The alcohol-soluble fractions were combined and concentrated by vacuum distillation at room temperature. Small samples of the extracts were then chromatographed as described by Bassham, Calvin, Goodale, Haas and Stepka (1950) using phenol-water as the first solvent and

n-butanol-propionic acid-water in the second direction. The locations on the paper of spots containing radioactivity were detected by autoradiography. The activity in each spot was counted by means of a Scott type Geiger-Müller tube with a thin (1 mg./cm.^{2}) "Mylar" window. In experiments dealing with respiration or with the incorporation of $^{14}CO_2$ in the dark, the algal suspensions were contained either in Warburg vessels or in 10 ml. Erlenmeyer flasks and were shaken in the Warburg respirometer while in complete darkness. The killing procedure and subsequent manipulations of the extracts were the same as with samples in the light.

RESULTS

Nutrient solution and distilled water. The results from one typical experiment comparing the effect of distilled water and nutrient solution are shown in Table I.

(Please insert Table I near here.)

The total fixation of $^{14}CO_2$ by the algae in nutrient solution was increased by 89 per cent over that in the controls with distilled water. The patterns of incorporation of ^{14}C seen on the developed chromatogram differed mainly in a decreased activity in the sugar phosphates in the nutrient solution sample (50 per cent of the total soluble ^{14}C in the nutrient solution compared with 57 per cent in distilled water), and a large increase in the activity incorporated into the amino acids. It should be borne in mind that the figures for the incorporation of ^{14}C in the individual compounds are in terms of the percentage of the total soluble ^{14}C in the extract which is present in that compound. Thus, when the total fixation is different in two samples, the per cent of activity in any one compound may be equal in both the samples, though the absolute amounts may vary considerably. Thus, the sugar phosphates decreased in percentage of the total activity fixed, but the absolute amount increased from 5×10^6 dis./min. to 7.2×10^6 dis./min.

To determine which of the salts in the nutrient medium was having an enhancing effect on the fixation, and also which were affecting the distribution patterns

of the carbon, the same type of experiment was carried out with the individual salts of the nutrient solution. The micro-elements boron, manganese, molybdenum, zinc, copper, cobalt, and also iron and calcium, were not further examined as it was considered extremely unlikely that a deficiency for any one of these elements could be elicited by the procedures used. The results are shown in Table II. It

(Please insert Table II near here)

is seen that the fixation in nutrient solution was significantly higher than that in distilled water (increased by 45 per cent), and that the separate additions of potassium nitrate, potassium dihydrogen phosphate, and magnesium sulphate all increased the fixation, the latter two causing approximately the same fixation as the complete nutrient solution. The figures showing the per cent of the activity incorporated into the sugar phosphates and into the amino acids are interesting in that, compared with the controls, the addition of potassium nitrate increased the activity in the amino acids and decreased it in the sugar phosphates, while the addition of potassium dihydrogen phosphate had just the reverse effect, i.e., the activity in the sugar phosphates was high while the activity in the amino acids was decreased. Other experiments using potassium chloride, sodium nitrate, and sodium dihydrogen phosphate indicated that the potassium ion was without effect, and that the nitrate and phosphate groups were responsible for the observed effects. It is not surprising that responses were elicited by the addition of nitrogen and phosphorous salts, as these are usually the first elements which apparently become limiting for algal growth both in the laboratory and in their natural habitat of lakes and streams (Gerloff and Skoog, 1954). If the cells are short in their supply of nitrogen and phosphorous, the effects noticed are just those which would be expected to occur. As in the first experiment, the nutrient solution caused a shift in the radioactivity from the sugar phosphates to the amino acids.

Effect of ammonia. If a deficiency of nitrogen is limiting the conversion of sugar phosphates to amino acids, etc., then it might be expected that ammonium ion would have a much greater effect in these short-term experiments than nitrate, due both to its more rapid penetration into the algal cells and to the fact that it already is reduced and can be utilized directly in amination reactions (Syrett, 1956). Many preliminary experiments bore out the suggestion that ammonium nitrate might have a marked effect on the fixation of carbon. In Table III is shown the

(Please insert Table III near here)

effect of ammonium nitrate (0.001 M.) on the total fixation of carbon after the cells have been exposed to the ammonium nitrate for varying periods of time before the addition of the radioactive bicarbonate. The time of exposure to the ^{14}C was the same in all cases, namely 30 seconds. The longer the period of contact with ammonium nitrate before the addition of the ^{14}C, the greater was the amount of carbon dioxide which was fixed; ammonium nitrate still had a noticeable effect on the total fixation and pattern of ^{14}C distribution even when added simultaneously with the bicarbonate.

To separate out the effects of nitrate and ammonium ions, the effect of potassium nitrate and ammonium chloride were studied individually. The data presented in Table IV and Fig. 1 show the effects of nitrate and ammonium ions

(Please insert Table IV and Fig. 1 near here)

on the total fixation and distribution of the ^{14}C compared with algae in distilled water. Ammonium ion increased the total fixation almost threefold, while nitrate did not increase it significantly. The distribution of the carbon (Fig. 1) was markedly affected in the cells supplied with ammonium ion, as can be seen from the percentage of the total fixed activity incorporated into the sugar phosphates and into the amino acids. The per cent of the total activity found in the early photosynthetic intermediates (sugar phosphates) decreased from 64 per cent in

distilled water to 7 per cent when ammonia was present, while the activity in the amino acids increased from 10 per cent to 57 per cent. Nitrate had a similar, though much less marked effect.

Data showing the incorporation of carbon dioxide with varying times of exposure to the ^{14}C from five seconds to three minutes are presented in Table V.

(Please insert Table V near here)

In this experiment the presence of ammonium ion again increased the total fixation of ^{14}C (by 5-10 per cent), but not to the same extent as in previous experiments. There was no significant difference in the total fixation between the controls in distilled water and the cells suspended in nitrate. The distribution patterns of the incorporated ^{14}C were similar to those reported in previous experiments in that the addition of ammonium ion caused a greater percentage of the activity to be incorporated into the amino acids at the expense of the sugar phosphates. The patterns of ^{14}C in the presence of nitrate ion seemed to be intermediate between the controls and ammonium samples as they too showed some shift of activity from the sugar phosphates into the amino acids, but not nearly to the same extent as in the presence of ammonium ion. The ^{14}C content of the organic acids was also increased in the presence of added ammonium.

In order to determine whether the effect of ammonia on cells suspended in distilled water was due to the degree of nitrogen starvation, or to a more specific effect of ammonium ions _per se_, the incorporation patterns of $^{14}CO_2$ in the presence of ammonium chloride were examined in cells still suspended in the original nutrient solution, as well as in those in distilled water. Cells were removed from the culturing vessel and as rapidly as possible were placed in the photosynthesis vessels without any intervening centrifugation. Ammonium chloride was added to half the samples, and the cells photosynthesized in the presence of $^{14}CO_2$

for two minutes. Further aliquots of the cells from the culture vessel were centrifuged, washed, and resuspended in distilled water at the same concentration as those still in nutrient solution. After seven minutes of pre-adaptation, these cells were also exposed to $^{14}CO_2$ for two minutes in the light in the presence and absence of ammonium chloride. Table VI shows that the cells remaining in nutrient

(Please insert Table VI near here)

medium demonstrated little change when ammonia was added, while those in distilled water exhibited contrasting patterns in the presence and absence of ammonium chloride typical for this situation (Table V). The effect of ammonia on the photosynthetic incorporation patterns of $^{14}CO_2$ is thus primarily a result of nitrogen deficiency in the cells, and not of a specific effect of the ammonia itself.

Dark fixation of carbon dioxide. There exists the possibility that the enhancement of the fixation of $^{14}CO_2$ by the addition of mineral salts may not reflect a photosynthetic uptake of carbon dioxide, but may merely be brought about by one or more dark reactions. To test this possibility, the effects of the various salts hitherto mentioned were examined for their effect on the dark fixation of radioactive bicarbonate. The results are shown in Table VII. It is seen that ammonium chloride

(Please insert Table VII here)

increased the fixation about fourfold, while potassium dihydrogen phosphate, magnesium sulfate and nutrient solution also increased it by varying amounts. Even though ammonium chloride increased the fixation four times the amount fixed was still relatively small compared with the usual light fixation value (5×10^6 dis./min./min. of incubation /ml cells in the dark versus 900×10^6 dis./min./min. of photosynthesis/ml. cells). The quantitative aspects of this dark fixation invalidate the hypothesis that it is dark fixation reactions alone which account for the enhanced uptake of carbon dioxide in the light upon addition of the various salts. As the patterns of ^{14}C discussed hitherto reflect the interactions of the photosynthetic cycle and the respiratory mechanisms, it was also of importance to

ascertain if the salts under consideration had any effect on the respiration rate of Chlorella. This was tested by the usual manometric methods (Umbreit, Burris and Stauffer, 1949) and it was found that the addition of ammonium chloride did increase the dark respiration rate by about 80 per cent (cf. Syrett, 1953), while nutrient solution increased it by about 40 per cent. Addition of potassium dihydrogen phosphate and magnesium sulfate apparently had no effect on respiration.

DISCUSSION

The results presented in this paper demonstrate that the total fixation of radioactive carbon dioxide, as well as the patterns of ^{14}C incorporation, by a suspension of Chlorella cells in distilled water may be altered by the addition of various mineral salts. When a readily assimilable nitrogen source such as ammonium chloride is added to the photosynthesizing cells, there is a greater incorporation of the labelled carbon into amino acids, especially aspartate, glutamate, alanine, glutamine, and citrulline, and to a lesser degree, into the organic acids such as citric, fumaric, malic, etc. There is concomitantly a decrease in the ^{14}C usually found in the sugar phosphates and in sucrose. The interrelationships of some of the metabolic pathways involved here may be illustrated by Fig. 2. The addition of ammonium ion, and, to a lesser extent, nitrate ion,

(Please insert Fig. 2 near here)

accelerated the conversion of photosynthetic intermediates into other products as witnessed by the increase in the ^{14}C content of many of the organic acids and amino acids which are involved in, or formed by, action of the Krebs cycle. Although all the experiments that have been performed on this problem have consistently shown that addition of a nitrogen source increases the ^{14}C incorporated into many amino acids,

the quantitative results have not always been consistent with regard to an increase in total fixation, or even at times, in increasing significantly the activity is some of the organic acids. The causes of these fluctuations are not understood, but may reflect some unsuspected variation in the growing conditions of the algal cultures or in subsequent manipulation.

The possibility exists that the addition of these salts such as ammonium chloride will cause an alteration in the pH of the medium which may partially, at least, be responsible for both the apparent increased fixation and changes in the pattern of carbon dioxide incorporation. According to Ouellet and Benson (1952), the pH region for maximum photosynthetic activity in Scenedesmus (and it can reasonably be assumed that the same will hold approximately true for Chlorella) was between 6.0 and 9.0, with decreased activity in either more acidic or more alkaline conditions. Further, at lower pH's (2.0-3.0), the main change in the distribution pattern of the ^{14}C was an increase in the activity in sucrose, polysaccharaide material, alanine, and serine. At high pH's (10), incorporation into aspartic acid was favoured. The effects of salts discussed in this paper thus do not fit into the predictions of a pH effect, either with regard to the total fixation or to the distribution of the radiocarbon. In the experiment described in Table II the pH of all the added salt solutions had been adjusted to pH 5.3. After flushing the algal suspension with 1 per cent CO_2-in-air for ten minutes, the pH values ranged from 5.3 in the nutrient solution to 5.9 in the magnesium sulphate sample and in the distilled water control. Some elevation in the pH can be expected from the addition of the bicarbonate (pH about 8.8), but this should be small considering the volumes and molarities involved. Another point that wamants further mention is the possibility that the total fixation of ^{14}C is not indicative of the absolute rate upon different treatments within any one experiment. It may simply

reflect differences in the size of the reservoir of unlabelled carbon dioxide due to changes in pH, ionic strength, etc. This will undoubtedly have some effect in the samples with ammonium chloride, but it cannot be of much importance in experiments such as shown in Table III, where the pH values are all quite similar.

It has been regularly noticed in other experiments that when Chlorella or Scenedesmus were suspended in distilled water and the rate of photosynthesis continually measured in the steady-state apparatus (Wilson and Calvin, 1953; Bassham, Shibata and Calvin, 1955), the rate of photosynthesis decreased about 5-10 per cent per hour (personal communication from J.A. Bassham). This may mean that the cells were limited by a deficiency of one or more nutrient elements which resulted in a decreased rate of carbon dioxide uptake. The term "steady-state", as employed in this laboratory (Calvin and Massini, 1952) means that the concentrations of the intermediates and enzymes involved in the carbon reduction cycle remain constant and do not change during the course of the experiment. There is thus no assumption inherent in this definition that all the other metabolic processes are in a "steady-state" during the experiment. It is possible to conceive of changes occurring in some of the other metabolic pathways while the photosynthetic cycle itself is not materially influenced. Thus, the results presented in this paper show that some metabolic pathways may be influenced by the addition of salts, without greatly affecting others, particularly the pattern of compounds of the photosynthetic cycle. It is obvious that in time all photosynthetic activity will ultimately cease after the cells are placed in distilled water, but the time at which the first effect would be noticeable in the photosynthetic intermediates cannot easily be predicted from these results. It appears that there is no major change in the photosynthetic pattern in as long as an hour or more.

Thus, if one wished to obtain the best reproducibility from experiment to experiment, it would be advisable to eliminate this possibility of mineral salt deficiency by suspending the cells in dilute nutrient medium of some sort. The addition of salts to the suspension complicates the subsequent chromatography, but we have found that, using the standard nutrient solution, one can place on the chromatogram origin at least 100 µl. of the nutrient medium. Using the amount of $^{14}CO_2$ commonly employed, the limitation caused by this volume of extract is not serious with photosynthesis times of half a minute or more, as there is enough fixed activity in samples of this size to give good radioautograms in a few days, or possibly a week or two. For short exposure times of a few seconds or less, the whole problem of mineral deficinecy is not so serious, so that in these cases it might be advisable to use a medium of distilled water alone, as large aliquots of the extract must often be chromatographed.

If one were concerned about maintaining as steady a state as possible in the cells during a photosynthesis experiment, one of the best ways to minimize a change of conditions for the cells following their removal from the culture vessels would be to take them directly out of the growth tubes or flasks, pipette samples immediately into the small shaking vessels described previously, flush with the same aerating mixture as is used in all the growth vessels, and inject the labelled bicarbonate in the usual fashion. This technique could avoid the production of unknown transient phenomena which can be expected to occur if the suspension is flushed with air, nitrogen, etc., immediately before addition of the bicarbonate. This procedure would also eliminate the intervening centrifugations and other manipulations which are liable to have unknown effects on the metabolic activities.

The experiments described in this paper provide some insight into the capacity of Chlorella cells for the storage of such basic raw materials as nitrogen and phosphorous compounds. This capacity appears to be quite small, for within a short time (about 30 minutes) following the removal of the algal cells from the complete culture medium, signs of nitrogen(nitrate)and phosphorous (phosphate) deficiency can be observed when the cells are placed in conditions suitable for the rapid photosynthetic assimilation of carbon dioxide. Under optimal conditions newly assimilated carbon is presumably immediately converted to storage materials (polysaccharide) and to general cellular constituents, particularly protein, and the intracellular concentrations of soluble metabolic intermediates remains at a fairly low level. A deficiency of phosphorous, and especially of nitrogen, which are macro-constituents of cells, would limit many of the biosynthetic activities and lead to an accumulation of intermediates on the synthetic pathways, unless the assimilation of carbon dioxide were curtailed. Experiments have shown that both these effects can be demonstrated: when cells are suspended in media containing ammonium or nitrate ions there is an enhanced total fixation of $^{14}CO_2$ and a relative increase in amino acids with a corresponding fall in the per cent of the activity in the sugar phosphates, compared with cells in distilled water.

TABLE I

Effect of nutrient solution on total uptake and distribution of carbon-14 during photosynthesis in Chlorella

1.0 ml. of 1 per cent *Chlorella* suspension in distilled water. After 10 min. adaptation, 50 µl. of concentrated nutrient solution (final concentration equal to the usual growth medium) were added to the appropriate flasks and 50 µl. of water added to the controls. After another additional 10 min., the radioactive bicarbonate (20 µC.) was added and photosynthesis allowed to continue for 2 min. before addition of 4 ml. of boiling ethanol.

	Cells in Distilled Water	Cells in Nutrient Solution
Total Fixation (dis./min.)	9.2×10^6	17.4×10^6
Total Soluble Activity (dis./min.)	9.0×10^6	14.4×10^6
Radioactivity in the following compounds as per cent of total soluble activity:		
Diphosphates	15.2	3.3
Uridine diphosphoglucose	2.1	3.5
Hexose monophosphates	30.1	35.6
Phosphoglyceric acid	6.5	4.3
Glucose cyclic phosphate	2.1	2.0
Sucrose	27.2	19.8
Alanine	4.6	15.1
Aspartic acid	2.2	5.0
Serine and glycine	3.7	2.7
Citrulline	---	0.01
Glutamic acid	---	0.07
Malic acid	3.5	---
Glycolic acid	0.1	0.85
Unknown 1, probably amino acid	0.5	0.88
" 2, " organic acid	0.8	2.3
" 3, " " "	---	---
" 4, " amino acid	---	2.8
" 5, " sugar phosphate	1.1	0.95
" 6, " " "	0.3	---
Sugar phosphates as per cent of total soluble radioactivity	57.4	49.8
Amino acids as per cent of total soluble radioactivity	11.0	26.5

TABLE II

Effect of the individual salts of the nutrient solution on the total uptake and distribution of carbon-14 during photosynthesis in Chlorella

1.0 ml. of 1 per cent *Chlorella* suspension in distilled water. After 10 min. adaptation time on the shaker, 50 µl. aliquots of the appropriate solutions were added and the adaptation continued for another 10 min. The radioactive bicarbonate (20 µC.) was then added, followed by 4 ml. of boiling ethanol after 2 min. The pH of the added salt solutions was 5.3 in all cases.

	Cell suspension medium					
	Distilled water	0.012 M. KNO_3	0.008 M. KH_2PO_4	0.012 M. KNO_3 + 0.008 M. KH_2PO_4	0.01 M. $MgSO_4$	Nutrient solution
Total fixation (dis./min. x 10^{-6})	5.15	5.70	7.40	8.13	7.62	7.47
Total soluble radioactivity (dis./min. x 10^{-6})	4.07	4.50	6.28	7.03	6.55	6.10
Total radioactivity (as per cent of total soluble radioactivity)						
Diphosphates	22.4	23.3	14.5	15.3	16.5	22.7
Uridine diphosphoglucose	2.4	1.8	2.5	1.7	2.6	2.0
Hexose monophosphate	37.1	28.6	48.6	40.3	36.6	21.0
Phosphoglyceric acid	9.3	9.5	14.5	14.3	14.0	15.5
Glucose cyclic phosphate	2.0	--	1.2	0.94	0.96	1.0
Sucrose	9 .3	9.6	12.3	14.0	13.7	17.9
Alanine	2.9	7.0	2.3	5.2	5.7	6.9
Serine	1.3	2.1	1.0	1.3	1.2	1.8
Aspartic acid	0.56	2.6	0.24	1.6	1.6	1.9
Glycine	0.59	1.1	0.31	0.34	0.60	--
Malic acid	2.4	7.5	1.2	2.9	[illegible].1	3.6
Glycolic acid	0.52	1.6	0.35	0.16	0.34	0.55
Fumaric acid	3.9	1.5	--	0.50	0.73	0.59
Glutamic acid	--	--	--	0.68	0.61	0.88
UK 1, probably sugar phosphate	0.23	2.5	0.18	0.34	0.23	2.7
UK 2, probably sugar phosphate	4.2	0.26	0.69	0.60	0.50	0.67
Sugar phosphates (as per cent of total soluble radioactivity)	77.6	66.0	82.2	73.5	71.4	65.8
Amino acids (as per cent of total soluble radioactivity)	5.4	12.8	3.9	9.1	9.7	11.5

TABLE III

Effect of varying the length of the adaptation period with ammonium nitrate on total uptake of carbon-14 during photosynthesis in Chlorella

3.0 ml. of 1 per cent algal suspension. Photosynthesis time in the presence of $^{14}CO_2$ 30 sec. Adaptation period before injection of $^{14}CO_2$ 30 min. Final concentration of ammonium nitrate, 0.001 M. 50 µl. $NaH^{14}CO_3$ added (20 µC.). Total time elapsed between harvesting and killing, 2 hrs.

		Total fixation (dim./min. x 10^{-3})	Soluble activity (per cent of total fixed)
A.	Control (algae in distilled water)	2,800	90
B.	$^{14}CO_2$ plus NH_4NO_3 injected simultaneously	3,540	90
C.	NH_4NO_3 adaptation period of 30 min.	4,070	91
D.	Cells in NH_4NO_3 during all rinsings, etc.	4,770	88

TABLE IV

Effect of potassium nitrate and ammonium chloride on total uptake and distribution of carbon-14 during photosynthesis in Chlorella

1.0 ml. of 1 per cent *Chlorella* suspension. Photosynthesis time in presence of ^{14}C, 2 min. Cells originally in distilled water and adapted on the shaker for 10 min., at which time 50 µl. of distilled water (as control), NH_4Cl solution (final concentration in algal suspension, 0.002 M.), or KNO_3 solution (final concentration in algal suspension, 0.002 M.) were pipetted into the separate vessels. After a further period of 10 min. adaptation, the radioactive bicarbonate (20 µC.) was added. The cells were killed by the addition of 4 ml. of boiling ethanol.

	Distilled water	0.002 M. KNO_3	0.002 M. NH_4Cl
Total fixation (dis./min. x 10^{-3})	6,700	7,000	18,500
Total soluble radioactivity (dis./min. x 10^{-3})	5,320	5,250	14,500
Sugar phosphates (as per cent of total soluble fixation)	64.4	58.6	7.1
Amino acids (as per cent of total soluble fixation)	9.9	16.2	56.8

TABLE V

Effect of potassium nitrate and ammonium chloride on total uptake and distribution of carbon-14 after varying times of photosynthesis with labelled carbon dioxide.

1.0 ml. of 2 per cent *Chlorella* suspension in 0.067 M. phosphate buffer, pH 7.0. After 10 min. adaptation, 50 µl. of the appropriate solution (water, ammonium chloride or potassium nitrate; final concentrations, 0.001 M.) were added, followed by another 12 min. of adaptation before the addition of the labelled bicarbonate (20 µC.). Photosynthesis times with the carbon-14 were as indicated below. The cells were killed by the addition of 4 ml. of boiling ethanol.

Incubation periods	Total fixation (dis./min. x 10^{-3})	Per cent of the total soluble radioactivity found in the more important compounds, together with the total activities in the various groups of substances.												
		Totals in												
		sugar-P	amino acids	organic acids	PGA	HMP	Di-P	UDPG	Sucrose	Alanine	Aspartate	Glutmate	Ser.+ Glyc.*	Citrulline
A) Controls														
5 sec.	84	92		7.0	73	12	7.0							
10 sec.	195	94		7.0	54	29	11							
30 sec.	908	92	3.0	4.0	30	47	15			1.7	0.1		1.1	
60 sec.	1,800	90	5.0	4.0	24	50	14	1.1	0.3	3.6	0.2		0.9	
120 sec.	2,920	84	7.0	4.0	17	53	11	2.8	2.5	3.3	0.6		2.6	
180 sec.	3,900	79	9.0	5.0	14	47	14	3.8	4.7	4.3	1.2	0.2	2.8	
B) With NH_4Cl														
5 sec.	79	81		18	46	29	6.0							
10 sec.	214	74	7.0	19	35	31	8.0			2.0				2.9
30 sec.	970	74	12	12	22	41	11		1.0	6.0	1.9			4.2
60 sec.	1,920	60	24	13	18	32	9.0	1.2	2.9	12	3.2		1.6	4.0
120 sec.	3,330	50	33	13	10	29	10	15	2.3	17	6.0	3.0		6.0
180 sec.	4,600	42	44	14	7.0	24	9.0	2.0	0.7	22	5.0	4.0	3.0	4.0
C) With KNO_3														
5 sec.	126	97		3.5	68	21	8.0							
10 sec.	246	92		3.7	51	28	13							
30 sec.	870	92	4.0	3.0	29	47	16			2.2	0.5		1.2	
60 sec.	1,530	88	7.0	4.0	22	48	17	0.9	0.4	4.0	1.0		1.0	
120 sec.	2,920	80	13	5.0	17	45	16	3.0	2.2	7.6	2.6	0.6	1.6	
180 sec.	3,900	76	14	5.0	14	42	17	3.0	4.8	8.0	3.0	1.0	2.0	

* Not separated chromatographically.

Key: Sugar-P, sugar phosphates; PGA, phosphoglyceric acid; HMP, sugar monophosphates; Di-P, sugar diphosphates; UDPG, uridine diphosphoglucose; Ser., serine; Glyc., glycine.

TABLE VI

Effect of ammonium ion on total uptake and distribution of carbon-14 by *Chlorella* cells suspended in distilled water and nutrient solution

1.0 ml. 0.8 per cent *Chlorella* suspension. Suspension in nutrient solution was pipetted directly from the vessels in which the algae were grown, while the cells in distilled water were centrifuged and rinsed twice in distilled water before final resuspension in distilled water. After 7 min. adaptation, 50 µl. of 0.042 M. NH_4Cl (final concentration, 0.002 M.) were added to the appropriate flasks and 50 µl. of water added to the controls. After 5 more min., the radioactive bicarbonate (20 µC.) was added and photosynthesis allowed to continue for 2 min. before addition of 4 ml. of boiling alcohol.

	Cells in Distilled Water		Cells in Nutrient Solution	
	Controls	NH_4Cl	Controls	NH_4Cl
Total fixation (dis./min. x 10^{-6})	5.11	6.95	3.85	3.99
Total soluble activity (dis./min. x 10^{-6})	3.48	5.49	2.97	2.92
Radioactivity in the following compounds as per cent of total soluble activity:				
Phosphoglyceric acid	15.8	13.2	9.1	10.9
Diphosphates	8.5	13.0	4.1	4.6
Hexose monophosphates	53.3	28.6	45.8	43.2
Uridine diphosphoglucose	2.2	2.6	2.3	2.4
Malic acid	1.5	12.5	6.1	5.0
Citric acid	0.0	0.7	0.3	1.1
Sucrose	12.7	3.5	11.0	5.7
Aspartic acid	0.6	5.0	5.2	3.8
Glutamic acid	0.1	1.9	1.4	1.4
Serine and glycine	2.8	5.9	6.2	5.3
Alanine	2.5	13.2	8.2	16.6
Sugar phosphates (as per cent of total soluble activity)	79.8	57.4	61.3	61.1
Amino acids (as per cent of total soluble activity)	6.0	26.0	21.0	27.1

TABLE VII

Effect of mineral salts upon the dark fixation of carbon dioxide in Chlorella

5.0 ml. of 2 per cent *Chlorella* suspension in distilled water in 25 ml. Erlenmeyer flasks. Shaken in complete darkness at 25° in air. After 15 min. the appropriate additions were made (250 µl. of each solution), followed by 30 min. of further adaptation, after which the radioactive bicarbonate (100 µC.) was added. After 4 min. exposure to the labelled carbon, the cells were killed by addition of 20 ml. of alcohol.

	Control	NH_4Cl (0.002 M.)	KH_2PO_4 (0.008 M.)	$MgSO_4$ (0.01 M.)	Nutrient Solution
Total activity fixed (dis./min. x 10^{-3})	192	788	286	553	240

Figure 1

Effect of potassium nitrate and ammonium chloride on the distribution of carbon-14 during photosynthesis in Chlorella.

For experimental details, see Table IV. Key to abbreviations: UDPG uridine diphosphoglucose; Di-P, sugar diphosphates; HMP, sugar monophosphates; PEP, phosphoenolpyruvic acid; PGA, phosphoglyceric acid.

Figure 2

Interrelationships between the carbon reduction cycle and other metabolic pathways.

Footnotes to page 1.

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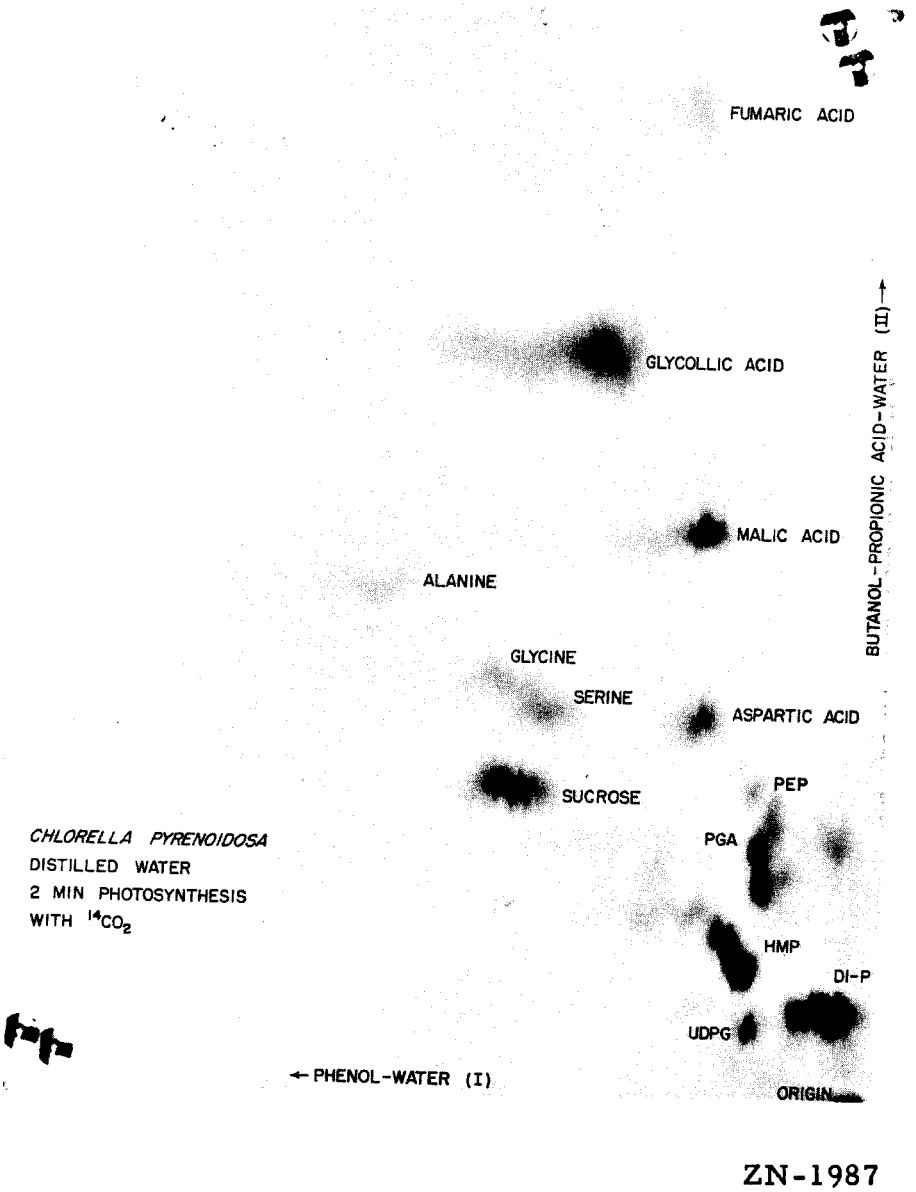
**** Present address: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GLYCOLLIC ACID
MALIC ACID
ALANINE
GLYCINE
SERINE
ASPARTIC ACID
SUCROSE
PEP
PGA
HMP
DI-P
UDPG
ORIGIN
BUTANOL-PROPIONIC ACID-WATER (II) →
← PHENOL-WATER (I)
CHLORELLA PYRENOIDOSA
DISTILLED WATER
2 MIN PHOTOSYNTHESIS
WITH $^{14}CO_2$

ZN-1987

Fig. 1

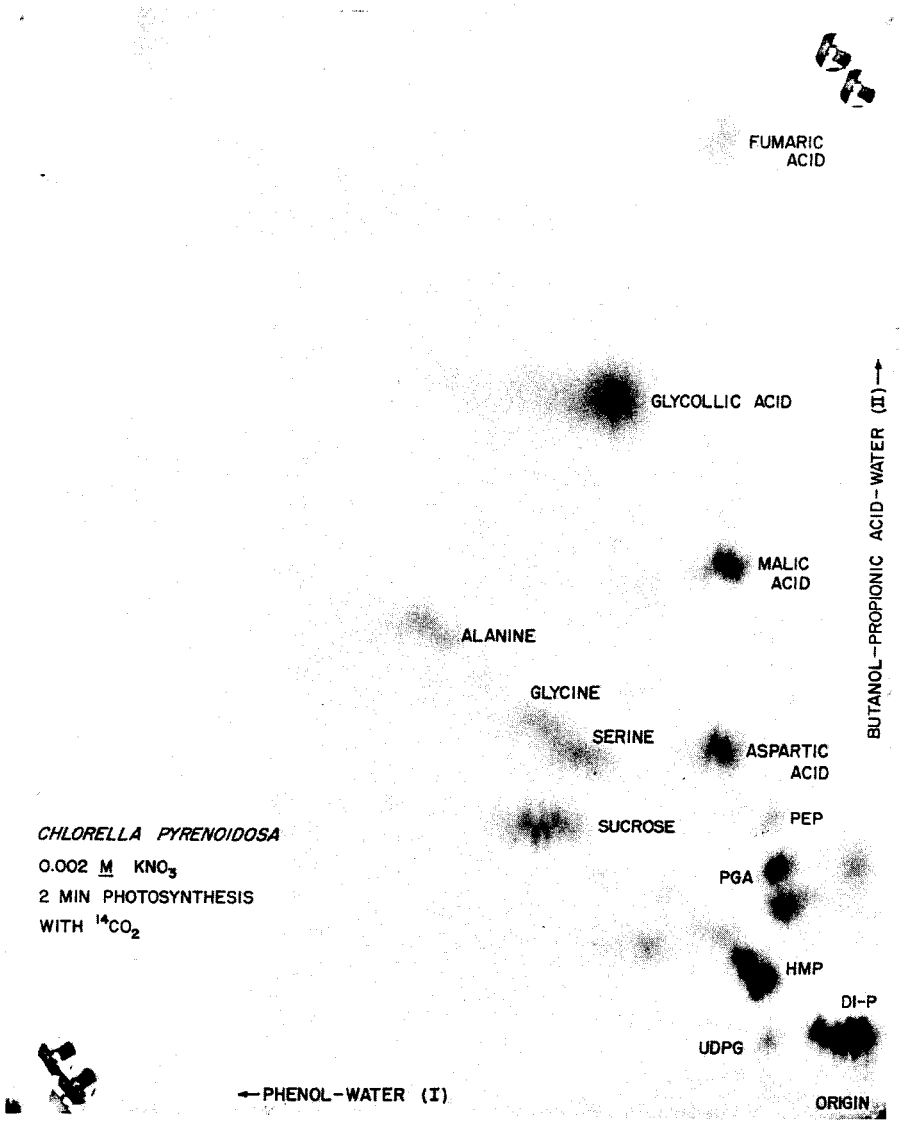

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Fig. 1 (cont'd)

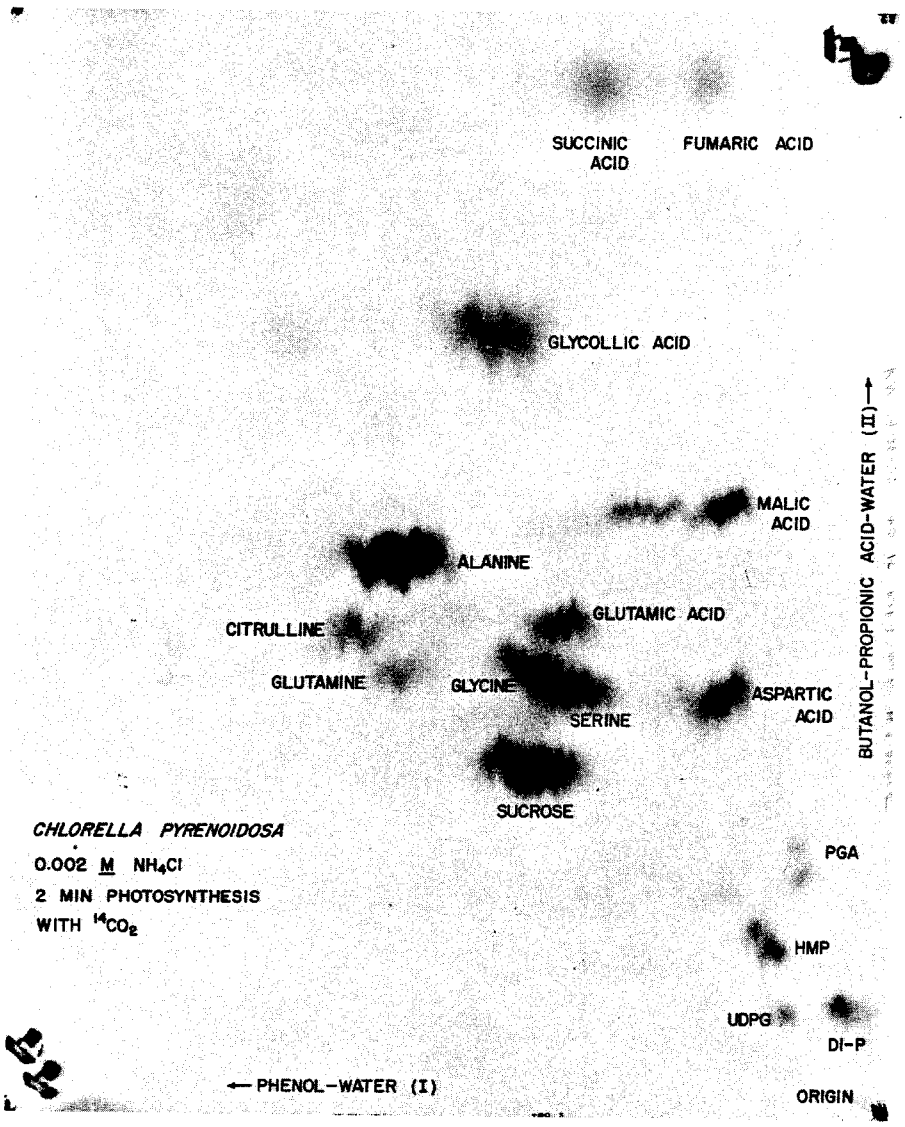

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Fig. 1 (cont'd)

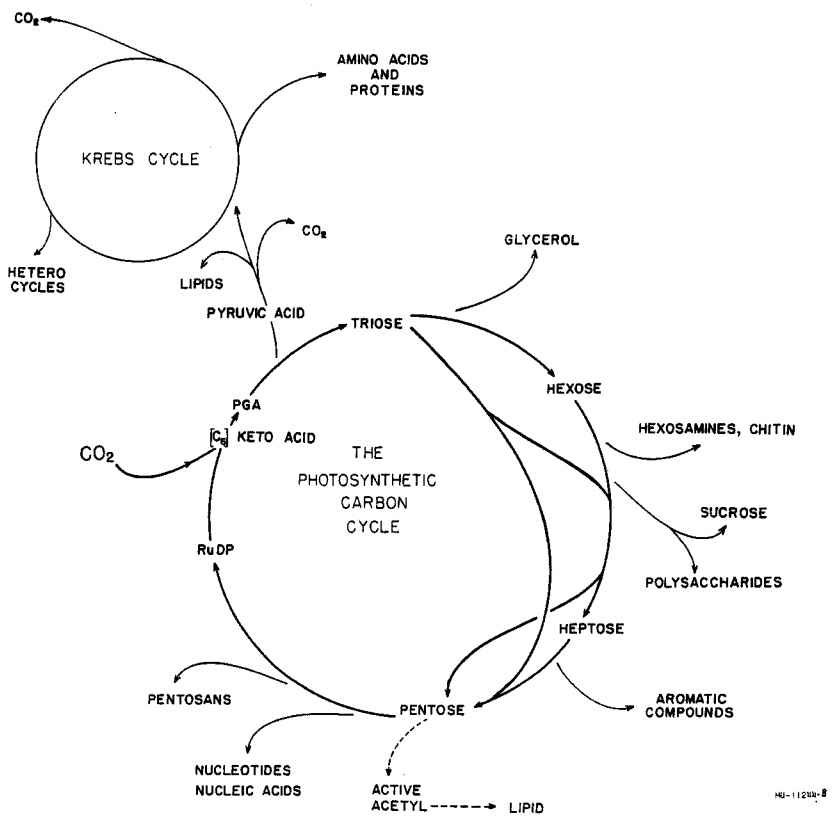
CO_2
AMINO ACIDS AND PROTEINS
KREBS CYCLE
HETERO CYCLES
CO_2
LIPIDS
PYRUVIC ACID
GLYCEROL
TRIOSE
HEXOSE
PGA
[C_3] KETO ACID
CO_2
THE PHOTOSYNTHETIC CARBON CYCLE
HEXOSAMINES, CHITIN
SUCROSE
POLYSACCHARIDES
RuDP
HEPTOSE
PENTOSANS
PENTOSE
AROMATIC COMPOUNDS
NUCLEOTIDES
NUCLEIC ACIDS
ACTIVE ACETYL
LIPID

Fig. 2