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Kanazawa, T.

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TRITIUM INCORPORATION IN THE METABOLISM OF CHLORELLA
PYRENOIDOSA

T. Kanazawa, K. Kanazawa and J. A. Bassham

Lawrence Berkeley Laboratory, University of California, Berkeley, California

Abstract

The incorporation of tritium into a large number of biochemical compounds in photosynthesizing Chlorella pyrenoidosa has been measured. Overall incorporation of tritium into the algae was about 70%. Tritium incorporation into individual compounds varied from 45% to 100% as compared with ordinary hydrogen. The highest tritium incorporation was found in compounds of the tricarboxylic acid cycle, suggesting that, once incorporated, tritium is preferentially retained during oxidative reactions.

Given the possibilities for the release of tritium (^3H or T) into the environment, detailed knowledge of the biochemical fate of T taken into living organisms is desirable. A primary point of incorporation of T from lakes and rivers would be through unicellular algae. Past studies of T incorporation into Chlorella pyrenoidosa indicated that the overall incorporation was subject to an isotopic discrimination leading to only 50% as much T incorporation as would be expected if T were taken up and retained at the same rates as ^1H . (Weinberger and Porter, 1953). However, the retention of T in specific compounds has not been studied. There could be substantial differences in the differential rates of uptake of the hydrogen isotopes by way of specific biochemical pathways. Once formed, T-labelled compounds might be more slowly broken down, leading to accumulation of T in such compounds.

The measurement of the concentrations of large numbers of compounds, both metabolic intermediates and end products, can be greatly facilitated if the algae are grown with $^{14}\text{CO}_2$ of constant specific radioactivity and total CO_2 tension. The resulting ^{14}C content then can be used as a measure of concentration. Since isotopic discrimination against ^{14}C is relatively small compared to that expected in the case of T, the concentrations can be estimated by dividing the ^{14}C content by the specific ratio of ^{14}C to total carbon, and further dividing by the number of carbon atoms per molecule. The accuracy of the assumptions can be tested by determining the concentrations of a few compounds such as amino acids, by quantitative colorimetric methods.

After removal of all exchangeable T from the biological material, analysis may be carried out by a variety of techniques, including two-dimensional paper chromatography and radioautography (Benson, Bassham,

et al., 1950; Pedersen, Kirk, et al., 1966), and the ^{14}C and T content of each substance can be determined. The T content is corrected for $\text{T}/(^1\text{H} + \text{T})$ ratio of the water in which the algae were grown. This T content is divided by the total hydrogen content in non-exchangeable positions of the molecule to give the incorporation ratio. The results of the present study show that this ratio varies from 0.41 to 1.05, depending on the specific compound.

Experimental

Apparatus. The algae were grown through several generations in a water-jacketed (20°C) cylindrical glass vessel (Fig. 1). A closed, gas-handling system, including a pump, 5-liter reservoir, and various stopcocks provided aeration of the algal suspension. The glass vessel (inner diameter 3 cm) was illuminated by four 6-watt fluorescent lamps. Vessel and lights were in a light-tight box, so that alternate light and dark periods could be provided. Gas samples could be taken by opening a stopcock on the high pressure side of the pump, and pressure equilibration was achieved by permitting air to flow in on the low pressure side through ascarite (to remove CO_2) and silicone oil. The entire apparatus, plus other equipment used in the initial stages of the workup (to remove most of the unused HTO) were contained in a large plastic and metal box with glove ports, double-door equipment ports, negative pressure venting, and other safety equipment needed for the handling of HTO .

Procedure. One ml of a suspension of Chlorella pyrenoidosa grown in a continuous apparatus (Bassham and Calvin, 1957), containing about 5 μl of packed cells, was taken into a sterile disposable syringe, and

injected into the culture apparatus containing 40 ml of sterile modified Myers medium (Kanazawa, Kanazawa et al., 1970), through the rubber serum cap attached to the culture apparatus. The culture apparatus was transferred into the tritium box. Within this box, 50 ml of the algal culture medium containing about 0.7 C of T in HT0 was poured into a syringe attached to a Millipore filter and needle. The needle was stuck through the attached rubber serum cap and the medium containing HT0 was forced through the Millipore filter and needle into the culture apparatus, after which another 5 ml of medium was forced in as a wash.

The culture apparatus was placed in the dark box and connected to the gas circulation system through ball joints. The system was closed and the pump was started, recirculating gas from the reservoir through the algal suspension. At the start of the experiment, the 5-liter reservoir contained 4% CO₂-in-air, with a ¹⁴C specific activity of about 0.8 μ C/ mole. A sample of gas was taken at the end of the experiment through the CO₂ sampling stopcock for analysis and determination of specific activity of ¹⁴C.

The algae were either continuously illuminated for three days (Exps. 2 and 4) or were given alternate periods of 16 hr light and 8 hr dark for four days (Exp. 5). By the end of the experiment, when the algae were harvested, the packed cell volume was found to be 240 μ l (Exp. 4), representing between five and six doublings. Thus, 98% of the cell material was formed during the culturing with the ¹⁴CO₂ and HT0.

Analysis of Labelled Algae. At the end of the culturing period, the algae suspension was removed through the bottom stopcock and divided into two portions. Several 5 ml portions used for the determination of labelling of the water-soluble intermediate compounds were quickly filtered through glass filter paper (Whatman GF/C). The algae on the filter were dipped into, first, 1 ml 80% methanol: 20% water, and then twice in 1 ml methanol. The extracts were combined and assayed with two-dimensional paper chromatography in phenol-water-acetic acid (PWA) and butanol-propionic acid-water (BPW). Two sets of chromatograms were prepared, one with 24 hr development in each direction and one with 48 hr development in each direction (Pedersen, Kirk et al., 1966).

For the analysis of the cellular macromolecules (starch, protein, nucleic acids, etc.), the larger portion of the algae suspension was centrifuged at low speed for 15 min, washed twice with water, and then killed by the addition of 5 ml 80% methanol to the pellet. The killed algae were further washed and extracted twice with methanol. These methanol extracts were combined to give 25 ml and a 50 μ l aliquot portion was chromatographed on paper for 20 hr with PWA. The front-running band was taken for the analysis of lipid labelling.

The residue from this extraction was suspended in 6 ml water, and three 1 ml aliquot portions (R-1, R-2 and R-3) were taken for hydrolysis of the components. R-1 was hydrolyzed for 1 hr at 100°C in 1 N HCl and analyzed by two-dimensional paper chromatography for 20 hr in PWA and BPW. After radioautography, the glucose derived from starch was removed for assay, and the areas containing nucleic acid compon-

ents were removed and rechromatographed for 18 hr in isopropanol-HCl-water (170:44:36) (Wyatt, 1951). After radioautography, the paper areas carrying nucleic acid components were removed and assayed.

Five mg of a commercial proteolytic enzyme preparation (Pronase) was added to R-2, and the suspension was incubated at 37°C (under a layer of toluene) for 24 hr. Then 2 ml of methanol was added, and the mixture centrifuged. Aliquot portions of the supernatant solution were analyzed by two-dimensional chromatography (PWA and BPW). To obtain good separations of all amino acids, two chromatograms were prepared for each sample, one for 20 hr in each direction and the other for 36 hr in each direction.

To the residue from Pronase treatment of R-2 were added 1 ml of water and 10 mg of diastase, and the mixture incubated under toluene for 24 hr at 37°C. After addition of 2 ml methanol and centrifugation, the supernatant solution was analyzed by two-dimensional paper chromatography for 24 hr each in PWA and BPW. Radioautography disclosed spots of glucose and maltose derived from starch, and these were assayed for T and ^{14}C .

R-3 was hydrolyzed in 6 N HCl for 24 hr at 115°C. The residue was evaporated to dryness, water added, evaporated to dryness again, and then taken up in 2.5 ml water. One portion was analyzed for amino acids with two-dimensional paper chromatograph (PWA and BPW). The other portion was analyzed with a commercial amino acid analyzer (Beckmann Model 120C).

Analysis for T and ^{14}C . After identification of the compounds on paper corresponding to the radioactive areas as revealed by radioautography, the areas carrying the radioactive compounds were cut out and combusted in the Packard Automatic Combustion Apparatus which converts carbon and hydrogen in organic compounds to CO_2 and water. These products are separated and added to vials containing scintillation fluid in a series of automated steps. The T and ^{14}C contents of each sample were determined in a Packard dual channel scintillation counter. The separation of T from ^{14}C before counting makes possible the use of relatively low levels of specific radioactivity of T in the original experiment.

Determination of the Specific Radioactivity of HTO and of $^{14}\text{CO}_2$. At the end of the experiment, a portion of the filtered culture medium was diluted, evaporated from the side arm of a Thunberg tube in vacuo, and trapped into the main tube imbedded in dry ice. In this way, any labelled, non-volatile compounds in the medium were removed. A sample of the distillate was then analyzed for T with the Packard Scintillation Spectrometer.

At the beginning and at the end of the experiment, samples of the gas in contact with the algae suspension were removed. The CO_2 in these was trapped in 25 ml of a saturated and filtered solution of $\text{Ba}(\text{OH})_2$ in a centrifuge tube. The precipitate was centrifuged, washed with CO_2 -free water twice and methanol once, and was dried over NaOH in a vacuum. The BaCO_3 was weighed, and then converted to CO_2 which was trapped in "Solubene". The ^{14}C content was determined in the scintillation counter.

Results

Of critical importance to this study is the accuracy of the assumption that ^{14}C content can be used as a measure of the amount of each compound. This assumption was tested with amino acids obtained by acid hydrolysis of the algae protein. The amino acids selected for this comparison were acid-stable compounds which are very well separated from other compounds by the two-dimensional paper chromatography used. The ^{14}C content of each of these compounds as isolated by paper chromatography was divided by the specific radioactivity of the $^{14}\text{CO}_2$. The resulting calculated concentrations (in $\mu\text{atoms of C}$) were compared with the concentrations as determined chemically by the amino acid analyzer, taking into account the number of atoms of C per molecule. The concentrations agree very well (Table I), with the chemically-determined concentrations averaging about 2% more than the concentrations determined by ^{14}C content. Thus, the assumption that isotopic discrimination against ^{14}C as compared with isotopic discrimination against T is small enough to be neglected seems to be valid.

The total carbon content of 7.01 millimoles for Exp 4 (Table II) gives a calculated dry weight of 213 mg per cm^3 algae, if one assumes an approximate molecular weight average of 30 per carbon atom for all the organic constituents. The calculated dry weight in Exp 5 is 226 mg. Dry weights determined gravimetrically in this laboratory for Chlorella pyrenoidosa usually are in the range of 180 to 240 mg per cm^3 algae.

Over 71% of the carbon was found to be in the methanol insoluble fraction, and of this, 74 to 81% was recovered in amino acids from

protein hydrolysis, glucose from starch hydrolysis, and nucleic acid components. Of the methanol soluble fraction, about 75% was recovered as the lipid band and about 10% as identifiable metabolites.

In the determination of R, the ratio of T to non-exchangeable hydrogen positions, the following operations were performed: (1) The T and ^{14}C contents of each compound as isolated were converted to T and ^{14}C content in the compound as $\mu\text{curies per cm}^3$ of algae. These were then divided by the specific radioactivities to give the concentrations of T and ^{14}C as measured by radioactivity. Under the assumption, just discussed, that there is negligible isotopic discrimination against ^{14}C , the ^{14}C content is equal to the actual concentration of carbon atoms in the given compound (atoms per molecule times molecular concentration) per cm^3 algae. (2) The T content, in $\mu\text{moles/cm}^3$ algae, was divided by the ^{14}C content in $\mu\text{moles/cm}^3$ algae to give the ratio (B) of concentrations of atoms of the two elements, calculated from radioactivity. (3) The ratio (B) was divided by the number (A) of ^{per carbon atom} hydrogen atoms/bonded to carbon atoms and hence considered to be non-exchangeable under the conditions of analysis. (The question of exchangeable hydrogen atoms bonded to carbon is considered later.) The resulting ratio, $R = B/A$, is taken as indicating the discrimination against T. In the case of no net isotopic discrimination, $T = 1.0$.

Under the assumptions just given, very low values for R were found for certain amino acids when liberated from protein by acid hydrolysis. Further treatment of these amino acids showed that glutamate, aspartate, tyrosine and histidine lose appreciable T from nominally non-exchangeable positions. Such loss of T with acid hydrolysis has been seen earlier

by Markley, Putter et al. (1963). Therefore, the amino acids used for the measurement of T were obtained from protein by enzymic hydrolysis. No evidence of T loss during hydrolysis was observed. For example, the R value of 1.05 for glutamate (Exp 4) from pronase treatment of protein agrees with the R value for glutamate (0.99), glutamine (1.01) and citric acid (1.04) in the methanol-soluble fraction in the same experiment. The recoveries of amino acids were not so quantitative by the pronase treatment, but quantitative recovery is not a requirement, provided the compounds are isolated in pure form. Thus, in Table II, amounts of amino acids are given as isolated by acid hydrolysis, and R values are for amino acids isolated after pronase treatment.

As a check on possible exchange loss of T during starch hydrolysis, starch was treated with diastase and the products were chromatographed. Both maltose and glucose were obtained. Upon analysis, the R values for both compounds agreed very closely with those shown in Table II for starch glucose from acid hydrolysis.

From the results shown in Table II, the following generalizations about T incorporation in Chlorella pyrenoidosa may be made:

1. Discrimination against T incorporation into specific compounds varies widely from one compound to another, in the range from 1.0 (no discrimination) to about 0.50 (50% discrimination against tritium).
2. For a given compound, the discrimination, or R value, is usually fairly constant from one experiment to another. Similar values to those shown were obtained in two other experiments (Exps 1 and 2 --- Exp 3 was not analyzed because of too dense algae growth).
3. R values for compounds related to the tricarboxylic acid cycle (malate, citrate, glutamate, glutamine, aspartate and asparagine)

are in the range 0.9 to 1.05. Thus, surprisingly, there is little or no net isotopic discrimination in the biosynthetic paths leading to these compounds.

4. R values for lipid were 0.5 to 0.6, assuming A values (ratios of non-exchangeable hydrogen atoms to carbon atoms) of slightly less than 2. Since the bulk of carbon atoms and hydrogen atoms in lipids are in the form of methylene groups ($-\text{CH}_2-$), this assumption is reasonable.

5. R values for amino acids not derived from the tricarboxylic acid cycle, and for sucrose, starch, nucleic acid components and some soluble metabolites, generally lie in the range 0.45 to 0.75.

6. R values for most soluble metabolites (sucrose, sugar phosphates, ADP, ATP, UDPglucose) range from 0.6 to 0.9. Sugar phosphates and 3-phosphoglycerate (PGA) seem to have significantly higher R values in Exp 5 (light-dark periods) than in Exp 4 (continuous light).

Discussion

An estimate of the overall isotopic discrimination against tritium can be made by assuming that the composition of the algae is approximately 50% protein, with an average A ratio (non-exchangeable H atoms/carbon atoms) of about 1.0, 25% starch, with an average A ratio of 1.17, and 25% lipid with an average A ratio of about 1.9. This gives an overall A ratio of 1.27, which divided into the measured B values ($\text{T}/^{14}\text{C}$) for the total sample (Table II) gives an estimated R value of 0.73 in Exp 4 and 0.66 in Exp 5. Comparison with the values of R for individual compounds given in Table II suggests that this is a reasonable estimate. These values are higher than the values around 0.47 previously reported for tritium content of combusted Chlorella pyrenoidosa compared with water in the medium (Weinberger and Porter, 1953).

The 1953 report made no allowance for the loss of tritium in the harvested sample (prior to drying) due to exchange. It was stated without explanation that tritium loss during repeated quick centrifugations in distilled water was shown to be less than 6% of the total tritium present in the cells. However, considering the large amounts of protein and starch in the cells, it seems possible that loss of tritium by exchange during washing might have been higher than 6%. A loss of about 25% would account for the difference between the earlier report and the present study. Full exchange of all exchangeable hydrogen atoms, such as is achieved in the present study, would lead to an even greater loss of tritium, perhaps as much as 40%. Nearly one-half of all the hydrogen atoms in carbohydrates and protein would be exchangeable under the conditions of the present study. It is concluded that the overall isotopic discrimination against tritium, by photosynthesizing Chlorella pyrenoidosa, is about 30%, tritium being incorporated to 70% of the extent of ^1H .

Within biochemical pathways, leading to specific end products, there are large differences in the discrimination against tritium, with incorporation and retention of tritium varying from 0.41 to 1.05. The probable maximum error in these figures is ± 0.05 .

In contrast to the incorporation of carbon, which is taken up mostly by a single carboxylation in the reductive pentose phosphate cycle in Chlorella pyrenoidosa (Bassham and Kirk, 1960), hydrogen is incorporated at many points along the biosynthetic pathways. The variation in tritium content of different compounds can be expected as a consequence of a variety of biosynthetic mechanisms.

The higher levels of tritium found in the intermediate compounds of the tricarboxylic acid cycle and derivative compounds of that cycle may be an indication of greater retention of tritium than of ^1H in oxidative reactions. Citric acid is formed by condensation of oxalacetate with acetyl CoA which is formed by oxidative decarboxylation of pyruvate. While it is true that the fatty acid moieties of the lipids are also formed from acetyl CoA and the lipids retain less tritium, some of the hydrogen atoms in fatty acids come from reductive reactions. Perhaps more important, the oxalacetate which condenses with acetyl CoA is formed by a series of four oxidative reactions from citrate. The reductive amination of α -ketoglutarate to give glutamate should discriminate against tritium. In fact, the R value for glutamate is significantly lower than the R value of citrate. Similarly, aspartate formed by transamination from oxalacetate has significantly lower R values than malate. It is interesting that in Exp 5, which ended with a dark period, the sugar phosphates, which in the dark would be formed by oxidative reactions, have higher R values than in Exp 4 (continuous light). Especially noteworthy is the difference in R values for 3-phosphoglycerate, 0.58 in Exp 4 and 0.88 in Exp 5.

The increases in tritium content during the transformations of sugar phosphates and 3-phosphoglycerate to malate and citrate (Exp 4) are significant, the R values rising from 0.78 and 0.58 to 1.04 and 0.97, or more. Thus, it appears that discrimination against tritium in oxidative reactions leads to retention of tritium in metabolic compounds. This conclusion may have some implications

for other organisms which are not autotrophic, and, therefore, depend on oxidative metabolism of food.

Once the amino acids are formed, there seems to be no significant further change in tritium content as the amino acids are incorporated into proteins. In the case of starch formation from sugar phosphates there may be some discrimination, since the R values for sugar phosphates and for sucrose range from 0.74 to 0.88, whereas the R values for glucose released from starch by hydrolysis are 0.69 and 0.75 in the two experiments.

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TABLE I. Carbon Found in Protein Amino Acids
(Acid Hydrolysis) by ^{14}C Content and by Amino
Acid Analyzer

Compound	From ^{14}C Deter. $\mu\text{moles C/cm}^3$ algae	From Amino Acid Analyzer $\mu\text{moles C/cm}^3$ algae
<hr/>		
Alanine		
Exp 4	186	196
Exp 5	245	252
Valine		
Exp 4	217	213
Exp 5	267	273
Glutamate		
Exp 4	300	317
Exp 5	378	378
Aspartate		
Exp 4	201	200
Exp 5	252	258
<hr/>		

TABLE II. Ratios of T/¹⁴C in Chlorella pyrenoidosa

Compound	<u>A</u> C-H _{n.e.} C	<u>B</u> T (μmoles) ¹⁴ C (μmoles)		Concentration μmoles C cm ³ algae		R = B/A	
		Exp. 4	Exp. 5	Exp. 4	Exp. 5	Exp. 4	Exp. 5
Total		0.92 ± 0.01	0.84 ± 0.01	7,010	7,540		
CH ₃ OH sol.		1.11 ± 0.02	1.01 ± 0.01	1,870	2,300		
CH ₃ OH insol.		0.83 ± 0.01	0.79 ± 0.00	5,000	5,200		
Lipid	< 2	1.18 ± 0.01	1.03 ± 0.03	1,406	1,494	>0.59	>0.52
Starch glucose	7/6	0.08 ± 0.06	0.75 ± 0.02	1,161	912	0.69	0.75
Uridylic ac.	8/9	0.63 ± 0.01	0.60 ± 0.04	54	65	0.71	0.68
Cytidylic ac.	8/9	0.67 ± 0.00	0.63 ± 0.00	52	68	0.75	0.71
Adenine	2/5	0.20 ± 0.01	0.21 ± 0.02	30	39	0.50	0.53
Guanine	1/5	0.12 ± 0.01	0.11 ± 0.00	26	34	0.60	0.55
Ribose	6/5	0.91 ± 0.01	0.85 ± 0.02	38	55	0.76	0.71
Protein amino acids							
Leucine:Iso- leucine:Phenyl- al: 2:1:1	10/6	0.78 ± 0.03	0.73 ± 0.00	A 536	A 715	(0.62)	(0.56)
Tyrosine	7/9	0.47 ± 0.01	0.42 ± 0.01	P 156	P 193	0.60	0.54
Valine	8/5	0.80 ± 0.01	0.74 ± 0.01	A 217	A 267	0.50	0.46
Alanine	4/3	0.96 ± 0.02	0.90 ± 0.01	A 186	A 245	0.72	0.68
Threonine	5/4	0.68 ± 0.01	0.55 ± 0.01	P 88	P 57	0.54	0.44
Glutamate	5/5	1.02 ± 0.02	0.91 ± 0.02	A 300	A 378	1.02	0.91

Glutamine	5/5	1.05 $\pm$ 0.01	0.94 $\pm$ 0.01	P 77	P 92	1.05	0.94
Serine	3/3	0.49 $\pm$ 0.01	0.49 $\pm$ 0.01	A 21	A 17	0.49	0.49
Glycine	2/2	0.45 $\pm$ 0.04	0.41 $\pm$ 0.01	A 31	A 124	0.45	0.41
Aspartate	3/4	0.71 $\pm$ 0.01	0.68 $\pm$ 0.02	A 201	A 252	0.95	0.91
Asparagine	3/4	0.70 $\pm$ 0.03	0.65 $\pm$ 0.01	P 56	P 77	0.93	0.87
Arginine	7/6	0.98 $\pm$ 0.04	0.90 $\pm$ 0.02	AA230	AA259	0.84	0.77
L6sine	9/6	0.92 $\pm$ 0.02	0.82 $\pm$ 0.03	AA200	AA257	0.61	0.55
Total Amino acids recov.				2,349	2,933	(0.73)	(0.66)

CH₃OH sol.

Alanine	4/3	1.00 $\pm$ 0.04	0.97 $\pm$ 0.02	23	23	0.75	0.73
Glutamate	5/5	0.99 $\pm$ 0.01	0.88 $\pm$ 0.01	66	63	0.99	0.88
Serine	3/3	0.58 $\pm$ 0.02	0.71 $\pm$ 0.01	6	9	0.58	0.71
Aspartate	3/4	0.69 $\pm$ 0.01	0.73 $\pm$ 0.01	19	20	0.92	0.97
Glutamine	5/5	1.01 $\pm$ 0.01	0.89 $\pm$ 0.01	9	5	1.01	0.89
Threonine	5/4	0.62 $\pm$ 0.03	0.59 $\pm$ 0.10	5	4	0.50	0.47
Sucrose	14/12	0.86 $\pm$ 0.02	0.92 $\pm$ 0.02	19	22	0.74	0.79
Malate	3/4	0.73 $\pm$ 0.01	0.81 $\pm$ 0.02	6	5	0.97	1.08
Citrate	4/6	0.69 $\pm$ 0.02	0.67 $\pm$ 0.02	4	7	1.04	1.01
3-PGA	3/3	0.58 $\pm$ 0.02	0.89 $\pm$ 0.05	3	4	0.58	0.89
F6P	7/6	0.91 $\pm$ 0.02	1.01 $\pm$ 0.03	2	2	0.78	0.87
G6P	(7.5/6.5)	0.95 $\pm$ 0.03	1.02 $\pm$ 0.01	5	4	(0.82)	(0.88)
S7P							
ADP	8/10	0.62 $\pm$ 0.02	0.63 $\pm$ 0.01	2	3	0.78	0.79
ATP	8/10	0.74 $\pm$ 0.02	0.68 $\pm$ 0.04	1	4	0.93	0.85
UDPG	15/15	0.82 $\pm$ 0.03	0.92 $\pm$ 0.04	5	4	0.82	0.92

TABLE II

$C-H_{n.e.}/C$ is the ratio of non-exchangeable H atoms per molecule to carbon atoms per molecule, at room temperature, pH 7. B is the ratio of T found, expressed as moles to ^{14}C found, expressed as moles. The moles of T found was obtained by dividing the T found in curies by the specific radioactivity of T in the HTO at the end of the experiment. The μ moles of ^{14}C was calculated as the curies of ^{14}C found divided by specific radioactivity of $^{14}CO_2$ found at the end of the experiment. In Experiment 4 (continuous light), T specific activity = $0.124 \mu C/\mu mole$. ^{14}C specific activity = $0.79 \mu C/\mu mole$. In Experiment 5 (16 hr light, 8 hr dark, killed at end of a dark period) T specific activity = $0.138 \mu C/\mu mole$, ^{14}C specific activity = $0.77 \mu C/\mu mole$. If there were no isotope discrimination for ^{14}C or T, $R = B/A$ would be equal to unity. Since discrimination against ^{14}C is negligible (see Table I) the discrimination against T is represented by values of R smaller than 1.

In the values given for amino acids from protein, A indicates concentration by acid hydrolysis and ^{14}C measurement, AA is by amino acid analyzer, and P is concentration from pronase treatment and ^{14}C content. For all R calculations, radioactive compounds from pronase treatment were used.

Error limits given under B are average deviations from the mean based on four or more separate analyses, including two-dimensional paper chromatography, where applicable.

Abbreviations: 3-PGA, 3-phosphoglyceric acid; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; S7P, sedoheptulose-7-phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; UDPG, uridine diphosphoglucose.

XBL 7110-5377

Fig. 1. Apparatus for Culturing Algae with HTO and $^{14}\text{CO}_2$

A - gas pump. B - bypass stopcock. C - pressure equilibrating device containing silicone oil. D - Ascarite trap for CO_2 . E - outlet for CO_2 sampling. F - light-tight chamber. G - bulbs containing cotton plugs. H - four-way stopcock for bypassing CO_2 reservoir. I - 5-liter reservoir for $^{14}\text{CO}_2$. J - fluorescent lights for illuminating algae. K - algae suspension. L - water jacket. M - stopcock for draining algae suspension. N - timer for controlling light-dark periods. O - thermostatted water bath and water pump.

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TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720