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September, October, November 1957

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Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

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BIO-ORGANIC CHEMISTRY

M. Calvin, Director

Edited by James A. Bassham

THE EFFECT OF RADIOCARBON ON THE RATE OF CARBON DIOXIDE UTILIZATION DURING PHOTOSYNTHESIS

Osmund Holm-Hansen, V. Moses, Christian F. van Sumere,* and M. Calvin

An isotope effect is a difference, in either the rate or the equilibrium of a reaction, induced by the difference in mass between the isotope under consideration and the one that is used for comparison, usually the natural mixture. The existence of such an isotope effect, both in simple chemical reactions and in the more complex reaction sequences found in metabolic studies, is well established.[1,2,3,4,5] Occasionally, it is most convenient to measure the rate of a reaction by using isotopic material and to depend upon the characteristics of the isotope (e.g., radioactivity) to provide the required sensitivity for the measurement. When the information desired is the absolute rate at which the reaction proceeds with the natural mixture of isotopes, it is necessary to apply a suitable correction factor to the rate thus measured; this will convert it from the rate of the reaction of the isotopic material to the rate of the reaction of the natural isotopes.

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*IRSIA Grant, 1957. Present address: Biochemical Department, Ruksuniversiteit Gent, Caspinoplein 11, Ghent, Belgium.

[1] J. W. Weigl, and M. Calvin, J. Chem. Phys. 17, 210 (1949).

[2] P. E. Yankwich and M. Calvin, J. Chem. Phys. 17, 109-110 (1949).

[3] S. Aronoff, Techniques of Radiobiochemistry (Iowa State College Press, Ames, Iowa, 1956), 1-15.

[4] D. A. Semenow and J. D. Roberts, J. Chem. Educ. 33, 2-14 (1956).

[5] Buchanan, Nakao, and Edwards, Science 117, 541-545 (1953).

Thus various investigators, studying the rate of photosynthesis, have found that carbon-14 is assimilated more slowly than carbon-12, by amounts varying from 6% to 17%.[6,7,8] Similar rate differences between C^{14} and C^{12} have been observed in single-step chemical reactions.[9,10] Although the reaction rate is altered by the use of different isotopes, the nature of the reaction product, apart from isotopic composition, is not changed. In a biological system the product is very often the result of a complex sequence of reactions that are related in several ways. If the isotopic difference is maintained throughout such a complex series of transformations, it is conceivable that the relationship between the starting material and the final products may be so different quantitatively as to appear to be qualitatively different. It is not to be expected, however, that the direction of an isotope effect in different reactions will always be the same, no matter what reaction is involved. Thus it is very unlikely that the situation would occur in which a unidirectional isotope effect would carry through a long sequence of reactions to produce a totally different metabolic pattern. It has been assumed, therefore, that the reactions of C^{14} in biological systems will not be qualitatively different from those of C^{12}. Recently, however, Boichenko and Zakharova[11,12] have reported that tracer amounts of C^{14} (in which 0.08% or more of the total carbon is present as C^{14}) reduced the rate of carbon dioxide uptake to 1/5 the control value (0.004% C^{14}) for 10 minutes of photosynthesis. When 0.72% of the total carbon was present as C^{14}, the rate was further reduced to 1/40 the control value. This implies that C^{14} somehow interferes with the assimilation of C^{12}. Furthermore, the pattern of C^{14} incorporation is reported to be altered to a marked degree as well. An effect like this is very different from the known isotope effect of C^{14}; as carbon dioxide containing 10% to 20% of $C^{14}O_2$ is commonly employed in our laboratory, these findings of Boichenko and Zakharova prompted us to re-investigate this point.

The experiments described below were designed to determine whether there is any effect on the rate of photosynthesis and the pattern of carbon dioxide incorporation in the presence of carbon dioxide containing 10% to 20% $C^{14}O_2$ when either algae or higher plants are used.

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[6] Weigl, Warrington, and Calvin, J. Am. Chem. Soc. 73, 5058-5063 (1951).

[7] R. W. Van Norman and A. H. Brown, Plant Physiol. 27, 691-709 (1952).

[8] E. Steeman Nielsen, Extrait du J. consul, Conseil permanent intern. exploration mer 18, 117-140 (1952).

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[10] G. A. Ropp and V. F. Raaen, J. Am. Chem. Soc. 74, 4492-4494 (1952).

[11] E. A. Boichenko and N. I. Zakharova, Conference of the Academy of Sciences of the USSR on the Peaceful Uses of Atomic Energy, July 1-5, 1955, 121-125.

[12] E. A. Boichenko and N. I Zakharova, Biochemistry (USSR) 21, 377-382 (1956).

To test the effect of increasing concentrations of C^{14} on the rates of photosynthesis and on the fixation patterns of carbon dioxide, Chlorella pyrenoidosa (1% suspension) was exposed, by techniques previously described,[13] to labeled sodium bicarbonate of specific radioactivity ranging from 0.003 to 15 μC/μmole. The experiment reported in Table I was performed in a new apparatus[14] in which several samples were shaken simultaneously over a light source. The radioactive bicarbonate solution, and later the boiling ethanol, were added without interrupting the shaking of the samples in order to ensure rapid and complete mixing. The results from the first experiment (Table I) show the total fixation of $C^{14}O_2$ by the cells after two minutes of photosynthesis, while in Fig. 1 is shown the fixation after varying times of exposure to $C^{14}O_2$ from 6 seconds to 3 minutes. In these experiments the volume and the molarity of the injected bicarbonate were constant. The data in Table I indicate that after 2 min there was no effect on the specific rate of C^{14} fixation caused by varying the specific activity of C^{14}, while Fig. 1 indicates that the same holds true for exposure times of 6 sec to 3 min. In both these experiments, the fluctuations in fixation from the theoretical values lie within the experimental error. The patterns of C^{14} fixation into the alcohol-soluble compounds were determined in the 6-sec, 40-sec, 2-min, and 3-min samples of Fig. 1. No significant changes were seen in the distribution of C^{14} among the various compounds in the samples supplied with varying specific activities of $C^{14}O_2$ for the times mentioned. The patterns for 3-min fixation time are shown in Fig. 2. These two experiments do not, however, indicate whether or not any change in the rate of total carbon dioxide utilization accompanies a change from an environment in which all the carbon is present as C^{12} to an environment containing some C^{14}. To obtain an answer to this question, the rates of gas exchange of Chlorella during photosynthesis were measured in an apparatus that automatically records the concentrations of oxygen, carbon dioxide, and radioactive carbon dioxide every 7 seconds.[15,16] The rates of gas exchange were first determined with an atmosphere of air containing 0.79% CO_2 being circulated through the suspension, and then again after the introduction of a known amount of $C^{14}O_2$ into the system. The amount of C^{14} introduced was sufficient to make a final isotopic concentration of about 5% $C^{14}O_2$ in the total CO_2 present, which was now raised to 0.90%. The rates of absorption of CO_2 before and after the introduction of the C^{14} were 21.5 and 20.5 arbitrary units of CO_2 per minute, while the corresponding oxygen-evolution rates were 16 and 15 arbitrary units of O_2 per minute. It is thus seen that there was no significant effect on the photosynthetic rate when C^{14} was introduced into the system. This same type of experiment was then performed with a leaf of a primrose-plant (Primula sp.), the plant

[13] Moses, Holm-Hansen, and Calvin, Biochim. et Biophys. Acta (in press) (1958).

[14] V. Moses, in this report, p. 17.

[15] A. T. Wilson and M. Calvin, J. Am. Chem. Soc. 77, 5948-5957 (1955).

[16] Bassham, Shibata, and Calvin, Biochim. et Biophys. Acta 17, 332-340 (1955).

Table I

Total incorporation of C^{14} by Chlorella after 2 minutes' photosynthesis in the presence of $NaHC^{14}O_3$ of varying specific activity

Sample	Specific activity of added $NaHCO_3$ ($\mu C/\mu mole$)	$C^{14}O_2$ (conc.(% total CO_2)	Total fixation of C^{14} (counts/min)	Ratio	Expected from spec. act.	Found fixed
A	1.5	2.2	517,000	A/C	547	530
B	0.057	0.084	22,000	A/B	26.3	23.5
C	0.00274	0.00405	974	B/C	20.8	22.6

1.0 ml of cell suspension containing 10 µl of wet packed cells was aerated in the light (intensity 2,000 ft-c) with air containing 1% CO_2 for 25 minutes, immediately after which the flow of air and CO_2 was stopped and 100 µl of 0.026 M $NaHCO_3$ (for specific activity see table) was injected. The cells were allowed to carry on photosynthesis in the presence of $NaHC^{14}O_3$ for 2 min. The reaction was then stopped by the addition of 4 ml of boiling alcohol.

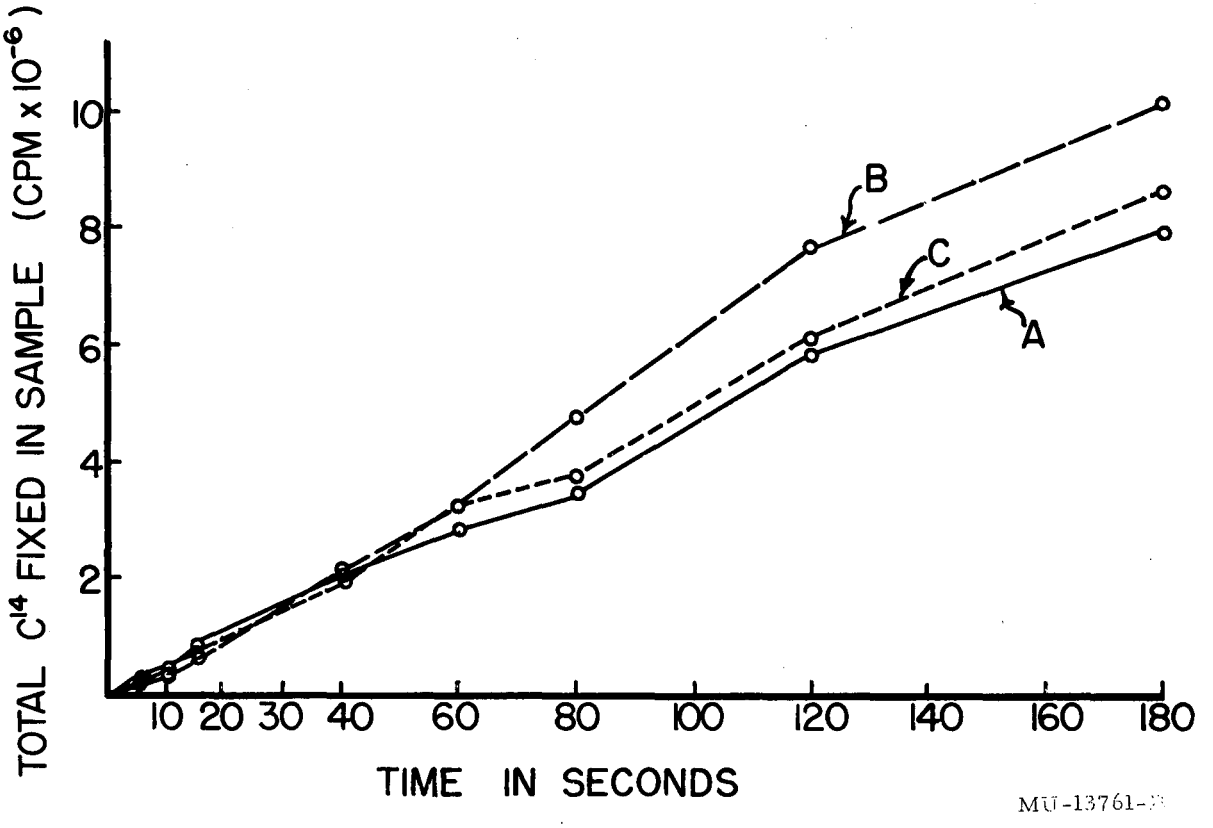

Fig. 1. Total incorporation of C^{14} by Chlorella at various times in the presence of $NaHC^{14}O_3$ of varying specific activity: 3.0 ml of cell suspension containing 30 µl of wet packed cells was aerated in the light (intensity about 15,000 ft-c) with air containing 1% CO_2 for 10 minutes, immediately after which the flow of air and CO_2 was stopped and 100 µl of 0.026 M $NaHC^{14}O_3$ was injected, the specific activity of which was 15 µC/µmole (Curve A), 1.5 µC/µmole (Curve B), and 0.15 µC/µmole (Curve C). The cells were allowed to carry on photosynthesis in the presence of $NaHC^{14}O_3$ for times ranging from 6 sec to 3 min, the reaction then being stopped by the addition of 12 ml of boiling alcohol. The ordinates for Curves B and C are multiplied by 10 and 100, respectively.

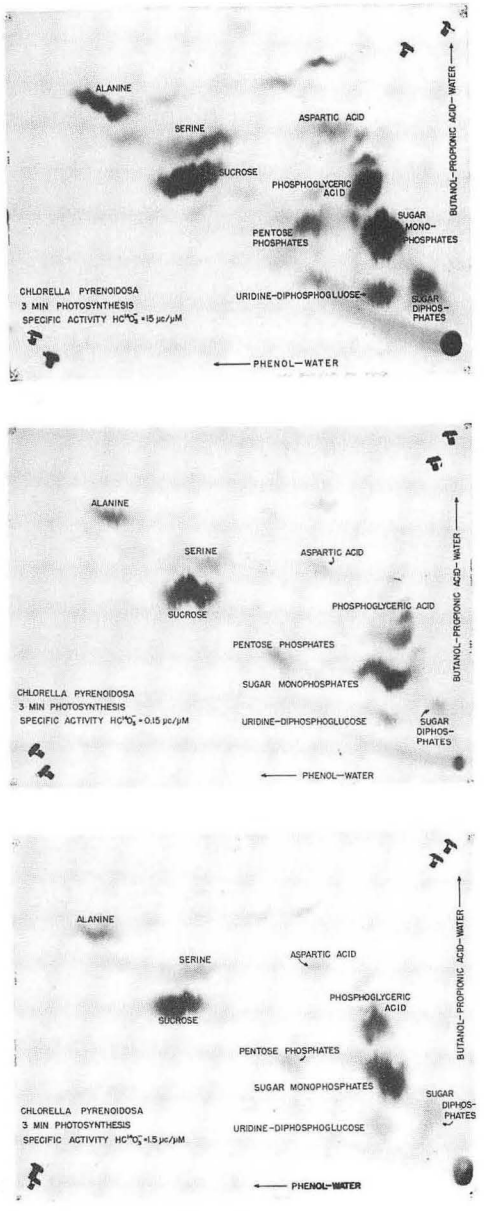

Fig. 2. Patterns of C^{14} incorporation by Chlorella after 3 min photosynthesis with $NaHC^{14}O_3$ of varying specific activity. A, specific activity 15 μC/μmole; B, 1.5 μC/μmole; C, 0.15 μC/μmole. For experimental details see Fig. 1.

genus used by Boichenko and Zakharova.[11] Immediately upon removal of the leaf from the plant, the base of the petiole was submerged in water in a small test tube and then placed in the illumination chamber.* The rates of oxygen and carbon dioxide exchange before and after the introduction of C^{14} (final $C^{14}O$ percentage about 5% of total CO_2) were 2.95 and 2.98 arbitrary units of CO_2 absorbed per minute and 2.50 and 2.90 arbitrary units of O_2 evolved per minute, respectively. Once again it is seen that there was no significant change in the rate of gas exchange caused by the introduction of C^{14}. It was noticed that after the introduction of C^{14} to both Chlorella and the primrose leaf the rate of utilization of the $C^{14}O_2$ was a little slower than that of $C^{12}O_2$ (the infrared CO_2 analyzer detects mainly $C^{12}O_2$, and not $C^{14}O_2$; the rate of $C^{14}O_2$ utilization was determined by measuring the radioactivity in the circulating atmosphere by use of an ionization chamber). Quantitatively this isotope effect is within the range mentioned at the beginning of this communication, but the important observation is that the presence of the $C^{14}O_2$ did not have any appreciable effect on the absorption of the $C^{12}O_2$, or upon the specific rate or pattern of $C^{14}O_2$ utilization.

The above experiments which are but a few done in our laboratory in an attempt to confirm the results of Boichenko and Zakharova,[11, 12] unequivocally demonstrate that aside from the usual isotope effect there is no effect of C^{14} that would invalidate its use as a tracer substance in biological systems at specific activity levels as high as 20% C^{14}, at least for short periods of time.

*The illumination vessel as depicted in Reference 15 was replaced by a modified illumination chamber which is similar in size and shape but more suitable for working with leaves, as one whole side can be removed.

IDENTIFICATION OF CARBOXY-KETOPENTITOL PHOSPHATES AS PRODUCTS OF PHOTOSYNTHESIS

V. Moses

Although it has been well established that the initial carboxylation reaction of photosynthesis is the combination of carbon dioxide with ribulose 1:5-diphosphate, the proposed six-carbon product, 2-carboxy, 3-keto pentitol 1:5-diphosphate,[1] has so far not been identified in experiments involving photosynthesis by green plants in the presence of radioactive carbon dioxide. The standard methods of analysis of the products of photosynthesis involve chromatography of the ethanol- and water-soluble components of the organism (following killing of the cells in boiling 80% ethanol) on sheets of washed Whatman No. 4 filter paper. The solvents (phenol-water in the first dimension, and butanol–propionic acid–water in the second dimension) are normally allowed to run only to the bottom edge of the paper (about 10 hours) or at most, twice that far, before the papers are dried. On such chromatograms the area near the origin containing principally the sugar diphosphates is not well separated, and it is known that the apparently single spots seen are often mixtures containing several diphosphate esters of the relevant sugars. In an attempt to better separate these components in extracts of cells that had been killed by ethanol at -15°, the solvents were allowed to run for 48 hours in each direction, and examination of the subsequent radioautogram showed that the "diphosphate area" was split into three separate areas (Fig. 3). One of these areas contained mainly ribulose diphosphate together with some minor unidentified components, while the second, much weaker, area was composed of fructose, glucose, sedoheptulose, and other diphosphates. However, there appeared a third spot never previously observed, which ran slower in the butanol–propionic acid solvent than the other two. It seemed possible that this spot might contain a component more acidic than the other sugar phosphates, and steps were taken to identify the nature of this spot.

On treatment of the third diphosphate spot with purified Polidase S, or with human seminal acid phosphatase, at pH 5 in both cases, a number of spots were seen on subsequent chromatography and autoradiography (Fig. 4). Two of these appeared of particular interest, and they now have been identified tentatively as 2-carboxy, 4-keto pentitol

$$(CH_2OH.COH(COOH).CHOH.C{:}O.CH_2OH)$$

and the lactone of 2-carboxy, 3-keto pentitol

$$(CH_2OH.COH(COOH).C{:}O.CHOH.CH_2OH).$$

These substances will be referred to henceforth as the γ-keto acid and the β-keto acid, respectively.

Investigation of the γ-Keto Acid

The following evidence has been obtained as a result of various chemical tests, supporting the suggestion that this substance is a 2-carboxy, 4-keto pentitol.

(a) Electrophoresis in 0.1 M ammonium acetate buffer at pH 9.1 (600 to 700 volts) for 3 hr together with gluconic acid, 2-ketogluconic acid,

[1] M. Calvin, J. Chem. Soc. 1895 (1956).

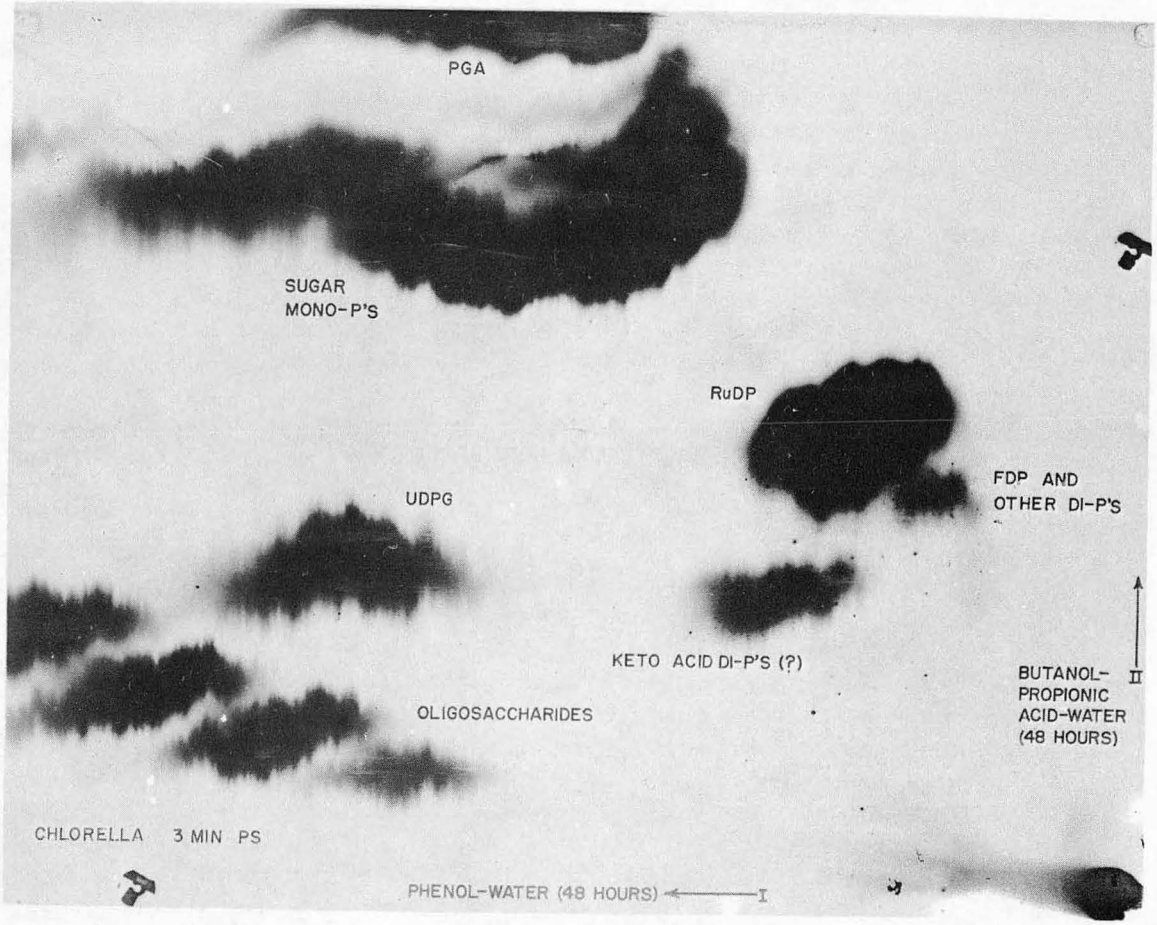

Fig. 3. Chromatogram of ethanol- and water-soluble fractions of Chlorella after 3 minutes' photosynthesis in the presence of $\overline{NaHC^{14}O_3}$. The solvents were run for 48 hours in each direction in order to separate the sugar phosphate area.

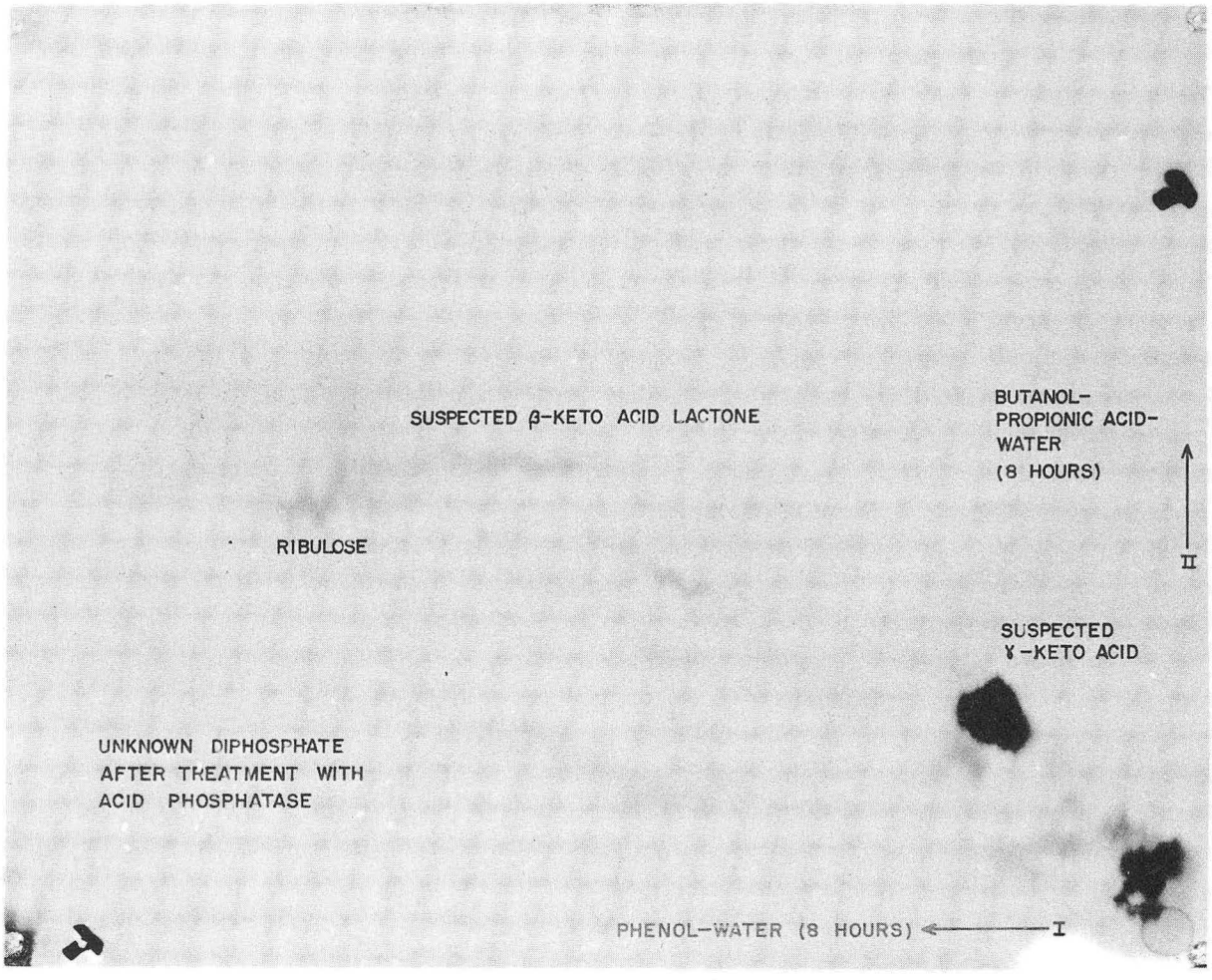

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Fig. 4. Chromatography of the third diphosphate spot after treatment with phosphatase.

5-ketogluconic acid, and hamamelonic acid showed that the substance ran the same distance as all these other acids. Chromatography in the two solvents mentioned above showed that these known acids could not be separated, and that the unknown ran at the same speed as the mixture of the known acids, suggesting a generally similar structure.

(b) Treatment with 0.07 N HCl at 100° for 30 min produced no lactone from the unknown substance, suggesting that no hydroxyl group was present at either the third or fourth carbon atom. Polyhydroxymonocarboxylic sugar acids all readily lactonize in acid solution. However, the keto acids, 2-keto-gluconic acid, and 5-ketogluconic acid undergo lactonization far more slowly than does gluconic acid.

(c) Standing for some hours at room temperature at pH 11 to 12, followed by acidification to pH 3 at 0°, resulted in the formation of some ribulose and a trace of glyceric acid, but apparently left most of the original material intact; there thus appeared to be no epimerization by alkali.

(d) Treatment with KBH_4 (about 0.0075 M) produced a substance capable of undergoing acid-lactone interconversion at appropriate pH levels, suggesting the reduction of a keto group to a hydroxyl group, which is then capable of lactonization.

(e) Treatment with 2:4-dinitrophenylhydrazine in 2 N HCl produced in low yield two 2:4-dinitrophenylhydrazones, which, on chromatography in butanol saturated with 6% aqueous NH_3, ran with the hydrazone of added carrier 5-ketogluconic acid; this also suggests the presence of an aldehyde of ketone grouping.

(f) The product found after reduction with KBH_4 chromatographed very nearly, but not identically, with hamamelonic acid, both in the acid and lactone forms. Two samples of hamamelonic acid were available; one, made by Schmidt and Heintz, was obtained by the action of HCN and ribulose followed by hydrolysis of the nitrile and separation of the epimeric acids produced by fractional crystallization of their phenylhydrazide derivatives.[2] The other, prepared by Rabin et al., was made by the action of KCN on ribulose diphosphate, followed by hydrolysis of the nitrile and removal of the phosphate groups.[3] This sample presumably contained hamamelonic acid and its epimer, in unknown proportion. The γ-keto acid, after reduction with KBH_4, chromatographed more nearly with Rabin's and co-workers' sample of mixed hamamelonic acid and epimer than with Schmidt's and Heintz's pure hamamelonic acid. Before reduction with KBH_4, the γ-keto acid ran quite distinctly from hamamelonic acid. The explanation for these findings may be that the γ-keto acid is reduced by KBH_4 to a mixture of two epimers of the hamamelonic acid type, neither of which is hamamelonic acid itself, but one of which is the same as the epimer in the sample from Rabin et al.

(g) Heating the γ-keto acid with 0.07 N HCl at 100° for 30 min converted some 35 to 45% into xylulose, presumably by decarboxylation, leaving some of the original compound apparently unchanged.

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[2] O. T. Schmidt and K. Heintz; Ann. 515, 77 (1935).

[3] Rabin, Shaw, Pon, Anderson, and Calvin, J. Am. Chem. Soc. (in press) (1958).

(h) Heating the γ-keto acid with 0.015 N NaOH at 100° for 30 min produced several substances, one of which was glyceric acid (the others were not identified), and again left some of the original compound apparently unchanged.

Investigation of the β-Keto Lactone

(a) Treatment with 10^{-4} N NaOH (i.e., pH 10) resulted in the formation of a substance running chromatographically in the same position as the γ-keto acid (i.e., just behind hamamelonic acid), together with a little glyceric acid. It appears that alkali treatment of the β-keto lactone converts the lactone partly into the β-keto acid, and also causes some breakdown to glyceric acid, as was found with the γ-keto acid.

(b) On treatment with KBH_4 at about pH 10 some glyceric acid was formed, together with a substance that ran chromatographically together with hamamelonic acid. However, the radioactivity was too weak to determine the degree of exact coincidence with hamamelonic acid.

(c) Treatment of the γ-keto acid with alkali at pH 11 to 12 for some hours, followed by acidification at ice temperature to pH 3, resulted in the formation of some glyceric acid and ribulose, while some of the original γ-keto acid remained unchanged. It has already been mentioned that acid treatment of the γ-keto acid results in the production of xylulose, not ribulose. It seems that at pH 11 some of the β-keto acid is produced from the γ-keto acid by enolization, and that the former then breaks down to glyceric acid and ribulose at alkali and acid pH, respectively.

(d) The chromatographic position of the mixed keto acid diphosphate spot (Fig. 3) suggests that both these compounds are present as the diphosphates. Although not providing rigid proof, these findings are consistent with the proposal that the structures of these two compounds are 2-carboxy, 4-keto pentitol and 2-carboxy, 3-keto pentitol, for the γ- and β-keto acids, respectively. The latter, having a keto group on the β-carbon atom, might be expected to be very unstable, and this is reflected by its decomposition under very mild alkalinity and acidity. The β-keto acid, having a hydroxyl group on the fourth carbon, might be expected to form a lactone, and evidence has been presented for acid-lactone interconversion with this compound. The γ-keto acid, on the other hand, might be expected to be quite stable, and far less likely to form a lactone; decomposition of this substance is incomplete even after 30 min in boiling acid or alkali, and no evidence has been found for lactonization.

Physiological Significance of These Keto Acids

Although nothing is definitely known of the physiological significance of these substances, it may be mentioned that the β-keto acid has been predicted as an intermediate in the carboxylation reaction that results in the formation of two molecules of phosphoglyceric acid from carbon dioxide and ribulose diphosphate.[1] The γ-keto acid may be formed from the β-keto acid when the cells are killed by ethanol, and thus be an artifact, or it may play some physiological role. Of interest in this connection is one

observation that more of the γ-keto acid is formed during photosynthesis when the cells are supplied with a source of nitrogen (NH_4NO_3) than when they are suspended in distilled water; there may thus be some interconnection between the γ-keto acid and nitrogen metabolism.

Kandler has reported the presence of a new diphosphate spot when cells photosynthesizing in the presence of labeled carbon dioxide were supplied with KCN a few seconds before being killed with boiling ethanol;[4] this substance was subsequently identified as hamamelonic acid diphosphate.[3] Kandler also claims to have found this substance in the absence of KCN.[4] However, each of the diphosphate spots shown in Fig. 3 has been intensively investigated to detect the presence of any other sugar acid diphosphates, including hamamelonic acid, which may be present; none have been found in any of the three diphosphate spots. This matter is more fully discussed by Rabin et al.[3]

[4] O. Kandler, in Chemistry Division Quarterly Report, UCRL-3710, March 1957, p. 9.

A NEW APPARATUS FOR TRACER STUDIES OF PHOTOSYNTHESIS

V. Moses

In a program involving the use of tritium oxide to follow the path of H in photosynthesis it became necessary to devise a scheme, other than the hand-shaken flattened test tubes previously in use,[1] for the incubation of small amounts of cells in the light of the presence of tracer. As the tritium oxide was to be greatly diluted by the water in which the algae were suspended, it was desirable to use small volumes of dense cell suspension (about 25%). However, such suspensions transmit very little light, and layers of suspension had to be thin in order to achieve a satisfactory degree of illumination throughout.

An apparatus was therefore designed to provide continuous shaking of six vessels while they were simultaneously illuminated from the bottom. The vessels were constructed from glass tubing of about 3.5 cm internal diameter and 7 cm length, to which a flat circle of glass was sealed to form a bottom. One ml of algal suspension in such a vessel forms a layer about 1 mm deep. A loose-fitting lid was provided with an inlet and an outlet tube, the former reaching nearly to the bottom of the vessel (Fig. 5). During the preadaptation period before a photosynthesis experiment the cells are shaken in the light, while air plus 1% CO_2 is blown over the surface of the cell suspension; this gas mixture is pre-wetted (by bubbling through water) to minimize evaporation from the cell suspension.

A rectangular water bath, fitted with inlet and outlet tubes to permit a constant stream of cooling water to pass through it, was arranged over a bank of eight 6-watt fluorescent tubes. At the top of the water bath two rails were fitted along which ran a small carriage consisting essentially of a four-wheeled plate in which were six holes, in which six of the glass vessels described above could be placed. The vessels were suspended below the plate so that the bottoms of the vessels reached nearly to the bottom of the water bath. The vessels were prevented from falling through by three glass "ears" on each vessel arranged near the top. A second plate, fitted to the carriage about 2 cm below the first and also containing six holes corresponding with those on the upper plate, prevented too much rattling of the glass vessels. The whole carriage was made to run back and forth along its rails by means of a connecting rod attached to an eccentric on a modified stirrer motor. The carriage oscillated about 3 cm at approximately 250 cycles per minute. Two views of the apparatus are shown in Figs. 6 and 7. The light intensity at the bottom of the vessels was about 2000 foot-candles.

As the whole apparatus needed to be as small as possible for use in a "hot" box, the fluorescent tubes were of a limited length, and although illumination to each vessel was fairly constant there were some variations between different positions on the shaker. For this reason the samples were supplied with the radioactive substrate, one at a time in a serial manner, each flask being moved to one particular position for the period of incubation with labeled substrate.

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[1] Moses, Holm-Hansen, and Calvin, Biochim. et Biophys. Acta (in press) (1958).

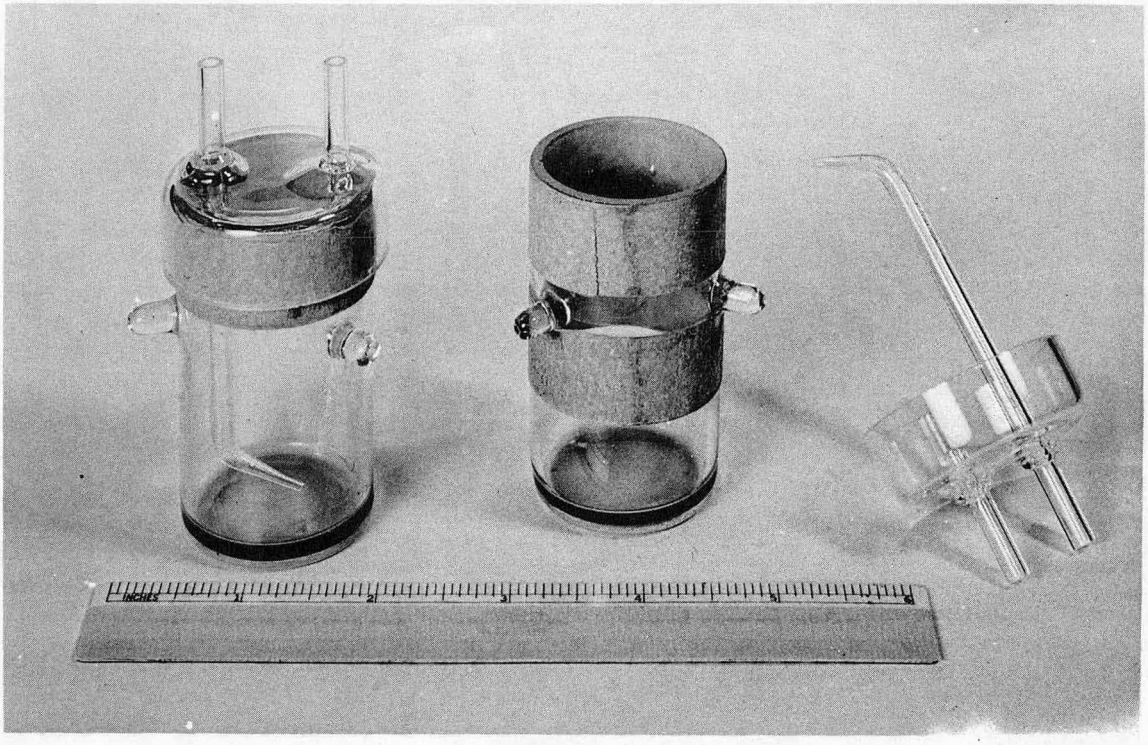

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Fig. 5. Close-up of glass vessels for algae, showing details of lids.

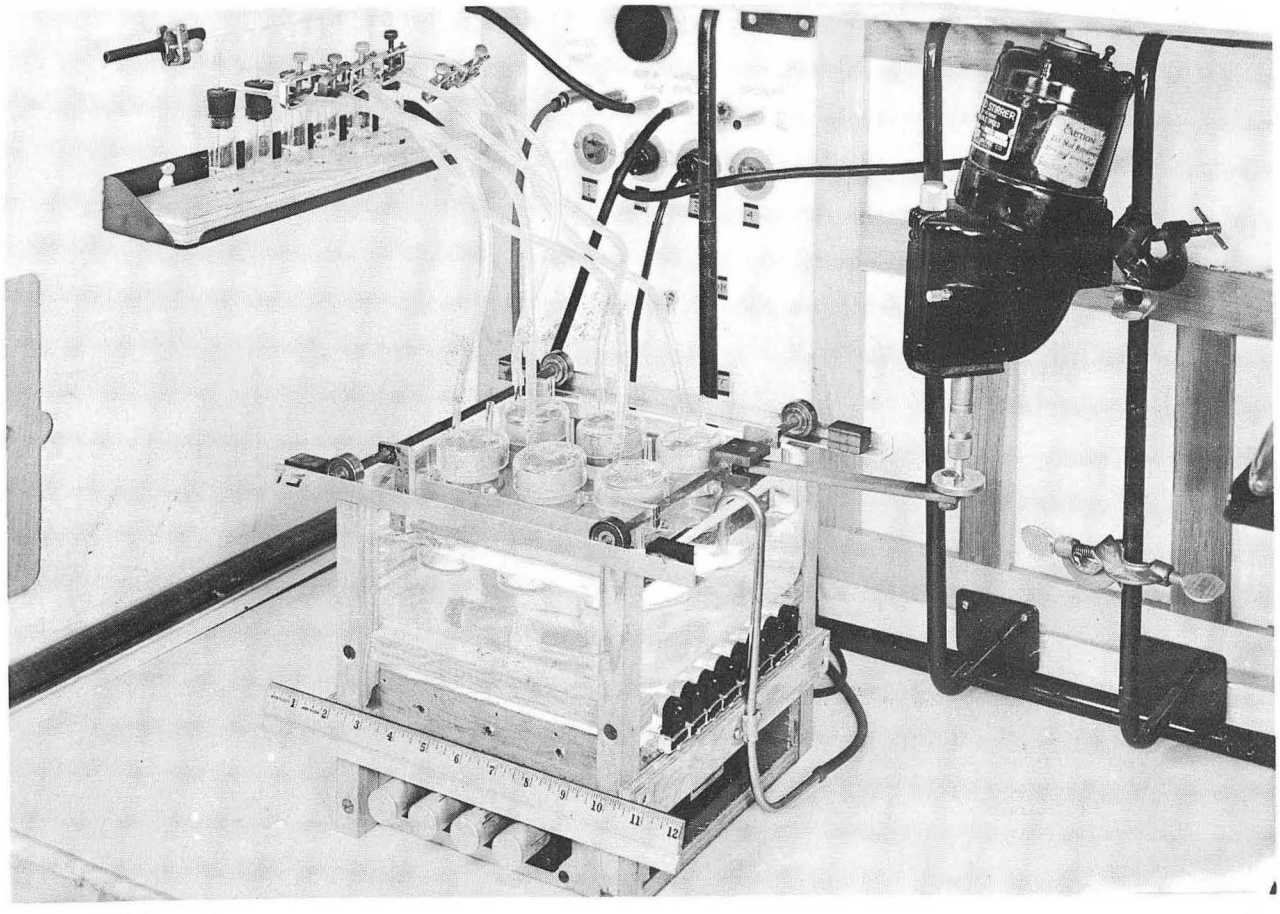

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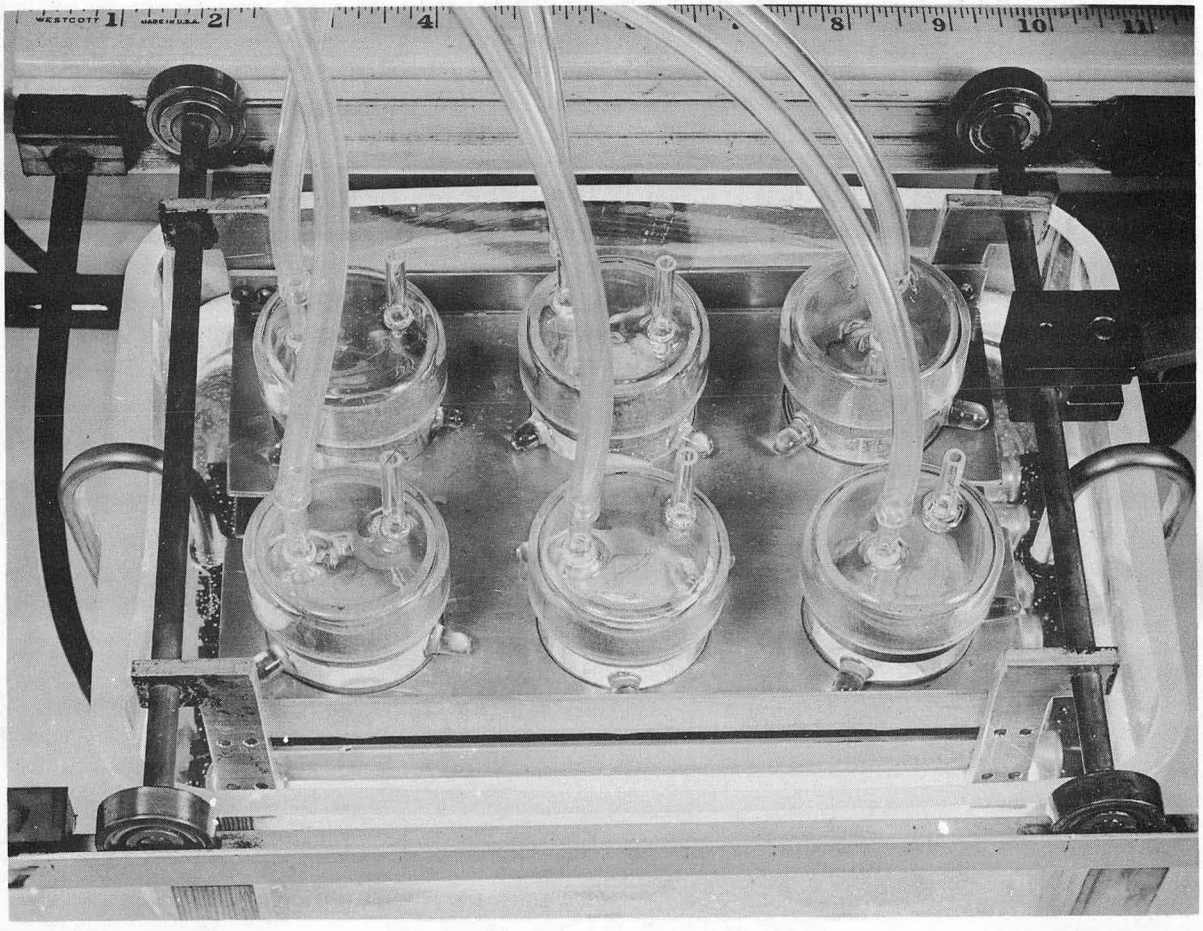
Fig. 6. General view of apparatus, showing shaker, motor, and bubbling apparatus.



ZN-1869

Fig. 7. Close-up of shaker from the top.

The experiments were performed as follows: After harvesting and washing with water, the cells were suspended in distilled water at the required concentration and 0.8 ml placed in each vessel. The vessels were shaken over the lights for 30 minutes during which period they were flushed with the air-and-CO_2 mixture in order to obtain the cells in a steady state of photosynthesis. Immediately before addition of the labeled substrate the motor was stopped and the lid removed from one vessel. The motor was restarted and 0.2 ml tritiated water was added to one vessel from a hypodermic syringe while the vessel was in motion in order to achieve rapid and complete mixing. The motor was again stopped, the lid replaced, the motor restarted, and the cells shaken for the desired period. A few seconds before the end of the experiment the lid was again removed from the stationary vessel, the latter set in motion again, and at the desired time 4 ml of ethanol was injected to kill the cells. After a few seconds for mixing, the motor was stopped once more, the vessel removed from the shaker, and the next vessel placed in the standard position.

Operation of the apparatus has been found by experience to be very satisfactory and the results far more reproducible than in experiments performed by the flattened-test-tube method.

THE EFFECT OF AMMONIUM NITRATE ON PHOTOSYNTHESIS IN CHLORELLA

William J. Toaspern, Osmund Holm-Hansen, and V. Moses

In most experiments on photosynthesis with algae conducted in our laboratory, the cells after harvesting are rinsed in distilled water several times and finally resuspended in distilled water with a small amount of phosphate, where they remain during the course of the photosynthesis experiment, which is usually completed within an hour (but sometimes has lasted as long as 12 hours). Because the algae have been growing in a nutrient solution that is fairly concentrated (0.012 M, KNO_3, 0.010 M $MgSO_4$, 0.008 M KH_2PO_4, plus other salts in smaller amounts), the question arises as to what happens within the cell during this period, and what the resulting effects are on the uptake and distribution of radioactive carbon. Instances are known where algal cells, when suspended either in distilled water or in synthetic sea water deficient in one or more mineral elements, show a marked response (photosynthetically, or by some other response such as phototaxis) upon the addition of small amounts (measured in ppm) of a metallic element.[1,2,3] It is to be expected that the distribution of radioactive carbon in the alcohol-soluble compounds formed during photosynthesis with $C^{14}O_2$ will be in some respects different when the cells are suspended in dilute phosphate from that which would be obtained with cells during growth in normal medium in continuous-culture tubes.[4] Such an indication of the effect of added salts has been noticed from time to time during studies of the carbon-reduction cycle, but in general there was no qualitative effect on the reduction of CO_2 through the sugar phosphates to sucrose. More particularly, Helmut Simon found that the addition of 0.001 M NH_4NO_3 to Chlorella not only enhanced the total uptake of carbon-14 but also altered the incorporation patterns of $C^{14}O_2$.[5] The work reported here was an attempt to confirm and extend these observations.

The general procedures employed were similar to those already described.[6] Flattened test tubes were used in all experiments, and contained either 2 or 3 ml of a 1% Chlorella pyrenoidosa suspension. The concentration

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[1] Clendenning, Brown, and Eyster, Canadian J. Botany 34, 943-966 (1956).

[2] Per Halldal (University of Oslo, Norway), personal communication.

[3] Kjell Baalsrud (University of Oslo, Norway), personal communication.

[4] O. Holm-Hansen, P. Hayes, and P. Smith, in Chemistry Division Quarterly Report, UCRL-3595, Oct. 1956, p. 56.

[5] Helmut Simon (UCRL), personal communication.

[6] Moses, Holm-Hansen, and Calvin: Biochim. et Biophys. Acta (in press, 1958).

of NH_4NO_3 was 0.001 M. Eight experiments were performed to investigate the effect of ammonium nitrate on photosynthesis in Chlorella; in these experiments, both the length of exposure time to radioactive carbon and the length of the adaptation period with ammonium nitrate were varied within wide limits. The results from a typical experiment indicating the effect of ammonium nitrate on the total fixation and also on the distribution of C^{14} within various alcohol-soluble compounds are shown in Table II. The radioautograms from these two samples are shown in Fig. 8; the aliquots put on paper were identical, therefore the densities of the spots on the film may be compared directly. To see if the length of exposure to ammonium nitrate before the addition of radiocarbon was of importance, an experiment was carried out that involved four different samples: cells in distilled water; cells that had been in 0.001 M NH_4NO_3 during all centrifugations, rinsings, etc., and final resuspension (total time 2 hours); cells suspended in distilled water, but given ammonium nitrate 30 minutes before C^{14}; and finally, cells that were given ammonium nitrate and C^{14} simultaneously. The length of time of photosynthesis with radiocarbon was 30 seconds. For some of the data from this experiment see Table III.

The over-all results from these experiments indicate that 0.001 M NH_4NO_3 has a marked effect on increasing the total fixation of radioactive carbon (usually close to 100% increase), and also greatly increases the activity found in amino acids such as aspartic, glutamic, alanine, citrulline, serine, etc., and in such organic acids as malic, lactic, succinic, glyceric, etc. Ammonium nitrate also has marked effects on the sugar phosphates and sucrose, but not always in the same direction. A full report of this work has been written describing each of these experiments separately, and will be filed in the Old Radiation Laboratory, Bio-Organic Chemistry Division's files. No discussion will be presented at this time, as this work is being continued by Kojiro Nishida and will be discussed in ensuing Quarterly Reports.

Table II

Effect of NH_4NO_3 on total uptake and distribution of carbon-14 during photosynthesis in Chlorella

3.0 ml of 1% Chlorella suspension; photosynthesis time in presence of C^{14} 30 seconds; adaptation time 5 minutes.

	Cell-suspension medium	
	Distilled water	NH_4NO_3
Total fixation (cpm)	474,000	775,000
Total soluble activity (cpm)	480,000	720,000
Total activity (cpm) in:		
Diphosphates	15,411	29,100
Uridine diphosphoglucose	2,061	15,666
Hexose-monophosphates	109,000	140,000
Phosphoglyceric acid	39,555	68,400
Aspartic acid	1,737	7,611
Citric acid	----	765
Glyceric acid	7,758	25,950
Glutamic acid	----	1,614
Serine and Glycine	12,510	36,750
Sucrose	9,726	28,440
Alanine	5,736	26,850
Citrulline	----	4,140
Glutamine	5,490	17,160
Malic acid	1,827	12,420
Glucose-cyclic-phosphate	8,355	3,354
Phosphoenolpyruvate	3,762	1,083
Phosphoglycolic	39,600	18,825

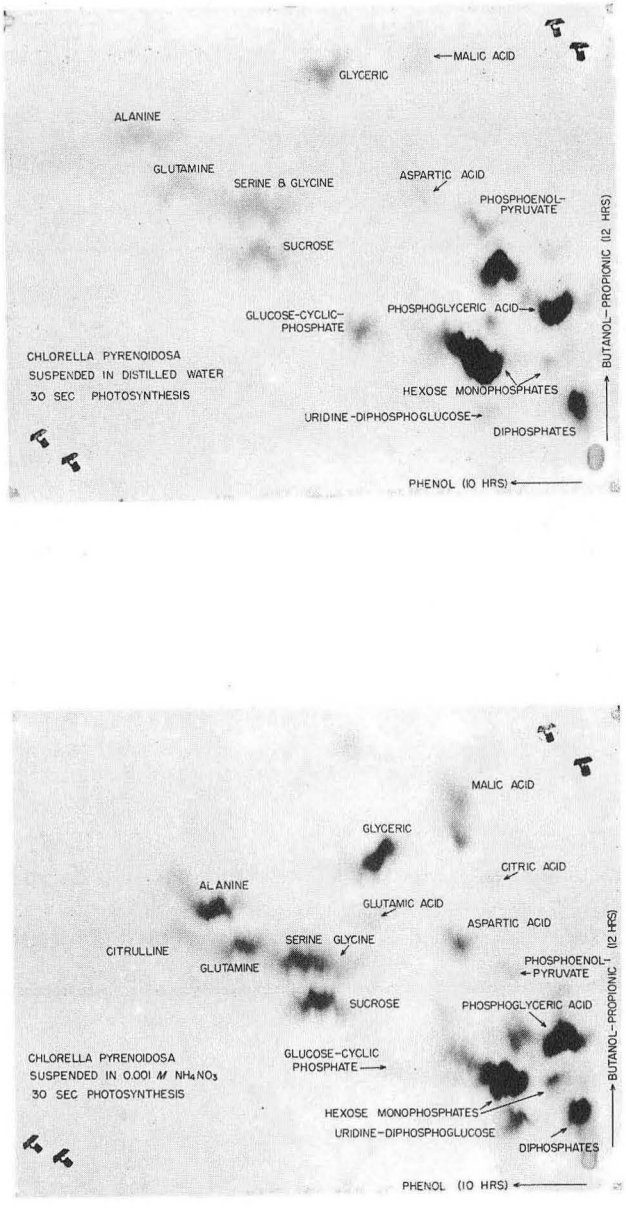

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Fig. 8. Radioautograms showing photosynthetic incorporation of C^{14} into alcohol-soluble compounds of Chlorella when the cells are suspended in distilled water (A), and in 0.001 M NH_4NO_3 (B). For details see Table II.

Table III

Effect of varying the length of the adaptation period with NH_4NO_3 on total uptake of carbon-14 during photosynthesis in Chlorella

3.0 ml of 1% algal suspension, photosynthesis time in the presence of C^{14} 30 seconds; adaptation period before injection of labeled carbon 30 minutes.

Treatment	Total fixation (cpm)	Total activity in alcohol-soluble fraction (cpm)	Soluble activity (% of total fixed)	Activity in insolubles (cpm)
A. Control (algae in distilled water)	756,000	686,000	90	70,000
B. C^{14} plus NH_4NO_3 injected simultaneously	958,000	867,000	90	91,000
C. NH_4NO_3 adaptation period of 30 min	1,100,000	1,007,000	91	93,000
D. Cells in 0.001 M NH_4NO_3 during all rinsings, etc. (total time in NH_4NO_3 before killing with alcohol about 2 hours)	1,288,000	1,130,000	88	158,000

EFFECT OF MINERAL SALTS ON PHOTOSYNTHESIS IN CHLORELLA

Kojiro Nishida, Osmund Holm-Hansen, and V. Moses

The preceding paper[1] describes the changes that addition of ammonium nitrate causes in the pattern of labeled compounds and total C^{14} fixation in Chlorella, photosynthesizing with $C^{14}O_2$. This report is concerned with two main considerations: first, whether or not the ammonium ion and (or) nitrate ion is responsible for this effect, and secondly, the effect of the constituent salts of the normal nutrient solution, either separately or in combination, upon the uptake and distribution of radioactive carbon.

All photosynthetic experiments were performed in the shaker apparatus described by V. Moses.[2] One ml of a 1% suspension of Chlorella pyrenoidosa was pipetted into each glass flask and adapted in the light (usually 10 min) before the addition of radioactive carbon (50 λ of 0.026 M $NaHCO_3$, 400 μC/ml). Boiling alcohol was used to kill the algae, and the subsequent chromatographic techniques were those in common use in the laboratory.

A. Effect of KNO_3 and NH_4Cl on Photosynthetic Carbon-14 Fixation

Results are shown in Table IV. It is seen that NH_4Cl had a great effect on the total fixation of $C^{14}O_2$ during photosynthesis, whereas KNO_3 did not show this effect. The low levels of radioactivity incorporated into the sugar phosphates in the algae supplied with NH_4Cl, and the very high amounts of C^{14} in their amino acids, were very striking in a comparison of these algae with algae in distilled water or in KNO_3. It is seen, however, that if cells with KNO_3 are compared with the controls in distilled water, the differences are much smaller than those with NH_4Cl, although mostly in the same direction.

B. Effect of Nutrient Solution* on Photosynthetic $C^{14}O_2$ Fixation

The results, shown in Table V, indicate that the total fixation of C^{14} by the algae suspended in nutrient solution was much greater than by the algae in distilled water, and also that--with few exceptions there was more activity incorporated (in nutrient solution) in all the soluble compounds that were counted.

C. Individual and Combined Effects of KNO_3, KH_2PO_4, and $MgSO_4$ on Photosynthetic $C^{14}O_2$ Fixation

The results are shown in Table VI. It is seen that the total fixation of C^{14} was markedly enhanced by the addition of KH_2PO_4, $MgSO_4$, and nutrient solution, while the addition of KNO_3 had little or no effect on the total fixation compared with that obtained in the distilled water control.

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[1] Toaspern, Holm-Hansen, and Moses, this report, p. 22.

[2] V. Moses, this report, p. 17.

*Composition of the nutrient solution is given by O. Holm-Hansen, P. Hayes, and P. Smith, in Chemistry Division Quarterly Report, UCRL-3595, Oct. 1956, p. 56.

Table IV

Effect of KNO_3 and NH_4Cl on total uptake and distribution of carbon-14 during photosynthesis in Chlorella.

1.0 ml of 1% Chlorella suspension; photosynthesis time in presence of C^{14} 2 min. Cells originally in distilled water and adapted on the shaker for 2 min. Cells originally in distilled water and adapted on the shaker for 10 min, at which time distilled water (as control), NH_4Cl solution (final concentration in algal suspension 0.002 M), and 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 was added.

	Cell-suspension Medium		
	Distilled water	0.002 M KNO_3	0.002 M NH_4Cl
Total fixation (cpm)	1,813,000	1,894,000	5,014,000
Total soluble radioactivity (cpm)	1,441,000	1,421,000	3,917,000
Total radioactivity (as % of soluble radioactivity			
Diphosphates	27.4	26.6	1.9
Uridine diphosphoglucose	3.1	1.7	0.9
Hexose monophosphates	20.3	15.5	2.3
Phosphoglyceric acid	4.1	6.0	1.0
Glucose-cyclic phosphate	3.4	1.8	1.0
Aspartic acid	3.6	5.8	5.2
Serine-glycine	4.6	6.8	15.0
Alanine	1.7	3.6	26.7
Fumaric acid	1.4	1.9	1.5
Malic acid	6.6	6.5	5.8
Glycolic acid	8.2	9.7	4.4
Sucrose	9.6	7.1	22.1
Citrulline	--	--	3.0
Glutamine	--	--	2.6
Glutamic acid	--	--	4.3
UK 1, probably phosphates	4.1	5.4	--
UK 2, probably phosphates	2.0	1.6	--
UK 3, probably organic acid	--	--	2.4
Sugar phosphates (as % of total soluble fixation)	64.4	58.6	7.1
Amino acids (as % of total soluble fixation)	9.9	16.2	56.8

Table V

Effect of nutrient solution on total uptake and distribution of C^{14} during photosynthesis in Chlorella

1 ml of 1% Chlorella suspension, photosynthesis time in presence of C^{14} 2 min. After 10 min adaptation photosynthesis in distilled water, distilled water (as control) and nutrient solution (final concentration in algal suspension about 0.03 M) were pipetted into separate flasks, and again after 10 min adaptation photosynthesis, the radioactive bicarbonate was pipetted into these algal suspensions.

	Cell-suspension medium	
	Distilled water	Nutrient solution
Total fixation (cpm)	3,420,000	4,746,000
Total soluble radioactivity (cpm)	3,132,000	3,900,000
Radioactivity (as % of total soluble radioactivity)		
Diphosphates	4.2	3.3
Uridine diphosphoglucose	1.9	3.5
Hexose monophosphates	17.8	35.6
Phosphoglyceric acid	5.9	4.3
Glucose-cyclic phosphate	1.3	2.0
Sucrose	22.9	19.8
Alanine	10.1	15.1
Aspartic acid	2.1	5.0
Serine/glycine	4.9	2.7
Citrulline	---	0.01
Glutamic acid	---	0.07
Malic acid	8.7	---
Glycolic acid	0.6	0.85
UK 1, probably amino acid	5.3	0.88
UK 2, probably organic acid	3.6	2.3
UK 3, probably organic acid	2.7	---
UK 4, probably amino acid	4.2	2.8
UK 5, probably sugar phosphate	1.6	0.95
UK 6, probably sugar phosphate	1.9	
Sugar phosphates (as % of total soluble radioactivity)	34.6	52.5
Amino acids (as % of total soluble radioactivity)	26.6	23.7

Table VI

Effects of KNO_3, KH_2PO_4, $MgSO_4$ on total uptake and distribution of C^{14} during photosynthesis in Chlorella

1.0 ml of 1% Chlorella suspension; photosynthesis time in presence of C^{14} 2 min. After 10 min adaptation photosynthesis in distilled water, distilled water (as control), KNO_3 solution (final concentration in algal suspension 0.012 M), KH_2PO_4 solution (final concentration 0.008 M), KH_2PO_4 plus KNO_3 solutions (final concentration 0.02 M), and $MgSO_4$ solution (final concentration 0.01 M), which are all components of nutrient solution, and nutrient solution (final concentration, 0.03 M) were pipetted into separate flasks. After 10 min adaptation, the radioactive bicarbonate was pipetted into these algal suspensions.

	Cell-suspension medium					
	Distilled water	0.012 M KNO_3	0.008 M KH_2PO_4	KNO_3 + 0.008 M KH_2PO_4	0.01 M $MgSO_4$	Nutrient solution*
Total fixation (cpm)	1,391,250	1,542,000	2,000,000	2,206,000	2,064,000	2,019,000
Total soluble radioactivity (cpm)	1,100,000	1,220,000	1,700,000	1,899,000	1,770,000	1,650,000
Total radioactivity (as % 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	4.1	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 % of total soluble radioactivity)	77.6	66.0	82.2	73.5	71.4	65.8
Amino acid (as % of total soluble radioactivity)	5.4	12.8	3.9	9.1	9.7	11.5

*The salts present on this chromatogram interfered with the separation of the sugar phosphates; hence the value here is probably lower than if the good separation had been achieved.

The amount of radioactivity incorporated into the sugar phosphates was markedly increased by the addition of KH_2PO_4. The amount of radioactivity incorporated into the amino acids was also sharply increased; the increase was most pronounced with the addition of KNO_3.

D. Effect of NH_4Cl, KH_2PO_4, and Nutrient Solution on Dark $C^{14}O_2$ Fixation

The data (Table VII) show that in no case was the dark fixation of $C^{14}O_2$ enhanced by the addition of salts; on the contrary, on the addition of NH_4Cl, either by itself or in combination with KH_2PO_4, the apparent fixation of $C^{14}O_2$ was decreased by about 26%.

E. Effect of NH_4Cl, KH_2PO_4, $MgSO_4$, and Nutrient Solution on Respiration

Since it was thought that the apparent decrease in dark CO_2 fixation by Chlorella brought about by NH_4Cl might be related to a change in the respiration level,[3] the actual effect of NH_4Cl, KH_2PO_4, $MgSO_4$, and nutrient solution on the respiration rate was measured in a Warburg respirometer. It was found that the addition of NH_4Cl increased the rate of oxygen uptake to twice that of the control (distilled water), but none of the other salts had any effect on the rate of respiration. It is thus possible that the decrease in the rate of dark fixation of $C^{14}O_2$ in Chlorella is caused by an increase in the internal concentration of $C^{12}O_2$ brought about by an increased rate of respiration.

The above results indicate that addition of mineral salts to a suspension of Chlorella in distilled water may have striking effects, both on the total rate of uptake of radioactive CO_2, and also on the distribution pattern of the labeled carbon. Further experiments on this problem are in progress.

[3] P. J. Syrett, Ann. Botany (London) 17, 1 (1953).

Table VII

Effect of NH_4Cl, KH_2PO_4 and nutrient solution on total uptake during dark $C^{14}O_2$ fixation in Chlorella

100 ml Erlenmeyer flasks, made lighttight by black tape and containing 5 ml of 1% Chlorella, were kept in a water bath in the darkroom and all flasks were flushed with air containing 1% CO_2. After dark adaptation of 10 min, distilled water (as control), NH_4Cl solution (final concentration in algal suspension 0.002 M), KH_2PO_4 solution (final concentration 0.008 M), NH_4Cl solution plus KH_2PO_4 solution (final concentration 0.01 M) and nutrient solution (final concentration about 0.03 M) were pipetted into separate flasks. After 24 min dark adaptation, 250 λ of radioactive bicarbonate was added and 3 min of dark fixation was permitted.

	Cell-suspension medium				
	Distilled water	0.02 M NH_4Cl	0.008 M KH_2PO_4	0.001 M NH_4Cl + 0.08 M KH_2PO_4	Nutrient solution
Total fixation (cpm)	30,800	22,600	30,800	22,200	28,400

SYNTHETIC AND BIOSYNTHETIC STUDIES ON MORPHINE

Melvin Look and Henry Rapoport

The scheme in which morphine is demethylated and then resynthesized[1] is shown to be adequate in removing completely the N-methyl group of morphine. Thus when 82 mg of morphine-N-methyl-C^{14} (0.054 μC/mg) was degraded and resynthesized, morphine containing no radioactivity was isolated as the product. The final scheme is outlined in Fig. 9. The scheme makes it possible to study the rate of formation of the N-methyl group morphine from poppies grown under radioactive carbon dioxide. The conversion of biosynthetic, randomly C^{14}-labeled morphine to morphine-C^{14}-N-methyl-C^{12} for drug-addiction studies is now possible.[2] Papaver somniferum (opium poppy) is being grown under a controlled radioactive carbon dioxide atmosphere. The description of the plant chamber and controls is given in another report.[3]

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[1] M. Look and H. Rapoport, in Chemistry Division Quarterly Report, UCRL-3836, June 1957, p. 19.

[2] ibid. UCRL-3415, May, 1956, p. 4.

[3] D. Baker and H. Rapoport, Biosynthesis of Morphine, in this report, p. 35.

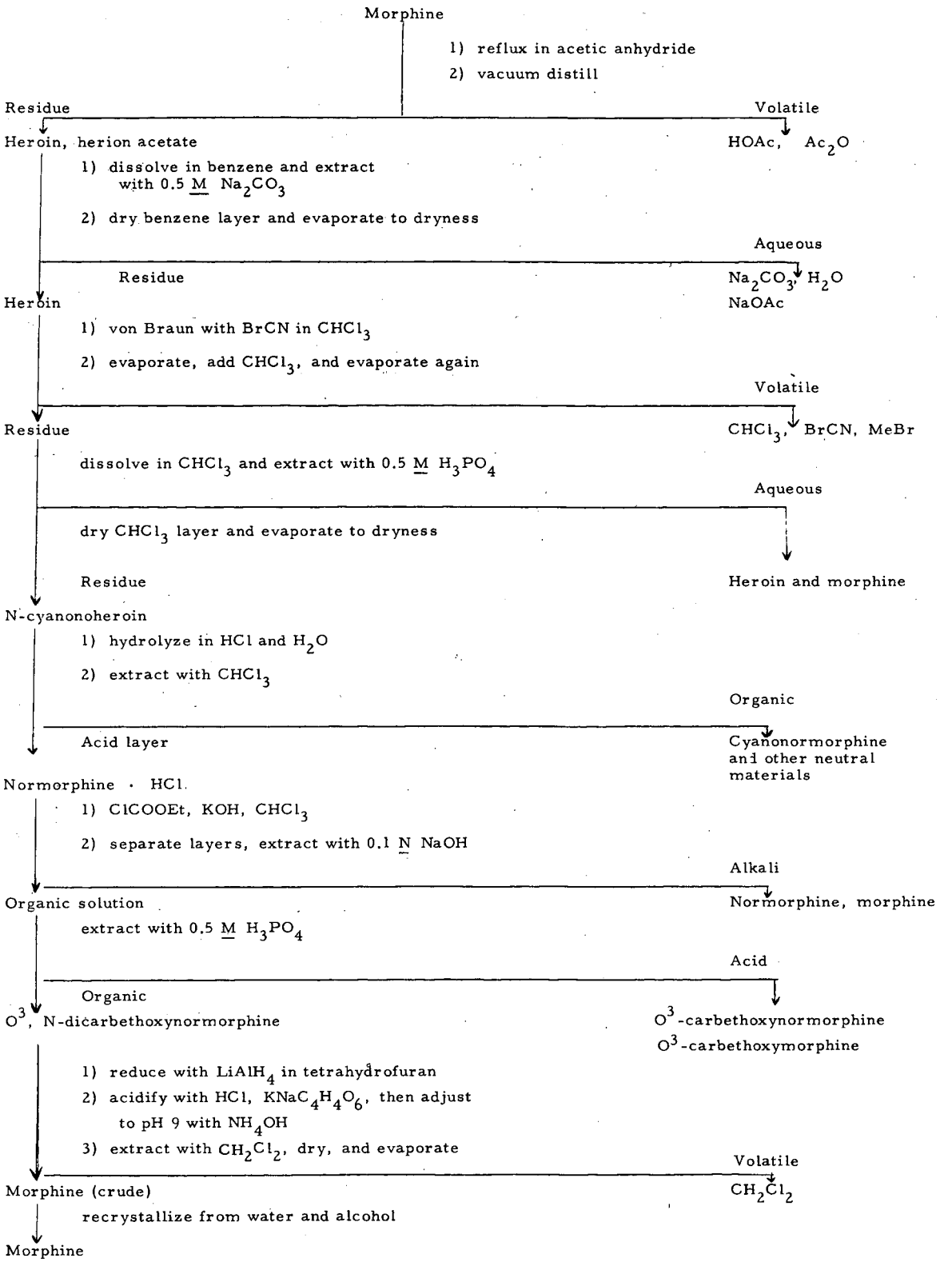

Fig. 9. Scheme in which morphine is demethylated.

BIOSYNTHESIS OF MORPHINE

Don R. Baker and Henry Rapoport

In the study of the biosynthesis of morphine by Papaver somniferum with radioactive carbon dioxide, it is necessary to raise the plants in a well-sealed container in order to eliminate the danger of escape of the gas. Such a plant chamber has been constructed of lucite in order to permit artificial lighting from outside the chamber. The chamber measures approximately 30 × 30 × 30 inches and is capable of containing nine pots at one time.

Illumination is furnished by two banks of nine 48-inch 40-watt fluorescent lights placed at opposite sides of the chamber. A bank of four fluorescent lights is suspended over the chamber. These lights give an intensity of approximately 600 foot candles at the center of the chamber. The artificial day length is controlled by a clock relay.

The humidity in the chamber is controlled by means of a stainless steel coil through which cold water is circulated. The water is cooled in a low-temperature bath by refrigeration. Temperatures as low as 4°C may be maintained by means of this cold water bath.

The room in which the plant chamber is located is insulated and its temperature is controlled by means of thermostatically regulated heating and refrigeration units. Circulation of the air around the box during the hours while the lights are on is maintained by a 12-inch electric fan. The temperature in the room is continuously recorded on a 3-day circular recorder. Pressure changes in the chamber due to temperature and barometric pressure are minimized by an expansion bag made of polyvinyl chloride.

The plants can receive water and nutrient solution by means of a system of watering lines inside the chamber which are connected to the source of the solution outside the chamber. Also it is possible to allow the condensate from the dehumidifying coils inside the chamber to flow back into the pots.

The concentration of the carbon dioxide in the chamber may be determined spectrophotometrically by means of the carbon dioxide's strong absorption at 4.3 microns.

Plants have been successfully grown in this chamber for periods of more than 2 months.

HUMAN METABOLISM OF MORPHINE-N-METHYL-C^{14}

Henry Rapoport, Mohindra Chadha,[1] and Melvin Look

The urine fractions[2] from the volunteers who had been injected with morphine-N-methyl-C^{14} were fractionated further. The fractions of interest are the ones containing the morphine (VII and H-VIII). Fraction VII was purified by ion exchange on Dowex 50×1 (cation exchanger), Dowex 1×1 (anion exchanger), and Dowex 50×1 Na^+.

The product isolated was morphine, but after subsequent purification, pseudomorphine (2, 2'-dimorphine) was isolated. The pseudomorphine must have formed after the ion-exchange sequence, as blank runs with morphine, tracing back to before the ion-exchange steps, did not convert morphine to pseudomorphine. Morphine added to the mother liquor of Fraction VII, however, was converted to pseudomorphine. A catalyst in Fraction VII is postulated, and research is now directed towards its isolation and characterization. It is not possible to say now if the catalyst is a normal metabolite, a morphine-induced metabolite, or an abnormal metabolite from one or more volunteers. The catalyst may be active only after interfering substances have been removed during the latter part of the scheme. Fraction H-VII is showing the same behavior. Preliminary work indicates that the pseudomorphine isolated has only 50 to 75% of the original morphine specific activity. Transmethylation in the metabolism of morphine is significant.

Fractions VIII and H-VIII are being further purified in order to detect normorphine and hence obtain proof of demethylation in the metabolism of morphine.

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[1] Department of Chemistry, University of California; present address: New Delhi, India.

[2] H. Rapoport, M. Chadha, and M. Look, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 70.

UTILIZATION OF ADENINE AND 5'-ADENYLIC ACID BY C_{57} MICE

Edward L. Bennett and Hilda Karlsson

Numerous investigations of the utilization of purines, especially adenine, by mice and rats have been reported by us[1] and by others.[2] Previously we have studied the extent of incorporation of adenine into nucleotides and nucleic acids by a number of tissues of C_{57} mice at numerous times ranging from $\frac{1}{2}$ hour to 29 days after administration of adenine-C^{14}. It has been shown that adenine is rapidly and extensively converted into soluble nucleotides, principally derivatives of 5'-adenylic acid, and in addition adenine is incorporated into the adenine and guanine of both RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) of the various tissues of the mouse. The pattern and magnitude of incorporation varies greatly from tissue to tissue. Evidence has been presented that 5'-AMP (5'-adenylic acid) may be a direct precursor of nucleic acids in the mouse. This evidence was primarily the close correspondence of the specific activity of RNA adenine and 5'-AMP adenine in both the small and large intestine from 2 to 29 days after administration of adenine-C^{14}.[1] Furthermore, in most tissues studied, a close relationship was found between the half lives of 5'-AMP and of RNA adenine.

Relatively few experiments have been reported in which adenine nucleotides were used as possible precursors of nucleotides and nucleic acids in mammals. Weinfeld et al. studied the incorporation in the rat of the 2'-, 3', and 5'-adenylic acids and 3'-guanylic acid after repeated subcutaneous injection at the level of 0.2 mmole/kg.[3] The incorporation of the nucleotides into the RNA and DNA adenine and guanine from the pooled internal organs and the incorporation into the ADP and ATP from the hind leg and back muscle were determined. Their results, expressed as relative specific activity (RSA),[4] are summarized in a table in

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[1] E. L. Bennett and B. J. Krueckel, Biochim. et Biophys. Acta 17, 503 (1955); 17, 515 (1955).

[2] G. B. Brown and P. M. Roll, Biosynthesis of Nucleic Acids, in The Nucleic Acids, E. Chargaff and N. Davidson, Eds. (Academic Press, New York, 1955).

[3] H. Weinfeld, P. M. Roll, and G. B. Brown, J. Biol. Chem. 213, 532 (1955).

[4] The relative specific activity (RSA) is expressed as

$$\frac{\text{Specific activity in tissue fraction} \times 100}{\text{Specific activity of injected compound}}$$

footnote.[5] The 2'- and 3'-adenylic acids were twice as effective as 5'-adenylic acid as precursors for RNA adenine and approximately $1\frac{1}{2}$ times as effective for RNA guanine. The amount of 5'-AMP incorporated into DNA was insufficient to detect. The RSA of the RNA adenine administration of 5'-AMP was approximately 1/10 of that obtained after administering adenine.

In the published data the utilization of nucleotides has been expressed in terms of the ratio

$$\frac{\text{specific activity of the nucleic acid purine}}{\text{specific activity of the injected nucleotide}}$$

There are no published data on the ratio

$$\frac{\text{specific activity of the nucleic acid purine}}{\text{specific activity of the nucleotide purine}}$$

obtained following labeled-nucleotide administration. Therefore, even if nucleotides within a tissue are true precursors of nucleic acid purines, low incorporation of labeled nucleotide administered may occur owing solely to elimination of a large amount of the administered nucleotide, particularly 5'-AMP, by intestinal or muscle deaminases and phosphatases before it can enter the tissue. Moreover, the distribution of an administered nucleotide among tissues may be different from the distribution of an administered purine. Extensive utilization of a nucleotide in one tissue may be masked by a relatively low utilization in another tissue when pooled organs are analyzed.

[5] Incorporation in the rat of adenine nucleotides (results expressed as RSA)

Tissue fraction		Compound injected			
		3'-adenylic acid	2'-adenylic acid	5'-adenylic acid	3'-guanylic acid
RNA	Adenine	1.89	1.89	1.01	0.09
	Guanine	0.64	0.69	0.42	1.04
DNA	Adenine	0.16	0.16	0	0.01
	Guanine	0.05	0.07	0	0.15
ADP		0.27	0.58		
ATP		0.30		0.06	0.01

We have administered 5'-AMP-8-C^{14} (a single injection) to C_{57} mice and have isolated the nucleotide 5'-AMP, RNA adenine, RNA guanine, DNA adenine, and DNA guanine of five tissues. The 5'-AMP-8-C^{14} was obtained from the pooled intestines, livers, and kidneys of two C_{57} mice that had been injected 2 hours previously with a total of 2.4 mg of adenine-8-C^{14} (25 μC).[6] The soluble nucleotides were extracted from the homogenized tissues with cold 10% trichloracetic acid, which was subsequently removed by continuous ether extraction. The adenylic acid derivatives were hydrolyzed to 5'-AMP, which was isolated and purified by chromatography on a Dowex-2 column in the formate form using 0.1 F formic acid for elution. The final yield was approximately 3.6 mg of 5'-AMP-8-C^{14} (≈1.4 mg adenine), specific activity 1860 dpm/μg adenine. The purity of the 5'-AMP was determined by two-dimensional paper chromatography on Whatman No. 1 filter paper using butanol–propionic acid–water followed by propanol–ammonium hydroxide–water as the developing solvents. The chromatogram was placed in contact with x-ray film for 4 months. The major spot was 5'-AMP. The main impurity was probably hypoxanthine, and it amounted to less than 1% of the total activity. Three other unidentified impurities were present; each contained not more than 0.5% of the 5'-AMP activity. A quantitative estimation of the 5'-AMP with muscle deaminase indicated that 90 to 95% of the uv absorption at 265 mμ was due to 5'-AMP.

The 5'-AMP-8-C^{14} dissolved in 0.9 ml of 0.9% NaCl (0.01 mmole, 0.4 mmole/kg, 2.5×10^6 dpm total activity, 1860 dpm/μg adenine) was injected intraperitoneally into a 4- to 6-month old C_{57} male mouse. The mouse was sacrificed 24 hours later and the soluble nucleotides, RNA adenine, RNA guanine, DNA adenine, and DNA guanine were isolated from the small intestine, large intestine, liver, kidney, and skinned carcass (including bone) by our standard procedures.[7] The specific activities of the 5'-AMP and of guanine were determined as previously described. The method for the determination of adenine specific activity has been modified. Adenase (isolated from Azotobacter vinelandii) and xanthine oxidase were used to estimate adenine; the estimation is based upon the oxidation of adenine to uric acid, with a resultant large increase in optical density at 292 mμ. This rapid and convenient method is more accurate than the previous method, which used only xanthine oxidase.

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[6] Miss Madeline Madsen, who participated in the Radiation Laboratory High School Teacher program during the summer of 1956, prepared the 5'-AMP used in this study.

[7] Mr. Ervin McDonald, who participated in the High School Teachers' program during the summer of 1957, performed the isolation and purification of the tissue fractions.

The data obtained from this experiment are summarized in Tables VIII and IX. In Table VIII are summarized the percentage of the injected 5'-AMP found in each tissue fraction, the specific activity, and the RSA of the 5'-AMP, RNA adenine, RNA guanine, DNA adenine, and DNA guanine. Comparable values obtained after adenine administration are also tabulated. These values are the averaged values from four to six previously reported experiments.

The utilization of 5'-AMP for nucleotides and RNA (expressed as % of the administered compound found in a tissue fraction) varies from 1/3 of to equal that of adenine (with the exception of the nucleotide fraction of the carcass). In general 5'-AMP utilization is somewhat less than adenine utilization for DNA formation. The utilization in liver of 5'-AMP is equal to or greater than the utilization of adenine given at the same dosage. A comparison of the RSA of the 5'-AMP and of RNA adenine and guanine after 5'-AMP administration and after adenine administration also indicated that 5'-AMP is utilized more than adenine in the liver and utilized about 1/3 to 1/2 as well as adenine in other tissues examined.

In Table IX, other ratios obtainable from the data are summarized. If 5'-AMP is the principal precursor of RNA in tissues, the RNA-adenine/5'-AMP specific activity ratio after administration of the two compounds should be an important and significant ratio to compare. For liver and carcass, this ratio is approximately the same after 5'-AMP is administered as after adenine is administered, whereas in kidney and small intestine this ratio is approximately 1/2 as large after 5'-AMP administration as after adenine administration. The ratio of RNA adenine to RNA guanine after 5'-AMP administration is approximately 1/2 of that obtained after adenine administration. These ratios may indicate that some adenine goes directly into nucleic acids, while additional adenine goes first into 5'-AMP and subsequently into nucleic acids. Other data we have obtained also suggest this method of incorporation.

The amount of 5'-AMP incorporated into DNA is very small, and no great significance can be attached to the data obtained so far. It appears, however, that the utilization of 5'-AMP for DNA formation is relatively small, as shown by a comparison of the RSA found in the DNA after 5'-AMP administration with that found after adenine administration, as well as by a similar comparison of the RNA-adenine/DNA-adenine ratios. These ratios are larger after 5'-AMP administration than after adenine administration.

This experiment is only preliminary, but it appears to offer good evidence that 5'-AMP is significantly utilized in the mouse for formation of nucleotide and nucleic acid. Further experiments are planned in which animals will be sacrificed at various times after administration of 5'-AMP.

Table VIII

Percentage incorporation, specific activity, and relative specific activity of

Tissue	Administered	% Incorp. cold TCA fraction	SA 5'-AMP	RSA 5'-AMP	% incorp. RNA fraction	SA RNA adenine
Liver	5'-AMP	4.7	219	11.5	1.7	58
	Adenine	4.6	1120	6.6	1.4	374
Small intenstine	5'-AMP	1.2	81	4.2	0.6	19
	Adenine	2.8	1410	8.3	2.1	715
Large intestine	5'-AMP	0.36	52	2.7	0.15	13
	Adenine	0.63	1190	7.0	0.38	920
Kidney	5'-AMP	0.54	77	4.1	0.16	26
	Adenine	1.2	990	5.8	0.22	490
Carcass	5'-AMP	0.9	7.1	0.38	0.4	8.6
	Adenine	7.8	204	1.2	0.9	204

*3.4 mg of 5'-AMP-8-C^{14} (equivalent to 1.3 mg of adenine) was injected into a 25-g C_{57} male mouse. The mouse was sacrificed 24 hours later. The specific activity of the 5'-AMP was 1860 dpm/μg adenine. All specific activities (SA) are expressed in terms of dpm/μg equivalent of adenine. The data for adenine administration are averaged data obtained from four to six

Table VIII

several tissues of C_{57} mice after administration of 5'-AMP-8-C^{14} or adenine-C^{14}*

RSA RNA Adenine	SA RNA Guanine	RSA RNA Guanine	% Incorp DNA Fraction	SA DNA Adenine	RSA DNA Adenine	SA DNA Guanine	RSA DNA Guanine
3.1	15.8	0.85	~ 0.04	3.8	0.2	~ 2	0.1
2.2	51	0.30	0.02	11	0.064	2.2	0.013
1.0	6.4	0.34	0.2	5.3	0.28	2.9	0.16
4.2	97	0.57	0.9	375	2.2	61	0.36
0.69	4.6	0.25	~ 0.03	2.6	0.14	~ 1	~ 0.05
5.4	165	1.0	0.14	365	2.1	61	0.36
1.4	10.4	0.56	< 0.01	--	--	~ 1	~ 0.05
2.9	109	0.64	< 0.005	8.8	0.05	3.7	0.022
0.46	3.0	0.16	0.03	0.08	0.04	~ 0.8	0.04
1.2	44	0.26	0.7	148	0.87	29	0.17

experiments in which adenine-C^{14} was administered at the rate of approximately 1.3 mg/25 g mouse. The mice were sacrificed 24 hours later. The specific activity of the adenine was 17,000 dpm/μg adenine. RSA is expressed as $100 \frac{\text{dpm/μg equivalent adenine in the isolated fraction}}{\text{dpm/μg equivalent adenine in the injected compound}}$.

Table IX

Ratios of specific activities of several tissue fractions after administration of 5'-AMP-8-C^{14} and adenine-C^{14} to C_{57} mice*

Tissue	Administered	RNA adenine / 5'-AMP	DNA adenine / 5'-AMP	RNA adenine / RNA guanine	RNA adenine / DNA adenine
Liver	5'-AMP	0.26	0.017	3.7	15
	Adenine	0.33	0.01	7.3	34
Small intestine	5'-AMP	0.23	0.065	3.0	3.6
	Adenine	0.51	0.27	7.4	7.4
Large intestine	5'-AMP	0.25	0.05	2.8	5.0
	Adenine	0.77	0.31	5.6	2.5
Kidney	5'-AMP	0.33	--	2.5	--
	Adenine	0.50	0.11	4.5	56
Carcass	5'-AMP	1.2	0.11	2.9	11
	Adenine	1.0	0.72	4.6	1.4

*These data have been calculated from the specific activity values given in Table VIII. See Table VIII for experimental details.

INCORPORATION OF ADENINE-C^{14} INTO NUCLEOTIDES AND NUCLEIC ACIDS IN RATS

Edward L. Bennett and Hilda M. Karlsson

The effect of the dose of adenine upon its distribution in the rat is being studied. The data obtained from previous experiments with rats have indicated that the incorporation of adenine into the deoxynucleic acid (DNA) of the thymus was significantly less than expected in comparison with incorporation into other tissues such as liver and small intestine and with corresponding data obtained from experiments using P^{32}-phosphate. In the experiment presented here, adenine has been administered to rats at three dosage levels, 0.065, 0.23, and 0.41 mmoles/kg and the relative distribution and specific activity in eight tissues have been determined.

The distribution of the radioactivity, expressed as a percentage of the injected adenine-8-C^{14}, in the acid-soluble fraction, RNA (ribonucleic acid) fraction, and DNA fraction was summarized in a previous Quarterly Report.[1] The data indicated that the percentage of the injected adenine in the nucleotide and nucleic acid fractions usually remained approximately the same or decreased with increasing dosage in the liver, small intestine, and muscle, and decreased markedly in the kidney. The percentage of the adenine found in the acid-soluble fraction, RNA fraction, and DNA fraction of the bone marrow, thymus, spleen, and brain increased markedly with an increase in administered adenine from 0.065 mmole/kg to 0.23 mmole/kg, and slightly with a further increase in administered adenine to 0.41 mmole/kg.

The specific activity of each tissue fraction has been determined. In Table X the results are expressed in terms of RSA

(relative specific activity =

$$\frac{100 \times \text{specific activity of purine in tissue fraction}}{\text{specific activity of injected adenine}}) .$$

The ratios of the RSA of the tissue fractions at the two lower dosage levels to the highest dosage level have been calculated. In Table XI the data are summarized for each tissue fraction arranged in rank order of this ratio. At the lowest dosage employed, brain, thymus, and bone incorporated significantly less adenine than would be expected if the distribution in each tissue fraction were independent of dosage. In addition, spleen incorporated less adenine into DNA than would be expected. Muscle incorporated markedly more adenine than would be expected at the lower dosage. Perhaps better stated conversely, the incorporation into muscle increased only slightly with dosage. Deviations from the expected incorporation were not as marked at two higher dosages. Brain incorporated less than a proportional amount at the 0.23-mmole/kg level, and muscle incorporated more than the proportional amount. The deviations from the expected ratio for other tissues did not show a consistent pattern. It should be emphasized that these results are based upon a single animal at each dosage, and further work must be done to confirm the validity of small deviations from the expected value.

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[1] Edward L. Bennett and Hilda Karlsson, in Chemistry Division Quarterly Report, UCRL-3836, June 1957, p. 33.

Table X

Relative specific activities of 5'-AMP, RNA adenine, RNA guanine, DNA adenine, and DNA guanine after administration of adenine-8-C^{14} [a]

Tissue	Dosage (mmole/kg)	Relative specific activity				
		5'-AMP	RNA adenine	RNA guanine	DNA adenine	DNA guanine
Liver	0.065	0.61 ?	0.47	0.16	0.03	< 0.01
	0.23	7.7	2.1	0.31	0.13	< 0.04
	0.41	13.0	4.2	1.0	0.17	< 0.04
Small intestine	0.065	2.4	1.1	0.15	0.50	0.081
	0.23	5.0	4.2	0.90	2.3	0.63
	0.41	11.0	7.6	2.1	2.7	0.91
Kidney	0.065	4.6	2.0	0.27	0.022	0.01
	0.23	8.7	3.5	0.81	0.16	0.086
	0.41	13.7	6.3	2.5	0.19	0.12
Muscle	0.065	0.35	0.21	0.091	0.044	0.013
	0.23	0.63	0.44	0.31	0.13	0.056
	0.41	0.73	0.48	0.26	0.16	0.066
Bone	0.065	0.56	0.26	0.039	0.077	0.013
	0.23	3.2	2.6	0.63	1.5	0.14
	0.41	4.9	4.4	1.12	3.0	0.86
Thymus	0.065	0.50	0.23	0.053	0.041	0.025
	0.23	4.0	3.1	0.68	1.0	0.22
	0.41	8.5	4.6	1.22	1.5	0.52
Spleen	0.065	1.56	0.66	0.11	0.055	0.012
	0.23	6.3	3.3	0.85	0.53	0.16
	0.41	9.5	4.6	1.25	0.95	0.38
Brain	0.065	0.06	0.02	0.017	~ 0.002	< 0.01
	0.23	1.1	0.26	0.15	~ 0.007	< 0.03
	0.41	3.3	0.76	0.39	~ 0.016	< 0.06

[a] Three female Long-Evans rats, weight 155, 165, and 200 g, age 2 months, were intraperitoneally administered 1.35, 5.05, and 11.1 mg of adenine containing adenine-8-C^{14} with 3.0, 2.6, and 2.7×10^7 dpm. The specific activity of the injected adenine was 22,200, 5150, and 2430 dpm/μg adenine.

The relative specific activity =

$$= 100 \frac{\text{specific activity of purine in tissue fraction}}{\text{specific activity of injected adenine}}$$

All specific activities have been calculated on the basis of μg equivalent of adenine.

Table XI

Rank-order comparison of ratios of relative specific activity at low adenine dosage to RSA at high adenine dosage

$$\text{RSA Ratio} = 100 \times \frac{\text{Relative specific activity obtained at an adenine dosage of 0.065 mmole/kg}}{\text{Relative specific activity obtained at an adenine dosage of 0.41 mmole/kg}}$$ [a]

5'-Adenylic Acid		RNA Adenine		RNA Guanine		DNA Adenine		DNA Guanine	
Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio
Brain	1.8	Brain	2.5	Bone	3.5	Bone	2.6	Bone	1.5
Liver	4.7 ?	Thymus	5	Thymus	4.3	Thymus	2.7	Spleen	3.1
Thymus	6	Bone	6	Brain	4.4	Spleen	5.8	Thymus	4.8
Bone	11	Liver	11	Sm. Int.	7.5			Kidney	8.3
				Spleen	8.8	Kidney	11.6	Sm. Int.	8.9
Spleen	16	Spleen	14			Brain	12		
Sm. Int.	22	Sm. Int.	15	Kidney	12	Liver	18		
				Liver	16	Sm. Int.	18.5		
Kidney	34	Kidney	32						
Muscle	48	Muscle	43	Muscle	35	Muscle	28	Muscle	20

$$\text{RSA Ratio} = 100 \times \frac{\text{Relative specific activity obtained at an adenine dosage of 0.23 mmole/kg}}{\text{Relative specific activity obtained at an adenine dosage of 0.41 mmole/kg}}$$ [b]

5'-Adenylic Acid		RNA Adenine		RNA Guanine		DNA Adenine		DNA Guanine	
Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio	Tissue	RSA ratio
Brain	33	Brain	34	Liver	30				
				Kidney	32	Brain	44	Thymus	42
Sm. Int.	45	Liver	50	Brain	38	Bone	51	Spleen	42
Thymus	47	Sm. Int.	55			Spleen	56	Bone	48
		Kidney	56	Sm. Int.	44				
Liver	59			Bone	56	Thymus	65	Sm. Int.	69
Kidney	63	Bone	60	Thymus	56	Liver	76	Kidney	71
Bone	65	Thymus	67			Kidney	83	Muscle	85
Spleen	66	Spleen	71	Spleen	68	Muscle	83		
Muscle	86	Muscle	91	Muscle	120	Sm. Int.	85		

[a] The expected ratio would be 12 on the basis of the absolute amount of adenine administered, and 16 on the basis of the amount per kg of rat.

[b] The expected ratio would be 45 on the basis of the absolute amount of adenine administered, and 55 on the basis of the amount per kg of rat.

Ratios of the specific activities of various nucleotide and nucleic acid fractions have been compared as a function of dosage. These results are summarized in Table XII. The RNA-adenine/RNA-guanine and DNA-adenine/DNA-guanine ratios showed a substantial decrease with increasing dosage in small intestine, kidney, bone, and spleen, and a questionable decrease in muscle. The RNA-adenine/DNA-adenine ratio showed a decrease with increasing dosage in kidney, muscle, bone, thymus, and spleen. The RNA-guanine/DNA-guanine ratio showed a corresponding decrease in all these tissues except thymus. No significant trends were noted for the RNA-adenine/5'-adenylic-acid ratio.

The results of this investigation indicate that the distribution and utilization of adenine and its interconversion to guanine are markedly affected by the dosage level of adenine. The RNA/DNA specific-activity ratio, often used as a measure of relative renewal of the two types of nucleic acids, is also markedly affected by dosage level.

One of the objectives of this experiment was to determine the effect of the level of adenine dosage upon its incorporation into spleen and thymus, to ascertain the optimal dosage conditions of adenine for x-irradiation experiments. The incorporation into spleen and thymus--organs of particular interest in irradiation experiments--is low. The determination of specific activity of the adenine incorporated into these tissues, particularly into the DNA purines, is difficult and not so accurate as desired. The results of this experiment indicate that it is desirable, when studying these tissues, to administer adenine at the dosage level of at least 0.2 to 0.4 mmole/kg, even if inactive adenine must be added to the radioactive adenine in order to minimize the amount of adenine-C^{14} required for the experiment. Thus higher specific activities will be obtained in the spleen, thymus, bone, and brain when a given amount of radioactive adenine with a total of 5 to 10 mg of adenine is administered to a 150- to 200-g rat than when the same absolute amount of radioactivity is administered with a smaller quantity of adenine.

Table XII

Ratios of specific activity of nucleotide and nucleic factions after adenine-8-C^{14} administration[a]

Tissue	Dosage mmole/kg	Specific activity ratio: RNA adenine / 5'-adenylic acid	RNA adenine / RNA guanine	RNA adenine / DNA adenine	DNA adenine / DNA guanine	RNA guanine / DNA guanine
Liver	0.065	0.76 ?	3.0	16	> 4.5	> 16
	0.23	0.27	6.7	16	> 3.4	> 8
	0.41	0.32	4.0	24	> 4.2	> 25
Small intestine	0.065	0.47	7.3	2.3	6.1	1.9
	0.23	0.83	4.6	1.8	3.6	1.4
	0.41	0.69	3.7	2.8	3.0	2.3
Kidney	0.065	0.44	7.5	93	2.0	27
	0.23	0.41	4.4	22	1.8	9.4
	0.41	0.45	2.5	33	1.6	5.1
Muscle	0.065	0.60	2.3	4.7	3.4	7.0
	0.23	0.70	1.4	3.4	2.3	5.5
	0.41	0.67	1.8	3.1	2.4	3.9
Bone	0.065	0.46	6.5	3.3	6.1	3.0
	0.23	0.81	4.1	1.7	3.7	1.5
	0.41	0.88	3.9	1.5	3.4	1.3
Thymus	0.065	0.45	4.2	5.5	1.6	2.1
	0.23	0.78	4.6	3.1	4.5	3.1
	0.41	0.55	3.8	3.0	2.9	2.3
Spleen	0.065	0.42	6.2	11.9	4.6	9.1
	0.23	0.52	3.8	6.2	3.3	5.3
	0.41	0.49	3.7	4.9	2.5	3.3
Brain	0.065	0.32	1.2	~ 8	--	--
	0.23	0.23	1.8	~38	--	--
	0.41	0.23	1.9	~46	--	--

[a] See Table X for experimental details.

GLUCOSE DISSIMILATION IN A FREE-CELL NEOPLASM. II

Karl K. Lonberg-Holm

Further studies have been made on glycolysis in suspensions of Ehrlich mouse ascites tumor cells (EMATC) with the aid of high-specific-activity glucose-C^{14} and $P^{32}O_4^{\equiv}$. These experiments have employed the "automatic rapid sampler"[2] for the killing of samples at 1.6-second or longer intervals, following the introduction of glucose substrate.

Radiocarbon Experiments

The results substantiate those reported previously[1] in most details. In the previous report, however, spot 3c (see map in previous report) was incorrectly assumed to contain only ribose phosphate, whereas it actually contained mainly phosphoglyceric acid (PGA). This error occurred because the phenol solvent tank used in the separations had been contaminated with acidic vapor so that spot 4b moved over to the area of spot 3c. The tanks have since been cleaned. Actually, ribose phosphate seems to be of low activity during the first 20 seconds following exposure to glucose-C^{14} (less than 3% of the total hexose phosphate activity). Therefore, the conclusion based on the appearance of "3c"—namely, that "3c" production depended upon hexose diphosphate production[1]—merely means that, as we know, PGA is made from hexose diphosphate.

By treating the phosphorylated compounds obtained from an experiment similar to the one previously reported[1] with phosphatase it has been shown that the identities of the labeled compounds in the other spots are:

Spot 1. 98% fructose diphosphate, at least during the first 14 seconds of exposure to C^{14}-labeled glucose. Traces of sedoheptulose, glyceric acid, and glucose are also found. (Sedoheptulose was identified by conversion to sedoheptulosan.)

Spot 3a. Mainly glucose-6-phosphate with a few percent sedoheptulose (identified as above). Also, when spot 4a is not resolved, it contains a few percent phosphogluconic acid.

Spot 3b. 60–70% fructose phosphate with some 20–30% mannose phosphate and 10–15% glucose phosphate.

Also, under normal conditions of phenol solvent separation:

Spot 4a. A mixture containing variable amounts of glucose and gluconic acid upon phosphatase treatment.

Spot 4b. PGA

Quantitative results of treating these spots with phosphatase will be reported at a later date.

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[1] Karl K. Lonberg-Holm, in Chemistry Division Quarterly Report, UCRL-3710, March 1957, p. 43.

[2] Karl K. Lonberg-Holm, in Chemistry Division Quarterly Report, UCRL-3415, June 1956, p. 36.

In the experiments described below, spots 4a, 3a, and 3b are counted and reported as "hexose phosphate." It must be kept in mind that under normal conditions of glucose exposure and during the first 25 seconds, these contain up to 80% glucose, 20% fructose, and 10% mannose, but also up to 2% sedoheptulose and 2.5% gluconic acid. There is no guarantee, however, that these limits will be maintained under conditions of anaerobiasis or methylene blue poisoning until such experiments have been treated with phosphatase.

Following these identifications an experiment was run under conditions similar to the ones previously reported (37°C) but without prior feeding of cold glucose. The results are shown in Fig. 10 and resemble those obtained when prefeeding of cold glucose is employed. Again we see that there is an initial transient in hexose monophosphates; then a slow increase in hexose diphosphate and PGA. These compounds fall off again with time, PGA first, as is confirmed in other experiments (but not shown in the previous report). This experiment serves for comparison for an experiment with P^{32} described later and also with an experiment shown in Fig. 11 in which cells were poisoned with methylene blue(MB)[3] before being given glucose. In MB poisoning as well as in anaerobiasis (Fig. 12) there is indication of a suppression of the initial high transient peak in hexose monophosphates, and also a slow buildup of compounds shown, indicating that the first step in the reaction sequence may be rate-limiting during the first 10 or 20 seconds.

The molar concentrations of these glycolytic intermediates within the cell may be calculated from the amount and specific activity of the glucose-C^{14} used and the cell volumes employed in each experiment. This calculation requires the assumption that the specific activity of the carbon in the sugar monophosphates is the same as that of the administered glucose carbon. Such an assumption follows from the results of the P^{32}-labeling experiment described later which shows that endogenous levels of glycolytic intermediates are very small compared with the levels resulting from administration of glucose. These concentrations are given in Table XIII for about 10 seconds' exposure to glucose. Experiments IX and XI may be compared with each other as can experiments XVI and XVIII; in the latter two there is some uncertainty in the exact specific activity of the glucose used and hence they can be only roughly compared with IX and XI. One must also bear in mind the variability of biological material.

Since each chromatogram represents 2 to 3 microliters of cells at the most, it is clear that only very small amounts of these compounds are involved in each determination, on the order of 0.05 to 5 millimicromole per spot.

Radiophosphorus Experiments

When EMATC suspensions are incubated with $P^{32}O_4^{\equiv}$ it is taken up into a variety of nucleotide compounds, and, if glucose is present, into sugar phosphates as well. To get a high enough specific activity of phosphate into the suspension, it was necessary to use a bicarbonate buffer ($LLCO_2I$) rather than the phosphate buffer heretofore employed ($NaSMPO_4$, Lockes[4]). The composition of $LLCO_2I$ is given as follows:

[3] E. Racker, Ann. N. Y. Acad. Sci. 63, Art. 5, 1017 (1956).

[4] R. W. McKee and A. J. Walker, Science 118, 133 (1953).

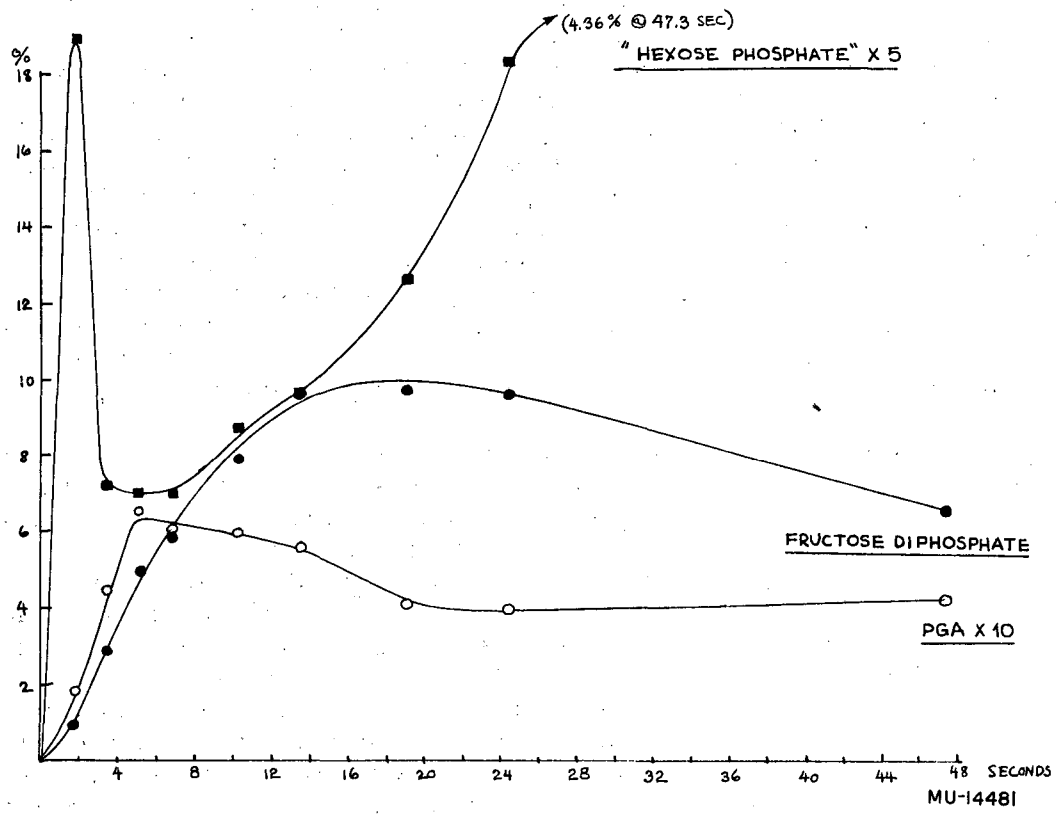

Fig. 10. Kinetics of appearance of some C^{14}-labeled compounds in EMATC following glucose-C^{14} exposure. Percentage total extractable C^{14} plotted against time following glucose addition. 113 microliters 2X washed cells incubated in $NaSMPO_4$ Lockes. After 7 minutes, 72 μC glucose (approx. 300 μg) added. Total volume about 1.1 ml. Temperature 37°C.

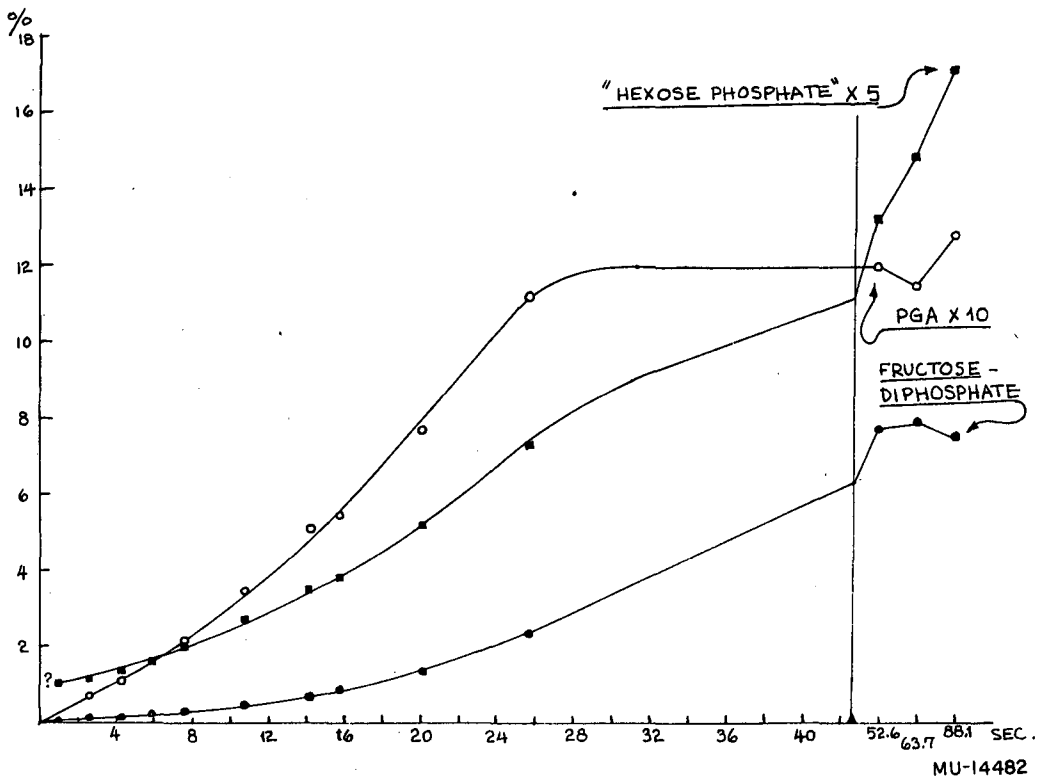

Fig. 11. Kinetics of appearance of some C^{14}-labeled compounds in EMATC following glucose-C^{14} exposure. Percentage of total extractable C^{14} plotted against time following glucose addition. 134 microliters of 2X washed cells in 1 cc $NaSMPO_4$ Lockes, containing 0.33 mM methylene blue hydrochloride (Eastman Kodak). After 5 minutes, 72 μC glucose (approx. 300 micrograms) added. Total volume about 1.1 ml. Temperature 37°C.

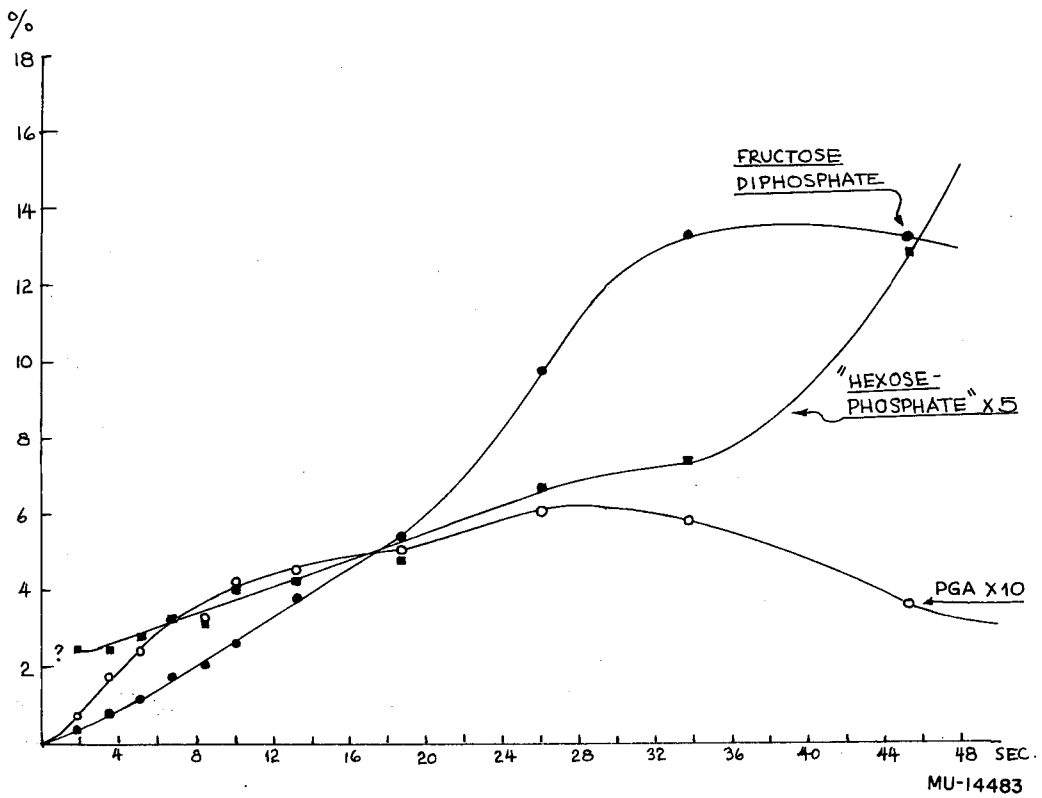

Fig. 12. Kinetics of appearance of some C^{14}-labeled compounds in EMATC following glucose-C^{14} exposure under nitrogen. Percentage of total extractable C^{14} plotted against time following glucose-C^{14} addition. 103 microliters of 2X washed cells in $NaSMPO_4$ Lockes, given 72 μg cold glucose; 3 minutes later 300 μg glucose (67 μC) administered. Total volume about 1.1 ml. Experiment run at 37°C under nitrogen flushing.

Table XIII

Approximate concentration of C^{14} compounds formed from glucose-C^{14} in about 10 seconds

Experiment	Concentration (millimoles per liter of cells)		
	Fructose diphosphate	Hexose phosphates	PGA[a]
IX (previous report). Cells preincubated with cold glucose. Sample at 9.9 seconds	1.9	0.70	0.13
XI(Fig. 12). Cells preincubated with cold glucose. Experiment under nitrogen. Sample at 10.0 seconds	0.42	0.12	0.14
XVI (Fig. 10). Cells given no cold glucose preincubation. Sample at 10.2 seconds	1.2	0.26	0.17
XVIII(Fig. 11). No cold glucose preincubation. Cells poisoned with MB. Sample at 10.8 seconds	0.062	0.068	0.088

[a]Assume three carbon atoms.

Salt	Concentration (mole/liter)
$NaHCO_3$	0.012
Na_2HPO_4	0.001
NaCl	0.132
KCl	0.0055
$CaCl_2$	0.001
$MgCl_2 \cdot 6H_2O$	0.0006

This is made up with glass--redistilled water in two solutions--one containing the sodium salts (which is gassed with 5% CO_2) and one containing the other cations. These are mixed before use so as to give the composition listed above. $LLCO_2I$ has the same pH and freezing point depression as has $NaSMPO_4$ Lockes, and similar cation concentrations, except that Ca^{++} and Mg^{++} have been increased to concentrations and to a ratio of concentrations that are closer to those of mammalian serum. This is possible because of their greater solubilities in bicarbonate buffer than in phosphate buffer.

Figure 13 shows approximate relative locations of spots obtained when the cell extracts are separated chromatographically on washed Whatman No. 4 filter paper for 40 hours with phenol-water followed by 25 hours with butanol–propionic acid–water solvent (200 ml per trough of two papers). The separation of these compounds is not always reproducible and the appearance of the chromatograms varies from experiment to experiment, or even from one chromatographic run to another with the same radioactive extract. The identifications of these spots as compiled from several experiments are given below:

Spot 1b. Fructose diphosphate plus a trace of contaminants.

Spot 1d. Mainly UTP, but could not be shown to be free of GTP. Not ITP.

Spot 1e. Unknown; not IPT; not UDPG.

Spot 2. PGA.

Spot 3a. Largely glucose phosphate.

Spot 3b. Fructose phosphate and some mannose phosphate.

Spot 3c. Ribose phosphate.

Spot 4a. Unknown, not ATP.

Spots 4b and 4c. These represent ATP, 4c being ADP caused by hydrolysis of ATP during second dimension of chromatographic separation. May have a trace of contaminant.

Spot 4d. ADP.

Spot 20. Unknown.

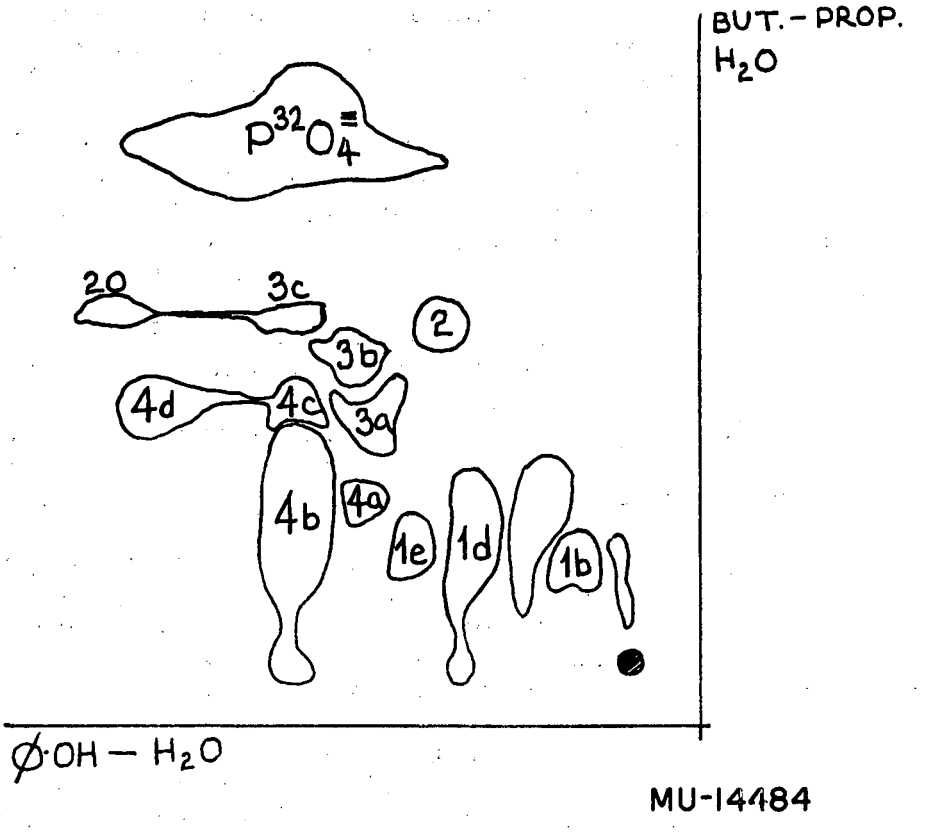

Fig. 13. Approximate relative locations of P^{32} spots near origin, obtained from chromatographic separation of $P^{32}O_4^{\equiv}$-exposed EMAT cells (glucose present).

The identification of these compounds is based upon (a) retention by acid-treated Darco G-60 as a criterion for nucleotides;[5] (b) electrophoretic separation in pH 3.4 propionate buffer for ATP and ADP; (c) co-chromatography with carrier standards in phenol-water, butanol–propionic acid–water, and propanol-ammonia-water,[6] done with all spots identified; and (d) C^{14}-sugar content following phosphatase treatment of a P^{32}-C^{14} doubly labeled run. It should be mentioned that without carrier the R_f of these spots was sometimes vastly altered, and their sharpness very much decreased, because the tracer amounts involved were exceedingly small. This caused much difficulty in identifying the spots.

An incubation of cells with lactate and $P^{32}O_4^{\equiv}$, followed by exposure to large amounts of glucose, produced the distribution of soluble P^{32} seen in Table XIV. In this experiment the identity of spots 1d and 1e was not confirmed but the activities are listed to give the activity distribution.

A kinetic experiment was then carried out. The conditions of incubation were close to those employed in glucose-C^{14} experiments, excepting that $LLCO_2I$ bicarbonate buffer was employed and the cells were kept under 5% CO_2. Figure 14 shows some of the results. The chromatograms showed good resolution, but in several ADP and PGA ran partly off the paper. Also, no attempt was made to identify either of the two spots that appeared in the region of 3c. The identity of other spots was confirmed by co-chromatography in three solvent systems (Method (c) above). As usual, the nucleotides showed considerable streaking, in contrast to the sugar phosphates, which were sharply resolved. Also about 2% of the radioactivity in spots 1d and 4b failed to move in the butanol–propionic acid–water dimension on the chromatograms.

As each chromatogram was counted, its radioactivity was related to a standard radiophosphorus spot on paper (one of the spots from this experiment) and could thus be corrected for decay. This also solved the problem of the contamination in unknown quantities of the P^{32} by P^{33}, which has a longer half life and also a much weaker radiation.

This experiment confirms the observations in glucose-C^{14} feeding experiments with respect to the general behavior of the sugar phosphates, including the transient peak of hexose monophosphates during the first 2 seconds of exposure. In addition, the P^{32} experiment shows the concomitant behavior of the adenosine polyphosphates, and in this respect confirms an earlier (unreported) experiment made without the use of the rapid sampler and employing larger amounts of glucose.

It should be mentioned that this method gives the upper limit of the ADP/ATP ratio. The in vivo concentrations of the ADP^{32} are, if anything, lower than those measured, owing to hydrolysis of ATP during the working up of the samples. To calculate such a ratio, we must make assumptions

[5] E. L. Bennett and B. J. Krueckel, Biochim. et Biophys. Acta 17, 515 (1955).

[6] E. L. Bennett, Biochim. et Biophys. Acta 11, 487 (1953).

Table XIV

Distribution of P^{32} following $P^{32}O_4^{\equiv}$ feeding of EMATC

Fraction	% total	% extractable (as counted from chromatograms)
Nonextractable	6	--
Extractable in 80% and 20% ethanol	94	--
Spot 1b Fructose diphosphate and trace of something else	--	0.7
Spot "1d" Probably largely UPT	--	3.8
Spot "1e" Unknown	--	1.1
Spot 3a Mainly glucose phosphate	--	2.2
Spot 3b Mainly fructose phosphate and probably some mannose phosphate	--	1.3
Spot 3c Ribose phosphate	--	0.4
Spots 4b and 4c Mainly ATP	--	13.9
Spot 4d ADP	--	1.5
Spot 20 Unknown	--	0.2
Orthophosphate	--	74.5

52 microliters of cells in $LLCO_2I$ at 37°C given 7.5 μM lactate and 60 μC of $P^{32}O_4$. After 15 minutes incubation, cells given 27,000 μg glucose. Total volume of the suspension 700 microliters. This cell suspension killed after 5 minutes glycolysis with hot 80% ethanol. Not all spots listed.

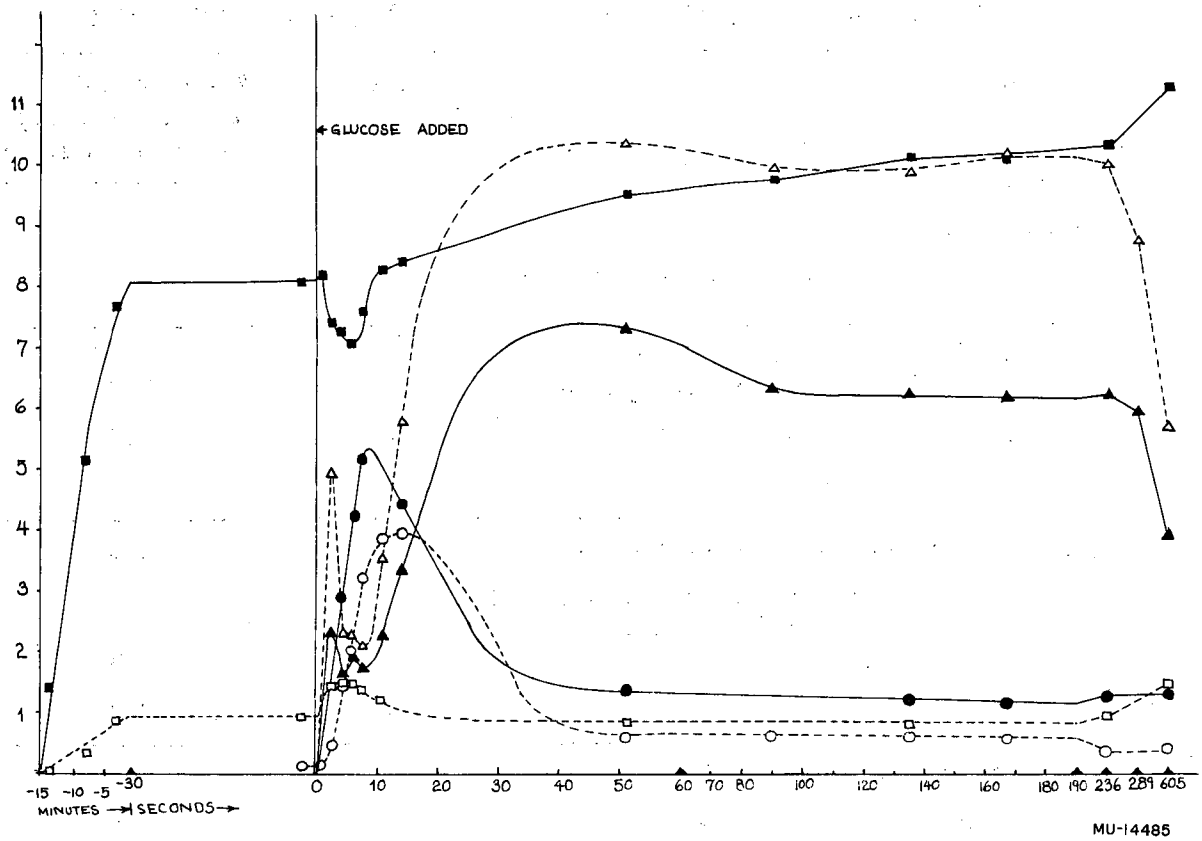

Fig. 14. Kinetics of some P^{32}-labeled compounds in EMATC following glucose exposure. Percentage of total extractable activity plotted against time relative to the addition of glucose. 107 microliters of cells incubated in 1.05 ml $LLCO_2I$ containing 7.5 μM lactate and 120 μC P^{32} (radiophosphate used had 2.7 γ carrier phosphate maximum by colorimetric determination[7]). After 15 min, 300 γ glucose added. (Note changes in time scale.)

- ■ ATP (spots 4b and 4c)
- □ ADP (spot 4d)
- ● PGA × 10 (spot 2)
- ○ Fructose diphosphate (spot 1b)
- △ Glucose-6-phosphate × 10 (spot 3a)
- ▲ Fructose and other phosphates × 10 (spot 3b)

[7] R. J. L. Allen, Biochem. J. 34, 858 (1940).

about the distribution of P^{32} in the ATP molecule. Colorimetric determinations indicate that about 40% of the total soluble phosphorus is in organic form. Since only about 20% of the total P^{32} activity can be recovered in organic form, we must conclude that P^{32} equilibration is incomplete. (Note that ATP^{32} and ADP^{32} still increase after 10 minutes.) For ATP^{32} we can assume that the first phosphate residue linked to the nucleoside is very slowly labeled. ADP^{32} hydrolyzes when eluted and rechromatographed to give some $P^{32}O_4^{\equiv}$, but no appreciable activity in the AMP area. Therefore, the ratio of activity ADP^{32}/ATP^{32} must be multiplied by two to give approximate values for the ADP/ATP ratio. That the P^{32} is evenly distributed in the last two phosphate residues only is substantiated by the fact that the decrease in ATP activity at 5 seconds is just twice the increase in ADP activity. We can calculate that the ratio ADP/ATP before glucose addition is 0.23; the ratio after 135 seconds' glucose addition is 0.17. Since, however, there may well have been hydrolysis of ATP, these values may be much lower and therefore may have been decreased by glucose to a much greater extent.

Discussion

The general behavior of phosphorylated glycolytic intermediates following glucose introduction is as follows:

1. Glucose enters the cell more rapidly than its rate of utilization, as was previously shown by Crane, Field and Cori.[8]

2. There is a sudden increase in hexose phosphates. This is because the rate of hexokinase activity is faster in the first one or two seconds than at later times. Possibly, hexokinase may be inhibited by glucose, but more likely the "extra-mitochondrial" supply of ATP[3] is rapidly decreased (see the sudden dip in ATP of about 14% during the first 8 seconds). As hexokinase activity falls off, there is a minimum in hexose monophosphates after 4 seconds. We see that the ratio of glucose to fructose-6 phosphates is close to the theoretical equilibrium of 7:3.[9] This means that isomerization is more rapid than phosphorylation.

3. ATP concentration in the cytoplasm decreases for 8 seconds following glucose addition, together with a concomitant increase in ADP. This may result in an increased rate of phosphohexokinase activity if it is inhibited by ATP.[10] Also ADP concentration is increased, resulting in a transient oxidation of DPN by stimulating cytochrome oxidative phosphorylation, as has been shown by Chance and Hess.[10] That both DPN and ADP are increased means that the sequence of reactions leading from hexose diphosphate to PGA is accelerated and there is a rapid rise in PGA concentration.

4. At 8 seconds (at 37°C) the DPN becomes reduced[10] and the ADP concentration returns to lower levels, so that there is a drastic reduction in the

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[8] R. K. Crane, R. A. Field and C. F. Cori, J. Biol. Chem. 224, 649 (1957).

[9] M. W. Slein, J. Biol. Chem. 189, 753 (1950).

[10] B. Chance and B. Hess, Ann. N. Y. Acad. Sci. 63, Art. 5, 1008 (1956).

rate of PGA formation, and its concentration peaks and falls off. There is a slowdown in phosphohexokinase activity that is even greater than the decrease in PGA formation as a result of increased ATP concentration, and hexose diphosphate decreases. (We know both from our own unpublished data and from the published data of Chance and Hess[10] that at about this time glucose disappearance falls off, and hence the falling off of hexose diphosphate represents a decreased rate of formation rather than an increased rate of utilization.) Also intracellular pH changes may affect the rates of each step.

5. Hexose monophosphates increase owing to decreased utilization. That they level off indicates that there is some sort of feedback control on hexokinase activity. This may be some sort of mitochondrial localization of ATP, as suggested by Racker[3] and Chance and Hess[10] (which would, however, make it impossible to declare that phosphohexokinase activity had been slowed down by increased ATP concentrations). More likely it is a product-caused inhibition resulting from hexose phosphate accumulation.[11,12]

6. Steady state is achieved; the first step is rate-limiting.

In anaerobiosis or methylene blue poisoning[3] the ATP concentrations are presumed to be markedly lowered (yet to be demonstrated). The first step (glucose phosphorylation) must proceed slowly until glycolysis produces a good supply of ATP. We see little evidence of the 1-second high transient in hexose monophosphate. Thus, the first step is limiting from the beginning and we cannot observe the interesting normal transient behavior; instead, radioactivity moves slowly into all the compounds. The concentrations of all intermediates are decreased initially, except for PGA. The over-all rate of glycolysis may be increased under these conditions so that the ultimate rate of the hexokinase reaction may not be decreased in an absolute sense but only in relation to the total reaction rate.

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[11] H. Weil-Malherbe and A. D. Bone, Biochem. J. 49, 339 (1951).

[12] R. K. Crane and A. Sols, J. Biol. Chem. 203, 273 (1953).

GENETIC INVESTIGATIONS OF ENZYME LEVELS IN THE BRAIN OF THE RAT

Thomas H. Roderick, Edward L. Bennett, Hilda Karlsson, and Ann Ohlander

(In collaboration with Professor E. R. Dempster, Department of Genetics, and Professors David Krech and Mark R. Rosenzweig, Department of Psychology.)

In the preceding quarterly report,[1] a selective breeding program to obtain strains of rats with high levels and with low levels of cortical cholinesterase (ChE) activity was discussed. Two highly heterogeneous foundation stocks were used, the Castle stock and the Dempster stock. These foundation stocks were obtained from the Department of Genetics small-animal colony.. From each of these lines, a subline high in cortical ChE activity (referred to as the Roderick C Hi line and the Roderick D Hi line) and a subline low in cortical ChE activity (referred to as the Roderick C Lo line and the Roderick D Lo line) have been selected, using a double-first-cousin mating system. The methods used and the results for the first four selected generations were summarized in the preceding quarterly report.

Rats from the fifth selected generation are now being analyzed. The results of the analyses for cortical ChE activity that have been completed for this generation are summarized in Table XV. At the fourth generation, the difference between the average value for ChE $(V+S)/2$ (average value for the visual and somesthetic areas) was 12.5 for the Roderick D Hi line–Roderick D Lo line and 7.0 for the Roderick C Hi line–Roderick C Lo line.

For those animals of the fifth selected generation which have been analyzed to date, the difference in the average ChE level, $(V+S)/2$, was 14.2 for the Roderick D lines, and 8.0 for the Roderick C lines. With the exception of the second generation of the Roderick D line, these are the highest differences so far obtained from these sublines. The differences in cortical ChE activity between the Hi and Lo sublines are significant at better than the 0.01 level of significance.

In some groups of animals of the fifth selected generation, we have determined the activity of the brain ChE (that is, brain minus cortex) and the activity of lactic dehydrogenase (LDH). The data are summarized in Table XVI. Significant differences were found between the ChE activity of the V (visual) and S (somesthetic) areas of the cortex of the Hi lines as compared to the Lo lines of rats in 3 out of 4 comparisons. However, no significant difference was found between the brain ChE levels of the Hi lines and the Lo lines. It thus appears that the breeding program for different ChE levels in the cortex has been very specific and has not resulted in a corresponding shift in the ChE activity of the rest of the brain. In these rats, as in the S_1 and S_3 rat strains, the ChE activity increases in the order $V<S<<B$. We have not, as yet, analyzed the motor area of the Roderick rats for ChE activity.

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[1] T. H. Roderick, E. L. Bennett, and H. Karlsson, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 93.

Table XV

ChE activity of rats selected for high and low cortical ChE activity.[a] (Reported in terms of moles of acetylcholine hydrolyzed $\times 10^{10}$/min/mg of tissue)

Statistical measures	Brain section			Brain section		
	V	S	$\frac{V+S}{2}$	V	S	$\frac{V+S}{2}$
	Strain: Roderick C Lo (N = 8 rats)			Strain: Roderick D Lo (N = 8)		
$\bar{X}$	44.2	48.8	46.5	44.9	49.3	47.1
σ	1.55	1.55	1.44	2.8	2.7	2.2
	Strain: Roderick C Hi (N = 4)			Strain: Roderick D Hi (N = 7)		
$\bar{X}$	51.6	57.3	54.5	59.1	63.5	61.3
σ	3.2	4.5	3.8	5.8	3.6	4.9
Δ_{Hi-Lo}	7.4	8.5	8.0	14.2	14.2	14.2
σ_{Hi-Lo}	1.34	1.7	1.47	2.3	1.6	1.9
t	5.5	5.0	5.4	6.2	8.9	7.5
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

a

$$\sigma = \sqrt{\frac{\Sigma(X_n - \bar{X})^2}{N-1}} \quad \text{or} = \sqrt{\frac{\Sigma x^2}{N-1}} = \text{estimated standard deviation of sample population}$$

$$\sigma_{Hi-Lo} = \sqrt{\frac{(N_1+N_2)(\Sigma x_1{}^2 + \Sigma x_2{}^2)}{N_1 N_2[(N_1-1)+(N_2-1)]}} = \text{standard error of the difference between means}$$

$$t = \frac{\Delta_{Hi-Lo}}{\sigma_{Hi-Lo}}$$

P = level of significance

Although a significant difference between the ChE activity of the Hi and Lo lines was found, the results of the LDH analyses obtained to date indicate that no difference exists between the LDH activity of the Roderick C Hi line and the Roderick C Lo line or between the Roderick D Hi line and the Roderick D Lo line. This is true of the cortical areas as well as of the brain. It thus appears that the selective-breeding program has been specific for one enzyme or perhaps a group of enzymes, rather than for the general enzyme level of the cortex.[2]

The pattern of LDH activity is also different from that of ChE activity. The subcortical brain has about the same LDH activity as the cortex. Although significant differences in LDH activity were apparently found between different areas of the brain, we are not yet prepared to attach a great deal of significance to these differences because the analyses of different areas were done on separate days. Analyses of like areas were done on the same day. As yet we have not done sufficient LDH determinations to assess the day-to-day reproducibility of analyses.

The selective-breeding program is being continued. In addition, reciprocal crosses between the two high lines are being made. Similar crosses are being made between the two low lines. Analyses for several enzyme systems of these animals will follow the establishment of the two cross lines.

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[2] Other data that we have obtained in S_1 and S_3 rat strains indicate that ChE activity varies markedly as a function of age, whereas LDH activity does not vary with age.

Table XVI

ChE and lactic dehydrogenase activities of rats selectively bred for high and low cortical ChE activity[a]
(Lactic dehydrogenase activity reported as 1000 × change in optical density at 340 mμ/mg tissue/min.)

Statistical measures	Cholinesterase				Lactic dehydrogenase			
	V	S	$\frac{V+S}{2}$	Brain	V	S	$\frac{V+S}{2}$	Brain
Strain: Roderick C Lo; N = rats								
$\bar{X}$	44.3	49.1	46.7	131	142.7	157.7	150.2	128.0
σ	0.9	1.3	1.0	5.0	1.1	4.6	3.8	3.5
Strain: Roderick C Hi; N = 4								
$\bar{X}$	51.6	57.3	54.5	132	141.3	161.5	151.4	126.2
σ	3.2	4.5	3.8	5.9	6.2	2.6	5.0	5.8
Δ_{Hi-Lo}	7.3	8.2	7.8	1	-1.4	3.8	1.2	-1.8
σ_{Hi-Lo}	1.7	2.4	2.0	3.9	3.1	2.7	3.1	3.8
t	4.4	3.4	4.0	0.26	0.45	1.4	0.39	0.47
P	< 0.01	< 0.02	< 0.01	0.8	> 0.6	0.2	0.7	> 0.6
Strain: Roderick D Lo; N = 3								
$\bar{X}$	43.4	50.3	46.8	122	136.5	150.7	143.6	137.2
σ	2.3	1.3	1.2	11.2	3.5	7.2	2.4	2.7
Strain: Roderick D Hi; N = 3								
$\bar{X}$	53.5	60.7	57.1	128	136.2	149.6	142.9	140
σ	3.4	6.1	4.8	5.0	1.7	4.0	2.7	2.1
Δ_{Hi-Lo}	10.1	10.4	10.3	6	-0.3	-1.0	-0.7	2.8
σ_{Hi-Lo}	2.4	3.6	2.9	7	2.2	4.8	2.1	2.0
t	4.2	2.9	3.5	0.9	0.14	0.23	0.33	1.4
P	< 0.02	< 0.05	0.02	> 0.4	0.9	> 0.8	> 0.7	> 0.2

[a] These rats are included in the larger groups of Table I.

THE EFFECT OF D_2O ON FERTILITY IN MICE

Ann M. Hughes

At the time that we were investigating the inhibition of ascites tumor with D_2O (UCRL-3852) we also initiated a study on the effect of D_2O on the fertility of mice. The first report on this project has been issued in a paper by Ann M. Hughes and M. Calvin, Sterility Produced in Mice by Deuterium Oxide, UCRL-8048, Nov. 1957, in which the abstract states:

> Sterility in C_{57} and Swiss mice has been produced by substituting D_2O for a part of the drinking water. The effective range lies between 5% and 30% D_2O. It appears that the effect is greater in C_{57} males than females, and that the size and viability of the litter is affected.

This first experiment is being repeated, and the study has been expanded to include the investigation of some metabolic changes in D_2O-treated mice. Preliminary data indicate that the respiratory metabolism of glycine is markedly affected.

ACTION AND EMISSION SPECTRA OF THE DELAYED LIGHT EMISSION OF VARIOUS PLANTS MATERIALS

Gordon Tollin and E. Fujimori

In order to learn more about the delayed light emission of plant materials, a study of the action and emission spectra of this luminescence was undertaken. The materials investigated to date are Chlorella, Nostoc, and whole spinach chloroplasts.

Experimental Procedure

The apparatus used in these studies was identical to that reported previously[1] except for the following changes. A time constant of 0.1 second was incorporated into the electronic system to permit working at higher gain. For the measurement of the action spectra, a Bausch and Lomb grating monochromator of 500-mm focal length (catalogue No. 33-86-45) was placed between the sample and the flash. For the measurement of the emission spectra, the monochromator was placed between the sample and the photomultiplier. The curves were measured by observing the intensity of the luminescence approximately 0.2 second after the excitation by the flash as a function of wave length of either the exciting light or the emitted light.

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[1] Gordon Tollin and Melvin Calvin, The Luminescence of Chlorophyll-Containing Plant Material, UCRL-3863, July 1957; and G. Tollin and M. Calvin, Proc. Nat. Acad. Sci. U.S. 43, 895 (1957).

The band pass of the monochromator was approximately 20 mμ. The curves were corrected for the spectral energy distribution of the flash (action spectra) and for the spectral sensitivity of the photomultiplier (emission spectra).

Results

Chlorella

The action spectrum of Chlorella luminescence follows the absorption spectrum of this organism very closely. It shows the typical chlorophyll bands both in the red and in the blue as well as the carotenoid bands, and is quite similar to the photosynthetic-action spectrum of this organism. The room-temperature emission spectrum is essentially identical to the chlorophyll-fluorescence spectrum of the algae.

Nostoc

The action spectrum of Nostoc luminescence is significantly different from the absorption spectrum of this organism. The action spectrum indicates that a good portion (probably around 50%) of the chlorophyll is inactive and that the carotenoids are almost completely inactive. The phycocyanin, however, is apparently quite active. The emission spectrum of this organism is apparently similar to that of Chlorella. The yield of luminescence, however, is significantly lower than that for Chlorella, as would be expected from the action spectrum. There are some indications that part of the emission may be due to phycocyanin.

Whole Spinach Chloroplasts

Both the action spectrum and the room-temperature emission spectrum of this material are very similar to those for Chlorella. The emission spectra at -45°C and at -70°C are identical to the room-temperature spectrum.

Discussion

The results demonstrate conclusively that the delayed light emission of Chlorella and of spinach chloroplasts, and at least part of that of Nostoc, is the result of a transition from the first excited singlet state to the ground state of chlorophyll. The low-temperature spectra suggest that the triplet state of chlorophyll is not involved at all in the delayed light emission of spinach chloroplasts. Moreover, the transfer of electronic excitation energy from carotenoids to chlorophyll may take place in Chlorella and in spinach chloroplasts, but not in Nostoc. However, transfer from phycocyanin to chlorophyll does occur in Nostoc.

PHOTOCONDUCTIVITY AND SEMICONDUCTIVITY OF ORGANIC COMPOUNDS

David Kearns and M. Calvin

As reported earlier,[1] a study is now being made of the photo- and semiconductivity of organic compounds that have conjugated ring systems. The conductivity apparatus existing at that time has been modified and used for photoconductivity as well as semiconductivity measurements.

The following substances have shown photoconductivity:

Cytochrome C (beef heart extract)
Scenedesmus (whole cells, dried)
New Zealand spinach chloroplast fragments (dried)
Phycocyanin
Phthalocyanin

In all cases the observed photocurrents were of about the same order of magnitude as the dark currents. Action spectra and temperature dependence of the photoconductivity of these compounds as well as others are to be measured.

Preliminary semiconductivity measurements on dried Scenedesmus whole cells indicate an activation energy for conduction of about 1 ev. After prolonged drying under a reduced pressure of 10^{-3} mm Hg the algae lost their semiconducting property.

A study of electron spin resonance and semiconductivity of metal-free phthalocyanin has indicated that an increase in free-radical content decreases the activation energy for conduction. A small increase in free-radical content decreased the activation energy by about 0.25 ev. More measurements will be made to relate quantitatively the radical concentration to the activation energy for conduction in phthalocyanin and other compounds.

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[1] David Kearns, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 68.

ELECTRON SPIN RESONANCE STUDIES OF IRRADIATED DEUTERATED CHOLINE CHLORIDE

Robert Lindblom and Richard M. Lemmon

The need for synthetic routes to selectively deuterated choline chlorides, for the purpose of studying their radiation-damage mechanism, was discussed in the preceding quarterly report.[1] These compounds have been synthesized, irradiated, and the irradiated samples examined with electron spin resonance (ESR). The ESR spectra indicate that the propagated radical does not contain a nitrogen atom. The unpaired electron seems to show nearly equal interaction with the α and β hydrogen atoms (indicated in the formula below):

$$[(CH_3)_3\ NCH_2CH_2OH]^+\ Cl^-$$

$$\beta \quad \alpha$$

Synthesis of Choline Chloride Deuterated in the α Position

This isotopically labeled compound was synthesized by reduction of methyl-N, N-dimethylglycine with $LiAlD_4$ in ether, followed by quaternization with CH_3I and exchange of the I^- by Cl^- on Dowex I resin. The over-all yield of this sequence was 29%. The methyl N, N-dimethylglycine was made in 65% yield by condensation, under anhydrous conditions in benzene, of dimethylamine and methyl bromoacetate. The methyl N, N-dimethylglycine was also made by dry distillation at 295°C of anhydrous betaine with a yield of 45%.

Synthesis of Choline Chloride Deuterated in the β Position

This compound was synthesized by the reduction and quaternization of the substituted ester of glycine in the same manner as the α-substituted choline. However, in this case, the substituted glycine ester was prepared from methyl bromoacetate-d_2 and the reduction was carried out with $LiAlH_4$. The methyl bromoacetate-d_2 was made from acetic acid-d_4 by consecutive treatment with PBr_3, Br_2, and MeOH in a modified Hell-Volhard-Zelinsky reaction. No yield figure was obtained, as spillage occurred during this synthesis.

Synthesis of Choline Completely Deuterated in the Methyl Position

This compound was synthesized by methylation of ethanolamine with methyl-d_3 bromide in ether in the presence of K_2CO_3, in 27% yield (based on acetic acid-d_4). The methyl bromide-d_3 was made from completely deuterated acetic acid by the Hundsdiecker (silver salt) reaction.

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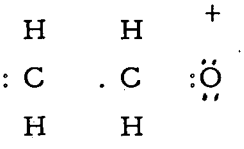
[1] Robert Lindblom, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 73.

Electron Spin Resonance Spectra of Irradiated Deuterium-Labeled Choline Chloride

The spectrum of the unpaired electron on methyl-deuterated choline chloride is almost indistinguishable from that of unlabeled choline chloride (see Fig. 15). Both have five lines and approximately the expected areas for a radical interacting with four protons. Both the α- and β-deuterated samples show three lines. Although the β-deuterated sample is better resolved than the α-deuterated one, the general features are the same. In none of the spectra is there any suggestion of nitrogen splitting (three equal peaks). One resonance form of a radical that would satisfy the above observations is



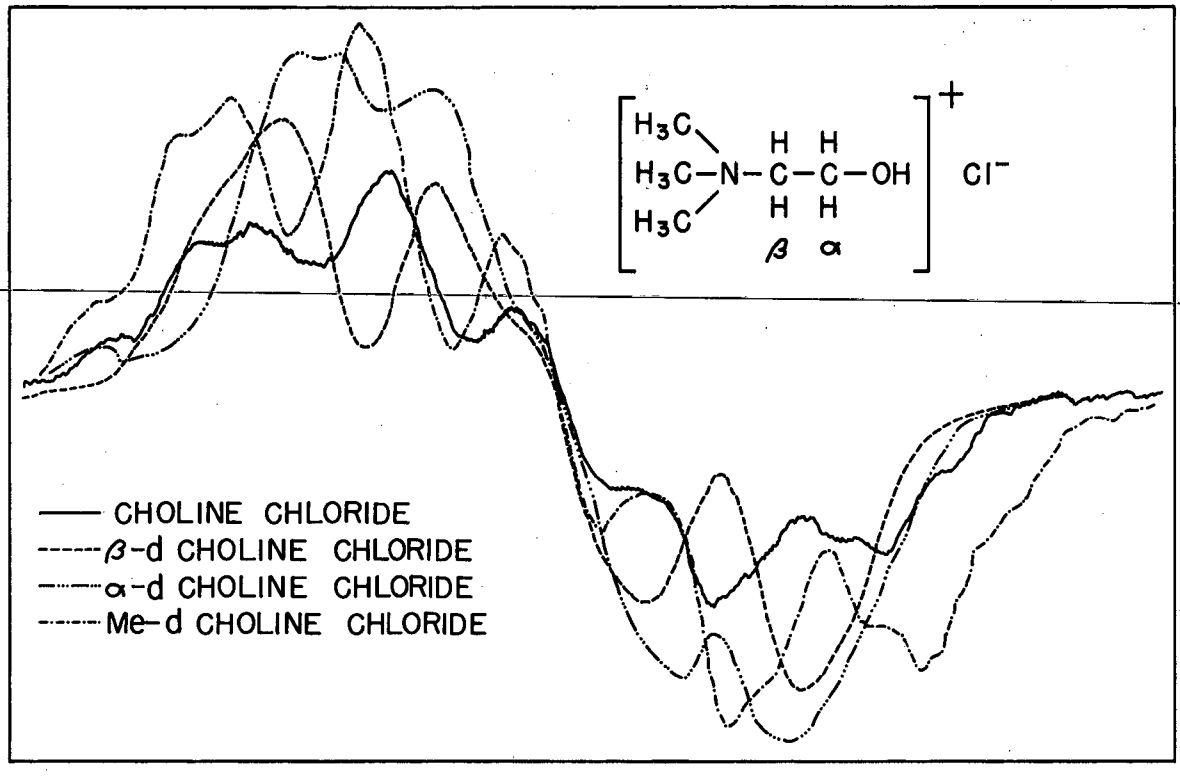


Fig. 15. Electron spin resonance spectra of undeuterated and deuterated irradiation-damaged choline chlorides.

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

THE CRYSTAL STRUCTURE OF RUTHENOCENE

George L. Hardgrove and David H. Templeton

The crystal structure of ruthenocene (dicyclopentadienyl ruthenium), $Ru(C_5H_5)_2$, has been determined by the x-ray crystallographic method. The orthorhombic unit cell has dimensions a = 7.13 ± 0.01 Å, b = 8.99 ± 0.02 Å, and c = 12.80 ± 0.02 Å with four ruthenocene molecules in the unit cell. The structure is reported in space group No. 62 Pnma.

The atomic positions were determined by three-dimensional Fourier calculations and least-squares refinements on 792 independent reflections of which 586 had observable intensities. The final parameters are the averages of those determined from the Fourier and least-squares calculations.

	x/a	y/b	z/c	σ x/a	σ y/b	σ z/c
Ru	0.23705	0.25000	0.49581	0.00026	--	0.00010
C_1	0.4113	0.3783	0.3864	0.0021	0.0021	0.0012
C_2	0.2492	0.3299	0.3317	0.0020	0.0024	0.0014
C_3	0.1481	0.3808	0.6316	0.0019	0.0021	0.0011
C_4	0.9892	0.3307	0.5815	0.0017	0.0020	0.0010
C_5	0.5156	0.2500	0.4212	0.0029	--	0.0017
C_6	0.2519	0.2500	0.6690	0.0027	--	0.0019

The molecular structure consists of a pair of aromatic five-membered rings situated in an eclipsed configuration about the ruthenium atom. The bond distances of interest are calculated from the atomic parameters.

$Ru-C_1$	2.200 ± 0.016 Å	Average Ru-C 2.208 ± 0.019 Å
$Ru-C_2$	2.223 ± 0.019 Å	
$Ru-C_3$	2.193 ± 0.016 Å	
$Ru-C_4$	2.204 ± 0.015 Å	
$Ru-C_5$	2.205 ± 0.021 Å	
$Ru-C_6$	2.220 ± 0.024 Å	

C_2-C_3	1.420 ± 0.021 Å	Average C-C in the rings 1.434 ± 0.027 Å
C_2-C_6	1.443 ± 0.021 Å	
C_4-C_5	1.378 ± 0.018 Å	
C_4-C_7	1.470 ± 0.021 Å	
C_3-C_3'	1.437 ± 0.044 Å	
C_5-C_5'	1.455 ± 0.036 Å	

It is interesting that the corresponding compound of iron, called ferrocene, has been found to be in a staggered configuration, rather than the eclipsed structure found here.

SPALLATION REACTIONS OF URANIUM-238 PLUS HELIUM-4

Joseph A. Coleman and T. Darrah Thomas

Spallation reactions for helium ions incident on U^{238} which yield U^{237}, U^{239}, Np^{238}, and Pu^{238} have been studied at bombarding energies of 20 to 48 Mev. It appears from their excitation functions that the (α, αn), (α, 2pn), and (α, p3n) reactions proceed by a direct-interaction mechanism at energies greater than that necessary for the alpha particle to surmount the Coulomb barrier. A typical compound-nucleus reaction mechanism is apparent from the (α, 4n) excitation function. Theoretical calculations based on the experimental results of the (α, 4n) reaction have yielded values for $\frac{\Gamma_n}{\Gamma_t}$ for the plutonium isotopes 239 through 242:

$$\left(\frac{\Gamma_n}{\Gamma_t}\right)_{Pu^{239}} = 0.23, \qquad \left(\frac{\Gamma_n}{\Gamma_t}\right)_{Pu^{241}} = 0.34,$$

$$\left(\frac{\Gamma_n}{\Gamma_t}\right)_{Pu^{240}} = 0.44, \qquad \left(\frac{\Gamma_n}{\Gamma_t}\right)_{Pu^{242}} = 0.57.$$

Experimental Cross Sections for U^{238} + He^4

E_α (Mev)	Cross Section (mb)			
	(α, αn)	(α, 2pn)	(α, p3n)	(α, 4n)
30.0-33.8	3.15 ± 0.30	0.66 ± 0.12		
31.0-34.6	3.84 ± 0.39	0.90 ± 0.26		
34.8-38.6	5.18 ± 1.30	0.91 ± 0.12		
37.4			2.71 ± 0.32	7.57 ± 0.93
37.7-42.0	15.12 ± 1.51	1.03 ± 0.15		16.8 ± 1.4
41.1			4.20 ± 0.93	19.22 ± 2.51
44.8			4.82 ± 0.53	16.40 ± 1.90
47.1			5.10 ± 2.20	8.64 ± 1.29

NEPTUNIUM-241

Richard M. Lessler and Maynard C. Michel

Uranium-238 was bombarded with helium ions on the Crocker Laboratory 60-inch cyclotron. A careful chemical separation of the resulting neptunium was carried out. This neptunium was then mass-separated in the time-of-flight separator with a collection full time at mass 241. The Np^{241} mass-separated plate yielded a single decay period of about 3.5 hours. The mass separation of Np^{241} previously reported had given two decay periods of 16 minutes and 3.3 hours.[1] The chemical separation time for this earlier experiment had been about 25 minutes, while that for the recent one was over an hour. Since a clean chemical separation was obtained in the recent experiment, it appears that 3.3- to 3.5-hour activity is due to Np^{241}. It was not possible to see the 15-minute activity again because of the longer separation time. Thus the 15-minute activity previously seen could be an isomer of Np^{241} or an impurity. Further mass separations of Np^{241} are planned in order to verify the assignment.

[1] Richard Lessler and Maynard C. Michel, Neptunium-240 and Neptunium-241, in Chemistry Division Quarterly Report, UCRL-2709 Sept. 1954.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

LIQUID-LIQUID EXTRACTION AND AGITATION: PERFORMANCE OF SPHERE-PACKED EXTRACTION COLUMNS

Gabriel L. Jacques and Theodore Vermeulen

Previous experimental data from this project have been correlated, and a thesis has been completed and a report prepared.[1] The results may be summarized as follows:

1. In uniform sphere packings of equal porosity but different permeability, the permeability has been found to have almost no effect on the Peclet number (velocity times particle diameter, divided by apparent dispersivity).

2. In two-phase flow, separate Peclet numbers are needed to describe the dispersion behavior of the continuous phase and of the discontinuous phase. These Peclet numbers are almost always less than the Peclet number for the same packing in single-phase flow, and thus correspond to a larger extent of back mixing. The values measured range between 15% and 95% of the single-phase Peclet number.

3. For either phase the Peclet number increases as the superficial velocity of that phase increases, or as the superficial velocity of the opposite phase decreases.

Holdup has been calculated for the two-phase runs, and these and other data are being examined with a view to possible correlation. Comparison of the experimental Peclet numbers with published extraction-column data indicates that dispersion may account for as much as 90% of the apparent mass-transfer resistance.

Performance of the experimental column in two-phase-flow runs has been improved by installation of a proportionating control valve on the bottom outlet, for better control of the interface level. A new photoprobe mounting, external to the column, is being installed, which will also increase the accuracy of dispersion measurements in the discontinuous phase.

LIQUID-LIQUID EXTRACTION AND AGITATION: THEORY OF LONGITUDINAL DISPERSION

Terukatsu Miyauchi, Alice K. McMullen, and Theodore Vermeulen

Calculation of concentration profiles for representative values of the dispersion and mass-transfer parameters, on the IBM 701 Computer, has been completed. The tabulated results will be issued as a supplement to an earlier report. It will be designated: Terukatsu Miyauchi, Longitudinal Dispersion in Solvent-Extraction Columns, UCRL-3911 (suppl.) January 22, 1958. Empirical correlation of the results is now in progress, in order to extend their usefulness.

[1] Gabriel L. Jacques and Theodore Vermeulen, Longitudinal Dispersion in Solvent-Extraction Columns: Peclet Numbers for Ordered and Random Packings, UCRL-8029, Nov. 1957.

ZONE MELTING

William R. Wilcox and C. R. Wilke

The work to date can be summarized as follows.

Experimental Work

Zone melting has been tried on approximately 50-50 mixtures of β-naphthol and naphthalene with zone travel rates of 1/2 to 3/4 inch per hour and stirrer speeds of 0 to 270 rpm. In all cases the separation was very small in comparison with that theoretically possible. The separation decreased with increasing rate of zone travel, as expected. The effect of stirrer speed has been slight so far. Experiments under wider conditions will be carried out.

One experiment revealed that the mixture decomposes slightly upon prolonged standing at 130°C. A dark product forms which appears to absorb heavily under the spectrophotometer used for analyses, giving the appearance of increasing % β-naphthol with time.

Theoretical Treatment

Equations for heat and mass transfer in zone melting under various conditions are being developed. Attempts to quantitatively explain the variation of separation with crystallization rate in a paper on the zone melting of salts and one on the progressive freezing of semiconductors have so far been unsuccessful.

Literature Search

An extensive survey of the literature on crystal growth has been completed and promises to be of value in the theory and understanding of zone melting.

STABILITY OF PERFORATED-PLATE TRAYS

Robert S. Brown, Donald N. Hanson, and C. R. Wilke

Recent experiments on gases bubbling from horizontal orifices have shown that there are at least two types of bubbling. The first occurs when the average gas flow through the holes is laminar and yields pressure fluctuations that are uniform and equal to one another. The second type occurs when the average gas flow through the holes is turbulent and yields pressure fluctuations that are random. The dumping rate and the transition between the two types is a complicated function of the volume under the plate and of the hole diameter.

Also, some data have been taken on a plate which three holes and the resulting dumping rates are markedly lower than those obtained from a one-hole plate thus indicating an averaging of the pressure fluctuation. This effect has also been detected experimentally.

MULTICOMPONENT DISTILLATION STUDIES USING AN IBM-701 TWO-FRAME DIGITAL COMPUTER

John H. Duffin, Donald N. Hanson, and C. R. Wilke

Trial runs on the unsteady-state approach to industrial distillation-column calculations have been made, but as yet have been unsuccessful. Work continues along these lines in an attempt to get an over-all solution to the problem. The matter of convergence in the unsteady-state approach is currently giving difficulty, as it did in the steady-state solutions.

ELECTRICAL CONDUCTIVITY OF HETEROGENEOUS MIXTURES

Robert E. Meredith and Charles W. Tobias

Experiments on the electrical conductivity of two-phase liquid mixtures indicate that it is possible to obtain equivalent specific conductivities of the two phases by proper choice of the components in the system. The water–propylene carbonate system with tetraethyl ammonium iodide as the electrolyte has been found to give conductivity ratios near unity. Fabrication of equipment for the purpose of studying the conductivity of liquid dispersions as a function of volume fraction is being continued.

ELECTROCHEMICAL STUDIES IN CYCLIC ESTERS

William S. Harris and Charles W. Tobias

Electrodeposition studies in these solvents have indicated that some of the alkali and alkaline earth metals may be electrodeposited from solutions of their halides. Several other more noble metals have also been deposited.

CORRELATION OF LIMITING CURRENTS AT HORIZONTAL ELECTRODES UNDER FREE-CONVECTION CONDITIONS

Eugene J. Fenech and Charles W. Tobias

It was originally thought that the use of mica particles suspended in a moving fluid would satisfactorily serve to outline the velocity distribution in the fluid. In actual practice, however, the platelet form of these particles turned out to be more detrimental than helpful to the experiment, because at certain orientations the particles became invisible. Finely powdered rosin has been substituted, and preliminary indications are that it will prove satisfactory. By making time exposures, it is possible to measure the fluid velocity by observing the displacement of these particles. In this manner it has been shown that the fluid velocity over the center of an electrode has a maximum of about 1.25 cm/sec. To improve lighting techniques, a cell allowing direct side illumination and having a black background (i.e., dark-field illumination) will be used in the future.

CORRELATION OF HEATS OF FORMATION OF INORGANIC COMPOUNDS

Howard Anderson and Leroy A. Bromley

The heats of formation of the halides have been correlated using two empirical parameters per element. Equations were derived, and by use of experimental heats of formation, the best values of the constants were evaluated. The calculation involves the setting up and solving, simultaneously, of a set of cubic equations. An iterative method of solution is utilized. This was programmed and the solution performed on an IBM 701 computer.

The correlation for the halides is quite satisfactory and will be summarized in a forthcoming UCRL Report which is Mr. Anderson's Master's Thesis.

The method was extended to oxides, sulfides, nitrates, sulfates, acetates, and carbonates. Except for the nitrates and possibly the acetates the results are not satisfactory, but suitable modifications in the method may be found.

HIGH-SPEED BOILING IN TUBES

Roger Wright and Leroy A. Bromley

Approximately 80% of all equipment to be purchased outside the Radiation Laboratory has been received. Approximately 50% of all equipment to be constructed in Radiation Laboratory shops has been completed and is ready for installation. Actual unit construction and assembly is now in progress and approximately 25% completed.

GENERAL CHEMISTRY

Leo Brewer, Robert E. Connick, and Kenneth S. Pitzer in charge

METALS AND HIGH-TEMPERATURE THERMODYNAMICS

Spectroscopic Studies of C_2 and C_3

John Engelke

An apparatus for modulated absorption-spectrum determinations has been successfully applied to the carbon vapor system. The absorption measurements confirm the previous emission measurements and indicate that some species other than C, C_2, or C_3 must be responsible for a large part of the strong ultraviolet continuum observed in carbon systems. Further study will be necessary to identify this species.

New Light Sources

Earl Worden

Work is continuing on the arc for production of strong CN emission.

Absolute-Lifetime Apparatus

Richard Brewer and Fred Stafford

The radiative lifetime of an excited electronic state of the I_2 molecule has been determined. The apparatus for extension of these measurements to high-temperature molecules has been almost completed.

Temperature Scale at High Temperatures

John Engelke, E. Worden, Richard Brewer, and Fred Stafford

Further checks of the high-temperature scale in the $2000^{\circ} - 3000^{\circ}$ K region are in progress with the U. S. National Bureau of Standards in an effort to find the source of serious discrepancies that have been uncovered in the past.

BASIC CHEMISTRY

Kinetics of Rapid Reactions

Claude Coppel

The study of the kinetics of the ferric chloride complexing reaction has been completed. The rate law has been determined and the heats and entropies of activation have been measured. A complete UCRL report is being issued: Claude P. Coppel, The Kinetics of the Complexing of Ferric Ion by Thiocyanate and Chloride Ions (thesis), UCRL-8059, Dec. 1957.

Chemistry of Iodine in the +1 Oxidation State

Y. T. Chia

The rate of formation of hypoiodite ion from iodide and hypochlorite ions in 1 M sodium hydroxide solution was investigated spectrophotometrically, and was found to be first-order dependent on both hypochlorite and iodide concentrations; in 1 M hydroxide solution the rate constant was found to have a value of $60 \pm 5\ M^{-1}\,sec^{-1}$. The dependence of the above reaction on basicity was also studied at an ionic strength of one, and the rate was found to be inversely proportional to the hydroxide concentration. Therefore the rate law of the reaction studied is

$$d(IO^-)/dt = k(I^-)(OCl^-)/(OH^-).$$

Species in Refluxed Chromic Nitrate Solutions

James Finholt

Attempts have been made to determine the charge per species of some of the products produced by refluxing chromic nitrate solutions. A number of batch equilibrations using ion-exchange resin have been made. For reasons as yet not understood, low apparent values were obtained, i.e., less than +3. Possible sources of error such as decomposition of the product, complex formation, etc., are being checked by varying conditions of temperature, anion concentration, acidity, type of resin, and the competing cation.

An experiment to determine the number of OH^- groups per chromium atom in chromium polymers is also in progress. It should be possible to do this by oxidizing the Cr(III) to Cr(VI) and titrating to determine the OH^- produced.

Nuclear Magnetic Resonance Study of Hydrogen Bonding

Jefferson C. Davis, Jr.

A specially designed nuclear magnetic resonance probe is being constructed for the purpose of high-resolution studies of chemical shifts in hydrogen-bonding materials over the temperature range 80^o to 450^oK.

Ruthenium Studies

Dwight Fine

Investigation of neutral (uncharged) ruthenium(III) chloride species is being continued. Efforts are being directed toward finding a source of these species which yields concentrated solutions reasonably free of other substances. The best source to date has been a preparation made by refluxing commercial ruthenium chloride with 0.5 M HCl in the presence of mercury. Spectra of solutions obtained from this preparation indicate the presence of at least three neutral species. During the course of the investigation a new method of analysis for Ru(III) in chloride solutions has been developed.

Dispersion Forces Between Atoms and Molecules

Wilm E. Donath

The work discussed in the preceding progress report is completed and is being written up as a UCRL report.

Determination of the Molecular Structure of Aluminum Hydride

Robert F. Nickerson

This work has been completed and a UCRL report is being issued: Robert F. Nickerson, Studies Related to the Structure of Aluminum Hydride, (Thesis) UCRL-8089, December 16, 1957.

Low-Temperature Heat Capacity of Ag_2O

Roger Gerkin

The heat-capacity apparatus previously described has been put into operation. Heat-capacity data for the empty calorimeter have been obtained for the 15-to-300°K region, and a plot of

$$\left(\frac{\Delta H}{\Delta R}\right)_{corr} \text{ vs } R_{Av}$$

has been made; this plot is quite smooth, as required.

Prior to the calculation of $\left(\frac{\Delta H}{\Delta R}\right)_{corr}$, several components of the apparatus were calibrated.

Construction of a cryostat and associated equipment for intercomparison and calibration of strain-free resistance thermometers and thermocouples has been initiated.

Low-Temperature Heat Capacity of MgO

Norman E. Phillips

Some preliminary measurements on the heat capacity of powdered MgO at liquid helium temperatures have been made, with the intention of investigating the effect of particle size on the temperature dependence of the heat capacity. Thermal equilibrium within the powder was attained in about 10 minutes following the end of each heating period even though no helium gas was present in the calorimeter to aid in heat transfer. These equilibrium times could be tolerated because of the low heat leak to the calorimeter. The results show that the heat capacity increases more slowly than T^3 in the range 1.2 to 4.2°K.

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