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UCRL-1959
Chemistry-Transuranic Elements

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August, 1952

September 25, 1952

Berkeley, California

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I QUARTERLY PROGRESS REPORT. Project 48

A. Nuclear Chemistry

G. T. Seaborg and I. Perlman

Chemistry of Curium

B. B. Cunningham and D. C. Feay

In line with this laboratory's interest in the chemistry of the actinide elements, recent attempts have been made to study the crystal structure of the compounds of curium. Approximately 40 μg of curium (principally Cm^{242}) were purified by the method of Stephanou and Penneman[1] using lanthanum as a carrier in the fluoride precipitation. The lanthanum and curium were separated using a concentrated hydrochloric acid cation column packed with Dowex-50 resin.[2]

Because of the limited supply of curium it was necessary to devise an efficient method of preparing samples for x-ray diffraction study. In order to develop a suitable method of mounting the samples, several preliminary trials were made with lanthanum in place of curium. The method adopted consisted of transfer of a few microliters of lanthanum nitrate solution to the end of a 10 mil platinum wire, partial evaporation by heating the opposite end of the wire, treatment with gaseous ammonia, followed by complete evaporation and ignition of the sample at ca. 800° C. The final deposit consisted of a coating of oxide around a section of wire about 2 mm in length. The tip of the platinum wire with the sample was cut off and mounted in a thin-walled capillary tube for x-ray analysis by the powder method. Various amounts of lanthanum were used, and it was discovered that approximately 3 μg of lanthanum, as the sesquioxide, gave a clearly recognizable pattern. Also 2 μg gave a pattern that could be recognized as the correct pattern of La_2O_3. All pictures showed the diffraction lines of platinum, which served as an internal standard.

Sample 55A. Approximately 2 to 3 μg of curium were ignited to the oxide and mounted as described above. The ignited oxide had a very light cream color in contrast to the pure white of freshly purified Cm_2O_3. The color could be due to a plutonium impurity which had grown into the curium. Two x-ray pictures of this sample, a 5 hr. and a 10 hr. exposure, each gave one line which was in about the right place for the strongest line of the hexagonal sesquioxide structure.

1. S. E. Stephanou and R. A. Penneman, J. Am. Chem. Soc. 74, 3701 (1952).

2. K. Street, Jr. and G. T. Seaborg, J. Am. Chem. Soc. 72, 2790 (1950).

Sample 56A. Approximately 5 μg of curium were treated as above. The film from the first exposure of 7 hours was too dark due to curium radiations for any lines except those due to the platinum wire to appear. A subsequent 2-1/2 hr. exposure gave no lines other than those due to platinum.

Sample 57A. Sample 56A was removed from the capillary, reheated to approximately 800° C for 5 min. and then remounted in another capillary. The first exposure was for 5 hours and gave the same line as mentioned before. Subsequent photographs of this sample on the same day and the two following days gave no positive indications of any structure other than that of the platinum wire.

The results obtained seem to indicate that not only does the extreme activity of the curium sample cause darkening of the film, but it also destroys the crystal structure. Consequently, x-ray pictures need to be taken very shortly after the sample has been prepared or heated to allow crystal formation.

Complex Alpha Spectra

Frank Asaro

The alpha spectra of a number of samples containing varying ratios of $Cm^{242,243,244}$ have been studied in detail. An alpha group at 5.86 Mev previously tentatively assigned to Cm^{243} has been definitely assigned to Cm^{242}. Table I shows the best values for the alpha spectra of Cm^{242} and Cm^{243} now available.

Table I

Nuclide	Alpha Decay Separation from Highest Energy Alpha Group (kev)	Abundance (%)	Alpha Particle Energy (Mev)
Cm^{243}	0	8	
	211 ± 5	76	5.779 ± 0.008
	257 ± 6	16	
Cm^{242}	0	73 ± 2	6.110 ± 0.003
	45 ± 2	27 ± 2	
	150 ± 8	0.036 ± 0.002	

The decay scheme of Cm^{242} is shown in Fig. 1.

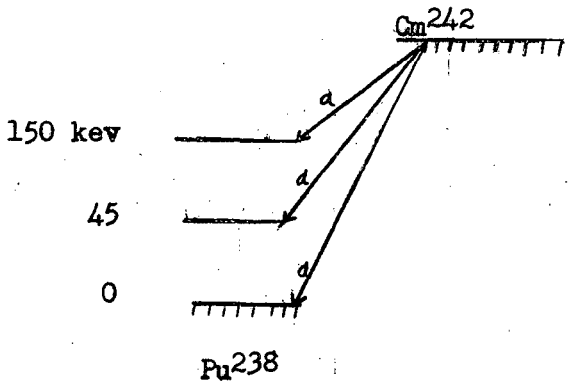


Fig. 1

In investigating the conversion electron spectrum of Np^{238}, which also decays to Pu^{238}, Freedman, Jaffey, and Wagner[1] found eight conversion electron lines which they interpreted as the decay scheme of Fig. 2.

1. M. S. Freedman, A. H. Jaffey, F. Wagner Jr., Phys. Rev. 79, 410 (1950).

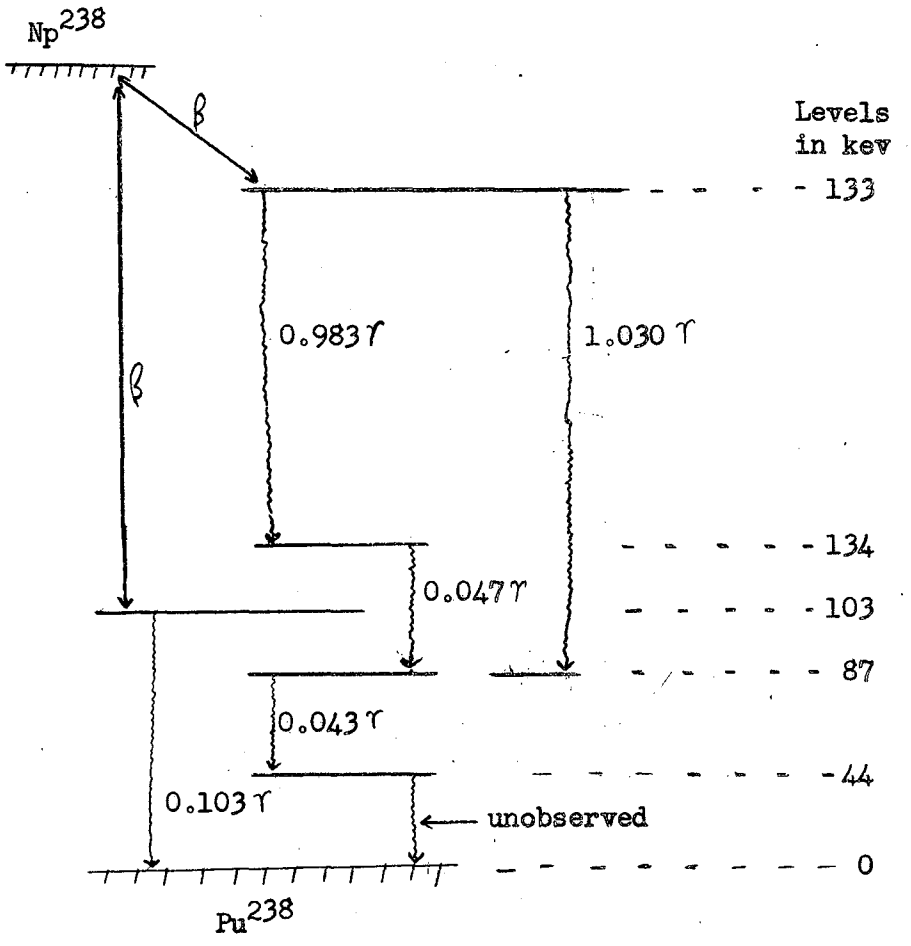


Fig. 2

The above authors, however, used an overall binding energy for the L shell and also for the M shell. By using binding energies obtained from a Mosely extrapolation, the gamma rays shown in Table II are obtained from the data of Freedman, et al.

Table II

Energy of Conversion Line	Binding Energy	Gamma Energy (Mev)
0.0208	0.0223 (L_{II})	0.0431
0.0247	0.0181 (L_{III})	0.0428
0.0374	0.0060 (M_I)	0.0434
0.0419	0.0016 (N_I)	0.0435
0.0802	0.0223 (L_{II})	0.1025
0.0979	0.0046 (M_{III})	0.1025
0.859	same interpretation as Freedman et al.	
0.913		

The above interpretation of the data is in good agreement with the results from Cm^{242} alpha decay shown in Fig. 3.

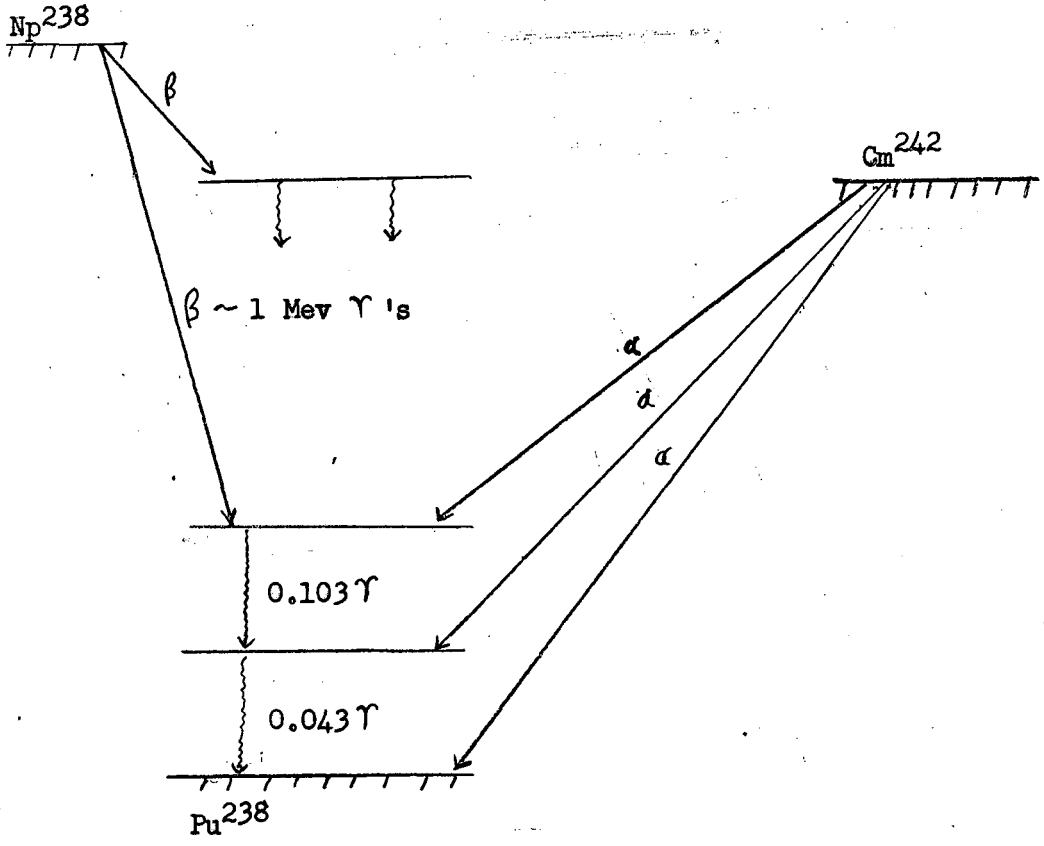


Fig. 3

The alpha spectrum of Ra^{223} has been observed. The four alpha groups found by Rosenblum[2] were verified and a new alpha group at 5.750 Mev was discovered. This group is of higher energy than any yet reported. The results are given below in Table III.

Table III

Nuclide	Alpha Decay Energy Separation from Highest Energy Alpha Group (kev)	Abundance (%)	Alpha Particle Energy (Mev)
Ra^{223}	0	11	
	32	53	5.719[3]
	146	25	
	214	9	
	323	2	

X Radiation from the Decay of Np^{238} and Pu^{238}

H. Jaffe and I. Perlman

A sample of Np^{238} was prepared by neutron irradiation of Np^{237}, and was investigated on the bent crystal x-ray spectrometer. The x-ray lines list in Table IV were observed.

2. S. Rosenblum, M. Guillot, M. Perey, Compt. Rend. 202, 1274 (1936).

3. W. B. Lewis, B. V. Bowden, Proc. Roy. Soc. A 145, 235 (1934).

Table IV

Line	Energy (kev)	Extrapolated Energy	Intensity
Np $L\gamma_1$	21.49 ± 0.02	21.38	40
Np $L\gamma_6$	22.28 ± 0.04	22.13	15
Np $L\beta_1$	18.37 ± 0.01	18.27	100
Np $L\beta_2$	17.36 ± 0.03	17.25	15
Np $L\alpha_1$	14.32 ± 0.02	14.28	30

The ratio of L_{II} lines, $L\beta_1 : L\gamma_1 : L\gamma_6 \cong 100:40:15$, and the ratio of L_{III} lines, $L\alpha_1 : L\beta_2 = 100:50$, are both in good agreement with previously acquired data. No L_I lines were observed.

A sample of more than 10^{10} α c/m of Pu^{238} was examined on the x-ray spectrometer and the x-ray lines given in Table V were observed and identified.

Table V

Line	Energy (kev)	Siegbahn's Energy	Intensity
U $L\beta_1$	17.28 ± 0.01	17.22	100
U $L\gamma_1$	20.22 ± 0.03	20.16	40
U $L\gamma_6$	20.91 ± 0.05	20.85	10
U $L\beta_2$	16.46 ± 0.02	16.43	15
U $L\alpha_1$	13.63 ± 0.02	13.61	20

The ratio of L_{II} lines, $L\beta_1 : L\gamma_1 : L\gamma_6 \cong 100:40:10$, and the ratio of L_{III} lines, $L\alpha_1 : L\beta_2 = 100:75$. No L_I lines were identified.

A careful search was made in the 30-50 kev region for the gamma ray (or rays) the conversion lines of which have been reported, but nothing

was found. This observation is consistent with the high conversion coefficient encountered for the similar transition in other even-even nuclides such as for Cm^{242} α-decay.

Tantalum Spallation and Fission

Walter E. Nervik

During the past quarter elemental fractions of tantalum, hafnium, barium, antimony, indium, palladium, rhodium, ruthenium, molybdenum, zirconium, and strontium have been isolated from tantalum targets bombarded with 340 Mev protons. Decay of all of these fractions is being followed at the present time and cross sections will be obtained in the near future.

Cross sections already calculated indicate that the cross section vs. mass number curve will have approximately the shape indicated in Fig. 4, with a broad peak of 0.1 mb near mass number 80-85, a minimum of 0.001 mb near mass number 105-110, and a maximum of 50 mb in the spallation region near tantalum.

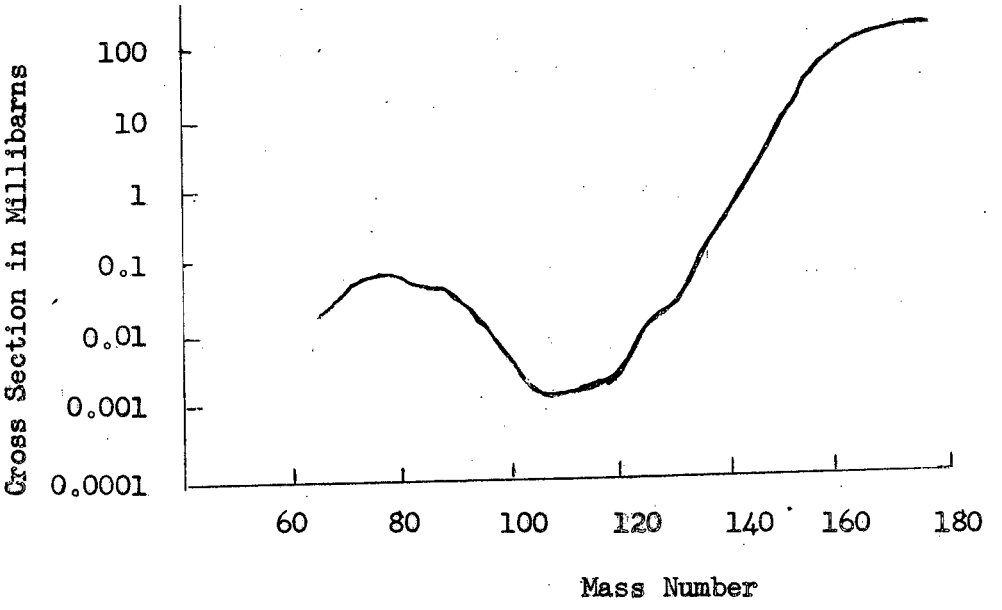


Fig. 4

Two methods have been discovered for separating tantalum and niobium which offer promise of preparation of target tantalum of extremely high purity. One involves the preferential extraction of tantalum into di-isopropyl ketone from a 4 M H_2SO_4 - 0.5 M HF aqueous solution, while the other involves preferential elution of tantalum from a Dowex-1 anion exchange resin column with 1 M HF.

Spallation of Silver

Per Kofstad

Studies have been continued on the spallation of silver with 340 Mev protons. To date the cross sections, shown in Table VI, have been calculated, some of which are corrections of earlier reported data:

Table VI

Isotope	Cross Section (mb)	Correction Which Was Applied for Copper Impurity
Ni^{65}	1.2×10^{-3}	no correction
Cu^{61}	2.2×10^{-2}	1×10^{-3} mb
Cu^{64}	2.5×10^{-2}	6×10^{-3} mb
Zn^{62}	2×10^{-2}	essentially no correction
Zn^{63}	2×10^{-3}	1.5×10^{-3} mb
Ga^{67}	2.5×10^{-2}	
Ga^{72}	2.3×10^{-2}	no correction for Ga
Ga^{73}	9×10^{-3}	
Se^{72}	2.6×10^{-2}	
Se^{73}	5.1×10^{-2}	
Br^{76}	9.2×10^{-2}	
Sr^{82}	0.86	
Sr^{83}	0.67	
Y^{91}	0.1	
Ru^{95}	4.9	
Ru^{97}	9.2	
Rh^{100}	0.1	
Rh^{101}	2.1	
Pd^{101}	7.6	
Cd^{105}	19.4	

The silver bombarded had a copper impurity of approximately 0.05 percent. Corrections for this amount of copper impurity have been made according to Batzel's work on spallation of copper. The copper impurity, however, is too large to make the corrections reliable, and in that respect silver of higher purity has been obtained from Johnson Mathey's.

Improved Values in the Branching Decay of the Long Lived Bi^{210} Isomeric State

Harris B. Levy

Bi^{210} has a long lived isomeric state that decays chiefly by alpha emission with a half-live of $\sim 10^6$ years. In the last quarterly report a report was made on the finding of a branching decay to Po^{210}. Further experiments have since been carried out, and have resulted in new values for the branching ratio in the decay of the long lived Bi^{210} isomer. The latest figures show that for every 270 nuclei that undergo alpha emission, one decays to Po^{210}. This means a half-life for the decay to Po^{210} of $\sim 2.7 \times 10^8$ years. These figures are believed to be more accurate than those previously reported.

There is still the same uncertainty that was reported last time as to which of the two states, the long lived isomer or RaE, is the ground state of Bi^{210}. Therefore, we still cannot say whether these figures on the decay to Po^{210} correspond to direct β^- decay of the long lived Bi^{210} isomer or to an isomeric transition to RaE with subsequent β^- decay to Po^{210}. However, these latest figures are not appreciably different from those originally reported, and the arguments expounded in the last report concerning the various modes of decay remain unaffected.

Search for Sm^{146} Alpha Activity

Dean C. Dunlavey

A water cooled target holder bearing 13 mg. of neodymium metal has been bombarded with 35 Mev helium ions in the internal beam of the 60-inch cyclotron. The samarium fraction has been isolated in an attempt to find the alpha activity predicted to be associated with Sm^{146}. Limited alpha activity has been found with a mechanical counter. Alpha energy measurements using nuclear emulsions are to be tried following decay of the associated beta decay.

Anti-coincidence Circuit for G-M Shield Cell

A. E. Larsh, Jr. and M. I. Kalkstein

To provide a lowered background for G-M counting, twenty 20 inch long, 2 inch diameter G-M tubes were arranged into a shield cell into which an end-window G-M tube and stand could be inserted. The entire assembly is shielded by 8 inches of steel on all sides.

All the shield tubes are connected in parallel and operate into a standard Nuclear scaler. The sample G-M tube is connected to a scaler from which the second amplifier (which drives the scaling circuits) is removed. The sample pulse is taken out after the first amplifier (cathode follower) and fed to the anti-coincidence (AC) circuit. The shield cell AC pulse is taken from the "Scope" connections on the panel of the shield cell scaler.

In the AC circuit the sample pulse is delayed 15 μsec (to account for possible delay in shield cell G-M tube firing), then fed to a 6BN6 mixer tube. If there is no pulse from the shield cell counters, the sample pulse is able to be counted (Fig. 5). If a pulse from the shield cell counters occurs with the signal pulse, there is no output from the AC circiut that can be counted (Fig. 6).

The shield cell pulse blocking period is 60 μsec. The signal pulse is made 5 μsec long. The 15 μsec signal delay allows ample time for the shield cell tubes to fire and block any background count which is common to both shield cell G-M and sample G-M tubes.

The output of the AC circuit is returned to the sample tube scaler, where it is counted in the usual fashion.

By means of the AC circuit, the G-M sample tube background in the shield cell has been reduced from 20 c/m to 8.5 c/m. Of the remaining 8.5 c/m, about 7 c/m appear to be due to nearby gamma activity.

The AC circuit is shown in Fig. 7.

New High Stability Pulse Height Analyzer

A. Ghiorso, A. E. Larsh, Jr., and H. P. Robinson

A new type pulse height analyzer has been designed around the 6BN6 Gated Beam tube. The circuits are simple, non-critical, use few components (three tubes per channel), and draw low power (5 ma per channel at B+ $\cong$ 150 v).

FIGURE 5

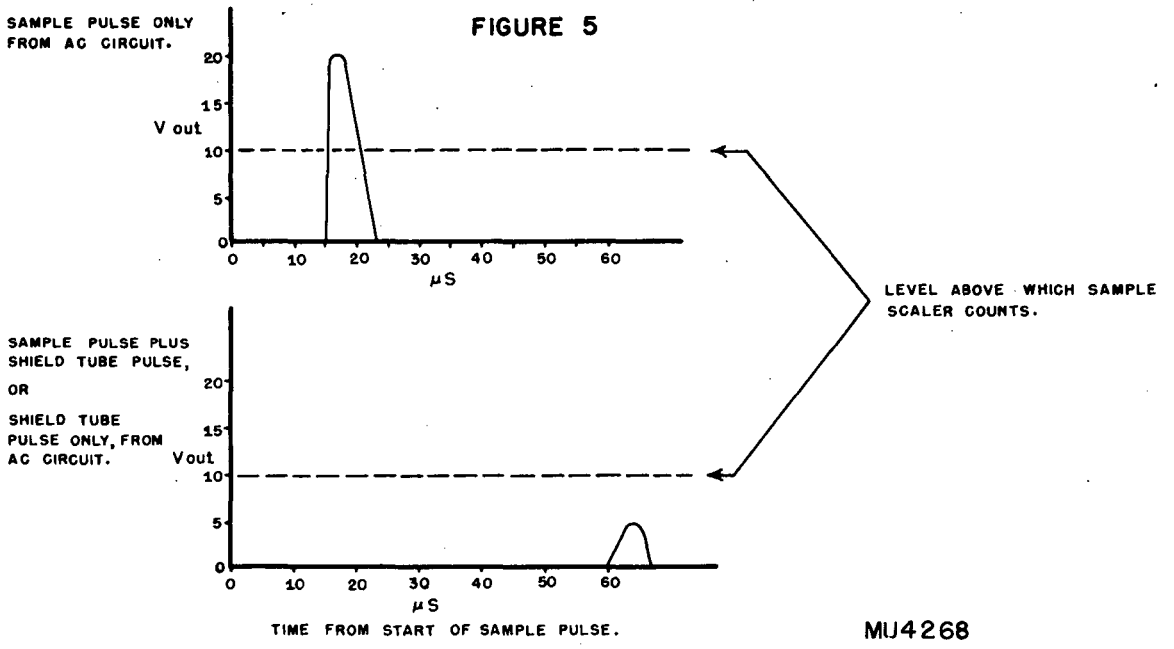


FIGURE 6

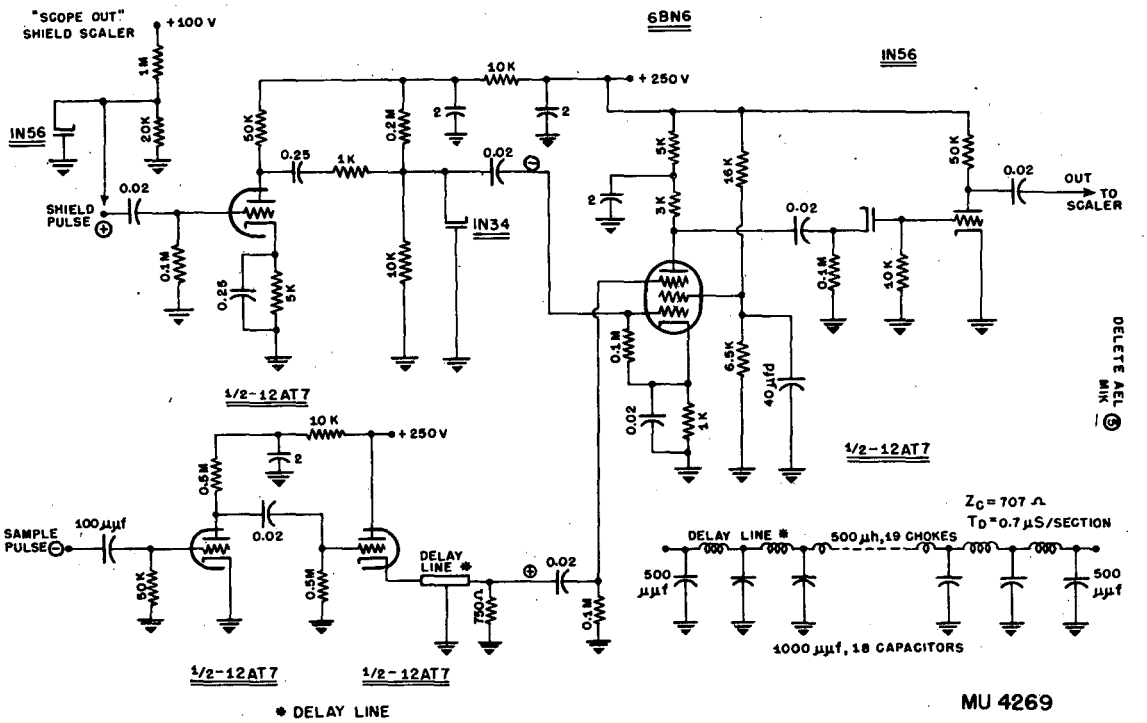

"SCOPE OUT" SHIELD SCALER
+100 V
1M
20K
IN56
0.02
SHIELD PULSE
0.1M
0.25
5K
50K
0.25
1K
0.2M
10K
2
2
10K
+250V
0.02
IN34
6BN6
IN56
5K
3K
2
16K
0.02
0.1M
10K
50K
0.02
OUT TO SCALER
0.1M
6.5K
40µµf
0.02
1K
1/2-12AT7
1/2-12AT7
DELETE AEL MK I
10K
+250V
0.5M
2
SAMPLE PULSE
100µµf
0.02
50K
0.5M
DELAY LINE *
750Ω
0.02
0.1M
1/2-12AT7
1/2-12AT7
* DELAY LINE
Z_C = 707 Ω
T_D = 0.7 µS/SECTION
DELAY LINE *
500µh, 19 CHOKES
500 µµf
500 µµf
1000 µµf, 18 CAPACITORS
MU 4269

FIGURE 7

The pulse height selection is performed by a chain of 6BN6's biased at successively increasing negative voltages on the signal grids. These biases sort the pulses into an integral picture corresponding to an energy spectrum obtained from a radiation detector.

The new and important feature of this system is the use of the 6BN6 as half of a gated multivibrator. When the signal pulse exceeds by a few millivolts the bias level of the 6BN6, the multivibrator effect takes control and the circuit "snaps" into a pulsed state.

The 6BN6 circuit drives a 30 millisecond multivibrator which turns on the register driving tube. At the same time a cancelling pulse is fed from each 6BN6 multivibrator to the next register multivibrator below to turn it off before it has a chance to fire and actuate the register driving tube. In this manner a differential pulse analysis is achieved, since only the highest energy 6BN6 multivibrator to be triggered will actuate a register.

A narrow delayed gate pulse is provided to allow the flat topped signal pulse to reach maximum value before the 6BN6's are allowed to operate.

A ten channel model of this analyzer has been constructed. It has been aligned and retained good short term stability with only 10 millivolts channel width during a test. In normal operation it would run with 5 v channel widths. This "window" width can easily be adjusted to within 10 mv. The long term stability of channel width is expected to be excellent, but can only be determined by field tests over a period of time.

Three 50 channel models are to be assembled immediately.

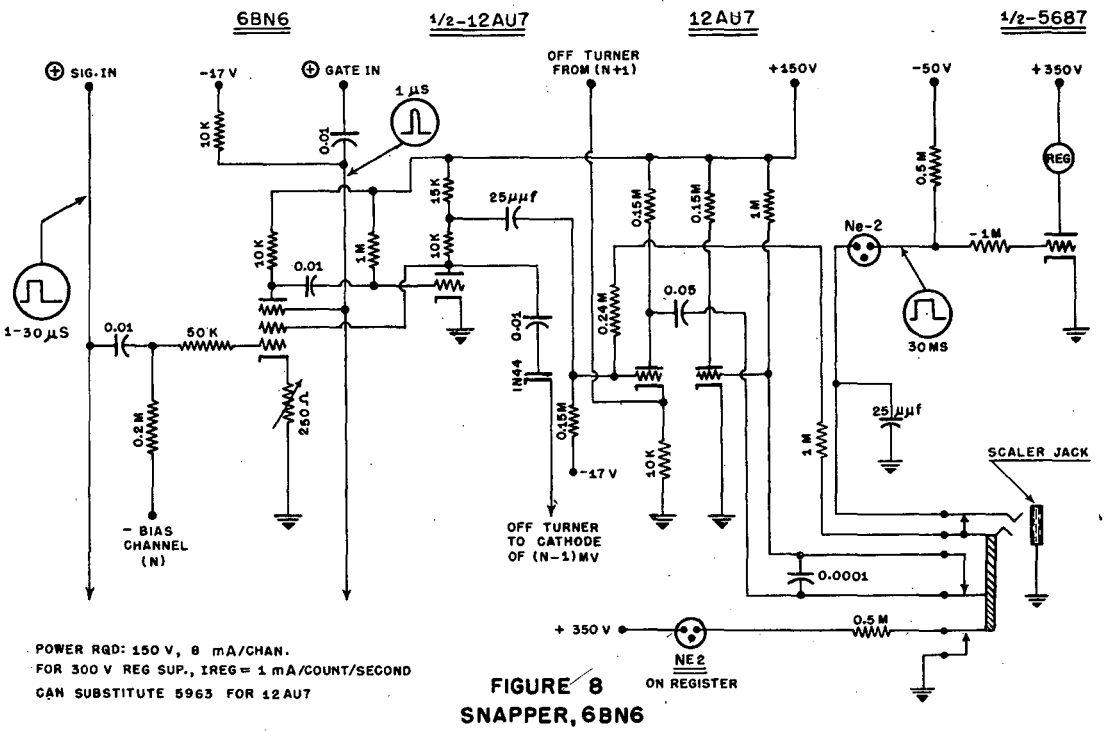
The circuit, shown in Fig. 8, is easily adapted to many types of coincidence and anti-coincidence work by using the second, or gate, grid of the 6BN6 tubes.

Intracyclotron Spectrometer and Intracyclotron Decay Analyzer

Rex H. Shudde

Plans have been drawn for two instruments: the intracyclotron spectrometer, and the intracyclotron decay analyzer. The construction of the first instrument has been completed. The purpose of these instruments is to determine alpha particle energies and half-lives (respectively) of short lived alpha emitters produced during bombardments in the 184-inch cyclotron.

The spectrometer has been successfully calibrated with a Ra^{226} sample obtained from Frank Asaro.



POWER RQD: 150 V, 8 mA/CHAN.
FOR 300 V REG SUP., IREG = 1 mA/COUNT/SECOND
CAN SUBSTITUTE 5963 FOR 12AU7

FIGURE 8
SNAPPER, 6BN6
PULSE HEIGHT ANALYZER

MU 4270

Figure 9 shows a top view of the spectrometer which utilizes the magnetic field of the cyclotron to bend the alpha particle trajectories. The trajectories are impinged on nuclear emulsions; the energies of the particles are obtained from the particle range in the emulsion.

Beta-Gamma, Gamma-Gamma Coincidence Counter

Thomas O. Passell

The past three months has seen development work on converting the double focusing beta spectrometer for use as a beta-gamma, gamma-gamma coincidence spectrometer. Investigations are proceeding for the use of a proportional counter for beta counting and the NaI-5819 photomultiplier tube combination for counting gamma rays. Pulses from each should be similar enough in time resolution to justify coincidence experiments.

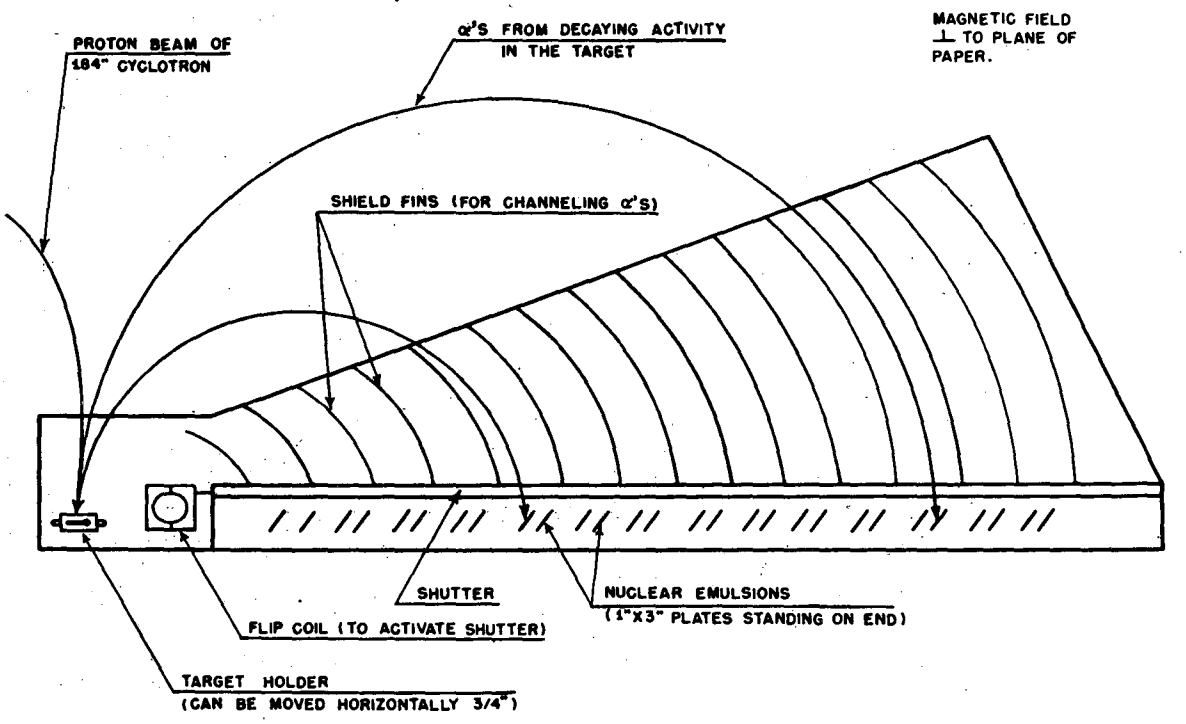


FIGURE 9
INTRACYCLOTRON SPECTROMETER (TOP VIEW)

MU4271

B. Bio-Organic Chemistry

M. Calvin

Search for Photosynthetic Intermediates

J. A. Bassham and D. F. Bradley

The search for additional products of carbon dioxide reduction during photosynthesis was continued in the hope of finding missing intermediate compounds. The method of killing described in the last report, 80 percent alcohol at room temperature, has been continued. A typical experiment in which this method of killing was used gave the radiogram shown in Fig. 10. It can be seen that in this experiment virtually all of the radiocarbon incorporated into compounds stable under the conditions of this method of analysis are phosphate compounds. Furthermore, enzymatic hydrolysis of each phosphate area gave only the free sugars from the phosphate esters named in Fig. 10. (There was overlapping of some areas, obviously.) Consequently it is possible in this particular experiment to identify virtually all of the radioactive stable compounds. This was found to be the case in 10 sec. PS Scenedesmus and 30 sec. PS Chlorella also. This means that in these particular experiments at least, if there are other intermediates involved in the reduction of carbon dioxide to carbohydrates during photosynthesis, as for example, two-carbon and four-carbon compounds, such compounds must fall into one or the other of two classes: Those which occur in such small concentrations that they are not seen by this sensitive method, and those which are too unstable or volatile to be seen by this method. It is known, of course, that many other labeled compounds are actually present in these experiments and would be seen if the x-ray film were exposed for a longer time. Known compounds that fall in this class include malic acid, sucrose and various amino acids. Such a low concentration of a labeled compound does not in itself eliminate the compound as a possible intermediate.

Evidence for volatile radioactive compounds is given elsewhere in this report. In addition to this, another experiment has been performed which indicates the possible existence of volatile or unstable intermediates. Scenedesmus were suspended in a "lollipop" in the usual way and, after reaching a steady-state of photosynthesis, were darkened for 15 minutes. C^{14}-labeled bicarbonate was then added in the dark and after five seconds mixing, an aliquot portion of the algal suspension was run into alcohol. The light was then turned on and aliquot portions taken every few seconds. The radioactive carbon present in each portion was measured within a few minutes of completion of the experiment. The insoluble material from each fraction was then centrifuged, re-extracted with 20 percent alcohol and centrifuged again. The insoluble material was then counted and in no case found to exceed 10 percent of the total radioactivity fixed in the initial count. However, when the radioactivity in the combined extracts was determined, it was found to be only 20 to 30 percent of the activity

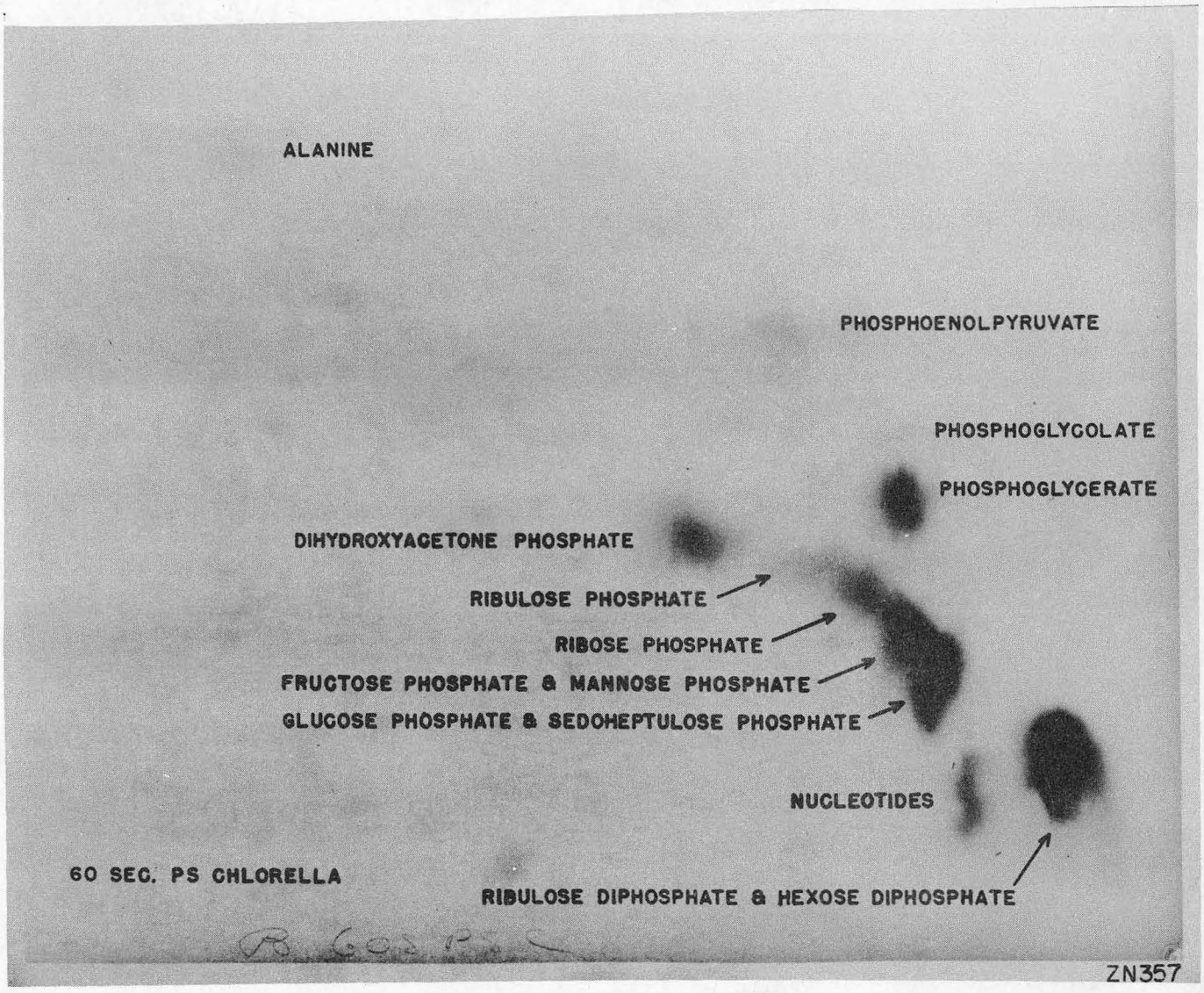

ALANINE
PHOSPHOENOLPYRUVATE
PHOSPHOGLYCOLATE
PHOSPHOGLYCERATE
DIHYDROXYACETONE PHOSPHATE
RIBULOSE PHOSPHATE
RIBOSE PHOSPHATE
FRUCTOSE PHOSPHATE & MANNOSE PHOSPHATE
GLUCOSE PHOSPHATE & SEDOHEPTULOSE PHOSPHATE
NUCLEOTIDES
60 SEC. PS CHLORELLA
RIBULOSE DIPHOSPHATE & HEXOSE DIPHOSPHATE
ZN357

Fig. 10

initially fixed in the shortest light times (see Fig. 11). This effect was not seen in the "dark" aliquot. The plates made of the original material, when recounted after several hours, were found to have decreased in activity to values approaching those of the plates of the extracts. The extracts were then concentrated to a small volume and their radioactivity found to be undiminished, indicating that nearly all the volatile or unstable compounds had disappeared prior to the concentration. The extracts were then analyzed by chromatography and it was found that the "dark" compounds were malic acid, aspartic acid and citric acid. The compounds formed after the light was turned on showed the same rates of formation as those reported in previous kinetic studies.

The most interesting aspect of this experiment is the apparent formation of some unstable or volatile radioactive compound in the light. This "compound" may be nothing more than a complex of CO_2 with some plant constituent or perhaps CO_2 itself if the light produces a rapid change of pH within the cell. Further work in which the pH of the algal suspension is more carefully controlled is in progress and is described in this report.

Steady-State Reservoir Sizes in Photosynthesis

P. Massini

Experiments have been performed to determine the concentration of intermediates in carbon reduction during photosynthesis. This has been done by measuring the saturation value of radioactivity incorporated into these compounds during steady-state photosynthesis.

The apparatus used for these experiments was constructed to permit the algal suspension to be left under controlled external conditions (illumination intensity, temperature, carbon dioxide and oxygen concentration) while photosynthesizing for at least one hour. Furthermore, it was required that the change from natural to radioactive carbon dioxide, which was to be circulated in a closed system, and the withdrawal of several samples at given time intervals be accomplished with a minimum of change in these conditions.

The apparatus consisted of:

(a) A square illumination vessel A (Fig. 12) made out of Lucite (polyacrylic plastic), 49 cm. high, 11 cm. wide and 0.7 cm. thick (inside dimensions). The bottom was provided with a gas inlet tube with five small holes to allow good contact between gas and liquid and a drain tube closed with a screw clamp. The top of the vessel was provided with a gas outlet tube. A water-alcohol mixture from a constant temperature bath was allowed to flow over the outer surfaces of the vessel in order to control the temperature of the suspension.

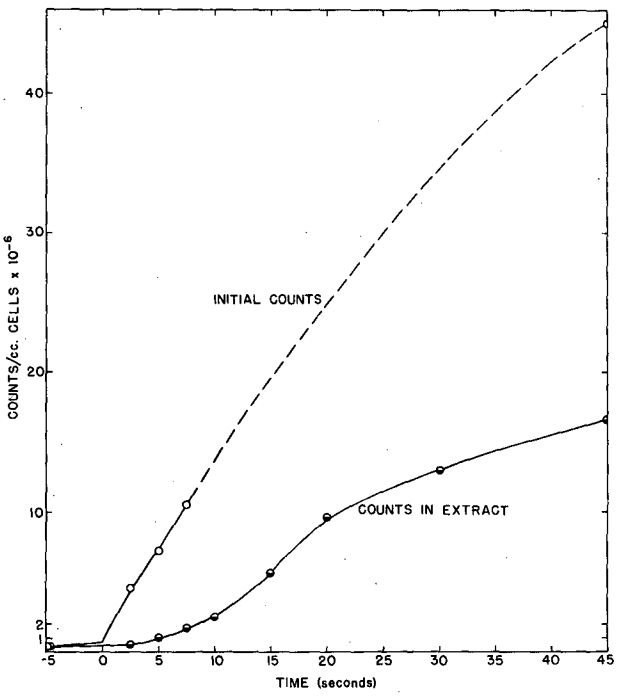

COUNTS/cc. CELLS x 10^{-6}
40
30
20
10
2
1
0
INITIAL COUNTS
COUNTS IN EXTRACT
-5
0
5
10
15
20
25
30
35
40
45
TIME (seconds)

Fig. 11

(b) Two illumination banks (represented by B), each with four fluorescent tubes (General Electric, quality white, 20 watts each), providing an almost uniform illumination over the whole surface of the vessel, of 7 x 10^4 ergs/(cm.^{2})(sec.); (roughly 700 footcandles).

(c) An ionization chamber C, connected to a recording vibrating reed electrometer, to record continually the activity of the gas leaving the vessel during the run.

(d) Three gas traps D, to permit the addition of a known amount of radioactive carbon dioxide to the system, and to trap the remaining radioactivity after the run.

(e) A flask E, 5 liters in volume, containing a mixture of 1 percent radioactive carbon dioxide in air. The reservoir contained so much carbon dioxide that the algae assimilated no more than 20 percent of it during a run.

(f) A gas circulating pump F of the rubber tubing type, and a flow meter G.

(g) A system of four-way stopcocks H, which permitted the vessel to be flushed with a mixture of one percent ordinary carbon dioxide in air, from the cylinder I.

A photograph of the entire assembly is shown in Fig. 13.

In a typical experiment, 2 cc. (wet packed) of one-day old Scenedesmus, washed and re-suspended in 200 cc. of de-ionized water, were placed in the vessel and aerated with the ordinary gas mixture for at least one-half hour, while the mixture of radioactive carbon dioxide circulated in the gas system for thorough mixing, without passing through the vessel. The suspension was kept at 24° C. After this time, during which a steady state of photosynthesis had been reached, the radioactive mixture was passed through the vessel in place of the ordinary gas mixture, by a manipulation of the pair of stopcocks at H, and samples of 20 cc. of the suspension withdrawn at intervals of five or ten minutes. These samples were run into 80 cc. of alcohol at room temperature, thus giving an 80 percent alcohol solution. After thirty minutes of photosynthesis, the lights were turned off and the suspension allowed to remain in the dark for a period of five minutes, during which time several samples were again withdrawn and treated in the same manner. In one experiment another light period followed the dark period.

The samples were shaken for one hour and centrifuged. The residue was re-extracted in 50 cc. of 20 percent alcohol at room temperature, centrifuged, and re-extracted again with 20 cc. of water. The extracts were concentrated together to 0.5 cc. The extracts were analyzed by the usual procedures of chromatography and radioautography and the individual compounds counted by means of a thin-window Geiger tube as described in previous reports.

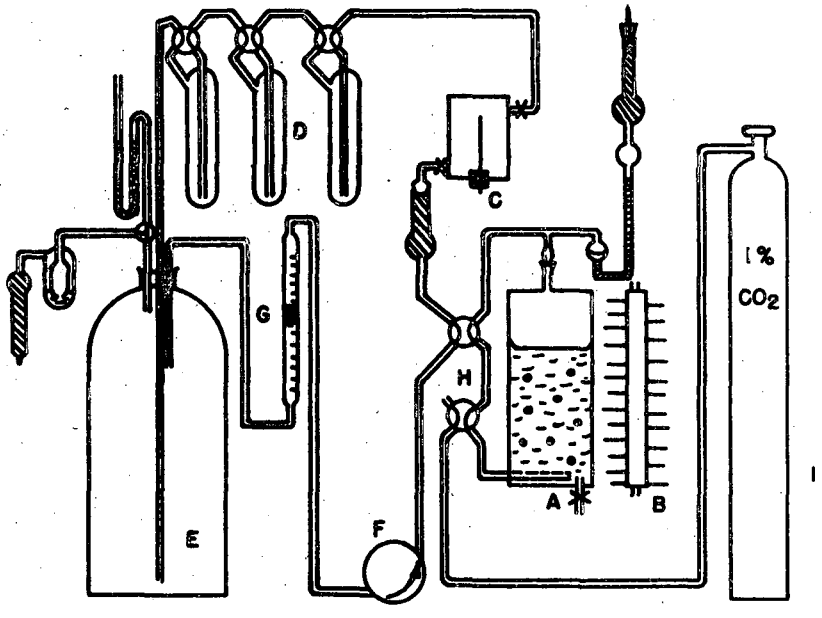


Fig. 12

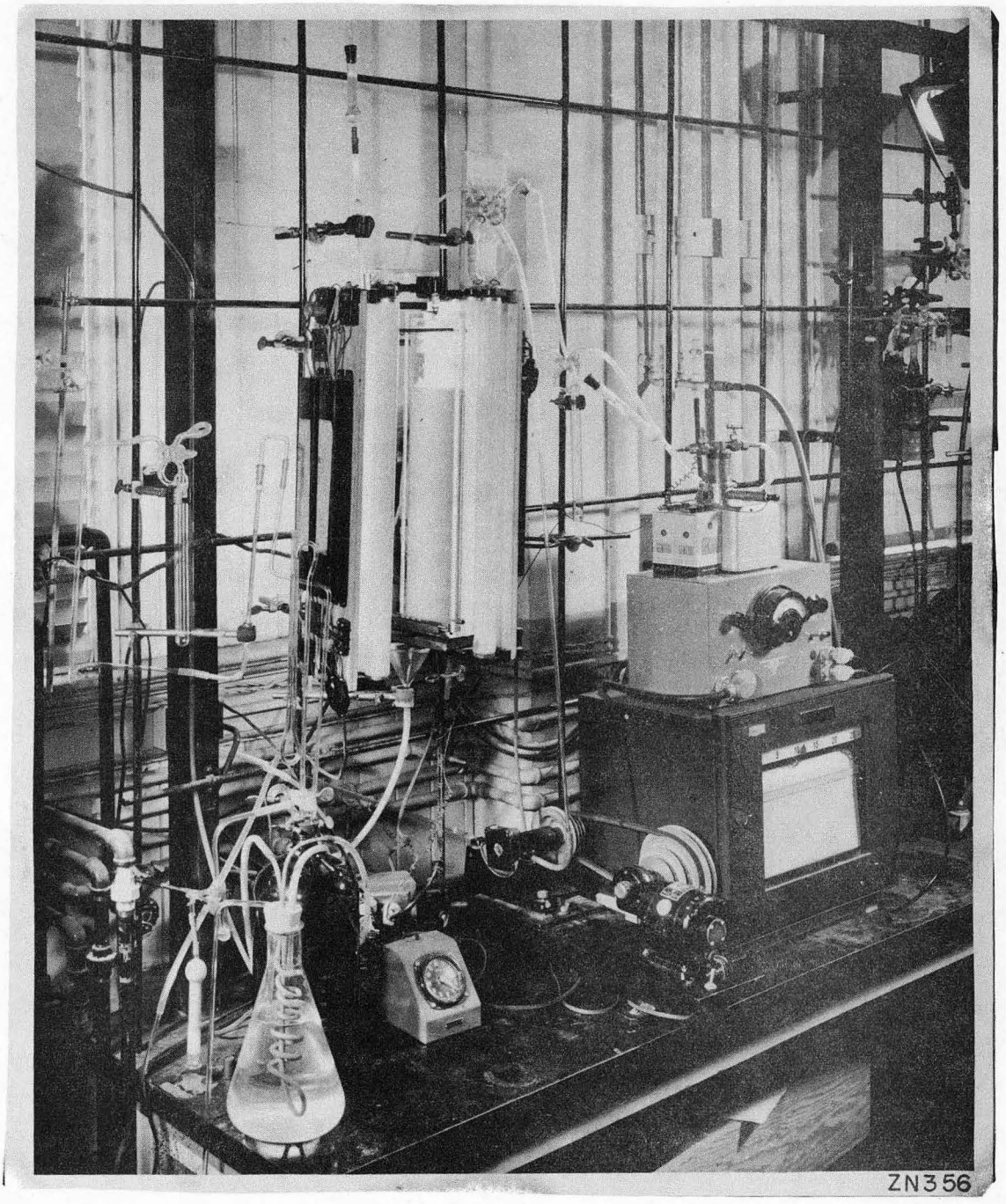


Fig. 13

Figure 14 shows the total and the extracted amounts of radiocarbon fixed by 1 cc. cells during thirty minutes of photosynthesis followed by five minutes of darkness. The slope in the total fixation curve in the light corresponds to an assimilation of 13 cc. CO_2 (NTP) per hour.

Figure 15 shows the amount of radioactivity incorporated into sucrose and three phosphorus compounds for the experiment of Figure 14.

Figure 16 gives the number of counts in sucrose and glutamic, malic and citric acids, for a different experiment of fifteen minutes photosynthesis, followed by ten minutes in the dark, and again five minutes of photosynthesis.

Although the variation between experiments is great, there are some striking features which are common to all:

(1) The curves of some of the compounds show a marked decrease in slope after five minutes of photosynthesis. These curves are: the diphosphates (mainly ribulose diphosphate), the hexose-monophosphates (50 percent glucose-, 26 percent sedoheptulose-, some fructose- and mannose-monophosphate), and phosphoglyceric acid. The decrease in slope clearly indicates the presence of rapidly turning-over reservoirs in the photosynthesis cycle which are then thoroughly labeled and reach the specific activity of the fed carbon dioxide. The leveling off of these curves permits the calculation of the concentration of the reservoirs of those compounds in the photosynthesis cycle. This is done by dividing the measured amount of radioactivity per carbon atom by the specific activity of the fed carbon dioxide.* Table VII gives the steady state concentrations during photosynthesis for some compounds determined by this method.

* The efficiency factor of the counting of spots on papers has been determined by converting three cut out spots to barium carbonate and measuring their activity in an ionization chamber. It is 19 disintegrations per count.

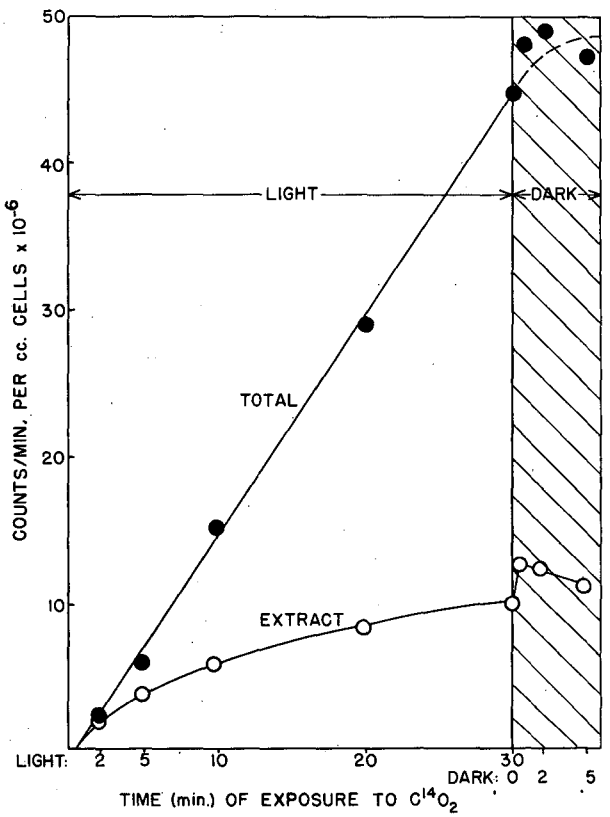

50
40
30
20
10
LIGHT
DARK
TOTAL
EXTRACT
COUNTS/MIN. PER cc. CELLS x 10-6
LIGHT: 2 5 10 20 30
DARK: 0 2 5
TIME (min.) OF EXPOSURE TO C14O2

Fig. 14

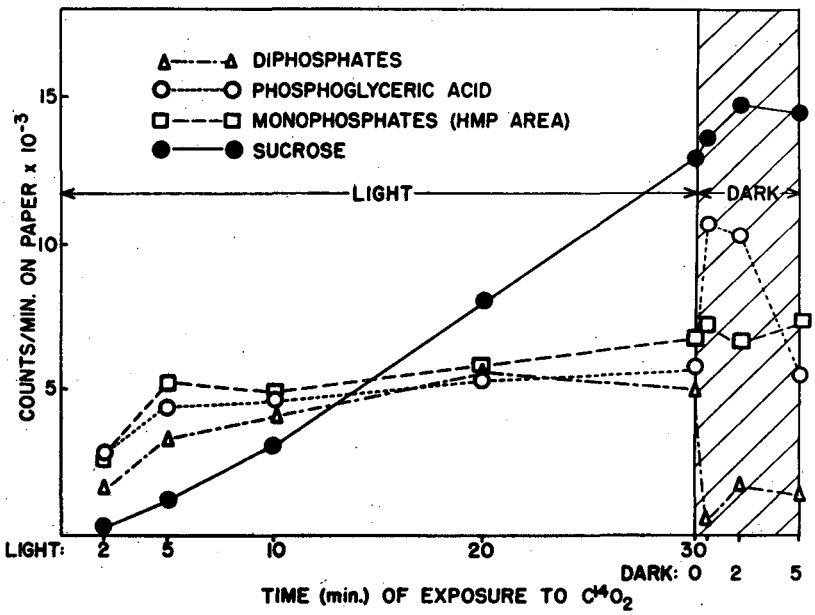

DIPHOSPHATES
PHOSPHOGLYCERIC ACID
MONOPHOSPHATES (HMP AREA)
SUCROSE
LIGHT
DARK
COUNTS/MIN. ON PAPER x 10⁻³
15
10
5
LIGHT: 2 5 10 20 30
DARK: 0 2 5
TIME (min.) OF EXPOSURE TO C¹⁴O₂

Fig. 15

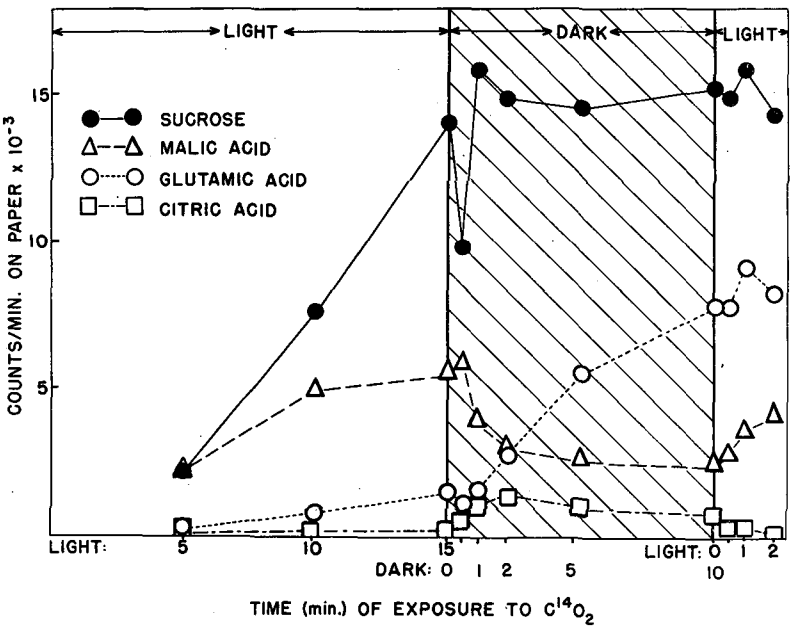

LIGHT
DARK
LIGHT
SUCROSE
MALIC ACID
GLUTAMIC ACID
CITRIC ACID
COUNTS/MIN. ON PAPER x 10⁻³
15
10
5
LIGHT: 5 10 15
DARK: 0 1 2 5 10
LIGHT: 0 1 2
TIME (min.) OF EXPOSURE TO C¹⁴O₂

Fig. 16

Table VII

Steady-State Concentrations of Some Compounds Involved in the Photosynthesis Cycle

Scenedesmus, experimental conditions as in Figure 14

Substance	μ moles/cc. cells*
Phosphoglyceric acid	1.4
Dihydroxyacetone phosphate	0.17
Fructose phosphate	0.12
Glucose phosphate	0.4
Mannose phosphate	0.05
Sedoheptulose phosphate	0.18
Ribulose diphosphate	0.5
Alanine	0.2

* Volume measured as wet packed cells

(2) The fact that the activity vs. time curves show a definite yet low slope for as long as thirty minutes can be taken to indicate that the breakdown of carbohydrates continues throughout the illumination, i.e., their formation from photosynthetic intermediates is reversible. Thus, there are two sources of the intermediates: (a) the carbon dioxide fed; the amount of compounds formed from this source reaches the maximum specific activity in five to ten minutes; (b) the carbohydrate pool of the cells; the amount formed from this source is labeled only slowly since the specific activity of the carbohydrate pool rises slowly due to the large size of the pool.

(3) Other compounds (sucrose, malic acid and glutamic acid) show almost constant rate of labeling during the whole period of photosynthesis. For this and other reasons it is clear that these compounds are not in the photosynthesis cycle, but are formed during the photosynthesis at a constant rate. Their large reservoirs in the cells are labeled only slowly.

(4) When illumination is interrupted there appears a sudden great increase in the concentration of phosphoglyceric acid (followed by a slow

decrease after two minutes), and an almost complete depletion of the diphosphate area. Analysis of the monophosphate area showed that the amount of sedoheptulose phosphate decreased also (cf. Table VIII.) The concentration of malic acid decreases as well. The rate of labeling of glutamic acid is increased greatly after a short induction period; citric acid, which contains little activity during the whole light period, shows a sudden increase in the dark, followed by a slow decrease. The labeling of sucrose continues at the same rate as in light for about two minutes, after which it is stopped almost completely.

Both experiments gave essentially the same picture for most of the compounds. However, in the second experiment the diphosphate area, which in the first contained almost the same number of counts as phosphoglyceric acid during the light, had only about 15 percent of it in this second run. This value dropped to 5 percent in the dark. The phosphoglyceric acid showed a hardly significant rise in the dark during the first two minutes, but again a slow decrease after five minutes. Although we do not know why in this experiment the concentration of ribulose diphosphate was so low in the light, the coincidence with the lack of increase of phosphoglyceric acid points to a connection between both effects.

(5) In the light following the dark, the diphosphates, phosphoglyceric and malic acid increase again.

Table VIII

Phosphatase Treatment of HMP Area after 30 Minutes Photosynthesis and 30 Minutes Photosynthesis Followed by 5 Minutes Dark

Substance	Number of counts/min. on Paper	
	30 min. PS	30 min. PS 5 min. D
Glucose	3140	4280
Fructose	910	1040
Sedoheptulose	1600	1210*
Mannose	460	

* An appreciable fraction of this count is certainly hexose so that one may estimate a maximum value of the heptose at around 800 counts/min.

The effect of dark on the labeling of glutamic and citric acid was studied in the following experiments: 0.2 cc. wet packed algae (Chlorella pyrenoidosa) were suspended in 200 cc. distilled water, illuminated in a flat circular vessel of 1 cm. thickness by incandescent lights through an infra red filter (intensity 1.6×10^5 ergs/cm.^{2})(sec.) and aerated with 0.08 percent carbon dioxide in air. The low concentration of cells was chosen to avoid shading of cells in the suspension, so that during the light period all the cells were illuminated continually.

After one-half hour, the aeration bubbler was taken out and a suitable amount of radioactive sodium bicarbonate solution added. (The algae, which were grown in slightly acid medium, had enough buffering capacity to convert the bicarbonate to carbon dioxide.) The vessel was immediately stoppered and shaken in the light. After one minute, the suspension was drained into a darkened flask, and after another minute poured into four times its volume of boiling alcohol. Control samples were treated in the same way, but kept in the light, in contact with radioactive carbon dioxide for one and two minutes, respectively. The analysis of the fixed radioactivity was performed by paper chromatography and radioautography with the technique already described. The results are shown in Fig. 17.

Discussion

It has already been pointed out that photosynthesis is not a mere reversal of respiration; this was supported by the observation that the carbon of newly formed photosynthetic intermediates is not available for respiration while the light is on.[1,2] We may thus represent the relationship between photosynthesis and respiration by the following scheme (see Fig. 18): The labeling of the Krebs cycle intermediates through the storage products (carbohydrates, fats, proteins) of the cells is a slow process, due to the relatively large size of the storage pools. The fact that the photosynthesis intermediates find their way into the tricarboxylic acid cycle very rapidly after the light is switched off means that there is another connection between the two cycles which is blocked as long as the light is on but becomes accessible in the dark.

The sudden rise in phosphoglyceric acid and the decrease in ribulose diphosphate and sedoheptulose phsophate in the dark period, together with the observation that the dark rise in phosphoglyceric acid is absent when the ribulose diphosphate concentration was low during the light, confirms the earlier suggestion that the phosphates of the C_7 and C_5 sugars are precursors of the C_2 carbon dioxide acceptor.[3]

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1. M. Calvin, J. Chem. Education 26, 639 (1949).

2. J. W. Weigl, P. M. Warrington and M. Calvin, J. Am. Chem. Soc., 73, 5058 (1951).

3. A. A. Benson, J. A. Bassham, M. Calvin, A. G. Hall, H. E. Hirsch, S. Kawaguchi, V. H. Lynch and N. E. Tolbert, J. Biol. Chem., 196, 703 (1952).

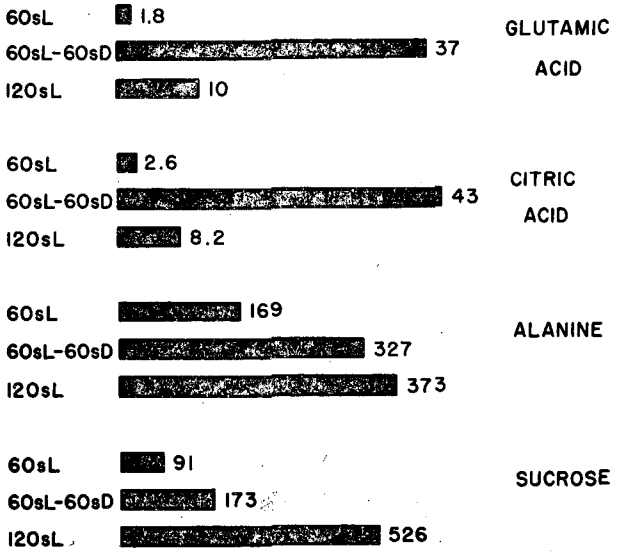
60sL
60sL-60sD
120sL
1.8
37
10
GLUTAMIC ACID
60sL
60sL-60sD
120sL
2.6
43
8.2
CITRIC ACID
60sL
60sL-60sD
120sL
169
327
373
ALANINE
60sL
60sL-60sD
120sL
91
173
526
SUCROSE

Fig. 17

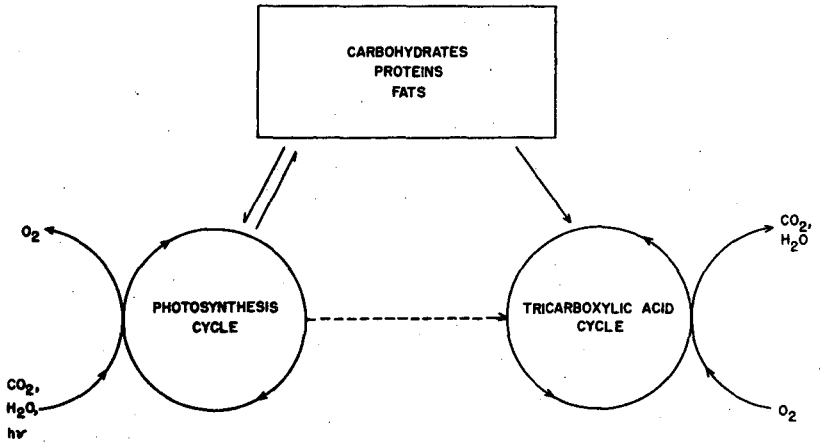
CARBOHYDRATES
PROTEINS
FATS
O_2
PHOTOSYNTHESIS CYCLE
CO_2, H_2O, $h\nu$
TRICARBOXYLIC ACID CYCLE
CO_2, H_2O
O_2

Fig. 18

The Degradation of Sedoheptulose

L. Daus, A. Zweiffler Harris, M. Calvin and J. A. Bassham

The problem of a complete degradation of sedoheptulose is still being attacked in the hope of eventually finding the rates at which individual carbon atoms in the sugar are labeled during photosynthesis. Treatment with periodate liberates formic acid from carbon No. 4 of sedoheptulose and previously reported experiments indicate that the percent of carbon in position 4 increases as the time of photosynthesis decreases. A new degradation of sedoheptulosan from 6 sec. photosynthetic soy beans gave 24 percent of the activity in carbon No. 4, a much lower value than expected from the previous results. Since the reproducibility of growth rate in leaves grown under different conditions has not been investigated, it is now proposed to conduct a time series on leaves grown at the same time with a number of points in the 1 second to 10 second PS range, and to examine the activity in carbon 4.

Attempts to obtain other carbon atoms as isolated fragments have taken several directions. Direct periodate oxidation of sedoheptulose as reported previously gives some indication of distribution, but is not a clean-cut reaction. Thus far no method has been found to hydrolyze the ring structure resulting from periodate oxidation of sedoheptulosan in good enough yield to use in small scale degradations. Periodate oxidation of the osazone gives carbon 7 alone, carbons 4, 5 and 6 together and carbons 1, 2 and 3 together. Oxidation of the mesoxaldehyde osazone containing carbons 1, 2 and 3 gives 1 phenyl-4-phenylhydrazone-pyrazolone-5. It is hoped that hydrogenolysis of this compound may give distinguishable fragments. The literature reports oxidation of the mesoxaldehyde osazone with alcoholic KOH to give glyoxalosazone. However, the product obtained by us under these conditions was consistently 4-benzalazo-1-phenylpyrazole. A similar oxidation of glucosazone gave some glyoxalosazone but in too small a yield to be useful in a degradation procedure. Further investigation of possible ways to split the mesoxaldehyde osazone is under way since such a degradation would provide a method for examining not only sedoheptulose but also ribulose.

Identification of Sucrose Phosphate in Sugar Beet Leaves

J. G. Buchanan

The synthesis of sucrose in green plants has for long been a problem to plant biochemists. The sucrose phosphorylase enzyme from Pseudomonas saccharophila will catalyse the reaction,

$$\text{sucrose} + H_3PO_4 \rightleftharpoons \alpha\text{-D-glucose-1-phosphate} + \text{D-fructose}$$

and Hassid, Doudoroff and Barker[1] have isolated sucrose synthesized by this enzyme. It appears, however, that this is not the mechanism by which sucrose is synthesized in the green plant. It has not been possible to show the presence of this enzyme in higher plants. In Part IV[2] of this series it was shown that when Chlorella were allowed to photosynthesize in radioactive carbon dioxide, sucrose was the first free sugar to be formed. This was interpreted to mean that in sucrose synthesis in higher plants, only phosphorylated derivatives of sugars were involved, probably yielding a sucrose phosphate as the first sucrose-containing product. There is reason to suspect, from work on the utilization of sucrose by microorganisms, that there is a naturally occurring sucrose phosphate in nature and that it is the fructose moiety which phosphorylated. More recently, Putman and Hassid[3], working with leaf punches, have obtained evidence for the formation of a phosphorylated sucrose derivative in sucrose synthesis.

We have examined the "hexose monophosphates" produced during photosynthesis in $C^{14}O_2$. These were treated with an invertase-free phosphatase preparation and subjected to paper chromatography. In several cases, there were only minute traces of sucrose formed by this treatment but in sugar beet (5 minutes in $C^{14}O_2$) there was an appreciable quantity. It was identified by co-chromatography, and enzymatic hydrolysis to glucose and fructose, themselves identified by co-chromatography.

When this "hexose monophosphate" sample was subjected to chromatography in t-butanol/picric acid/water, radioactive areas corresponding to glucose-6-phosphate, fructose-6-phosphate, sedoheptulose and mannose phosphates, and sucrose phosphate were obtained. The sucrose phosphate gave sucrose on phosphatase treatment, and on acid hydrolysis, glucose and a fructose phosphate were produced. The latter did not co-chromatograph with fructose-6-phosphate.

The sucrose phosphate in sugar beet leaves has the probable structure as shown in Fig. 19.

It would seem that in sucrose synthesis in green plants there are two possible mechanisms. Glucose-1-phosphate might react with fructose-1-phosphate to give sucrose phosphate, which would be dephosphorylated to sucrose. Alternatively, sucrose phosphate synthesis might be envisaged to occur through uridine diphosphate glucose,[4] which becomes labeled shortly

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1. W. Z. Hassid, M. Doudoroff and H. S. Barker, J. Am. Chem. Soc., 66, 1416 (1944).

2. M. Calvin and A. A. Benson, Science, 109, 140 (1949).

3. E. W. Putman, Thesis, Ph.D., University of California, June, 1952.

4. J. G. Buchanan, et al., "Phosphorus Metabolism, II" Johns Hopkins Press, 1952.

before sucrose in kinetic experiments with $C^{14}O_2$.[5] The uridine diphosphate glucose may be formed from glucose-1-phosphate by a series of reactions analagous to those proposed by Kornberg.[6] These alternative schemes are summarized in Fig. 20.

Although it has not been possible to find appreciable quantities of the sucrose phosphate in many of the plants studied, we believe that this is probably due to its reservoir size being very small and that the mechanism of sucrose synthesis is very similar in green leaves and in algae.

Experimental

The chromatographic techniques were those described previously.[7] The solvent t-butanol-picric acid was that of Hanes and Isherwood,[8] as modified by Moyle and Mitchell to avoid crystallization of the picric acid of the chromatogram during chromatography: t-butanol (80 cc.), water (20 cc.), picric acid (2 g.). The enzyme preparations used were "polidase-S" (Schwarz Laboratories, Inc.) and "Phosphatase" (General Biochemicals, Inc.). Both were used in 1 percent solution, without buffer. The incubations were carried out at 35° C. under toluene, with 200 μg. of enzyme, for periods of 24-72 hours. The "Phosphatase" was shown to be devoid of invertase activity.

Detection of a Phosphorylated Sucrose Derivative

The source of radioactive compounds was an extract from sugar beet leaves which had photosynthesized for 5 minutes in $C^{14}O_2$. The radioactive area used was that designated "hexose monophosphates" on chromatograms in the standard solvents (Phenol: n-butanol-propionic acid).

The "hexose monophosphate" area was extracted and hydrolyzed with phosphatase. On re-chromatography, besides the usual monosaccharides, there appeared a spot in a position characteristic of a disaccharide (Fig. 21).

In one experiment, the unknown spot was rechromatographed with carrier sucrose (100 γ). After exposure to film, the chromatogram was sprayed with aniline-trichloroacetic acid and heated at 100° for 5 minutes. It was then sprayed with orcinol-trichloroacetic acid (oricinol (0.5 g.), trichloroacetic acid (15 g.), t-butanol (90 cc.), water (10 cc.)) and heated

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5. A. A. Benson, S. Kawaguchi, P. Hayes and M. Calvin, J. Am. Chem. Soc., in press.

6. A. Kornberg, "Phosphorus Metabolism, I" Johns Hopkins Press, 1951.

7. A. A. Benson, et al., J. Am. Chem. Soc., 72, 1710 (1950).

8. C. S. Hanes and F. A. Isherwood, Nature, 164, 1107 (1949).

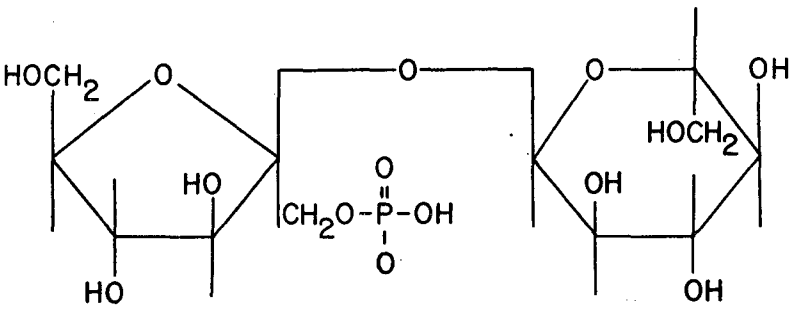

Fig. 19

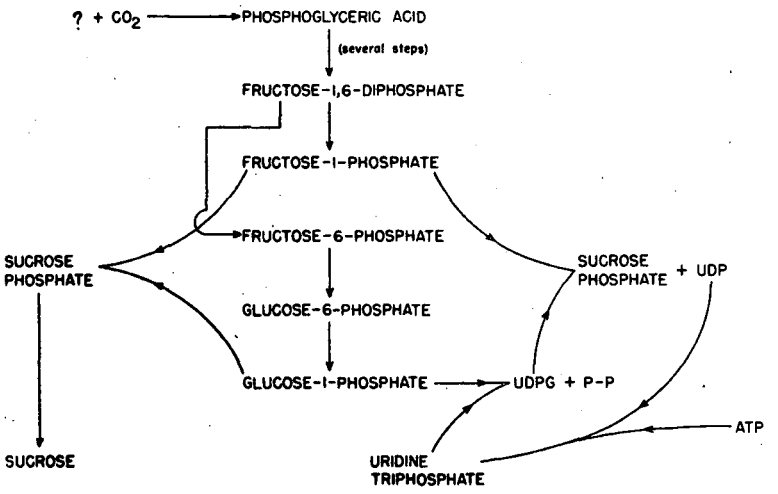
? + CO_2
PHOSPHOGLYCERIC ACID
(several steps)
FRUCTOSE-1,6-DIPHOSPHATE
FRUCTOSE-1-PHOSPHATE
FRUCTOSE-6-PHOSPHATE
SUCROSE PHOSPHATE
GLUCOSE-6-PHOSPHATE
GLUCOSE-1-PHOSPHATE
UDPG + P-P
SUCROSE PHOSPHATE + UDP
ATP
SUCROSE
URIDINE TRIPHOSPHATE
SCHEME 1
SCHEME 2

Fig. 20

in the same way as before. The brown spot which appeared was completely coincident with the darkened area on the radioautograph.

In another experiment the disaccharide spot was cut out and treated with "Polidase-S" (known to have invertase activity). The hydrolysate was chromatographed with glucose and fructose carrier. After exposure to film, the chromatogram was sprayed with ammoniacal silver nitrate (5 percent $AgNO_3$ in methanol) and heated. The black spots were coincident with the dark areas on the film. (Fig. 22).

The Separation of the Sucrose Phosphate from Monosaccharide Phosphates

The "hexose monophosphate" area was extracted and treated with a small quantity of the acid form of Dowex-50. Glucose-6-phosphate (250 γ of Ba salt, treated with acid Dowex-50) was added as carrier and the solution chromatographed as a band in t-butanol-picric acid. Four bands were produced. (Fig. 23.) The slowest moving band was extracted and chromatographed in phenol to remove the picric acid and any free sugars which might be present. The only free sugar observed had the same Rf value in phenol as glucose. When the phosphate was extracted from the phenol chromatogram and treated with "Phosphatase," followed by chromatography, three sugars, with the chromatographic coordinates of glucose, fructose and sucrose were produced. (Fig. 24.)

In another experiment, using the same sample of radioactive hexose monophosphates, the sucrose phosphate, freed from picric acid by chromatography in phenol after partial separation from the other phosphates in t-butanol-picric acid, was divided into two parts (a) and (b). Sample (a) was rechromatographed in two dimensions: phenol (first direction), t-butanol-picric acid (second direction). Under these conditions, three major spots were produced (Fig. 25). One co-chromatographed with glucose-6-phosphate, and another with fructose-6-phosphate. The other, the most radioactive of the three, behaved as sucrose phosphate.

Sample (b) was mixed with the same carriers as (a) and hydrolyzed with 0.1 N HCl at 100° for 5 minutes. The hydrolysate was then chromatographed in the same way as above. Four major (and several minor) spots were visible on the radioautograph: glucose-6-phosphate, fructose-6-phosphate, glucose (all identified by co-chromatography) and another phosphate close to but not coincident with the fructose-6-phosphate (Fig. 26). Only a very small amount of radioactive fructose was present, although spraying of the chromatogram showed that practically all of the carrier fructose was present. Table IX shows the relative radioactivities of the compounds (approximate).

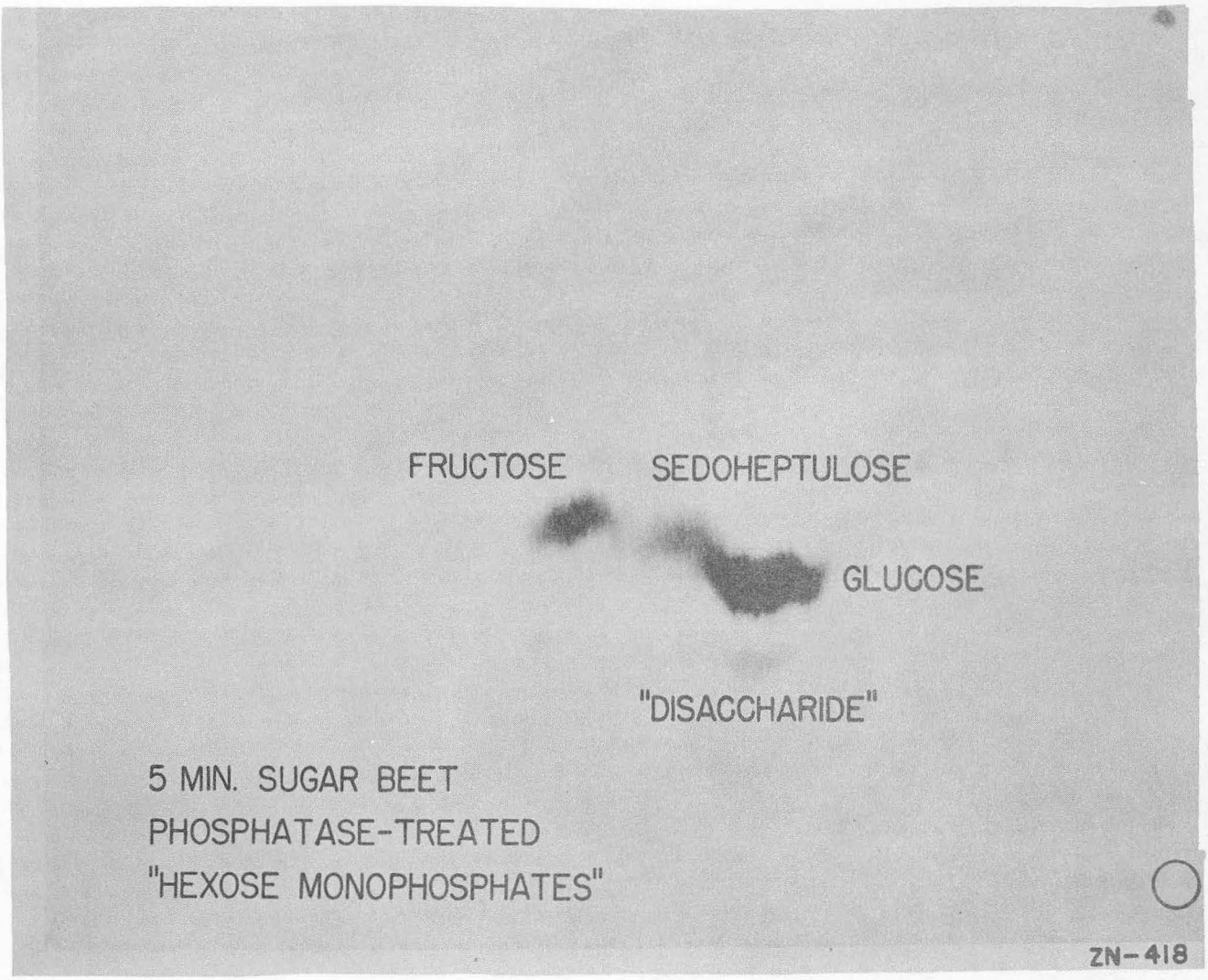
FRUCTOSE
SEDOHEPTULOSE
GLUCOSE
"DISACCHARIDE"
5 MIN. SUGAR BEET
PHOSPHATASE-TREATED
"HEXOSE MONOPHOSPHATES"
ZN-418

Fig. 21

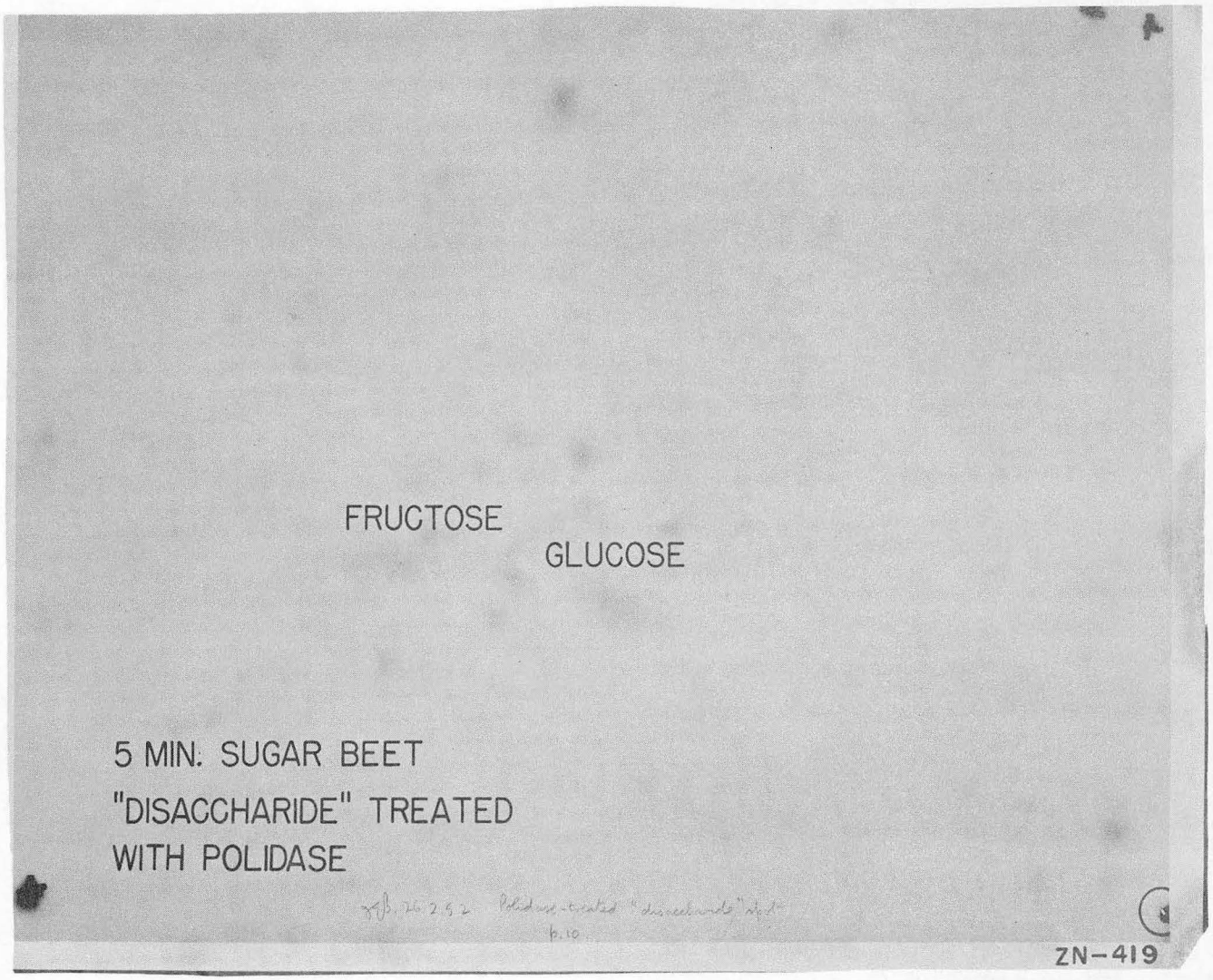
FRUCTOSE
GLUCOSE
5 MIN. SUGAR BEET
"DISACCHARIDE" TREATED
WITH POLIDASE
ZN-419

Fig. 22

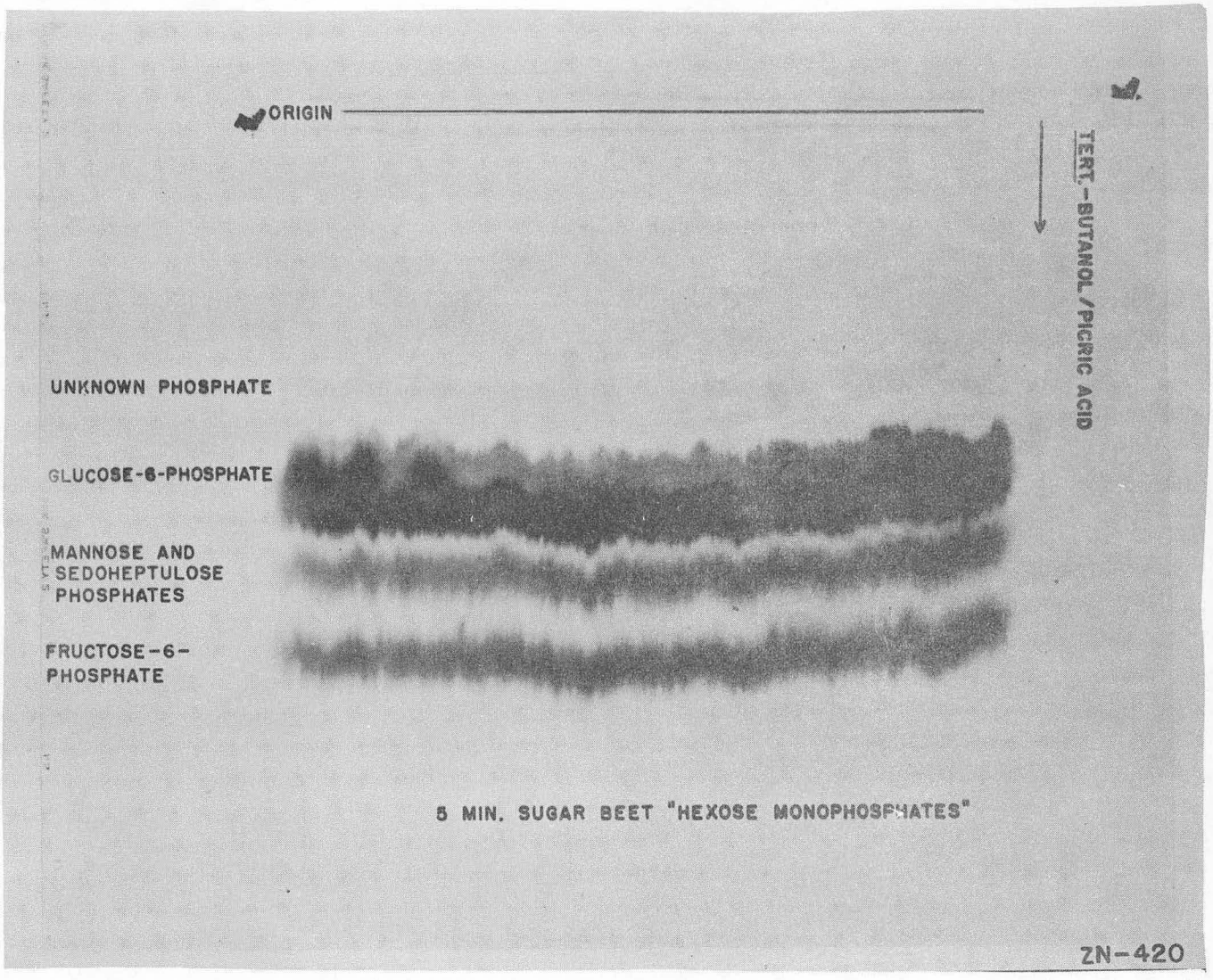
ORIGIN
TERT.-BUTANOL/PICRIC ACID
UNKNOWN PHOSPHATE
GLUCOSE-6-PHOSPHATE
MANNOSE AND
SEDOHEPTULOSE
PHOSPHATES
FRUCTOSE-6-
PHOSPHATE
5 MIN. SUGAR BEET "HEXOSE MONOPHOSPHATES"
ZN-420

Fig. 23

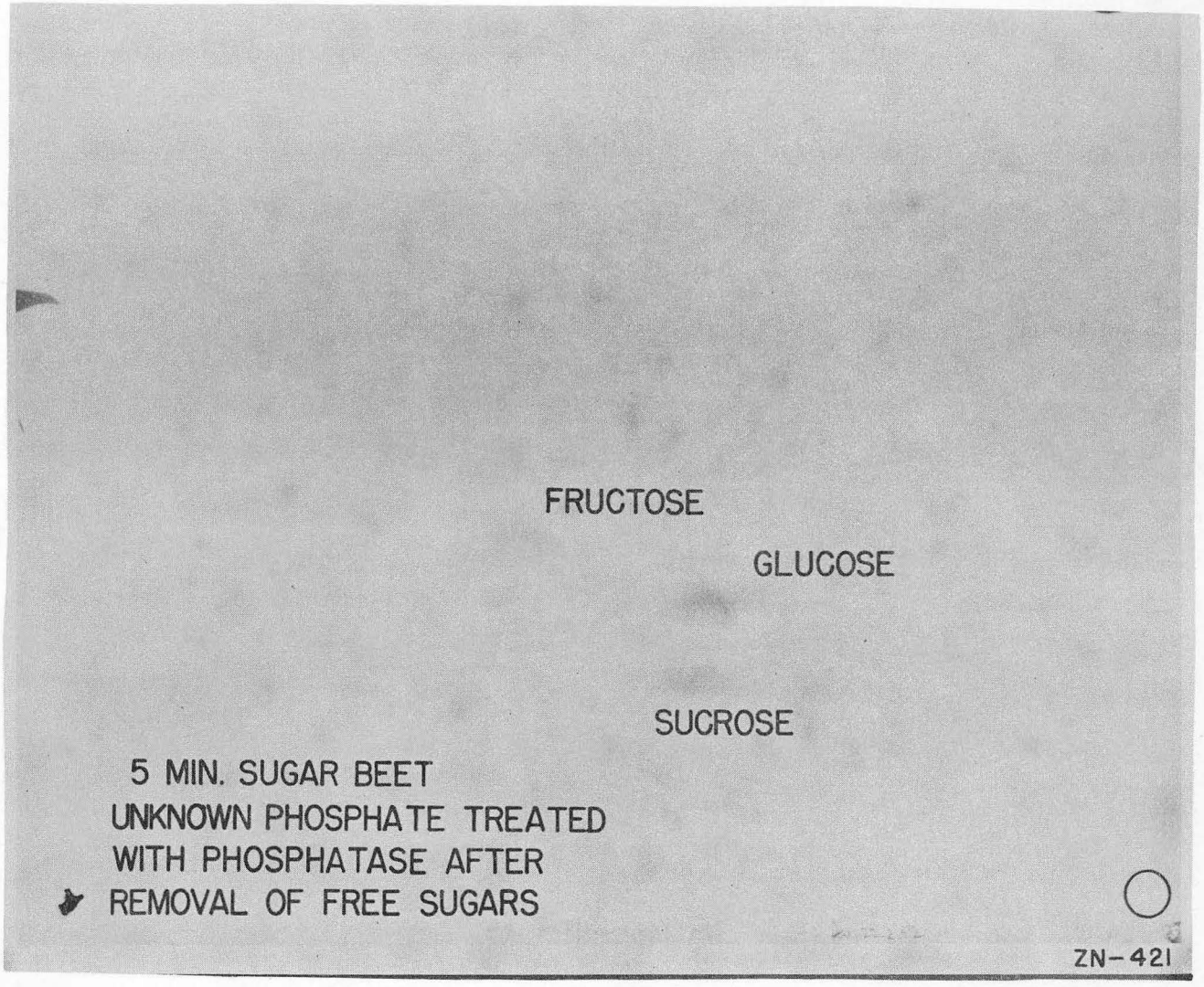
FRUCTOSE
GLUCOSE
SUCROSE
5 MIN. SUGAR BEET
UNKNOWN PHOSPHATE TREATED
WITH PHOSPHATASE AFTER
REMOVAL OF FREE SUGARS
ZN-421

Fig. 24

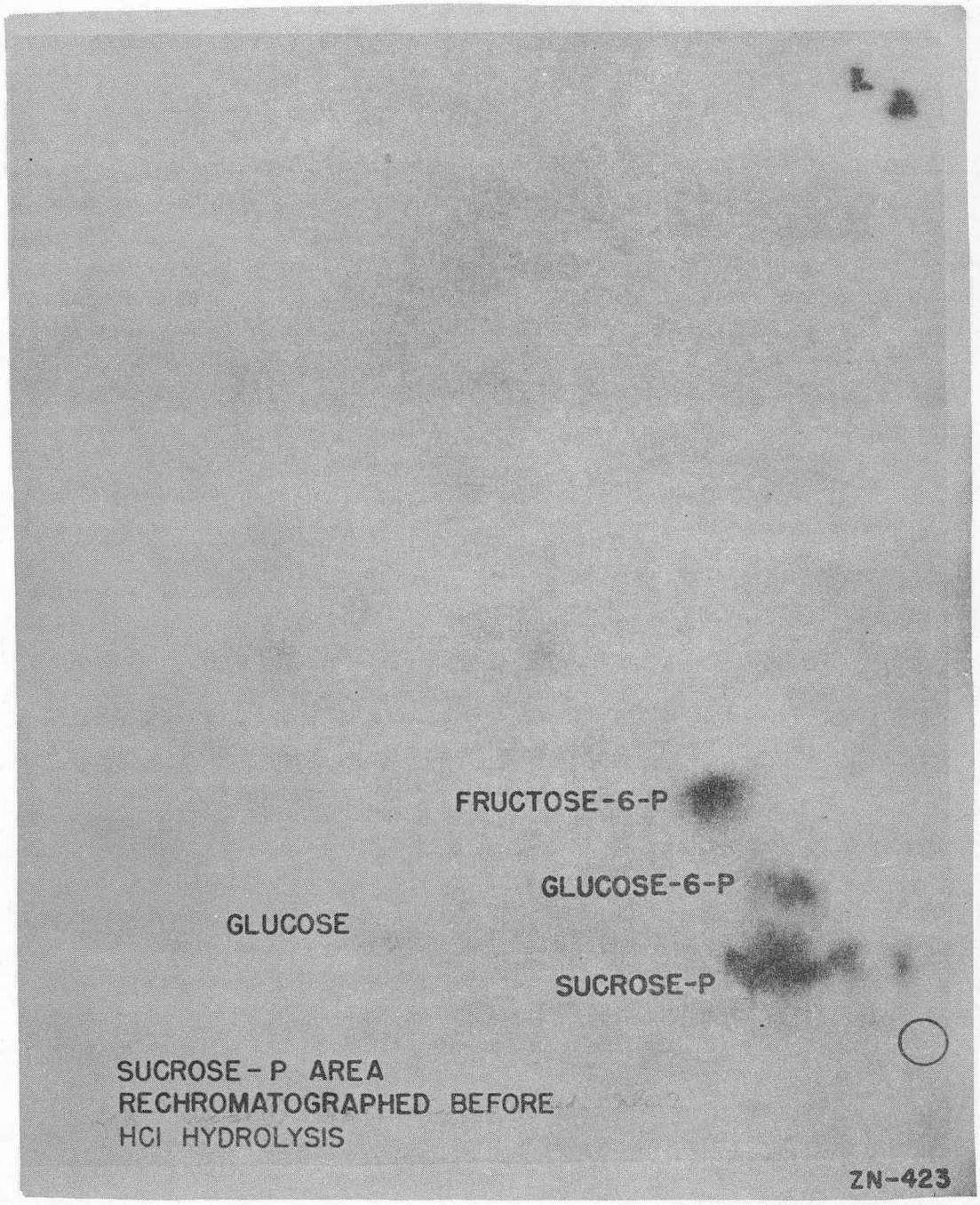
FRUCTOSE-6-P
GLUCOSE-6-P
GLUCOSE
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED BEFORE
HCl HYDROLYSIS
ZN-423

Fig. 25

Table IX

Compound	c.p.m. (a)	c.p.m. (b)
Sucrose phosphate	180	0
Glucose-6-phosphate	80	80
Glucose	20	120
Fructose-6-phosphate	110	110
Fructose-1-phosphate(?)	0	90
Fructose	0	20

The glucose-6-phosphate and the fructose-6-phosphate probably arise from a small amount of cross contamination between them and sucrose phosphate on the original picric acid chromatogram. Since practically all of the sucrose phosphate had been hydrolyzed and only half of the radioactivity originally present in it appears as glucose, almost all of the rest being in the new phosphate appearing next to fructose-6-phosphate, it follows that this phosphate is a fructose phosphate different from fructose-6-phosphate. The amount of free fructose present is roughly about 1/10 of that of the fructose phosphate suggesting that the rate of hydrolysis of this fructose phosphate corresponds to that of fructose-1-phosphate. This would lead to the tentative structure for the sucrose phosphate shown in Fig. 19.

Separation of Phosphorus-Containing Algae Metabolites

M. Goodman

An examination of some of the phosphorylated compounds in algae has been undertaken as follows: 60 cc. of Scenedesmus were killed in 500 cc. of 80 percent boiling ethanol. This 80 percent extraction was repeated once more. The cell residues were then extracted with five 500 cc. portions of 20 percent ethanol.

Both the 80 percent and 20 percent extracts were concentrated separately under vacuum at room temperature to 20 ml. The 80 percent extract was then treated with petroleum ether and the purified 80 percent

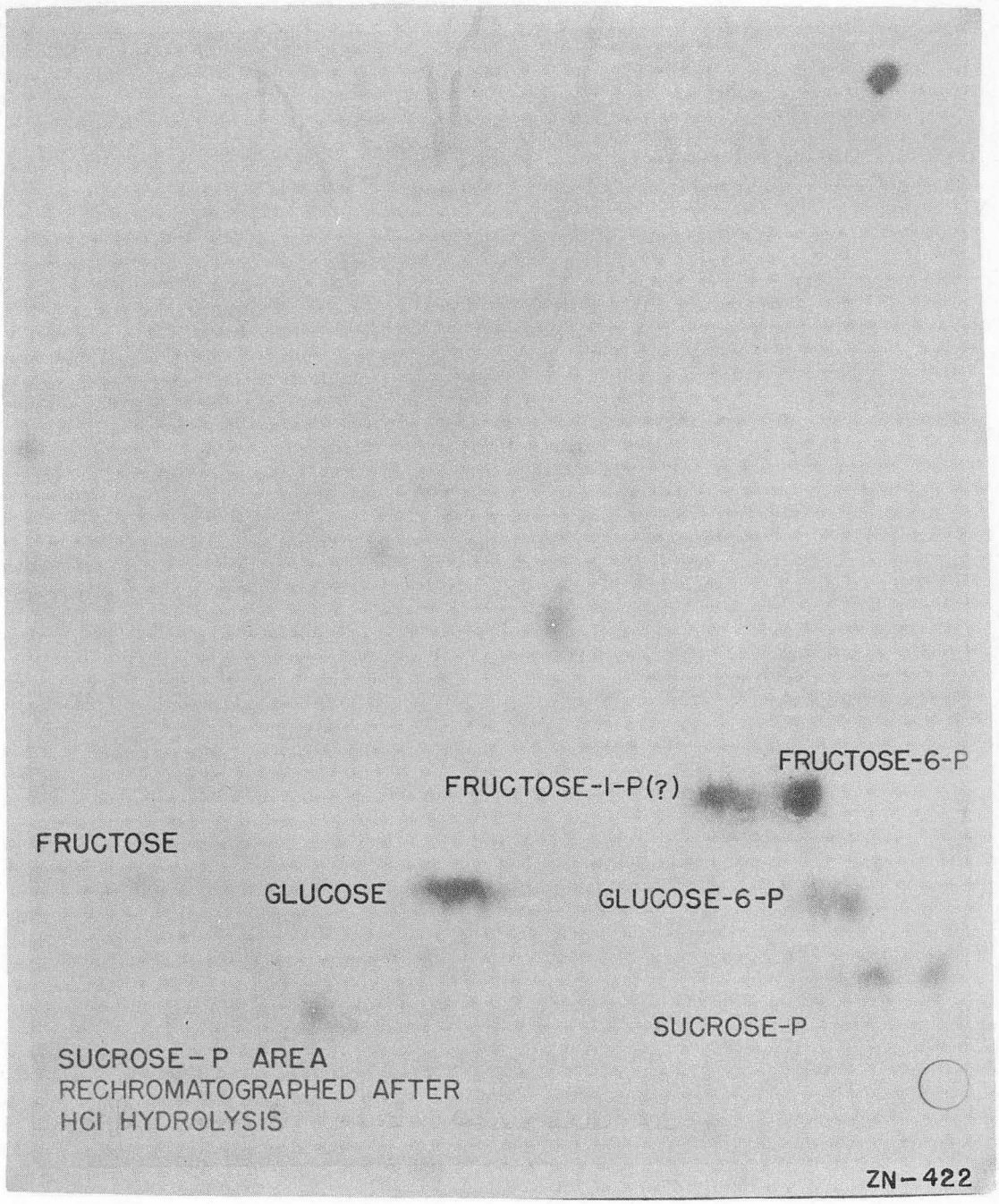
FRUCTOSE-6-P
FRUCTOSE-1-P(?)
FRUCTOSE
GLUCOSE
GLUCOSE-6-P
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED AFTER
HCl HYDROLYSIS
ZN-422

Fig. 26

extract was mixed with the concentrated 20 percent extract. In addition, the extracts obtained from 10 and 25-minute experiments during which Scenedesmus were exposed to radiophosphate were added.

The combined extracts were placed on a Dowex-1 column (chloride form) and eluted with 0.2 N Cl^- + 0.05 N H^+. Four phosphorus-containing peaks resulted. These are, in the order in which they came off the column: Peak (1) sugar monophosphates and nucleotides; peak (2) ortho phosphate; peak (3) phosphoglyceric acid; peak (4) unknown.

The material from (4) was subjected to hydrolysis in 0.1 N hydrochloric acid at 100° C. It hydrolyzed much more rapidly than fructose-1, 6-diphosphate under the same conditions. The unknown hydrolyzed to the extent of 50 percent of the phosphorus and then gave essentially no further hydrolysis showing it is a diphosphate. Paper chromatography of the diphosphate after exposure to phosphatase activity indicates that is might be ribulose diphosphate.

Distribution of Phosphorus Radioactivity in Algae Metabolites

D. F. Bradley and M. Goodman

The distribution of radioactivity in metabolities of a photosynthetic alga (Scenedesmus) exposed to $KH_2P^{32}O_4$ in light and dark has been determined by paper chromatography and radioautography. The following compounds have been identified: orthophosphate, phosphoglyceric acid (PGA), fructose-6-phosphate, glucose-6-phosphate, glucose-1-phosphate, phosphodihydoxyacetone, phosphoenolpyruvate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine diphosphate glucose (UDPG) and sugar diphosphates.

The rate of incorporation of radioactivity into the above organic compounds has been studied. The only two which show significant changes are ATP and PGA. ATP has a higher percentage of the radioactivity in the dark than in the light while PGA is larger in the light than in the dark. This indicates that the recently incorporated radiophosphate is involved in the photosynthetic cycle. Furthermore, if one plots that percentage of the total fixed activity which is ATP activity against time, one obtains a curve with an initial negative slope. This points to ATP as being a primary product of phosphate incorporation.

In addition, the early labeling of UDPG and ADP indicates that they too are involved in phosphate metabolism.

Radioactive Constituents of Ethanol Distillates after Photosynthetic Fixation of Labeled Carbon Dioxide

D. MacDougall and P. M. Hayes

A preliminary study of the amount of radioactivity and types of radioactive compounds in the distillate obtained on concentrating after photosynthetic fixation of carbon has been carried out. Samples of a 1 percent suspension of algae in distilled water were preilluminated for 15 minutes and allowed to fix radioactive carbon for three minutes. The cellular material was killed by mixing with five volumes of absolute ethanol previously cooled to 0° C. The mixture was immediately cooled in an acetone-dry ice bath to 0° C. and stored in the deep freeze. Approximately 75 percent of the fixed carbon was extracted from the algae by this method. The cell free extract was concentrated at a pressure of ca. 5 mm., the receiver being held in a dry ice-acetone bath. Material not trapped by the dry ice bath was caught in a trap placed in liquid nitrogen. A study of the contents of this trap with an ionization chamber showed that most, if not all, of the unassimilated radioactive carbon dioxide was present in this trap. Three carbon fixation experiments were carried out, two on Scenedesmus and one on Chlorella.

Samples of each distillate were combusted and the carbon dioxide formed was trapped in sodium hydroxide and precipitated as barium carbonate. The radioactivity of these samples was determined by the use of ionization chambers. Although the results are variable, they indicate that an amount of activity amounting to approximately 0.5-1.5 percent of the total fixed is present in these distillates.

Samples of each of the above distillates were treated with carrier formaldehyde and acetaldehyde and the dimedon and 2,4-dinitrophenylhydrazine derivatives were precipitated. These were dissolved in ethanol, plated and counted. A small amount of activity was found in these precipitates even after recrystallization. This represented only a portion of the total activity in the distillates. Samples of each distillate were made alkaline with sodium hydroxide and plates. Two of the distillates showed no activity while the third showed a small amount of activity. This means that in two cases, at least, there was no radioactive carbon dioxide in the distillates.

The positive identification of the small amount of aldehydic material present would be an exceedingly difficult task. Although this may be important in the photosynthetic mechanism there is, at present, no evidence that the material is actually associated at all with photosynthesis and it may well be that it has arisen from the breakdown of the other radioactive compounds.

The Effect of pH on Carbon Fixation by Scenedesmus

D. MacDougall and P. M. Hayes

During the course of experiments designed to determine the effect of certain biologically active chemicals on the rate of carbon fixation by photosynthesizing algae it was found that there was considerable variation due to changing the pH of the suspending medium with hydrochloric acid or with sodium hydroxide. Furthermore, experiments carried out on several different days showed that the maximum fixation was very variable. Therefore, it was deemed necessary to investigate this effect before proceeding to the examination of various types of chemicals.

A study of carbon fixation by Scenedesmus in phthalate buffers has been carried out. A final buffer concentration of 0.01 M was found to be sufficient. It was found that the length of time of contact with the buffer solution (from ten minutes to three hours) had no effect on the amount of carbon fixed. Samples of algae were suspended in distilled water, the pH of which had been changed with dilute hydrochloric acid or sodium hydroxide. Carbon fixation experiments were carried out on each sample at four different pH's adjusted with phthalate buffers. The initial pH of the suspending medium did not appear to affect the final fixation of carbon when the pH was altered by adding a suitable buffer. At present an attempt is being made to grow Scenedesmus in phthalate buffers at various pH's.

Reductive Carboxylation Study

W. McRary and M. Calvin

A preliminary study has been made of a postulated reaction in which certain metabolically important keto compounds may undergo reductive carboxylation. "Malic" enzymes catalyzing such a reaction for pyruvate have been prepared from wheat germ, spinach, sugar beet and barley leaves by fractional precipitation with ammonium sulfate. In tests using a system containing manganous ions, malate and TPN (Triphosphopyridine nucleotide), "malic" activity has been demonstrated by following the reduction of TPN spectrophotometrically. However, TPN-destroying enzymes appear to be so closely associated with "malic" enzymes that demonstration of the reversibility of the malic-pyruvic reaction has not proved feasible by this method.

Incorporation of the $NaHC^{14}O_3$ into the above system, containing malate, results in the fixation of C^{14}. This fixation is TPN-dependent and in all probability indicates reversibility of the malic-pyruvic reaction. Inclusion of dihydroxyacetone phosphate does not increase the fixation. If glucose-6-phosphate and its dehydrogenase are used to provide reduced TPN, and dihydroxyacetone phosphate included, no increased

fixation occurs as a result of the addition of the phosphate. Similarly, reduced DPN in the presence of the wheat germ enzyme does not appear to effect reductive carboxylation of this compound. However, final conculsions as to the reactivity of the triose must await trials with purer samples.

Degradation of Ribulose Diphosphate

A. T. Wilson and M. Calvin

The degradation of ribulose diphosphate to phosphoglyceric acid and phosphoglycolic acid is being studied. The two prinicpal problems are: (1) The adsorption of the degradation products on the origin when an attempt is made to separate them by chromatography; (2) difficulty in getting good yields on the oxidative degradation of the diphosphate. The first problem has been solved by saturating the chromatographic box with water and allowing the paper to come to equilibrium with this atmosphere before applying the degradation mixture. The second problem has been attacked by assuming that the reaction is a reverse aldol type followed by the oxidation of the aldehydes formed. At first, when high pH's and low volumes were used, there seemed to be much polymerization of the products. This has been improved considerably by diluting the degradation mixture and reducing the concentration of alkali.

It has been established that the 80 percent ethanol extract of the dead algae removes very little ribulose diphosphate and that the following 20 percent ethanol extract contains principally ribulose diphosphate. This observation is of great help in isolating the starting material for the degradation.

Incorporation of Carbon-14 into Ribonucleic Acid of a Photosynthesizing Alga

James L. Fairley, Barbara J. Krueckel and Lorel L. Daus

The elucidation of the pathway of biosynthesis of ribonucleic acid (RNA) has interested many workers in recent years. The nature of the precursors of the carbon atoms of uric acid in the pigeon (glycine, formic acid and carbon dioxide) has been well established by Sonne, Buchanan and co-workers[1,2], and these compounds have been shown by Heinrich and Wilson to play a similar role in the synthesis of nucleic acid purines in the

1. J. C. Sonne, J. M. Buchanan and A. M. Delluva, J. Biol. Chem., 173, 69 (1948).

2. J. M. Buchanan, J. C. Sonne and A. M. Delluva, J. Biol. Chem., 173, 81 (1948).

rat[3]. The utilization of preformed purines for nucleic acid synthesis in the rat has been extensively studied by Brown and his co-workers[4,5,6]. The synthesis of the pyrimidine components of RNA has received less attention. It has been shown that carbon dioxide is a precursor of the ureide carbon of uracil (3). Orotic acid, first studied in connection with nucleic acid synthesis by Mitchell et al.[7] has been shown by Hammarsten's group to be incorporated into the pyrimidines of the nucleic acids of the rat[8], and Reichard has reported experiments[9] indicating that orotic acid is a natural intermediate in the synthesis of RNA pyrimidines. Work with pyrimidine-deficient Neurospora mutants[10] has indicated that the free pyrimidine bases are not precursors of the polynucleotides and that the interconversion of uracil and cytosine took place only when these bases were combined with ribose. Rats, too, do not use dietary uracil[11] or cytosine[12] for RNA synthesis but do use the nucleosides[13]. Many tracer experiments with the various precursors of the purine ring have shown that in a wide variety of organisms nucleic acid guanine becomes labeled more rapidly than adenine[3,14,15]. With orotic acid--C^{14} as a pyrimidine precusor the nucleic acid uracil was found to be labeled two to four times as fast as cytosine.[16]

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3. M. R. Heinrich and D. W. Wilson, J. Biol. Chem., 186, 447 (1950).

4. G. B. Brown, Cold Spring Harbon Symposium Quant. Biol., 13, 43 (1948).

5. S. S. Furst, P. M. Ross and G. B. Brown, J. Biol. Chem., 183, 251 (1950).

6. A. Bendich, S. S. Furst and G. B. Brown, J. Biol. Chem., 185, 423 (1950).

7. H. K. Mitchell, M. B. Houlahan and J. F. Nyc, J. Biol. Chem., 172, 525 (1948).

8. H. Arvidson, N. A. Eliasson, E. Hammarsten, P. Reichard, H. von Ubish and S. Engstrom. J. Biol. Chem., 179, 169 (1949).

9. P. Reichard, J. Biol. Chem., 197, 391 (1952).

10. H. S. Loring and J. G. Pierce, J. Biol. Chem., 153, 61 (1944).

11. A. A. Plentl and R. Schoenheimer, J. Biol. Chem., 153, 203 (1944).

12. A. Bendich, H. Gettler and G. B. Brown, J. Biol. Chem., 177, 565 (1949).

13. E. Hammarsten, P. Reichard and E. Saluste, J. Biol. Chem., 183, 105 (1950).

14. R. Abrams, E. Harrasten and D. Shemin, J. Biol. Chem., 173, 429 (1948).

15. E. P. Tyner, C. Heidelberger and G. A. LePage, Cancer Research 12, 158 (1952).

16. L. L. Weed and D. W. Wilson, J. Biol. Chem., 189, 435 (1951).

The present article reports studies on the incorporation of carbon-14 into the RNA of the green alga, Scenedesmus, during photosynthesis in the presence of $C^{14}O_2$. Methods have been developed employing paper chromatography and modifications of the ion exchange techniques of Cohn[17,18] for the determination of the specific activity of each purine, each pyrimidine nucleotide and each pyrimidine base of the algal RNA. This work represents the first reported studies on the rate of incorporation of a common precursor into each of the bases and the ribose of RNA.

Experimental

Photosynthetic Procedures. Day-old cells from a continuous culture of Scenedesmus were packed by centrifugation. One milliliter of the wet-packed cells was suspended in a thin water-jacketed vessel in 100 ml. of distilled water to which 1 ml. of phosphate buffer (3.2 μM/ml. KH_2PO_4) was added. The suspension was pre-illuminated for 15 minutes while a constant stream of air was bubbled through. One ml. of a radioactive sodium bicarbonate solution (400 μc, 0.025 mμ) was then added, and the vessel was stoppered and shaken vigorously in the light for ten minutes of time. The cells were killed by immersion in four volumes of boiling ethanol and were collected by centrifugation.

Isolation of RNA Nucleotides. The Schneider procedure[19] was used to remove acid-soluble and lipid materials not previously removed by the hot 80 percent ethanol. Three extractions of the alga were made with 2 ml. portions of ice-cold 7 percent trichloroacetic acid. The crude nucleoprotein was sedimented each time by centrifugation and the supernatant solution discarded. Similarly, the sediment was extracted once with 1:4 water-ethanol, once with ethanol, and three times for five-minute periods with boiling 3:1 ethanol-ether. Two final extractions with ether were made to facilitate drying the protein mixture.

A modified Schmidt-Thannhauser[20] procedure was used to separate the RNA as nucleotides from the protein and desoxyribonucleic acid. The sample from the previous extractions was incubated at room temperature for twenty-four hours with 1.0 ml. of 1 N potassium hydroxide in order to hydrolyze the RNA. After the suspension was cooled with ice, 100 λ of 72 percent $HClO_4$ was added to precipitate the protein, the DNA and the bulk of the potassium ion as $KClO_4$. The use of $HClO_4$ rather than other mineral acids in combination with trichloroacetic acid provides a real advantage in giving a more nearly salt-free solution for later chromatographic procedures.

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17. W. E. Cohn, Science, 109, 377 (1949).

18. W. E. Cohn, J. Am. Chem. Soc., 72, 1471 (1950).

19. W. C. Schneider, J. Biol. Chem., 161, 293 (1945).

20. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).

The protein was allowed to coagulate for ten minutes on ice and then was packed by centrifugation. The supernatant liquid was filtered through a thin celite layer to remove traces of suspended protein. The sediment was washed twice with 0.4 ml. portions of ice-cold 5 percent $HClO_4$. To the combined filtrates was added N potassium hydroxide until the solution was about pH 10 to 11 as shown by universal check indicator paper. After cooling on ice, the precipitate of $KClO_4$ was removed by centrifugation. The supernatant solution, containing the RNA nucleotides, was allowed to percolate into a Dowex-2-chloride ion exchange column (2 cm. in height, 4 cm. in diameter) and the resin was then washed with about 75 ml. 0.01 M sodium chloride and about 25 ml. of water to remove non-anionic impurities. The nucleotides were then eluted with 25 ml. of 2 N hydrochloric acid, and this eluate was then evaporated to dryness by an air stream at room temperature.

Separation and Purification of the Purine Bases and the Pyrimidine Nucleotides. The nucleotide sample was heated on the steam bath for one hour with 200 λ N hydrochloric acid, thus freeing the purine bases. The solution was diluted to about 2 ml. with water and allowed to filter into a Dowex-50 ion exchange column (hydrogen form, 12 cm. x 0.7 cm.). Elution was begun with distilled water at a flow rate of about 1 ml. per minute. Optical density measurements in the ultraviolet range were made with a Beckman spectrophotometer in order to follow the elution of the various components. After elution of the uridylic acid (in the first ten ml.) and the cytidylic acid (15-50 ml.), 4 N hydrochloric acid was used to elute the guanine (10-35 ml.) and the adenine (35-85 ml.). Preliminary measurements of radioactivity showed poor correlation between optical density and radioactivity; therefore, the fractions comprising each individial component from several columns were combined and evaporated to dryness under an air stream and a heat lamp. Each compound was dissolved in 100 λ of 0.1 N hydrochloric acid and applied individually as a streak about four inches in length near the edge of a sheet of Whatman No. 1 filter paper. The isopropyl alcohol-hydrochloric acid solvent of Wyatt[21] was used to chromatograph the materials. The resulting bands of cytidylic acid, adenine and guanine, as located by examination of the chromatogram under an ultraviolet lamp, were coincident with the radioactive areas which were located by counting thin strips across the paper with a thin window Scott tube. The uridylic acid band, however, was highly contaminated with radioactive impurities.

The paper strips comprising each compound were cut out and the compounds eluted by dipping a blank end of each paper strip into a trough of 0.1 N hydrochloric acid and allowing the solvent to be drawn through the paper and drip off into a small beaker until about 0.5 ml. was collected.

The uridylic acid fraction was evaporated to dryness, the residue was dissolved in 100 λ water, and the solution was re-applied to filter paper as a streak six inches long. The chromatograph was developed by

21. G. R. Wyatt, Biochem. J., 48, 581 (1951).

the ascending method using the ammonium isobutyrate-isobutyric acid solvent of Magasanik, et al.[22]. The uridylic acid band was eluted with water as previously described and the solution again applied to filter paper. The ethanol-ammonia solvent of Leloir[23] was used to develop the chromatogram (ascending). After this procedure, the ultraviolet absorbing band and the radioactive area were apparently coincident.

The uridylic acid was eluted from the paper with 0.5 ml. of water, a few tenths of a milliliter of 0.5 N ammonium hydroxide were added, and the compound was chromatographed on a Dowex-2-chloride ion exchange column (12 cm. x 0.7 cm.) using 0.02 N hydrochloric acid as the eluting agent. The three remaining compounds were chromatographed on the Dowex-50 column, hydrogen form, using 0.1 N hydrochloric acid to elute cytidylic acid, 3 N hydrochloric acid for the guanine and 4 N hydrochloric acid for the adenine. Flow rates of about 1 ml. per minute were used throughout and the fractions collected were about 5 ml.

The elution of each compound was followed by measurement of the ultraviolet absorption at 260 mμ for the uridylic acid, 278 mμ for the cytidylic acid, 249 mμ for guanine and 262 mμ for adenine. The extinction coefficients used to calculate the concentrations of the compounds were respectively 9800, 13,000, 11,000 and 11,600. The first two values are from the literature; the latter two were determined by comparing the optical densities of solutions of each purine in 0.1 N hydrochloric acid with the corresponding densities in 3 N hydrochloric acid for guanine and 4 N hydrochloric acid for adenine. The literature values in 0.1 N hydrochloric acid which were used as reference were 11,400 for guanine and 13,100 for adenine. Aliquot portions of representative tubes, usually 0.50 ml., were pipetted into platinum cups, evaporated to dryness by warming slightly with a heat lamp and counted in a Tracerlab windowless flow Geiger counter. In every case, radioactivity and ultraviolet absorption gave curves which were practically coincident when adjusted so that the highest points coincided, thus demonstrating the purity of the compounds. Specific activities of the compounds were calculated for the three or four fractions of the greatest concentration from the value of the concentration as determined by the ultraviolet absorption measurements and from the measurements of radioactivity.

Determination of Activity of Uracil and Cytosine. The cytidylic acid and uridylic acid fractions remaining from the previous steps were evaporated to dryness and hydrolyzed to the free bases by heating at 100° on a steam bath with 25 λ of 72 percent $HClO_4$ as described by Marshak and Vogel[24]. The carbon resulting from decomposition of the ribose moiety of each nucleotide was removed by centrifugation after dilution of the solutions to about 0.5 ml.

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22. B. Magasanik, E. Vischer, C. Doniger, D. Elson and E. Chargaff, J. Biol. Chem., 186, 37 (1950).

23. A. C. Paladini and L. F. Leloir, Biochem. J., 41, 426 (1952).

24. A. Marshak and H. J. Vogel, J. Biol. Chem., 189, 597 (1951).

The cytosine solution resulting from this step was chromatographed on the Dowex-50 column, using 1 N hydrochloric acid as eluting agent, and following the elution by measuring the optical density of the fractions at 275 mμ. By experiments similar to those described for the purines it was ascertained that the molar extinction of cytosine was the same at 275 mμ in 1 N as in 0.1 N hydrochloric acid. Therefore, in calculating concentrations the value given by Ploeser and Loring[25] of 10,200 was used. Specific activity values were derived as given previously for the other constituents.

The solution of uracil in $HClO_4$ was treated with 25 λ of 10 N potassium hydroxide, the solution cooled in ice, and the precipitate of $KClO_4$ removed by centrifugation. The solution was then applied to filter paper in three spots and the uracil chromatographed using the isopropyl alcohol-hydrochloric acid solvent previously described. The ultraviolet-absorbing uracil spots were eluted by placing the cut out paper squares in 0.1 N hydrochloric acid over night. Contiguous paper areas were similarly treated to serve as reagent blanks in the determination of uracil concentration. Measurements were made at 259 mμ and a molar extinction of 8100 was used[25].

Results and Discussion

Values obtained by the previously described methods for the specific activities of the purine and pyrimidine bases and the pyrimidine nucleotides of the RNA of Scenedesmus after ten minutes in the presence of light and $C^{14}O_2$ are presented in Table X. Results are given for two different complete experiments carried out under similar conditions.

The values for the specific activity of the various components of Scenedesmus RNA cannot be assumed to represent actual turnover rates of these constituents as pool sizes were not determined. It is of interest that guanine was labeled more rapidly than adenine, in agreement with results for other organisms and other tracer compounds. Whatever the explanation of this phenomenon, a difference in the route of synthesis of the two purines, a different "turnover" rate in the nucleic acid, or merely a difference in size of the metabolic pools, its widespread occurrence seems noteworthy.

The most significant data appearing in the table are the figures showing the relatively low rate of incorporation of carbon-14 into cytosine as compared with uracil, and the apparently close relationship between uridylic and cytidylic acid synthesis.

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25. J. M. Ploeser and H. S. Loring, J. Biol. Chem., 178, 431 (1949).

The ratio of activity in each base to the ribose associated with it was 1.8 and 2.2 for the cytidylic acid and 1.5 and 2.0 for the uridylic acid, a very close agreement considering the fact that the ribose values are obtained by difference and that the amount of isotope incorporated into the uridylic acid was about four times that for the cytidylic acid. The significance of these results is not obvious and must await further experiments of different time periods before a full explanation can be offered. It would appear, however, that the synthesis of uridylic and cytidylic acids are closely related, either by means of the conversion of uracil combined with ribose to cytosine-ribose compound, or by means of conversion of a common precursor, such as orotidine, to both uracil-ribose and cytosine-ribose compounds. The high rate of incorporation of C^{14} into uracil as compared with cytosine may mean that a large pool of cytosine-ribose precursor of RNA exists or may represent actual differences in turnover. A similar difference in labeling of the two pyrimidines has formerly been found with orotic acid as a precursor in the rat, and as in the case of adenine and guanine may represent a fundamental difference in metabolism of these compounds.

Table X

Specific Activities of RNA Components of Scenedesmus after Ten Minutes Photosynthesis in $C^{14}O_2$

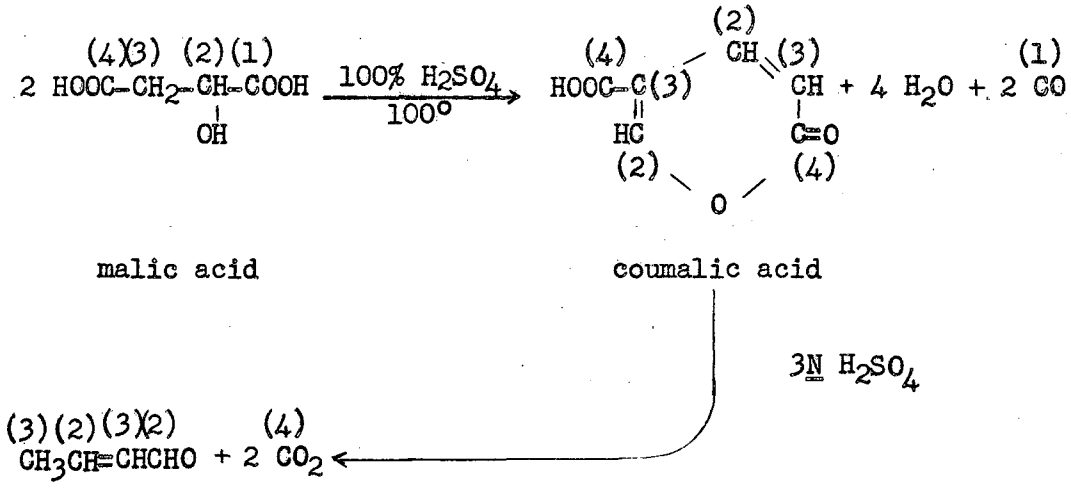
Component	Specific Activities 1 cpm/μM	2 cpm/μM
Adenine	4800	6500
Guanine	7000	8000
Cytidylic acid	2400	4500
Cytosine	1650	2900
Cytidylic acid ribose*	750	1600
Uridylic acid	9900	16500
Uracil	6000	11000
Uridylic acid ribose*	3900	5500

* The ribose values are obtained by differences only.

Degradation of Malic Acid

Per Koch Christensen

As part of the research program on the metabolic pathways of carbon in photosynthesis, it is desirable to have available a method for the systematic degradation of the labeled malic acid which is obtained from photosynthesizing algae. The distribution of activity between carbon atoms 1 and 4 of the labeled malic acid was investigated by the following reactions (the numbers in parentheses indicate the positions of the carbon atoms in the starting malic acid):



Thus, by measuring the activities of the evolved CO and CO_2 of the two separate reactions, one may determine the activities of the (1) and (4) positions of the initial malic acid.

Experimental

The degradation was carried out in a three-necked flask to which were attached (1) a dropping funnel for the introduction of the reactants, (2) an inlet tube for the introduction of nitrogen and (3) an outlet tube which passed successively through a gas bubble tower (I) filled with N NaOH, a CuO-filled tube at 650° and a second tower (II) filled with the same alkaline solution. The outlet tube was finally connected to the house vacuum line and, during a reaction, a pressure slightly below 1 atmosphere was maintained in the system. The total activity of the evolved CO (as CO_2) was measured; therefore it was unimportant if there should be a small leak of atmospheric CO_2 into the system.

The reaction mixture (n grams of malic acid of known specific activity and 3 n grams of 100 percent H_2SO_4) was added through a separatory

funnel and heated at 100° for two hours. Then, the carbonate in the two towers was precipitated with $BaCl_2$-NH_4Cl solution, dried, plated and counted in a Nucleometer. The barium carbonate from tower II should contain all the activity originally found in carbon atom 1 of the malic acid. In our experiments with synthetic malic acid-4-C^{14} a few percent of activity (highest amount: 4 percent) was always found in tower I. This is not enough to disturb greatly a determination of carbon 1, and is probably due to an unknown side reaction.

On completion of the reaction, crude coumalic acid is obtained by the addition of 4n grams of water to the reaction mixture and filtering off the product on the following day. One recrystallization from methanol gives essentially pure coumalic acid.

The reaction between coumalic acid and 3N H_2SO_4 was performed in essentially the same reaction system, this time, however, without the CuO tube and the second tower. The carbon dioxide evolved in this reaction should contain half the total activity originally present in carbon atom 4 of the malic acid.

In three test experiments using pure synthetic malic acid-4-C^{14}, the values for the specific activity of the resultant recrystallized coumalic acid were 100 percent, 95 percent and 95 percent of theory. In these experiments small amounts of activity (1 to 4 percent of the total) were recovered from tower I; no activity was found in tower II. In the degradation of coumalic acid from the same malic acid, three experiments gave values of 60 percent, 43 percent and 52 percent for the radioactivity found in the resulting $BaCO_3$ (since all the activity was in carbon atom 4 and since only 1/2 of carbon 4 appears as CO_2 in this reaction, the theoretical value is 50 percent).

Although this degradative procedure can, at present, be considered at best only a roughly-quantitative one, we have made the following determinations of the percentages of activities in carbons 1 and 4 of malic acid obtained from various photosynthesizing (PS) plant materials as well as from two algae which were exposed to the radioactive CO_2 in the dark.

Experiment	% of Total Act. in Carbon 1	% of Total Act. in Carbon 4
5 min. PS sugar beet	24	19
20 sec. PS soy bean	33	44
30 sec. PS barley	31	70
40 min. Dark Scenédesmus	48	52
40 min. Dark Chlorella	39	54
40 min. Dark Chlorella	47	53

Valeric Acid Metabolism in Mouse Liver Slices

James B. Gilbert

(see Quarterly Report for December, 1951 - February, 1952, p. 10, 28)

The several postulated intermediates in classical beta-oxidation of a fatty acid--α,β-unsaturated acid, β-hydoxy acid, β-keto acid--are oxidized by enzyme systems. However, these compounds have never been demonstrated as intermediates. The finding by Daus et al. (L. Daus, M. Meinke and M. Calvin, J. Biol. Chem., 196, 77 (1952)), that β-hydroxyvaleric acid is formed on incubation of propionic acid with mouse liver slices, suggests that this β-hydroxy acid may be demonstrable in the metabolism of valeric acid by this same tissue. Furthermore, the possibility exists that a precursor-product relationship may be demonstrable using doubly labeled valeric acid. With this idea in mind and with the hope of establishing the broad pattern of valeric acid metabolism as revealed by paper chromatographic techniques, the following work was undertaken.

Experimental

For further details on all experimental procedures, see UCRL-1476 or J. Biol. Chem., 196, 77 (1952).

Incubation of Liver Slices. Valeric-1-C^{14} acid was incubated with mouse liver slices in Krebs-Ringer buffer solution at pH 7. The reaction was carried out in a Warburg apparatus at 37° and the evolved carbon dioxide was absorbed in the center well base.

Fractionation of Incubation Mixtures. The carbonate in the center well was determined by precipitation as barium carbonate followed by the usual plating and counting methods. The incubation mixture was ground in mortar and pestle and distilled to dryness in vacuo. Volatile material was discarded. The non-volatile material was taken up in water and centrifuged to remove insoluble protein. The supernatant was continuously extracted with ether for 18 hours yielding two fractions, designated an non-ether extractable and ether extractable. These fractions were chromatographed on paper using phenol-water-butanol, propionic acid-water, ammonia-propanol-water solvent systems.

Identification of Compounds. Radioactive spots were eluted and placed on a silicic acid column with known carrier and developed with chloroform-butanol. Fractions of the eluate thus obtained were titrated with 0.01 N sodium hydroxide, and the C^{14} activity determined by direct plating. Coincidence of radioactivity curve with titration curve is provisional evidence for identity of unknown and known compound. In the case of β-hydoxyvaleric acid, the radioactivity and known carrier were, in addition, acetylated and co-chromatographed on a silicic acid column.

Synthesis. β-Hydroxyvaleric acid was prepared by a Reformatsky reaction from propyl aldehyde, ethyl bromoacetate and zinc and isolated as the barium salt. β-Hydroxybutyric acid was synthesized by hydrogenation of ethyl aceto-acetate using Rainey nickel catalyst and isolated as the sodium salt. A low-activity preparation of valeric-3-C^{14} acid was made from sodium propionate-1-C^{14} on a 10 mmole scale as follows:

$$CH_3CH_2C^{14}OONa \xrightarrow{HCl\ (g)} CH_3CH_2C^{14}OOH \xrightarrow[LiAlH_4]{[H]} CH_3CH_2C^{14}H_2OH \xrightarrow[P]{I_2} CH_3CH_2C^{14}H_2I$$

73%

$$CH_3CH_2C^{14}H_2I \xrightarrow{NaCH(COOC_2H_5)2} CH_3CH_2C^{14}H_2CH(COOC_2H_5)2 \xrightarrow[2)\ H^+]{1)\ OH^-} CH_3CH_2C^{14}H_2CH_2COOH$$

57%

The yield at the malonic ester condensation step was improved considerably by maintaining anhydrous conditions, i.e., using a continuous stream of dry nitrogen and employing alcohol distilled over magnesium as the solvent. It was also found that a 100 g. silicic acid column gives adequate separation of valeric acid and contaminating acetic acid. The data on the titration of valeric acid with sodium hydroxide, and the weight of resulting sodium salt, agree within 1 percent. The microanalysis showed: C (calc'd.) 48.38 percent; found, 45.80 percent. This discrepancy between equivalent weight and analytical values was observed repeatedly.

Results

The distribution of activity in the various fractions is shown in Table XI.

Table XI

General Distribution of Activity

Substrate	Time of Incubation, Hrs.	% as CO_2	Non-volatile water soluble	
			% non-ether extractable	% ether--extractable
Valeric-1-C^{14} acid 8.9 μc	2	26.0	3.1	8.2
Valeric-1-C^{14} acid 24.9 μc	2.5	16.3	2.0	5.3

The identification of specific compounds was attempted only on the "ether extractable" fraction. Three acids were identified by co-chromatography on silicic acid with known compounds: lactic acid, β -hydroxyvaleric acid and β -hydroxybutyric acid. The relative activities of these three compounds on a paper chromatogram are indicated in Table XII.

Table XII

Relative Distribution of Activity in Labeled Compounds Resulting from the Incubation of Valeric-1-C^{14} Acid

Compound	Activity*
Lactic Acid	13
β-Hydroxyvaleric acid	22
β-Hydroxybutyric acid	63
Unidentified spots	17

* The radioactivity was determined by separate measurements with a Scott tube on the separated spots of a paper chromatogram made directly from an aliquot portion of the ether extractable material.

Discussion

Since labeled β-hydroxyvaleric acid is obtained after administration of labeled valeric acid, it will now be of interest to ascertain if there exists a direct product-precursor relationship between these compounds. This, as was earlier suggested, could be done by using double-labeled valeric acid and determining the distribution of activity in the derived β-hydroxy acid.

The other finding of interest is the considerable radioactivity present in lactic acid. Two possible mechanisms for the introduction of the label into this compound are: (1) The fixation of $C^{14}O_2$ by the Wood-Werkman reaction with symmetrical distribution of the isotope in oxalacetate after equilibration with fumarate and succinate; (2) condensation of $[-CH_2C^{14}OOH]$ with oxalacetate and passage through the citric acid cycle to oxalacetate. Both of these mechanisms yield lactate-1-C^{14}. Thus, a degradation of the derived lactic acid would be of considerable interest.

Summary

β-Hydroxyvaleric acid has been definitely identified, and lactic acid and β-hydroxybutyric acid provisionally identified, as products of valeric acid metabolism in mouse liver slices.

The Effect of Heparin on the Rate of Fatty Acid Metabolism

M. Kirk

A method for continuous determination of breath carbon-14 dioxide eliminated by rats injected with labeled compounds was described in a previous report. This method is being applied to determine what effect, if any, administration of heparin may have upon rate of metabolism of fatty acids of various chain lengths to $C^{14}O_2$.

Dean M. Graham, et al.[1] found that heparin administered to humans and rabbits causes a profound reorientation in the distribution of low density lipoproteins. The present work is an attempt to gain some knowledge of the extent to which heparin enters into complete fatty acid metabolism.

Experimental

Long-Evans female rats weighing 180-200 g. were used in all experiments. Fatty acids were administered as their sodium salts in aqueous solution. In general, the dose was 2 mg. of salt containing 10-20 μc. C^{14} in 0.2 ml. water given by intraperitoneal injection. Those rats receiving heparin were given 1.0 mg. heparin sodium (Upjohn) in 0.1 ml. saline mixed with the fatty acid solution. Immediately after injection animals were placed in metabolism cages and radioactivity of the breath measured by means of a vibrating reed electrometer and a Brown recorder over a period of seven hours.

Results

Table XIII summarizes the results obtained in a series of experiments using various fatty acids; also glucose-1,6-C^{14} and glycine--2-C^{14}.

$CH_3(CH_2)_5C^{14}OONa$ was eliminated very rapidly (2.85 percent per minute in seven minutes after injection) either with or without heparin. A number of animals were injected with sodium myristate-1--C^{14} (both with and without heparin), but the difficulties involved in keeping the compound in solution caused the results to be quite variable and indeterminate.

Conclusions

1. Apparently heparin speeds up the metabolism of fatty acids to carbon dioxide in varying degrees for different acids, but whether by some actual biochemical action or because it facilitates transport of the acid remains to be determined.

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1. D. M. Graham, T. P. Lyon, J. W. Gofman, H. B. Jones, A. Yankley, J. Simonton and S. White, "The Influence of Heparin Upon Lipoprotein Metabolism," Circulation, IV, 466 (1951).

2. The effect may be seen almost immediately.

3. As far as observations on the appearance of radioactivity in the breath are concerned, heparin has no effect on the metabolism of glucose-1,6-C^{14} or glycine-2-C^{14}.

Table XIII

Compound	Without Heparin				With Heparin			
	Total % Activity Recovered		Maximum %/min.	Time After Injection	Total % Activity Recovered		Maximum %/min.	Time After Injection
	3 hr.	7 hr.			3 hr.	7 hr.		
$C^{14}H_3COONa$	69.27	77.39	1.02	20 min.	80.14	88.79	1.36	20 min.
$C^{14}H_3CH_2COONa$	67.35	74.57	1.18	20 min.	76.86	85.25	1.29	23 min.
$CH_3(CH_2)_4COONa\text{-}2\text{-}C^{14}$	62.02	71.37	0.949	17 min.	83.01	92.87	1.41	12 min.
Glucose-1,6-C^{14}	73.57	83.33	0.991	40 min.	71.47	81.35	0.900	40 min.
Glycine-2-C^{14}	17.84	24.48	0.210	30 min.	16.32	23.40	0.200	30 min.

Preparation of Labeled Organic Compounds

P. Adams, R. Noller and D. Pack

During the past quarter the following high specific activity preparations have been completed (all yields are based on $BaC^{14}O_3$): methyl-C^{14}-iodide, 60 mc. in 79 percent yield; sodium acetate-1-C^{14}, 33 mc. in 95 percent yield; sodium acetate-2-C^{14}, 60 mc. in 65 percent yield; ethanol-1-C^{14}, 5 mc. in 30 percent yield; isobutyl-1-C^{14} iodide, 44 mc. in 67 percent yield; and aspartic-4-C^{14} acid, 15 mc. in 36 percent yield.

The aspartic-4-C^{14} acid was prepared by the following reactions:

$$C^{14}O_2 \xrightarrow{\text{Grignard reaction}} CH_3C^{14}OOH \xrightarrow{Cl_2} ClCH_2C^{14}OOH$$

$$\xrightarrow{Et_2SO_4} ClCH_2C^{14}OOC_2H_5 \xrightarrow[3)\ \text{decarboxylation}]{1)\ \text{sodium acetamido-malonate}\ 2)\ \text{hydrolysis}} HOOC^{14}\text{-}CH_2\text{-}\underset{NH_2}{CH}\text{-}COOH$$

The first purification of the aspartic acid product was accomplished by elution from a Dowex-50 ion exchange column. However, in order to remove contaminating metal ions, the aspartic acid was further purified through preparation of its copper salt.

Δ^7-Cholesterol Content of Serum Cholesterol

R. M. Lemmon and Margaret Anderson

In pursuance of the program outlined in the last quarterly report the possibilities of a large scale (300-400 g.) synthesis of Δ^7-cholesterol ("lathosterol") from cholesterol has been investigated. The reactions for this synthesis are as follows:

$$\text{Cholesterol} \longrightarrow \text{Cholesteryl benzoate} \xrightarrow[2)\ Na_2CO_3]{1)\ \text{N-bromosuccinimide}}$$

$$\text{7-dehydrocholesteryl benzoate} \longrightarrow \text{7-dehydrocholesterol}$$

$$\xrightarrow[3\ \text{atm.}]{\text{Raney nickel hydrogenation}} \text{lathosterol}$$

It has been found that the hydrogenation of 7-dehydrocholesterol to lathosterol takes place with considerable facility. However, the preparation of the 7-dehydrocholesterol from cholesterol has proven quite

troublesome. Initially, the conditions of Bernstein, et al.[1] for this reaction were followed. These authors have reported a yield of 24 percent in going from cholesteryl benzoate to 7-dehydrocholesteryl acetate; however, we have not been able to exceed a yield of 10 percent.

More recently an improved synthesis of 7-dehydrocholesterol has appeared in the literature[2]. The principal inovation of this procedure is the addition of certain N-containing compounds (especially α-picoline) in the allyl-bromination step. Using the conditions of this procedure, 7-dehydrocholesteryl benzoate has been obtained from cholesteryl benzoate in a yield of 24 percent. Considering the cheapness and ready availability of the starting material (cholesterol) the synthesis of lathosterol in amounts large enough for an animal feeding program now appears feasible. This synthesis is now in progress.

The program for the further determination of the lathosterol content of various lipoprotein fractions of blood serum is still underway. Several such samples have been sent to Prof. Fieser's group at Harvard for the lathosterol analysis.

Metabolism of Morphine-N-methyl-C^{14} in Humans

Elton M. Baker and B. M. Tolbert

In an earlier report (Quarterly Report for December, 1951-February, 1952) there was described an experiment with the Division of Pharmacology and Experimental Therapeutics in the University of California Medical School at San Francisco, in which 20 μc. of the morphine was injected into a cancer patient, who was a non-user of the drug.

Succeeding experiments have been carried out with both male and female cancer patients and normal humans. Apparently the metabolism follows essentially the same path of excretion in all persons; we have found that the relative amounts of C^{14} which are excreted in breath, urine and feces are nearly the same. The rate of excretion is slightly greater in females than in males; however, little difference is noted between cancer and normal patients. The total value of the excreted C^{14} as carbon dioxide is about 5 percent of the amount injected. Only 1 μc. was injected in the normal humans.

Our part of the work has been concerned with the direct determination of the activity of the respired carbon dioxide from the normal humans. This has been accomplished by collecting samples of respired breath from the person directly into a large balloon, then deflating the balloon and

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1. S. Bernstein, et al., J. Org. Chem. 14, 443 (1949).

2. U. S. Patent No., 2,568,025 as reported in Chem. Abstracts, 46, 5096b (1952).

passing the gas into a liquid nitrogen-cooled trap, where the oxygen and nitrogen are eliminated. Water is removed by evaporation of the solid carbon dioxide through a dry ice-cooled trap, and collecting it in another liquid nitrogen-cooled trap. An ionization chamber of approximately 500 cc. is rapidly filled through a tube filled with activated charcoal, which removes radon. The presence of the high alpha activity of the radon would interfere with the $C^{14}O_2$ activity determination.

The following are data taken from the break of normal male and female humams by the above method. One μc. of the morphine was used in each case. (See Table XIV).

These studies are being continued.

Table XIV

Time	Specific Activity in dis./min./cc at 250° C	
	Female	Male
0	0.049	0.030
15 min.	0.670	0.460
30 min.	1.428	0.726
60 min.	1.928	0.984
2 hrs.	1.517	0.863
4 hrs.	1.351	0.782
6 hrs.	0.574	0.460
8 hrs.	0.347	--
9 hrs.	--	0.331
10 hrs.	0.304	--
12 hrs.	0.226	0.105
24 hrs.	0.201	0.064

Studies on Morphine Metabolism

H. Rapoport and C. H. Lovell

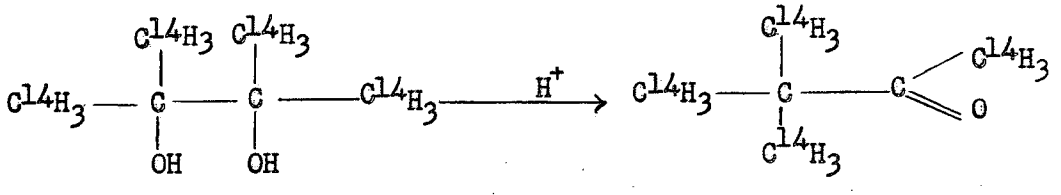
As part of a program which is being undertaken to trace the metabolic pathways of morphine in the animal body, the desirability of having available some ring-labeled morphine has become evident. Therefore, the synthesis of morphine- and codeine-7-C^{14} is being undertaken as follows: Codeine will be oxidized at the alcoholic hydroxyl group to give codeinone; this latter compound will be converted to 6-methylcodeine by treatment with methyl-C^{14} lithium. Hydroxylation of the double bond of the 6-methyl derivative with osmium tetroxide gives the vicinal triol, 6-methyl-C^{14}-dihydroxydihydrocodeine. The opening of ring C of the triol with the removal of C-7 by either lead tetraacetate or periodate would give the aldehydo-ketone; cyclization of the latter to re-form ring C, with subsequent dehydration, would give codeinone-7-C^{14}. This compound can be hydrogenated back to codeine-7-C^{14} which may, in turn, be hydrolyzed to morphine-7-C^{14}.

At the present time inactive preparations have been carried out in good yields of the 6-methyl-dihydroxydihydrocodeine and the opening of ring C of this compound is being investigated. Since periodate oxidations, in contrast to lead tetraacetate oxidations, may be run in aqueous solutions under a variety of acid conditions, particular attention is being paid to the use of periodate as the reagent for opening ring C.

The Isotope Effect in the Pinacol Rearrangement

Per Koch Christensen

The purpose of this work was to carry out the pinacol rearrangement with pinacol labeled evenly in all methyl groups,



and to measure the size of the expected isotope effect involved, that is, to measure any difference in the migratory aptitudes of $-C^{12}H_3$ and $-C^{14}H_3$.

The preparation of the labeled pinacol and its rearrangement were carried out following the directions in Organic Syntheses, Coll. Vol. I, p. 459-463. The pinacolone was then split into trimethylacetic acid and bromoform following Organic Syntheses, Coll. Vol. I, p. 526-528. All yields were comparable to those reported in the literature. After careful purification of the products, the specific activities were determined by burning

to CO_2 using the wet-combustion method, trapping the CO_2 in sodium hydroxide solution, precipitating barium carbonate, generating CO_2 from the carbonate and determining the activity in an ionization chamber. The reactions involved are as follows:

$$C^{14}H_3COONa \longrightarrow (C^{14}H_3COO)_2Ba \xrightarrow{\text{heat}} C^{14}H_3-CO-C^{14}H_3$$

$$\xrightarrow[(2)\ H_2O]{(1)\ Mg,\ HgCl_2} \underset{\text{pinacol}}{\begin{matrix} C^{14}H_3 & & C^{14}H_3 \\ & C-C & \\ C^{14}H_3 & | \quad | & C^{14}H_3 \\ & OH \quad OH & \end{matrix}} \xrightarrow{H_2SO_4}$$

$$\underset{\text{pinacolone}}{\begin{matrix} C^{14}H_3 & & \\ C^{14}H_3-C & C-C^{14}O_3 & \\ C^{14}H_3 & \| \ O & \end{matrix}} \xrightarrow[(2)\ H_2SO_4]{(1)\ NaOH,\ Br_2} \underset{\text{tri-methylacetic acid}}{(C^{14}H_3)_3C-COOH} + \underset{\text{bromoform}}{C^{14}HBr_3}$$

The pinacol and pinacolone each have four labeled carbon atoms out of a total of six, the trimethylacetic acid (TMA) has three labeled carbons and a total of five, and the bromoform has only one labeled carbon atom. In order to make the results directly comparable, the specific activities determined for the barium carbonate from the pinacol and pinacolone are multiplied by 6/4 and that from the TMA is multiplied by 5/3. (Since the bromoform has only one carbon the specific activity of the barium carbonate is used directly.) These adjusted values for the specific activities of the barium carbonate then represent the specific activities of the methyl carbons in the cases of pinacol, pinacolone and TMA and of the single carbon of the bromoform. Thus, if there were no isotope effect involved in the rearrangement, all of the values would be equal; if there was an isotope effect the results for the two degradation products (TMA and bromoform) would be on either side of the value for the pinacol and pinacolone.

Early experimental results gave the following directly comparable values:

Sp. Act. $BaCO_3$ from pinacol, x 6/4.....2.04 dis./min./mg.
Sp. Act. $BaCO_3$ from TMA, x 5/3.....1.87 dis./min./mg.
Sp. Act. $BaCO_3$ from bromoform.....2.25 dis./min./mg.

If these values are reliable they show a tendency for $-C^{12}H_3$ to rearrange rather than $-C^{14}H_3$, with a k12/k14 value of about 1.09.

However, further experimental work with higher specific activity pinacol gave quite ambiguous results and, at the present time, no certain

statement can be made regarding the isotope effect in the pinacol rearrangement. Some of our results have led us to believe that the wet-combustion method is not altogether reliable. Other results point to possible ionization chamber contaminations. These sources of difficulties are being further investigated.

Chemical Actinometry of A Cobalt-60 γ -Ray Source

P. Adams and R. Noller

Because of the need for a more accurate measure of dosage in experiments involving gamma irradiation, two methods of chemical actinometry have been studied. The oxidation of ferrous ion and the reduction of ceric ion in acid solution under the influence of ionizing radiation both provide excellent methods for determining the dose rate from a Cobalt-60 γ -ray source. In both cases, the ratio of ions changed to energy absorbed is independant of acid concentration, initial substrate concentration and dose rate over a very broad range. An additional advantage of both systems is the ease and accuracy with which the concentrations of ceric and ferric ions can be assayed, namely, by measuring their ultraviolet absorptions in a Beckman spectrophotometer.

The ferrous-ferric couple is a very satisfactory tool for the measurement of gamma radiation dosage if one keeps certain conditions in mind. The solutions must be made with water that has been distilled from alkaline permanganate, and care must be taken to saturate the solutions with enough oxygen to insure that it will not be used up during the course of the irradiation. In addition, the samples should not be irradiated by a source delivering more than about 60,000 r/hr.

The advantages of the method are the relative stability of the standard solutions at the concentrations used (10^{-4}-10^{-1} $\underline{M}$), the relative efficiency of the oxidation process, and the ease with which the concentration of ferric ion can be measured in a Beckman spectrophotometer.

Standard Fe^{+++} in 0.8$\underline{N}$ H_2SO_4 was prepared by making standard Fe^{++} in 0.8$\underline{N}$ H_2SO_4 from analytical grade iron wire and then oxidizing to ferric ion with hydrogen perioxide. The ferric ion in 0.8 $\underline{N}$ H_2SO_4 was shown to have an ultraviolet absorption peak at 303 mμ and a molar extinction coefficient of 2201 at that wave length.

Since the radiation effect is noted by measuring the amount of product, it is not necessary to know accurately the concentration of ferrous ion. For this reason, the only check on the concentration of ferrous ion is the initial weighing of the ferrous ammonium sulphate.

The 0.8$\underline{N}$ H_2SO_4 was made up to contain about $10^{-3}\underline{M}$ ferrous ion and oxygen was bubbled through the solution for two hours at room temperature in order to reach saturation. Volumetric tubes were each filled with 2 ml. of solution and irradiated. Blanks identical to the samples were also prepared.

Two separate runs were made and a total of six samples were irradiated and analyzed. It was found that the average change in ferrous ion concentration was $6.34 \pm 0.71 \times 10^{-9}$ moles/ml./min. Analysis of the blanks showed that there was no measurable amount of ferric ion formed during the period of the experiment.

If for ferrous ions in 0.8$\underline{N}$ H_2SO_4 solutions, G = 20/100 (G = 20 ferrous ions changed per 100 e.v. of energy expended in solution; T. J. Hardwick, Can. J. Chem., 30, 23, 1952) and 1 rep equals 93 ergs per gram of water, then with the above data it may be calculated that the Cobalt-60 source is supplying about $19.7 \pm 2.2 \times 10^3$ rep/hr.

The reduction of ceric sulphate, because of its complete independence of the concentration of oxygen in the solution, is well adapted for use as an actinometric method at higher dose rates than is the oxidation of ferrous ion. However, in use with moderate or low dose rates where the initial concentration of Ce(IV) is low, extreme caution must be taken to protect the solution from reduction by trace impurities in the water or on the glassware or from ultraviolet light.

A stock solution, $4 \times 10^{-3}\underline{M}$ in ceric sulfate and 0.8$\underline{N}$ in H_2SO_4 was prepared using water which had been distilled from alkaline permanganate. The solution was initially standardized by titration against standard ferrous sulfate. The concentration of the stock ceric ion solution was found to remain quite constant over a period of three to four weeks, whereas acid solutions of less than $10^{-3}\underline{M}$ Ce(IV) showed decreases in concentration of as much as fifty percent per week.

Fresh dilutions of the stock solution in acid were used for analysis in the spectrophotometer. A broad maximum was found in the range 310-330mμ and the molar extinction coefficient found ($\epsilon_{320m\mu} = 5600$) was in good agreement with data recently published, (Medalia and Byrne, Anal. Chem. 23, 453 (1951)); for Ce(IV) in 1$\underline{N}$ H_2SO_4, ($\epsilon_{320m\mu} = 5580$). It was found that Beer's law is followed over a range of at least 3 -15 x $10^{-5}\underline{M}$ Ce (IV).

For the measurement of the strength of the Cobalt-60 source, samples were prepared by diluting the stock ceric sulfate solution with 0.8$\underline{N}$ H_2SO_4 to a concentration such that about half of the ceric ion present would be reduced during the time of exposure. Six 2-ml. volumetric tubes, scrupulously cleaned, were filled with the solution. Three of these tubes were placed in the radiation source and three were kept as blanks. After irradiation, aliquot portions of the six solutions were diluted with acid to a concentration of 6 - 10 x $10^{-5}\underline{M}$ and analyzed in the spectrophotometer. In all cases where the concentration of the solution was above $10^{-4}\underline{M}$, the blanks agreed within 1.5 percent. When attempts were made to work with solutions of lower Ce(IV) concentration, erratic results were obtained. Experiments indicated that light from an unscreened "Mineralite" ultraviolet source rapidly reduces ceric ion in solution and it is believed that the destruction of a high percentage of the ceric ion in dilute solutions may be due to the absorption of ultraviolet from the sunlight.

The G Value for the ceric-cerous couple in acid solution is 3.3 ions per 100 e.v., calculated on the basis of 93 erg/ml/rep. Over time intervals of 0.75, 1, 2, 3.5 and 16 hours in the Cobalt-60 source (found by air ion chamber measurements to deliver 2 - 2.5 x 10^4 rep/hr.) the ceric ion actinometer gave results of 2.2 ± 0.4 x 10^4 rep/hr.

II QUARTERLY PROGRESS REPORT. Project 48B

A. Metals and High Temperature Thermodynamics

Leo Brewer, LeRoy Bromley, Albert Rothman, James Kane
Richard Porter, John Margrave, Oscar Krikorian and Haakon Haraldsen

Silicides

The study of reactions of silicides of Ce, Ti, N, Np, Ta, Mo and W with N_2 and C has been largely carried out and the data are being used to fix the heats of formations of the silicides. The similar work with the borides has been written up for publication.

Vapor Pressure of Tin

The vapor pressure of tin has been determined and used to demonstrate that the linear Birge-Sponer extrapolation gives the correct D_o for SnO.

Volatility of Li_2O

The determination of the volatility of Li_2O in oxygen has demonstrated the existence of the Li_2O gaseous molecule and have indicated the existence of the LiO molecule.

Graphite - Hydrogen System

The graphite - hydrogen system was investigated at temperatures over 3,000° K for spectroscopic indication of CH_2 or CH_3 in absorption. No band spectra was observed.

Carbon Fluorides

Heating CF_4 with graphite in the King furnace has produced CF and CF_2 as indicated by absorption and emission spectra as well as a new molecule believed to be C_2F_2 which persists to room temperature.

Thermal Conductivity of Gases at High Temperatures

Construction of the conductivity cell has been completed. Construction of the constant temperature furnace should be complete within a month. The White potentiometer has been received and measuring circuits are being assembled. Electronic control equipment has been designed and construction has been started.

B. Basic Chemistry, Including Metal Chelates

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Thermodynamics of Thiosulfate

For the equilibrium:

$$S_2O_3^{=}(aq.) + 2\,Ag(c) \rightleftharpoons SO_3^{=}(aq.) + Ag_2S\,(c)$$

the equilibrium constant K at 123° C has been determined to be between 11.0 and 18.4. From a later set of samples that were originally made up to correspond approximately to K = 10 and 19 (instead of starting with pure materials as in all previous runs) the limits on K have been further set as 16.7 and 18.0. However, because of formation of tar in the oil bath and because of the unsatisfactory nature of a mercury temperature regulator for sustained operation at these temperatures, the temperature for these last two values is in considerable doubt. A proportional type, resistance thermometer controlled temperature regulator, is being constructed and installed.

Study of Hydrates

Attempts to prepare pure $CaCl_2 \cdot 2H_2O$ by adjusting the vapor pressure of water over the commercial salt in a vac. line, both from above and from below the equilibrium pressure, have finally been successful; the formula has been confirmed by chemical analysis. However the value of the monohydrate-dihydrate equilibrium vapor pressure at 65° C of 13 mm given in the literature by: P. Pascal: Traité de Chimie Minérale, is not well checked. With one sample so prepared, its heat of solution to a final state of $CaCl_2$ in 7918 H_2O has been measured as 10.832 K cal/mole. Further runs are to be made, and it is also desired to prepare some $CaCl_2 \cdot H_2O$ in order to measure a similar heat of solution for it.

Indium

Experiments designed to measure the extent of the equilibria

$$In^{+3} + HF \rightleftharpoons InF^{++} + H^{+}$$

$$InF^{++} + HF \rightleftharpoons InF_2^{+} + H^{+}$$

are being carried out at several temperatures. This work is nearly completed at 25°. In order to measure these equilibria at 15° and at 35°, it is necessary to know the equilibrium constants for the similar reactions involving Fe^{+++}. These have been obtained and the values at 25° of H. Dodgen and G. Rollefson have been checked. Work on Indium at 35° is proceeding and will begin presently at 15°.

Attempts to measure the equilibria between $In(m)$, In^{+}, In^{+2} and In^{+3} by the method previously reported are proceeding.

Entropy of Formation of Complex Ions

The work on the entropy of the six successive reactions of F^{-} with Al^{+++} to form AlF_6^{---} has lead to a general theory of the entropy of formation of complex ions in aqueous solutions. The total entropy change has been related to two general factors: (1) the entropy change involved in replacing a water molecule bound to the aqueous ion by the complexing ion or molecule and (2) the effect of changing the charge on the ion upon the entropy of the surrounding water.

The Hydrolytic Polymerization of Zirconium

The method used in the past for the determination of free acid in zirconium perchlorate solutions was to add excess sodium fluoride to complex the zirconium and titrate the free acid with sodium hydroxide to the methyl red end-point. Two other methods were tried. The first consisted of a direct titration of the zirconium perchlorate solution with sodium hydroxide using a pH meter. The results in general were slightly low, relative to those obtained by the fluoride method. Apparently a small amount of basic salt as well as hydroxide was precipitated.

In the second method excess sodium hydroxide was added to the zirconium perchlorate solution, which was then back titrated using a pH meter to locate the end-point. These results agreed well with the fluoride values.

Absorption Spectra in Liquid NH_3

The maximum in the infrared absorption of metallic sodium in liquid ammonia has been found to be at 1.50 ± 0.15 microns. This value is in agreement with Oggs value for the work function of 0.8 volt for the emission of an electron but such an interpretation would require an error of 16 kwl. in the experimental value for the heat of formation of the solvated electron.

Potential of the RuO_4^{-} - RuO_4 Couple

Further experiments at widely varying concentrations, have indicated that a steady state in RuO_4, rather than a true equilibrium was reached in the reaction:

$$2\ RuO_4^{-} + ClO^{-} + H_2O \rightleftharpoons 2\ RuO_4 + Cl^{-} + 2OH^{-}.$$

The consistency in the previously reported values for the equilibrium constant calculated on the assumption that equilibrium was reached was

fortuitous, and the resulting standard potential of -1.05 volts for Ru(VII) - Ru(VIII) must be considered merely as a lower limit to the true potential.

The steady state may result from reduction of RuO_4 by water or by minute amounts of impurities in the reagents used. Present evidence seems to support the latter possibility.

The following mechanism for the reaction is in conformity with data obtained on the rate of oxidation of RuO_4^- and $RuO_4^{=}$ by ClO^-:

(1) $2\ RuO_4^- \overset{K}{=} RuO_4^{=} + RuO_4$

(2) $RuO_4^{=} + ClOH \xrightarrow{k_2} RuO_4 + Cl^- + OH^-$.

Assuming this mechanism to be correct, the disproportionation constant K can be calculated from rate data and combined with the known Ru(VI) - Ru(VII) potential to give the Ru(VII) - Ru(VIII) potential. Early results using this method of attack are encouraging, and further experiments are in progress to confirm the mechanism and to obtain better precision in the tentative value of -0.96 volts.

Complexing of Rare Earth Ions with Fluoride

Measurements of the extent of complexing of the rare earth ions with fluoride ion at 15° C, 25° C, and 35° C are still in progress. The method has been previously described.

To measure these complexing constants at 35° C one needs to know similar constants for the ferric fluoride complexes. To determine these constants the method described by Dodgen and Rollefson[1] was employed.

The equilibria in question are:

$Fe^{+++} + HF = FeF^{+2} + H^+$ $\qquad K_1$

$FeF^{+2} + HF = FeF_2^+ + H^+$ $\qquad K_2$

the values obtained at an ionic strength of 0.5 are:

$K_1 = 178$

$K_2 = 10.1$

Information Division
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1. H. W. Dodgen and G. K. Rollefson, Jr. Am. Chem. Soc., 71, 2600 (1949).