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CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August, 1953

September 30, 1953

Berkeley, California

CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August, 1953

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*Previous Chemistry Quarterly Report: UCRL-2257.

CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August, 1953

NUCLEAR CHEMISTRY

G. T. Seaborg and I. Perlman

THE GAMMA SPECTRUM OF AcK

Earl K. Hyde

The gamma spectrum of AcK has been studied in a scintillation spectrometer consisting of a NaI detection head, a photomultiplier tube and a 50-channel pulse-height analyzer. The most prominent radiation observed was a 50-kev gamma ray (40 ± 10 photons per 100 disintegrations of AcK). Radiation of 80- to 83-kev energy is about half as prominent (24 photons per 100 disintegrations of AcK). Low-intensity gamma rays at 225 kev and 310 kev were observed (about 2 photons per 100 disintegrations and 0.4 photon per 100 disintegrations, respectively). Prominent gamma radiation observed at 80- to 85-kev may be K X-rays from the internal conversion of the 225- and 310-kev radiation, in which case these gamma rays are highly converted. This conversion has not been proved. L x-radiation is prominent and of about the same order of intensity as the 50-kev gamma radiation, but has not been carefully studied to date.

No other gamma radiation was observed.

An anthracene crystal was substituted for the NaI crystal in order to determine the beta spectrum of AcK. A visual end-point determination gave 1.15 ± 0.05 Mev as the energy of the most energetic beta ray.

This work may be compared with some previous absorption-curve studies by LeCoin, Perey, Riou, and Teillac, J. Phys. et Rad. 11, 227 (1950). These authors reported a gamma ray of 48.6 kev (27 photons per 100 disintegrations), L radiation (25 photons per 100 disintegrations), and a gamma ray of 350 kev (6 photons per 100 disintegrations). They made no mention of 80-kev radiation.

LOW-INTENSITY GAMMA RADIATION FROM Am^{241}

H. Jaffe and I. Perlman

A sample of Am^{241} was examined on a NaI(Tl) scintillation spectrometer with a 50-channel differential pulse-height analyzer. Silver absorbers were employed to decrease the intensity of the more abundant gamma radiation and four new lines were observed. The data, corrected for absorption, are summarized in Table I.

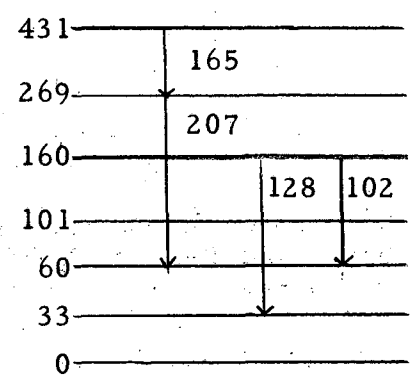
Table I

Gamma ray energy (kev)	102	128	168	207
Intensity (γ/α)*	5.6×10^{-4}	5.6×10^{-5}	6.4×10^{-6}	7.6×10^{-6}

*Based on figure of 0.40 59.6-kev gamma rays per Am^{241} alpha particle; J. K. Beling, J. O. Newton, and B. Rose, Phys. Rev. 86, 797 (1952).

Both half-width and intensity considerations indicate that little of the 102-kev radiation is due to K x-rays.

The 168- and 207-kev gamma rays were observed by Wagner et al.[a] in the beta decay of U^{237}. The other lines fit well into the previously proposed level scheme of Np^{237}.[a, b]



Current alpha-gamma coincidence studies in this laboratory[c] indicate the 102-kev gamma ray is from the 160-kev level and not from the 101-kev state.

---

a F. Wagner, Jr., M. S. Freedman, D. W. Engelkemeir, and J. R. Huizenga, Phys. Rev. 89, 502 (1953).
b F. Asaro, F. L. Reynolds, and I. Perlman, Phys. Rev. 87, 277 (1952).
c F. S. Stephens and F. Asaro, private communication (August, 1953).

BETA SPECTROSCOPY

T. O. Passell

The conversion electron spectrum of Am^{241} has been extensively investigated on the double focussing beta spectrometer. Some 25 conversion lines below 100 kev were observed, of which 20 were assigned to gamma rays of 26.4, 33.2, 43.4, 56.0, 59.6, and 99.5 kev, respectively. The following table gives a brief summary of the results. The summary includes lines of >100 kev whose interpretations are still in doubt.

e^- E Kev	Shell	"γ"	Intensity e^-/α	Best energy
25.0	N_{II}	26.3	0.0068	26.38 ± 0.04
10.6	L_I	33.1	0.067	33.24 ± 0.1
11.5	L_{II}	33.1	---	---
27.3	M_I	33.1	0.018	---
28.7	M_{III}	33.2	0.0012	---
31.7	N_I	33.2	0.004	---
20.8	L_I	43.3	0.017	43.4 ± 0.2
21.8	L_{II}	43.4	0.035	---
25.8	L_{III}	43.4	0.039	---
32.6	L_I	55.1	--	56 ± 1
33.7	L_{II}	55.3	--	---
38.8	L_{III}	56.4	0.0015	---
37.1	$L_I L_{II}$	59.6	0.236	59.62 ± 0.06
42.0	L_{III}	59.6	0.054	---
54.4	M_{II}	59.8	0.064	---
59.2	N_{III}	59.6	0.014	---
77.9	$L_I L_{II}$	99.5	0.00062	99.5 ± 1.0
81.5	L_{III}	99.1	0.00037	---
95	M_V	100.7	0.00031	---
118	---	---	0.00019	---
146.5	---	168.5	0.0001	168
187.8	$L_I L_{II}$	209.8	0.00016	209

NEUTRON-DEFICIENT ISOTOPES OF IRIDIUM, PLATINUM, GOLD, AND MERCURY

Warren G. Smith

I have been attempting to identify some of the neutron-deficient isotopes of iridium, platinum, gold, and mercury. Iridium, platinum, and gold metals have been bombarded with protons; chemical separations and milkings have then been made. (An excitation function was run previously to make the mass assignment of an 11-hour platinum activity.)

The following preliminary assignments have been made, some of which are in disagreement with previous assignments given in the literature:

Hg	188	189	190	191 45m	192 ~6h	193 150m
Au	187 ~10m	188	189	190	191 3h	192
Pt	186	187 ~2h	188 ~6h	189 11h	190	191
Ir	185	186	187	188	189 10d	190

The 230- and 560-kev gamma rays have been assigned to Pt^{189}, and 790- and 120-kev gamma rays have been assigned to Pt^{188}.

J. M. Hollander has observed a 35-minute activity in the bromine fraction of a carbon ion on copper bombardment. I have determined the relative yields of 35-minute, 1.7-hour and 16-hour bromine in alpha bombardments on arsenic from ~50 to 150 Mev. An excitation function for the production of 16-hour bromine will be run and from this it should be possible to make a mass assignment for the 35-minute activity.

HEAVY BOMBARDMENT OF TANTALUM

J. M. Hollander

The program of study of the neutron-deficient nuclides produced in heavy-ion bombardments is being continued.

Bombardments of tantalum with the carbon ion beam of the 60-inch cyclotron have resulted in the production of two new isotopes of gold and perhaps also a new platinum isotope.

In the gold chemical fraction from these bombardments, activities with half-lives of 42 ± 5 minutes and 9 ± 2 minutes have been observed, and in the platinum fractions activities with half-lives of 11 ± 1 hour and 2.2 ± 0.5 hours are seen. Work is being continued on the identification and study of these nuclides.

Bombardments of other elements with carbon ions are also planned.

THE ABSORPTION SPECTRUM OF CURIUM TRIFLUORIDE

D. C. Feay, J. G. Conway, and B. B. Cunningham

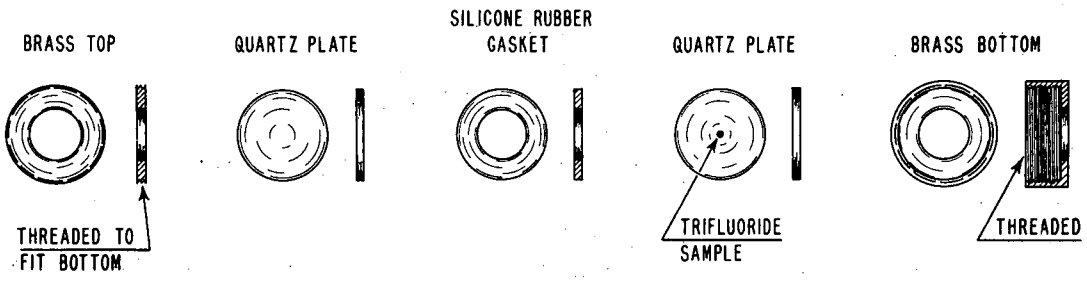
The curium solutions used in obtaining the absorption spectrum of tripositive curium reported earlier[a, b] were about 0.1 M in hydrogen peroxide. Solutions containing hydrogen peroxide have a very large extinction coefficient below 2800Å. Because of this, the obtaining of the absorption spectrum of tripositive curium would involve finding a relatively small difference between two large numbers; i.e., the difference between the extinction coefficients of a hydrogen peroxide solution and a solution of curium-containing hydrogen peroxide. In order to avoid the production of hydrogen peroxide, solid dry curium trifluoride was used as the absorber rather than an aqueous solution of curium.

The trifluoride was precipitated from solution and washed twice with dilute hydrofluoric acid. Using concentrated hydrofluoric acid, the precipitate was transferred as a slurry to a small platinum dish where it was dried and then heated at about 200° C for a few minutes. The sample was placed in the cell shown in Fig. 1 immediately after the last heating was finished. The kind and amount of impurities in the curium stock solution are listed in Table I.

---

a L. B. Werner and I. Perlman, "The Transuranium Elements: Research Papers," National Nuclear Energy Series, Plutonium Project Record, Vol. 14B, Paper No. 22.5. McGraw-Hill Book Co., Inc., New York, 1949.

b W. W. T. Crane, Some Physical and Chemical Properties of Curium, University of California Radiation Laboratory Report No. UCRL-1220 (Ph.D. Thesis), April 16, 1951.



THE CELL IS ASSEMBLED IN THE ORDER SHOWN ABOVE. THE BRASS TOP AND BOTTOM HOLD THE QUARTZ PLATES AND THE GASKFT TOGETHER. THE BRASS PARTS AND GASKET HAVE HOLES IN THEM FOR A LIGHT PATH.

CELL USED IN ABSORPTION SPECTROSCOPY OF SOLID TRIFLUORIDES.

MU-6636

Fig. 1

Table I

Spectrographic Analysis of the Curium Stock Solution

Impurity	Percent present by weight
Am	0.5
La	0.2
Fe	0.1
Al	0.4

The absorption spectrum was photographed on a 21-foot Wadsworth mount Jarrell-Ash spectrograph in the first order using 3.5 inches of a grating with 15,000 lines per inch. The optical system was composed entirely of quartz with a hydrogen discharge lamp as the light source. Eastman Kodak 103a0 ultraviolet-sensitive film was used. Several exposures of various times were made on two plates. Three or four densitometer tracings were made of each plate. The first plate was exposed before the sample was 1.5 hours old, and the second plate required another two hours.

A sample of gadolinium trifluoride was prepared in the same manner, and its absorption spectrum was also photographed. The observed lines and their relative intensities are given in Table II. The relative intensities are very rough, and the absorption in curium trifluoride is greater than in the gadolinium trifluoride. No attempt was made to calculate extinction coefficients because the path length of the light through the trifluoride samples was unknown.

The lines at the shortest wave lengths (2364 and 2334 Å) are both very broad. No absorption at wave lengths longer than 2850 Å was found in either curium trifluoride or gadolinium trifluoride. This is in sharp contrast to the absorption spectra of the other lanthanides and precurium actinides. While all the absorption lines that have been seen in the absorption spectrum of aqueous solutions of tripositive gadolinium were not found here, there is still considerable similarity between the two absorption spectra in the grouping and the relative intensities of the absorption lines. This would seem to indicate that curium and gadolinium occupy analogous positions in the actinide and lanthanide series; i.e., they both have half-filled f shells. Or more generally, that the two ions have the same ground state configuration as is required by the actinide concept.

Table II

Ultraviolet Absorption Spectra of Curium and Gadolinium in the Solid Trifluorides

CmF λ	I	GdF λ	I
2826 Å	2	2834 Å	4
2774	10	2759	10
2680	6	2666	4
2364	4	2334	4

The wave lengths are good to about 3 Å.

The intensities are only relative, and the absorption in the curium is stronger than in gadolinium.

THE VAPOR PRESSURE OF AMERICIUM TRIFLUORIDE

S. C. Carniglia and B. B. Cunningham

Using the twin effusion apparatus with which the vapor pressure of PuF_3 was recently measured,[1] the solid-vapor equilibrium of AmF_3 has been studied over the temperature range 850° to 1200° C. Two sets of points based on different samples were in essential agreement. The equilibrium pressures were in general about 2.5 to 3.0 times the values obtained previously by M. E. Jones,[2] although the calculated $\Delta H^{o}_{1000^{o}C}$ of 97 kcal/moles almost coincides with one of Jones's determinations.

A detectable curvature in the plot of $\log_{10}p$ vs. 1/T has permitted a rough estimate of the ΔC_p of vaporization for AmF_3. A preliminary estimate of the uncertainties fixes the limits as:

$$\Delta C_p = -20.0 \pm 15.0 \text{ cal/moles.}$$

A detailed analysis of the data is in progress.

1 S. C. Carniglia and B. B. Cunningham, University of California Radiation Laboratory Quarterly Report UCRL-2257 (June 25, 1953).

2 M. E. Jones, The Vapor Pressure of Americium Trifluoride, U. S. Atomic Energy Commission Declassified Report AECD-3270; UCRL-1438 (1951).

TRICHLORIDE HYDROLYSIS REACTIONS

C. W. Koch and B. B. Cunningham

Experiments have been continued on the vapor phase hydrolysis of rare earth trichlorides: $MCl_{3(s)} + H_2O_{(g)} = MOCl_{(s)} + 2HCl_{(g)}$. A summary of thermodynamic constants for the trichloride hydrolysis reactions is presented in Table I.[1] The calculated free-energy values listed are based on the function

$$\Delta F^o_T \text{ (kcal)} = \Delta H_o + (6.4T\log T/1000) + 0.18 \times 10^{-6}T^2 - 22/T + IT.$$

Calculations are currently being carried out to compare the present data with results obtained from various theoretical equations used to calculate the "crystal energy" from crystallographic data.

Table I

Summary of Thermodynamic Constants for the Trichloride Hydrolysis Reactions

Metal	ΔF^o_{785}	ΔH^o_{785}	ΔS^o_{785}	ΔF^o_{298}	ΔF^o_{298}	ΔS^o_{298}	ΔH^o_o	I
La	+1.4	25.6	30.8	17.0	27.0	33.5	27.9	-52.4
Pr	-2.1	21.6	30.2	13.2	23.0	32.8	24.0	-51.8
Nd	-3.0	20.8	30.4	12.4	22.2	33.0	23.2	-52.0
Sm	-5.4	19.2	31.4	10.4	20.6	34.0	21.6	-53.0
Gd	-6.2	17.8	30.6	9.3	19.2	33.2	20.2	-52.2
Tb	-6.7	18.8	32.6	9.7	20.2	35.3	21.2	-54.4
Am	-4.1	20.0	30.6	11.5	21.4	33.3	22.4	-52.3

1 C. W. Koch, Thermodynamics of the Trichlorides and Oxychlorides of Some of the Lanthanide and Actinide Elements, University of California Radiation Laboratory Report UCRL-2286 (Ph.D. Thesis), August, 1953.

ALPHA-GAMMA COINCIDENCE PULSE-HEIGHT ANALYSIS

A. E. Larsh, Jr., F. S. Stephens, and F. Asaro

Recently, use has been made of the gatable characteristic of one of our 50-channel 6BN6 Snapper Pulse-Height Analyzers (UCRL-1959) to investigate the coincident gamma spectra from several alpha-active isotopes.

A 2-microsecond delay-line-shaped gamma pulse was inserted into the signal input of the Snapper. The gate input was fed a 1-microsecond shaped and delayed pulse from the alpha detector.

For some experiments the energy selection of the alpha-gate range was done by an electronic single-channel analyzer. In other experiments the alpha energy to be examined for coincident gamma rays was determined with an alpha-ray spectrograph.

The block diagram shows the arrangement of circuit components (Fig. 2).

Gamma spectra curves are shown both with and without coincident alpha gating (Fig. 3).

For this work stock UCRL Linear Amplifiers were used to handle the narrow pulses. Their output is limited to 70 volts. Therefore, the Snapper was run with 1-volt channel widths instead of the usual 5 volts per channel.

Alpha-Gamma CPHA

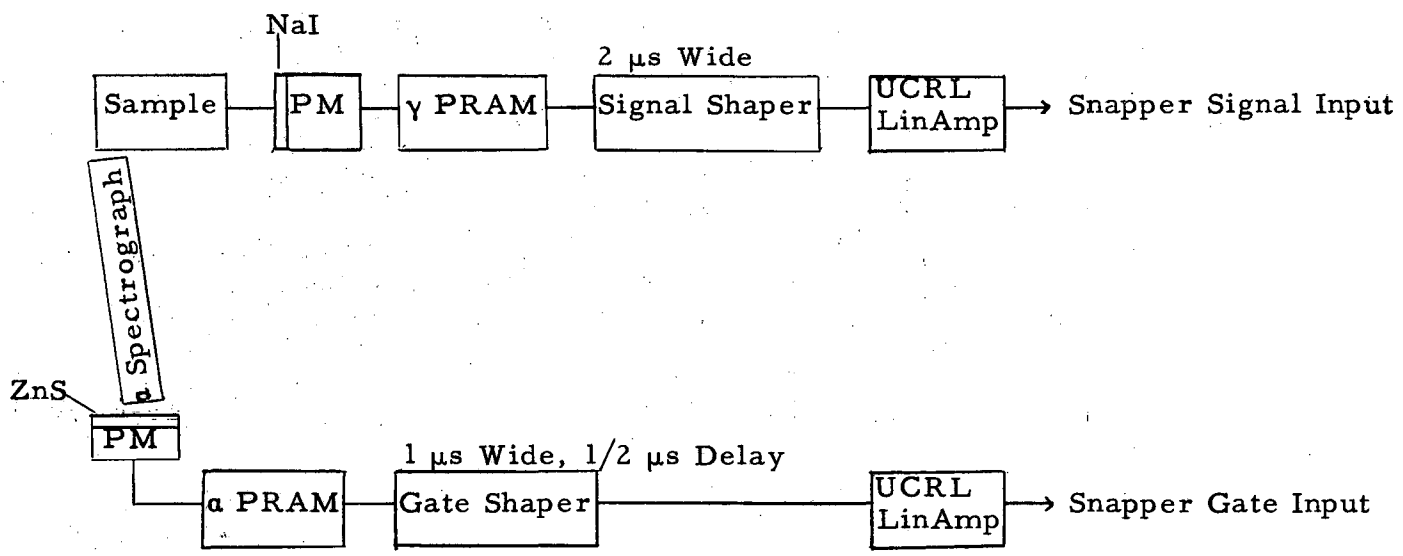


Fig. 2

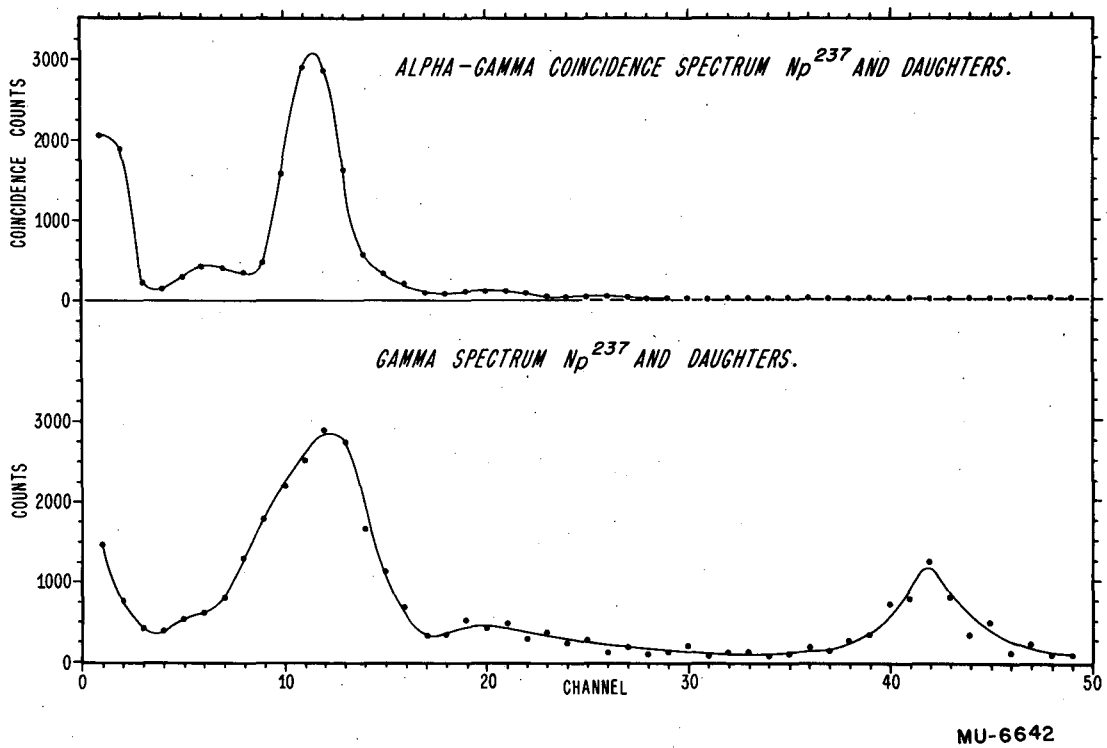

ALPHA-GAMMA COINCIDENCE SPECTRUM Np237 AND DAUGHTERS.
COINCIDENCE COUNTS
3000
2000
1000
0
GAMMA SPECTRUM Np237 AND DAUGHTERS.
COUNTS
3000
2000
1000
0
0
10
20
CHANNEL
30
40
50
MU-6642

Fig. 3

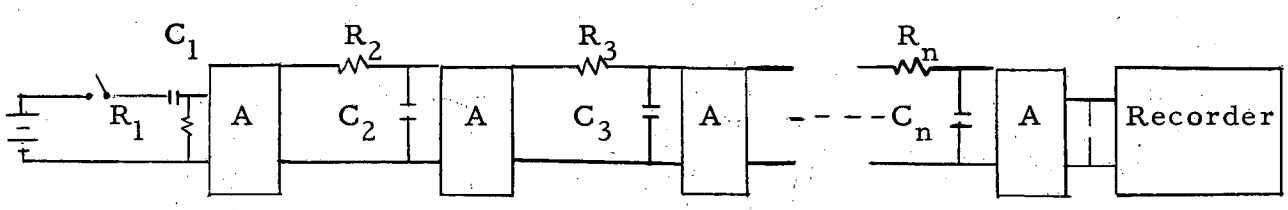
GADAC

H. P. Robinson and G. O. Brink

A very simple analog computer has been developed for plotting the growth and decay of members of a radioactive disintegration chain. It is equally applicable to the solution of problems involving pile bombardments in which elements are built up by successive neutron capture.

To use the computer one merely sets the several dials to values corresponding to the half-lives involved in the chain, or in the case of pile bombardments, to reciprocal capture cross sections, turns a switch to select the desired component in the chain, then turns on the "Start" switch. A recorder traces the growth and decay of that component on a convenient time scale, usually within a minute.

The electrical arrangement is as follows:



The A's are isolating amplifiers and ideally have unit gain. Then if R_1C_1 is made proportional to the half-life of A, R_2C_2 to that of B, etc., the above circuit is an exact analog of the decay chain:

$$A \rightarrow B \rightarrow C \rightarrow \text{--} N \rightarrow$$

If the chain is one of neutron capture, then R_1C_1 is made proportional to the reciprocal of the capture cross section (total cross section where more than one process is involved), etc. The recorder then gives a response that describes accurately the growth and decay of the nth component, in terms of disintegration rates.

The instrument can be used to solve for an intermediate half-life or cross section if the other constants are known, merely by trying various values until the computer produces a result in agreement with experiment. This method has the important property that an indication is obtained of the reliability of the desired half-life in terms of the reliability of the other parameters, i.e., the "sensitivity" of the measured results to the half-life is obtained.

Most of the problems handled with it to date have involved determining unknown cross sections in pile bombardments, and on the basis of this experience we plan to build a new unit capable of handling eight components instead

of the four used in the first two instruments. The recorder will be replaced by a meter, and a timer used to obtain accurate short intervals of time to operate a "hold" circuit so that the final reading can be observed on a meter of slow response, and at the same time the time constants in the computer can be relatively short.

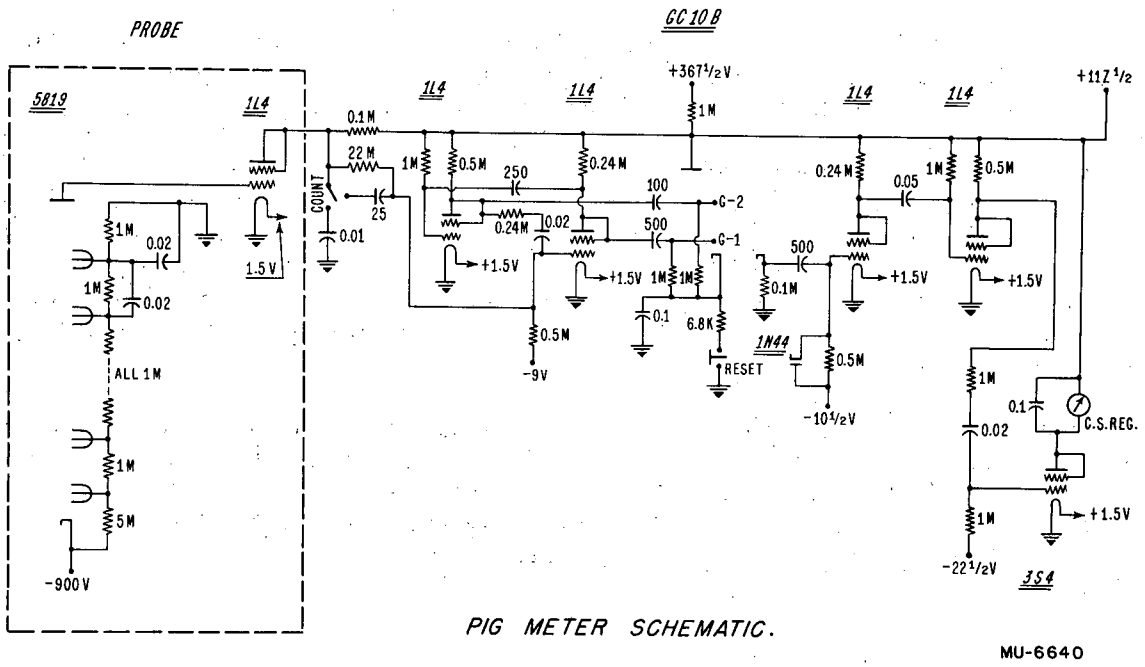
PIG METER

A. E. Larsh, Jr. and A. Ghiorso

A portable Indicating Gamma Meter (PIG Meter) has been built for use in making more accurate measurements during surveys of gamma-active material. The entire unit is battery powered to make possible the operation of the unit in the field, if need be. Figure 4 indicates the arrangement.

The PIG Meter has a Harshaw packaged NaI crystal for the gamma detector. A 5819, operated from a 900-volt supply, receives light from the crystal and produces voltage pulses which are amplified and used to trigger a univibrator. Two pulses are taken successively from the univibrator plates and fed to the guide electrodes of an Erickson Glow Transfer scaler tube, GC 10 B, which scales down the count rate by a factor of 10 and triggers a second univibrator which fires a register-driving tube. The Brower clock-type register was chosen for count readout because it required least driving power of the registers we had available. The higher possible counting speed of this register proves convenient since the initial count rate is being scaled down by a factor of only 10.

Curves showing constant-sample-activity count rates vs. time for both intermittent service and continuous monitoring are shown (Figs. 5 and 6). The curves indicate that for intermittent service the count reproducibility is very good. But during continuous monitoring the count rate drops off as the circuit is affected adversely by battery polarization.



PIG METER SCHEMATIC.

MU-6640

Fig. 4

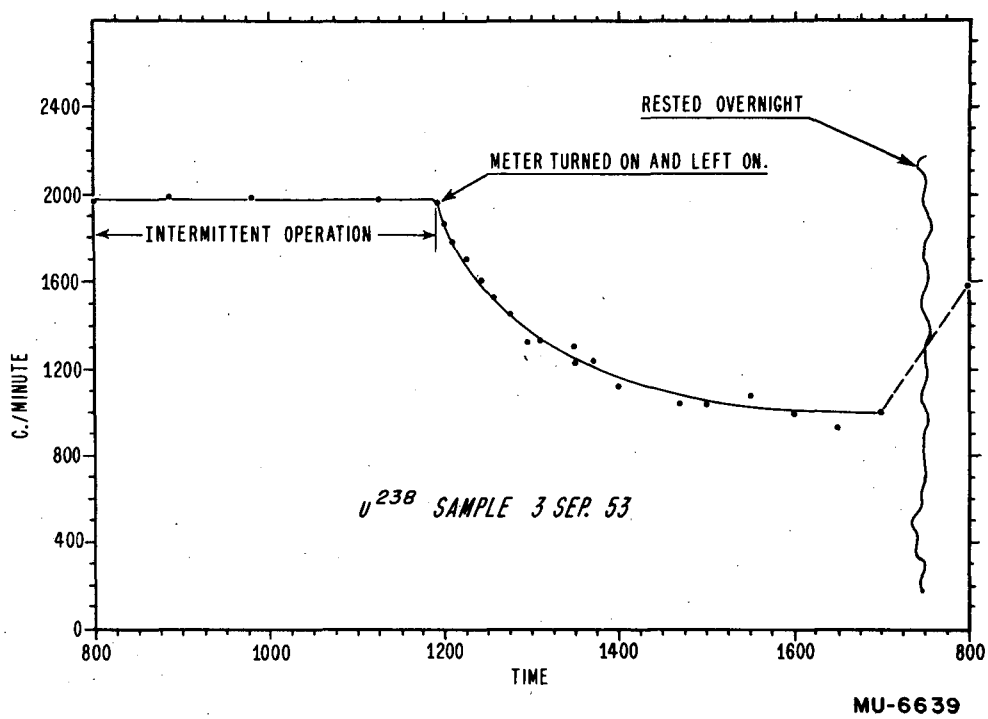

RESTED OVERNIGHT
METER TURNED ON AND LEFT ON.
INTERMITTENT OPERATION
C./MINUTE
2400
2000
1600
1200
800
400
0
800
1000
1200
1400
1600
800
TIME
U238 SAMPLE 3 SEP. 53
MU-6639

Fig. 5

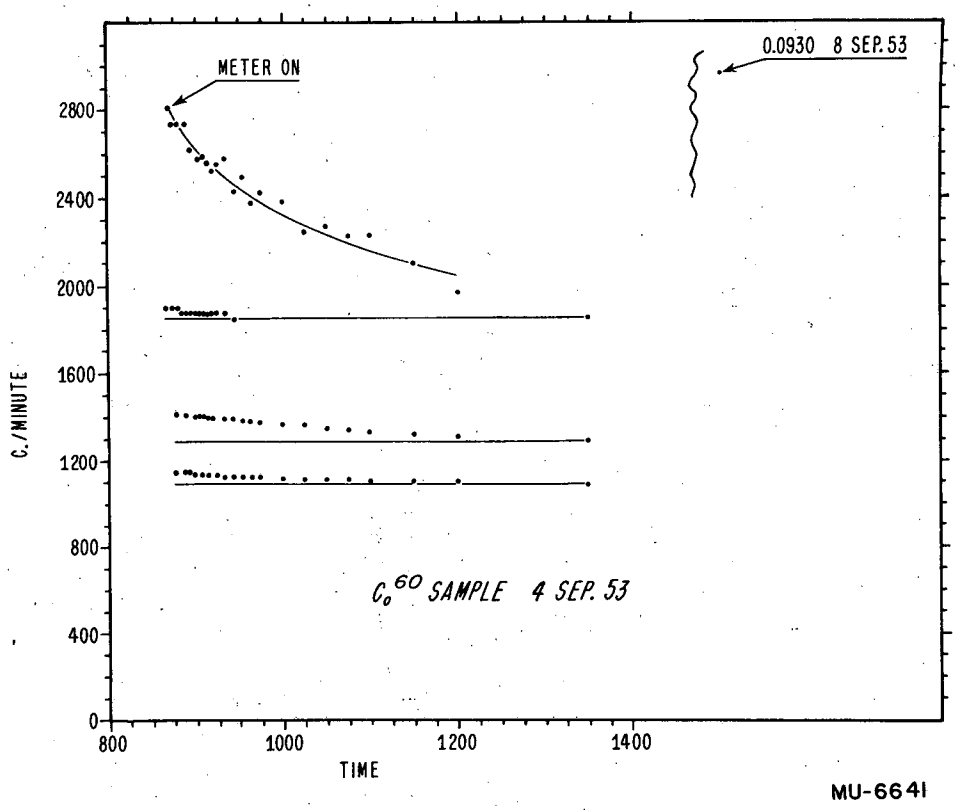

METER ON
0.0930 8 SEP. 53
2800
2400
2000
1600
1200
800
400
0
C./MINUTE
800
1000
1200
1400
TIME
C_o^{60} SAMPLE 4 SEP. 53
MU-6641

Fig. 6

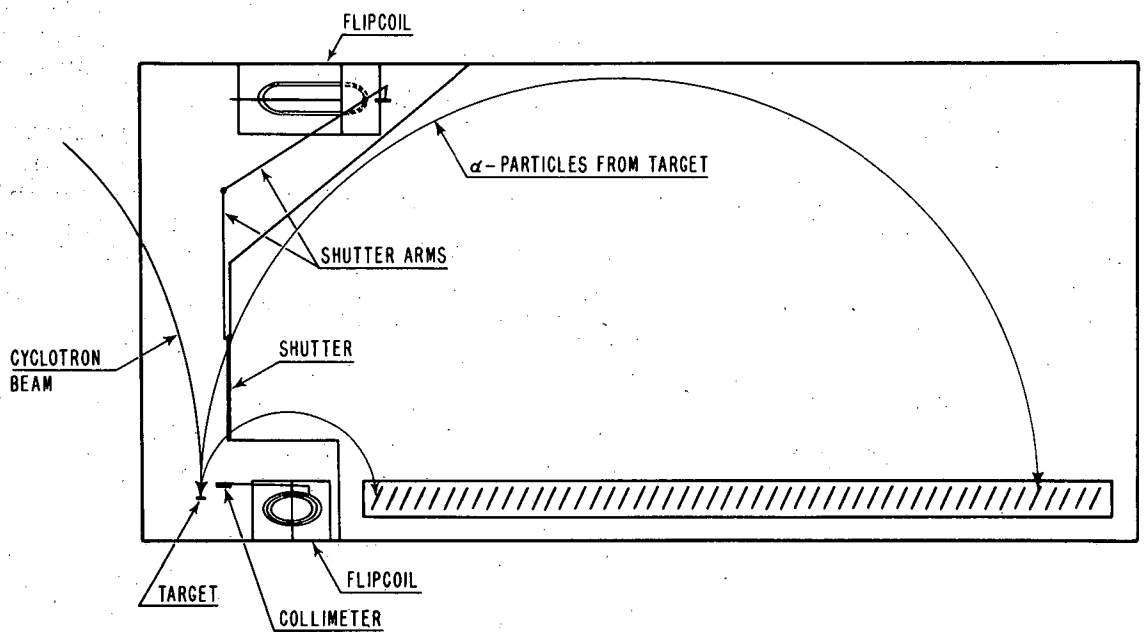
AN INTRACYCLOTRON ALPHA-RAY SPECTROMETER

R. H. Shudde

An intracyclotron alpha-ray spectrometer, which utilizes the magnetic field of the cyclotron, has been designed and constructed. The purpose of this instrument is to determine the alpha energies of heavy nuclides whose half-lives are too short to permit chemical separations (< 5 seconds). For example, the decay chains of Pa^{225} and Pa^{224} should be easily measured.

The spectrometer (see Figs. 7 and 8) consists of an aluminum box (~ 20 x 35 inches), a movable collimator, a shutter, a target holder, and a removable photographic plate holder. In operation, the spectrometer is put into the 184-inch cyclotron on the proton probe cart. The target is bombarded for a few seconds with the cyclotron beam. Using a special timer in the cyclotron control room, the beam is automatically turned off. Immediately after the beam is turned off, the timer, by means of a flip-coil, places a movable collimator in front of the target. When the collimator is in place, another flip-coil is actuated which opens a sliding aluminum shutter, thus exposing photographic plates to the collimated beam of alpha-decay particles from the target. The alpha particles are separated energetically by the magnetic field at the cyclotron, and are focused through 180° onto the photographic plates. After exposing the plates to the decaying target for a predetermined length of time, the timer closes the shutter. When the shutter is closed, the collimator flip-coil is actuated and the collimator is removed. Upon removal of the collimator, the cyclotron beam is automatically turned on for a predetermined length of time, and the whole cycle is repeated.

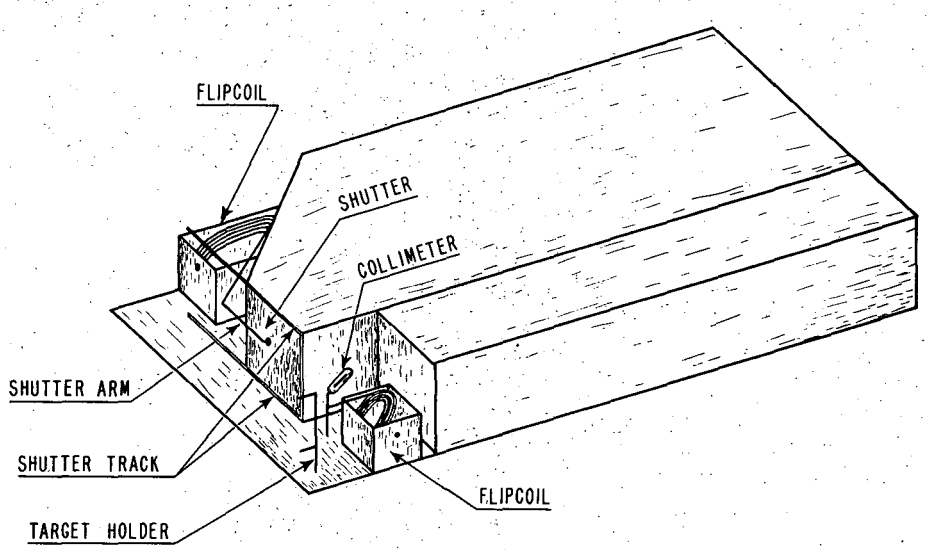
Ilford D-1 photographic emulsions are used. These emulsions are mounted on 1 x 3-inch microscope slides. For each bombardment 50 of these plates are placed upon end in the spectrometer and at an angle of 30° to the incident alpha particles. The plates are then scanned under a microscope. Plots of number of alpha particles vs. distance of alpha particles from the target reveal the intensity of alpha emitters with a given energy. The energies are determined from range-energy curves of alpha particles in D-1 emulsions, and are double checked from the known $H\rho$ of the particles.



TOP VIEW OF SPECTROMETER.
(MAGNETIC FIELD PERPENDICULAR TO THE PLANE OF THE PAPER)

MU-6638

Fig. 7



ISOMETRIC VIEW OF SPECTROMETER.

MU-6637

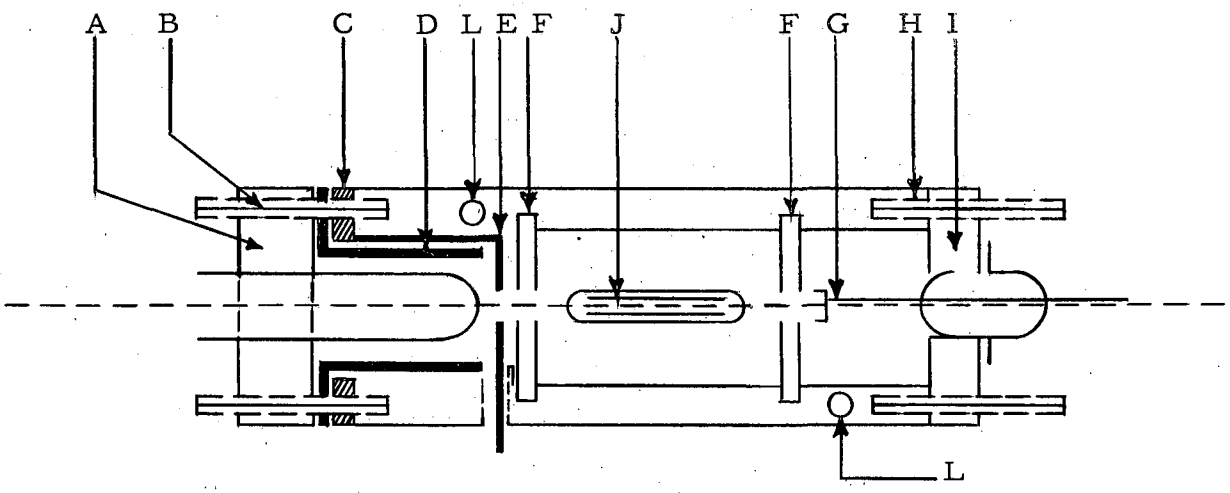
Fig. 3

MASS SPECTROSCOPY

F. L. Reynolds and M. C. Michel

A bombardment ion source similar in principle to that published by Winn and Nier, RSI 20, 773 (1949) has been constructed. The electron bombarding beam is run space charge limited and the emission is controlled by a biased grid which sees a changing potential with a change in the filament-to-grid emission. No vacuum tubes are used in this type of emission regulator. Experiments are in progress to ascertain the degree of regulation and other characteristics before employing the ion source in one of our spectrometers.

Construction details of our ion source are as shown in the sketch (which is approximately double actual size).



A. Filament support, removed by sliding off from pins B. Both filament leads (Kovar) and part A held to pins by 0-80 set screws.

B. Insulated pins, ceramic or glass.

C. Mica or glass insulated spacer.

D. Metal box, acts as both heat and electrostatic shield.

E. Insulated grid plate with 0.040 diameter hole.

F. Metal insert plates, with 0.040 diameter holes. Forms ion box in field free region.

G. Ion Trap.

H. Metal Pins.

I. Detachable plate, removed from pins H. Held in position by 0.80 set screws.

J. Sample filament, made by bending in a U shape a 0.030 wide tantalum filament. This operation is done in a special forming jig. The filament is spot welded to suitable Kovar leads which are supported from the top of the ion source box. The unit is then attached to a mounting plate through holes marked L.

BIO-ORGANIC CHEMISTRY

M. Calvin, A. A. Benson, and R. M. Lemmon

THE CYCLIC REGENERATION OF CARBON DIOXIDE ACCEPTOR IN PHOTOSYNTHESIS

J. A. Bassham, A. A. Benson, Lorel D. Kay, Anne Z. Harris, A. T. Wilson, and M. Calvin

Previously reported tracer studies of the path of carbon in photosynthesis[1] led to the conclusion that carbon is incorporated by a carboxylation reaction leading to phosphoglyceric acid (PGA), which is then reduced and condensed to fructose and glucose phosphates by a series of reductions similar to a reversal of glycolysis. These conclusions were supported by the observations that when carbon-14 is administered to the photosynthesizing plant as $C^{14}O_2$, the first radioactive compound isolated is carboxyl-labeled PGA, followed shortly by dihydroxyacetone phosphate (DHAP), fructose monophosphates (FMP), and glucose monophosphate (GMP), both hexoses being 3, 4-labeled. After longer exposures of the plant to $C^{14}O_2$, radiocarbon appears in other carbon atoms of PGA and hexose and the distribution of activity is in agreement with the above conclusions.

$$\begin{array}{cccccccccccccccccccccc}
 & & & & & * & & & & & * & & & & & * & & & & & * & * \\
* & & * & & * & & * & & * & & * & & * & & * & & * & & * & & * \\
C & - & C & + & CO_2 & \rightarrow & C & - & C & - & C & \xrightarrow{2[H]} & C & - & C & - & C & \rightarrow & C & - & C & - & C & - & C & - & C \\
A & & & & & & & & PGA & & & & & & TRIOSE & & & & & & HEXOSE & & & & & &
\end{array}$$

Observations on the rate and distribution of labeling of malic acid[2,3,4] showed it to be the eventual product of a second carboxylation reaction, which is accelerated during photosynthesis, and it was proposed that this second carboxylation played a part in the reduction of carbon in photosynthesis, leading eventually to the formation of the two-carbon CO_2 acceptor (A, above). Malic acid, itself, was apparently precluded as an actual intermediate by inhibition studies,[5] but was thought to be an indicator of an unstable intermediate which was actually the first product of the second carboxylation. The discovery[6] of rapidly labeled sedoheptulose monophosphate (SMP) and ribulose diphosphate (RDP) led to their inclusion in the proposed carbon-reduction cycle leading to the two-carbon CO_2 acceptor.

The reciprocal changes in reservoir sizes of RDP and PGA observed when algae were subjected to light and dark periods[7] indicated a close relationship, perhaps identity, between the RDP and the two-carbon CO_2 acceptor.

In order to test these conclusions, it was necessary to design experiments involving very short exposures of the plant to $C^{14}O_2$. In some of these experiments, the C^{14} was administered during "steady state" photosynthesis, the environmental conditions (light, carbon dioxide pressure, etc.) being kept as nearly constant as possible for the hour preceding and the time during the experiment. Degradation methods have been developed for sedoheptulose and ribulose and nearly complete distribution of radioactivity within these sugars obtained.

The results of these experiments seem to obviate the possibility that the carboxylation reaction leading to malic acid is a step in a carbon reduction during photosynthesis. Since no new evidence has been found for the second "photosynthetic" carboxylation, it now appears that a carbon-reduction cycle involving only one carboxylation (leading to PGA) is more likely than the previously proposed two-carboxylation cycle.

Experimental Procedure

Short "Steady State" Experiments

Algae (Scenedesmus obliquus) were grown under controlled conditions,[3] centrifuged from the growth medium, and resuspended in a 1%-by-volume suspension in distilled water. This suspension was placed in a rectangular, water-jacketed illumination chamber 6 mm thick, through which was passed a continuous stream of 4% CO_2-in-air (Fig. 1). From the bottom of the chamber, a transparent tube led to a small transparent pump constructed of appropriately placed glass valves and two 5-ml glass syringes mounted on a lever arm in such a position that the syringe plungers moved in and out reciprocally about 5 mm when the lever arm was moved back and forth by a motor-driven eccentric.

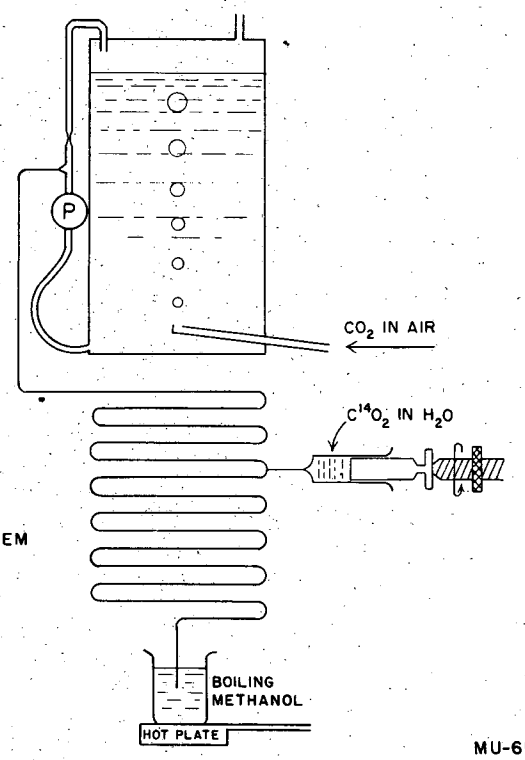
The output of the pump was divided, the major portion being returned to the illumination chamber and a smaller portion (20 ml/minute) forced to flow through a length of transparent "Transflex" tubing of about 1 mm diameter and thence into a beaker containing boiling methanol. This solvent was found to have an apparent killing time of less than 0.2 sec. as determined by the cessation of carbon fixation during photosynthesis. The linear flow rate of algal suspension in the tube was about 57 cm/second.

A solution of $C^{14}O_2$ in water (0.0716 M, 110 μc/ml) in a 30-ml syringe was injected through a fine hypodermic needle into Transflex tubing at a point a selected distance from the end of the tubing. From the known flow rate of algae suspension in the Transflex tubing and distance of flow from the point of injection of $C^{14}O_2$ to the killing solution, the time of exposure of the algae to C^{14} was calculated. The flow of the $C^{14}O_2$-containing solution was controlled by driving the syringe plunger with a constant-speed motor through a worm gear, and the flow rate was 0.5 ml/minute. The resultant dilution of the algal suspension was 2.5% and the increment in total CO_2 concentration less than 15%.

The entire apparatus was illuminated from each side by a nine-tube bank of 40-watt fluorescent lights (white) giving a uniform intensity of about 2000 foot-candles from each side. During an experiment the algal suspension was illuminated for an hour or more with 4% CO_2 before the start of the flow C^{14} exposures. Exposures to $C^{14}O_2$ ranging from 1.0 to 16 seconds were then carried out and the products of $C^{14}O_2$ reduction analyzed in the usual way[8] by paper chromatography and radioautography.

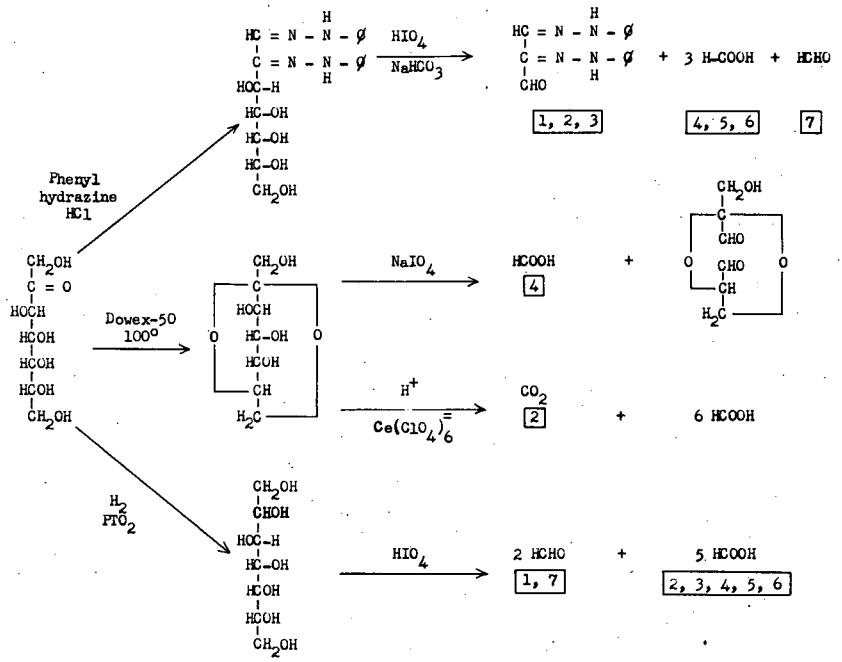
Short Soybean Experiments

A single excised trifoliate leaf from a soybean plant (var. Hawkeye) was placed in a circular flat illumination chamber with a detachable face. The chamber was equipped with two tubes, the lower one leading through a stopcock to an aspirator and the upper one through a two-way stopcock to a loop containing $C^{14}O_2$. A loosely tied thread led from the leaf stem under the detachable

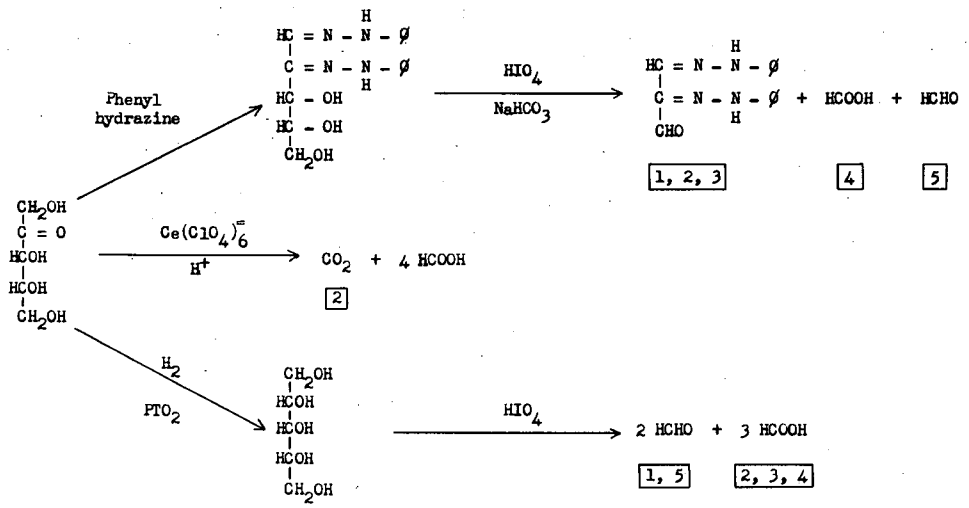


SCHEMATIC DIAGRAM OF FLOW SYSTEM FOR SHORT EXPOSURES OF ALGAE TO $C^{14}O_2$

Fig. 1



MU-6100



MU-6101

Eq. 1 Degradation of Sedoheptulose.

Eq. 2 Degradation of Ribulose.

face gasket, thence through a boiling ethanol bath and a glass tube to a weight. The illumination chamber was partially evacuated, both stopcocks were closed, and clamps removed from the chamber, the detachable face remaining in position through the atmospheric pressure. With the opening of the upper stopcock, the $C^{14}O_2$ was swept into the chamber by atmospheric pressure, the detachable face fell off and the leaf was pulled into boiling ethanol. An estimated exposure time of 0.4 sec. was obtained. The radioactive products were extracted and analyzed in the usual way. In other experiments, longer exposure times were obtained by holding the detachable face in position.

Degradation of Sugars

The reactions used for the degradation of the radioactive pentose and heptose are shown in Eqs. 1 and 2. All radioactive material was purified on two-dimensional paper chromatograms.[8] Radioactive sedoheptulose was converted to the anhydride by heating at 100° with Dowex-50 for one hour, followed by chromatography to separate the resulting equilibrium mixture.

Formation of the Osazones

The hexose and heptose osazones were made in the usual manner with phenylhydrazine hydrochloride, sodium acetate and acetic acid. Usually about 25 mg of sugar carrier was used for the reaction. Sedoheptulose osazone cocrystallized with glucosazone sufficiently well for fructose to be used as carrier with sedoheptulose activity.

The radioactive arabinosazone was made by the method of Haskins, Hann and Hudson[9] with 10 mg of arabinose carrier. The osazone was recrystallized once and diluted, as desired for each degradation, with pure crystalline, nonradioactive arabinosazone from a similar large-scale preparation.

Oxidation of Osazones

The recrystallized osazones were treated with periodate in bicarbonate buffer as described by Topper and Hastings.[10] The reaction mixture was fractionated to obtain all the products by centrifuging off and thoroughly washing the mesoxaldehyde osazone; distilling the supernate plus washings to dryness *in vacuo* and treating the distillate with dimedon to obtain the formaldehyde derivative; and acidifying and vacuum-distilling the residue to obtain the formic acid which was counted as barium formate. All products were recrystallized before counting.

Cerate Oxidation of Ketoses

The oxidation of the carbonyl carbon of a ketose to CO_2 by cerate ion was performed according to the method described by Smith.[11] To a solution of an aliquot portion of radioactivity plus weighed carrier (sedoheptulose carrier with sedoheptulose activity; fructose carrier with ribulose activity) was added a slight excess of 0.5 M cerate ion in 6 N perchloric acid, the final concentration of acid being 4 N. The resultant CO_2 was swept with nitrogen into CO_2-free base. The reaction was allowed to proceed for one hour at room temperature and then the CO_2 was precipitated and counted as barium carbonate. In all cases the theoretical amount of carbon dioxide was evolved.

Formation and Oxidation of Sugar Alcohols

The radioactive sugars were hydrogenated with platinum oxide as described previously[6] and chromatographed on paper for purification. Carrier ribitol or volemitol was added to an aliquot of radioactive alcohol and a slight excess of para-periodic acid was added. The reaction was allowed to stand at room temperature for 6 to 7 hours. Then the formic acid and formaldehyde were distilled off *in vacuo*. After the formic acid was titrated with barium hydroxide, the formaldehyde was redistilled and precipitated as formyldimedon. Both the residue of barium formate and the formyldimedon were recrystallized before plating and counting.

Oxidation of Sedoheptulosan

The radioactive sample plus carrier was treated with sodium periodate as described by Pratt, Richtmeyer, and Hudson[12] and allowed to stand at room temperature for 3 to 4 days to give time for most of the formate to be released from the intermediate ester. Then the mixture was acidified with iodic acid and the formic acid was distilled *in vacuo*. This was then counted as barium formate.

Results

In Fig. 2, the radiocarbon fixed in a "steady state" photosynthesis with *Scenedesmus* as shown as a function of time of exposure of the plant to $C^{14}O_2$ and appears to be reasonably constant over the period of the experiment. The distribution of radioactivity among various labeled compounds is shown in Fig. 3. The curve for the sugar diphosphates, principally ribulose diphosphate, is not shown but lies between the glucose monophosphate anf fructose monophosphate curves although individual points are more erratic, probably owing to the relative instability of the ribulose diphosphate.[6] The appearance of compounds other than PGA with a finite rate of labeling at the shortest times is demonstrated in Fig. 4, in which the percentage distributions of PGA and of the total sugar phosphates are shown. The extrapolations of the PGA and sugar phosphates to zero time would give about 75% and 17%, respectively. The remaining eight percent not shown is distributed among malic acid (3%), free glyceric acid (2%), and phosphoenolpyruvic acid (3%).[7] The percentage distribution among the sugar phosphates is shown in Fig. 5, where it is seen that no single labeled sugar phosphate predominates at the shortest times.

These data alone do not permit assignment of an order of precedence of the various labeled compounds in the path of carbon reduction. In order to make such as assignment it would be necessary to measure the relative rates of increase in specific activity of the various compounds. If the slopes of the curves shown in Fig. 3 are measured between 2 and 10 seconds, rates of increase in total radioactivity are obtained. If these rates are divided by the cellular concentration of the compounds involved, rates of specific activity increase are obtained. This has been done using measurements of concentrations made by two independent methods[7,13] which agreed fairly well in relative order (i.e., PGA concentration: GMP concentration = 4:1). The resulting values ranged from 0.3 for GMP to 1.0 for PGA, with FMP, DHAP, RDP and SMP falling between these values when the rates for PGA, DHAP, FMP, GMP, SMP and RDP were divided by 1, 1, 2, 2, 3 and 1, respectively, to allow for the number

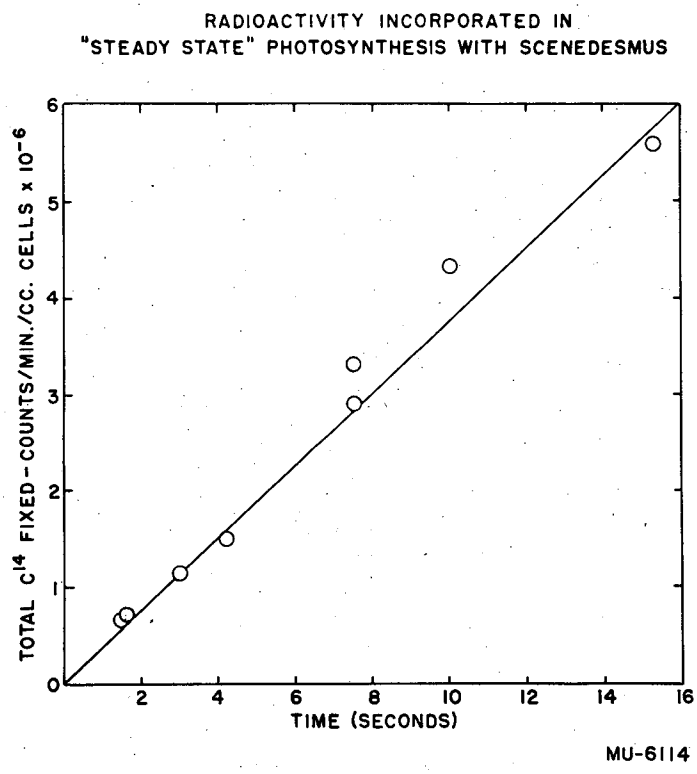
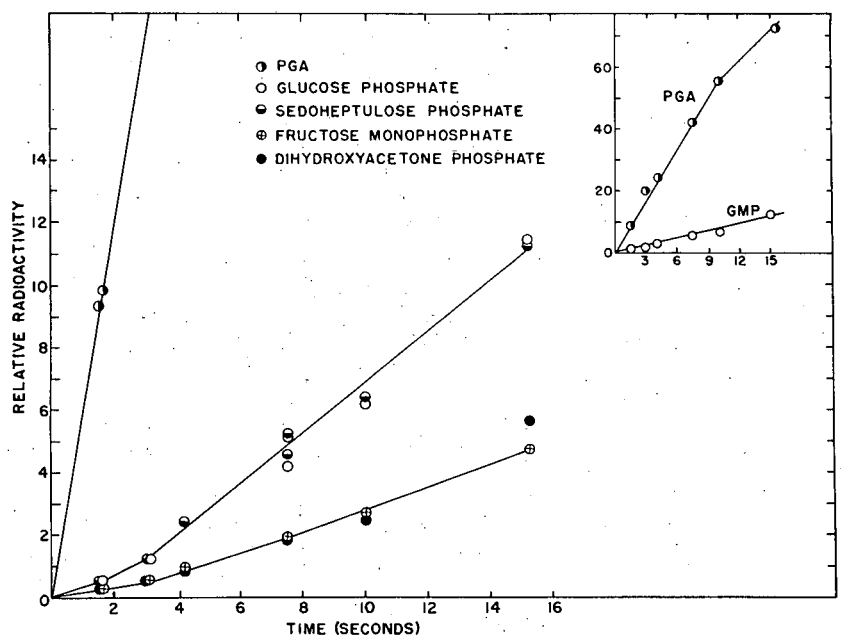

RADIOACTIVITY INCORPORATED IN
"STEADY STATE" PHOTOSYNTHESIS WITH SCENEDESMUS
TOTAL C14 FIXED-COUNTS/MIN./CC. CELLS x 10-6
0
1
2
3
4
5
6
2
4
6
8
10
12
14
16
TIME (SECONDS)
MU-6114

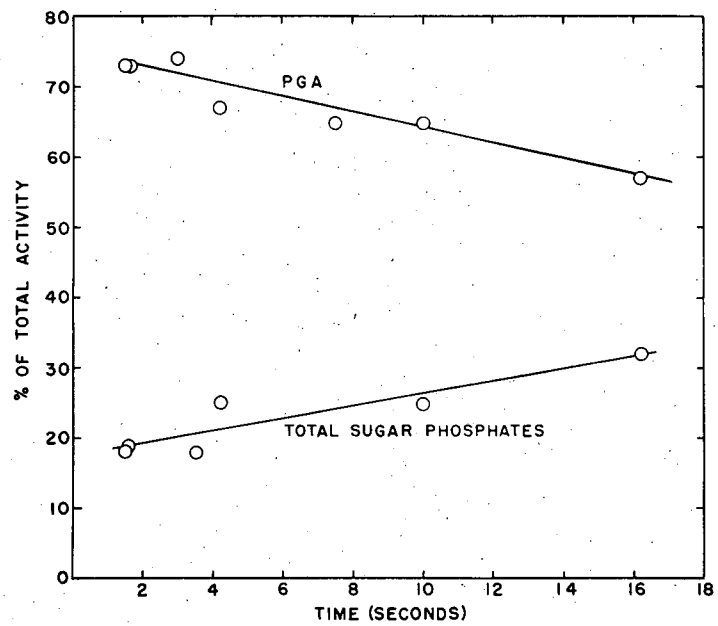
Fig. 2

DISTRIBUTION OF RADIOACTIVITY
AMONG COMPOUNDS FORMED DURING
"STEADY STATE" PHOTOSYNTHESIS WITH SCENEDESMUS



MU-6115

Fig. 3



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DISTRIBUTION OF ACTIVITY IN
"STEADY STATE" SCENEDESMUS

Fig. 4

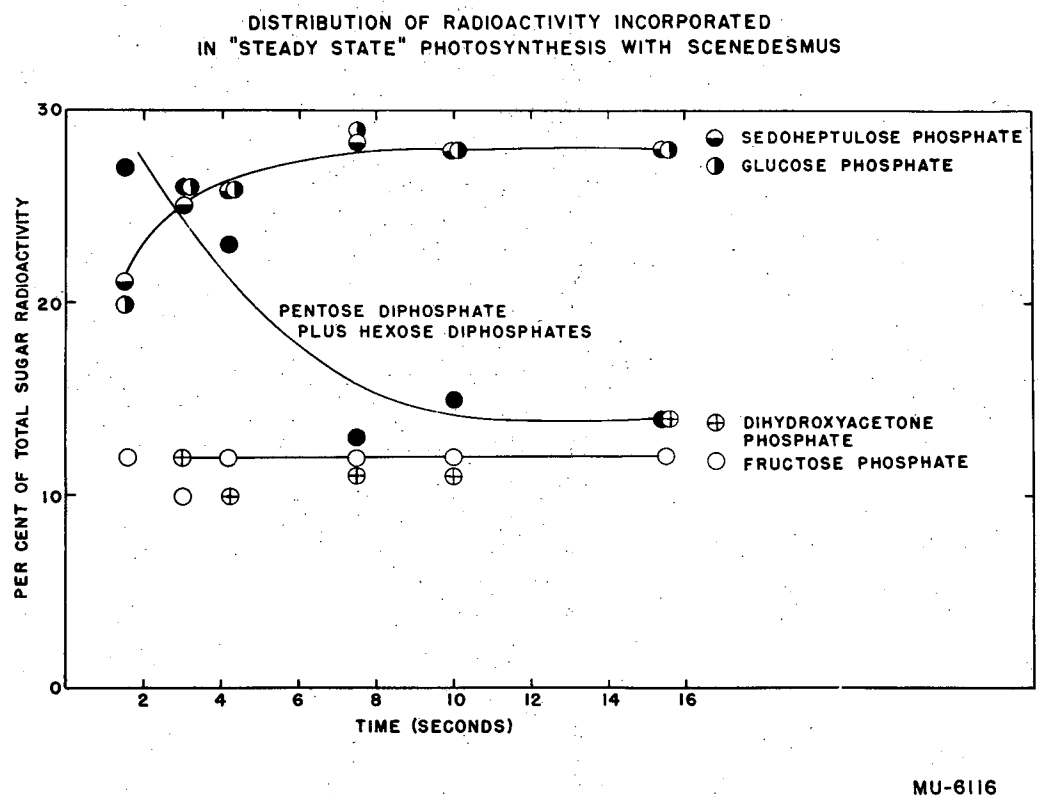

DISTRIBUTION OF RADIOACTIVITY INCORPORATED
IN "STEADY STATE" PHOTOSYNTHESIS WITH SCENEDESMUS
PER CENT OF TOTAL SUGAR RADIOACTIVITY
TIME (SECONDS)
SEDOHEPTULOSE PHOSPHATE
GLUCOSE PHOSPHATE
PENTOSE DIPHOSPHATE
PLUS HEXOSE DIPHOSPHATES
DIHYDROXYACETONE
PHOSPHATE
FRUCTOSE PHOSPHATE
0
10
20
30
2
4
6
8
10
12
14
16
MU-6116

Fig. 5

of carbon atoms being labeled significantly at these short times. This calculation is quite approximate, the concentration of compounds involved being measured in experiments with algae photosynthesizing under somewhat different conditions (i.e., 1% CO_2 instead of 4%). However, such a calculation does show more clearly the rapidity with which radiocarbon is distributed among the principally labeled carbon atoms and the difficulty in assigning an order of precedence of labeled compounds on the basis of labeling rates alone.

The fact that compounds besides PGA have finite initial labeling slopes (which results in their percentage activity's not extrapolating to zero at zero time) might be explained in several ways. One possibility is that during the killing time some of the enzymatic reactions (in this case reduction of PGA and rearrangement of the sugars) may not be stopped as suddenly as others (the carboxylation to give PGA), or may even be accelerated by the rising temperature prior to denaturation of the enzyme.

Another explanation is that some of the labeled molecules may be passed from enzyme to enzyme without completely equilibrating with the active reservoirs which are actually being measured. This sort of enzymatic transfer of radiocarbon could invalidate precedence assignments based on rates of increase in specific activities since the reservoirs would no longer be completely in the line of carbon transfer. That the equilibration between reservoirs and enzyme-substrate complexes is rapid compared to the carbon reduction cycle as a whole is indicated by the fact that all the reservoirs become appreciably labeled before there is an appreciable label in the α- and β-carbons of PGA, the 1, 2, 5, and 6 carbons of the hexoses, etc. In any event, it would appear to be safer to establish the reaction sequences from qualitative differences in labeling within molecules (degradation data) and from changes in reservoir sizes due to controlled changes in one environmental variable rather than from quantitative interpretations of labeling-rate data.

Table I shows the results of degradations on sugars obtained from the soybean series. The first column shows the variation in labeling of carbon number four of sedoheptulose obtained from soybean leaves exposed to $C^{14}O_2$ for very short periods. These soybean leaf experiments are, of course, not intended to represent "steady state" photosynthesis, since the carbon dioxide is depleted just prior to the administration of $C^{14}O_2$. Included in the table is a complete degradation of a sedoheptulose sample from sedum s. grown in radioactive carbon dioxide for two days (kindly supplied by N. E. Tolbert, Oak Ridge National Laboratory). Assuming this sample is uniformly labeled, its degradation indicates the probable limits of accuracy of the other degradations--about ± 10% of the obtained value, mainly owing to plating and counting errors because of the low amount of radioactivity available for degradation. The four degradations on sedoheptulose make it possible to obtain separate values for all the carbon atoms except 5 and 6 which are left together. However, since labeling of carbons 1, 2, and 7 is quite low in short-time experiments, it seems reasonable to assume that the labelings of these positions are equivalent to those at the α- and β-carbons in PGA and that of carbon 6 is therefore also low. In calculating the values given in Table II for sugars from Scenedesmus flow experiments, this assumption has been made. It is also assumed here that carbon atom 1 labeling is low, since it was low in short time soy-leaf experiments. The labeling of each carbon for ribulose can be individually obtained from the three degradations performed. The distributions in Table II should be interpreted as a clear qualitative picture of the position of the radioactivity within

Table I

Radioactivity Distribution in Sugars - Sedoheptulose and Hexose from Soybean Leaves

Time (Seconds)	Carbon Atoms							
	Sedoheptulose						Hexose	
	4	1, 2, 3	4, 5, 6	7	2	1 + 7	1, 2, 3	4, 5
0.4	8	33	57	0			47	52
0.8	18	43	60	2			48	51
1.5	24							
3.5	26					3		
5.0	29	36	64	2	4			
8.0	24							
10.0	28							
20.0	21	44		5	7			
300	14				12.5			
Sedum	12	37	35	12	12.5	28		

Table II

Radioactivity Distribution in Sugars from Flow Experiments

5.4 Seconds			8.5 Seconds	
Fructose	Sedoheptulose	Ribulose	Sedoheptulose	Ribulose
	2**			
3**	2		3	11*
3**	28*	11*		11
43*	24	10		
42*	27*	69*		64*
3**	2**	5		8
3	2	3		5

*Value by difference.
**Assumed.

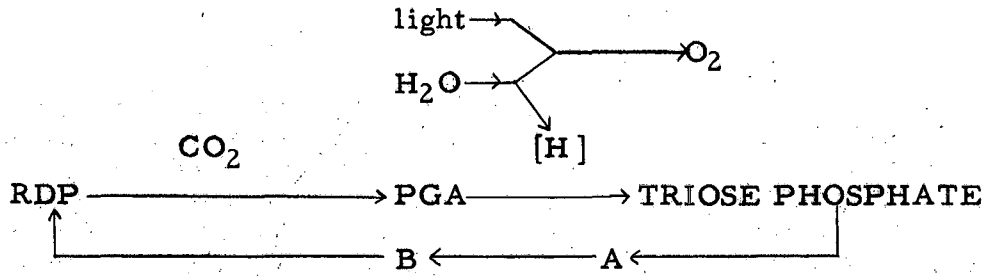
the molecule rather than as a quantitative picture. Fewer points were taken in this "steady state" flow experiment than in the one described earlier in order to obtain more labeled sugar per point for degradation purposes.

In other experiments,[14] the Scenedesmus have been kept at a steady state of light, temperature, CO_2 pressure, etc., and constant $C^{14}O_2$ specific activity until successive samplings of the suspension showed uniform labeling ("saturation") of all the common photosynthetic reservoirs (PGA, RDP, GMP, etc.). The total CO_2 pressure was then rapidly changed from 1% CO_2 in air to 0.003% in air, all other environmental conditions, including the specific activity of $C^{14}O_2$, being kept constant. The conditions of this experiment were, therefore, similar to those used previously[7] to study changing steady state except that CO_2 pressure was changed instead of illumination. In the case where the CO_2 pressure was lowered, the initial effects on the reservoir sizes of PGA and RDP were just the opposite of those observed when the illumination was stopped. Lowered CO_2 pressure resulted in an increase in RDP concentration and a decrease in PGA concentration. After a time the RDP concentration dropped to a lower level than before (as did the concentrations of all other intermediates) but the new steady state RDP concentration was higher relative to that of PGA than before. The labeled phosphoglycolic acid present, though a rather small percentage of total activity, increased significantly when the CO_2 pressure was lowered. The concentration of phosphoglycolic acid increased more slowly than the RDP and reached its maximum at a later time, thus eliminating the possibility that most of the labeled phosphoglycolic acid was formed by thermal decomposition of RDP subsequent to killing of the cells.

Discussion

Origin of PGA

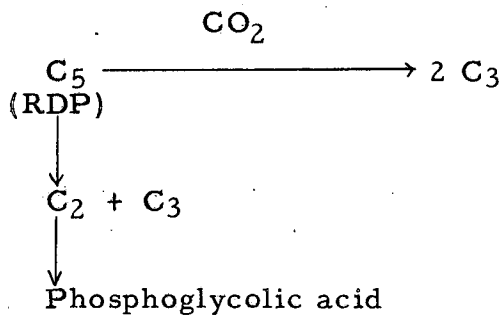
It has been suggested that RDP is the compound which supplies the two-carbon atoms for the carboxylation reaction leading to PGA.[7] If the reactions of these compounds are represented by



Sugar Rearrangements

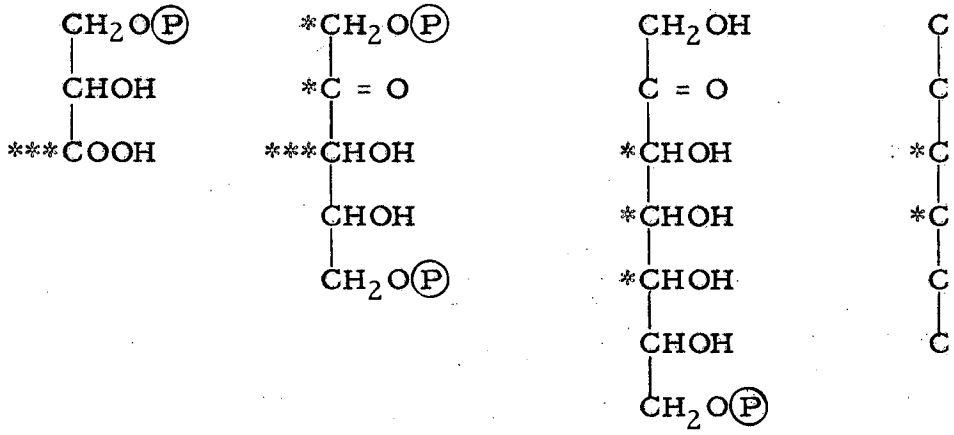
then the initial changes in reservoir sizes which would accompany changes in light or CO_2 pressure can be predicted. When the light is turned off, reducing power [H] decreases, so PGA would increase and RDP decrease. If CO_2 pressure decreases then RDP would increase and PGA would decrease. Both effects, as well as those opposite effects which would be expected to accompany a resumption of light or increase in CO_2 pressure have been observed. These results support the proposal of a carboxylation of RDP to give two molecules of PGA or the reductive carboxylation to give one PGA and one phosphoglyceraldehyde as the first step in the path of carbon dioxide reduction. It is possible

that the RDP first splits to give a three-carbon molecule and a free two-carbon fragment which is then carboxylated. However, if the phosphoglycolic acid is an indication of the free two-carbon fragment, then its slower increase in concentration (following reduction in CO_2 pressure) as compared with the increase in RDP concentration suggests that the appearance of C_2 is a consequence of the increased splitting of C_5 resulting from the increased C_5 concentration.



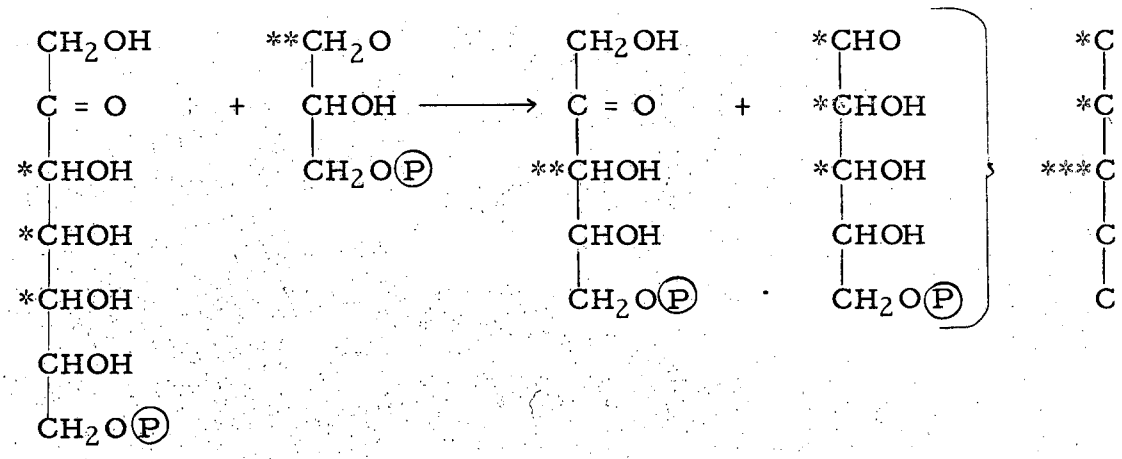
Origin of Ribulose Diphosphate

If one considers the principal labeling at short times of PGA,[1] RDP, SMP and the two hexose monophosphates[1] as, respectively,



it appears that the ribulose is not derived entirely from a $C_6 \rightarrow C_1 + C_5$ split or a $C_7 \rightarrow C_2 + C_5$ split. No five-carbon fragment of the hexose or heptose molecules contains the same distribution of radiocarbon as ribulose. The combination of C_3 with a labeled C_2 fragment could account for the observed radioactivity. However, some mechanism for the labeling of the C_2 fragment would be required. One such mechanism would be the breakdown of hexose into three C_2 fragments,[15] since carbon atoms 3 and 4 of hexose are labeled.

Another way of accounting for the observed distribution of radioactivity which seems quite plausible in view of the rapidly accumulating enzymatic evidence for the reverse reaction[16,17,18,19] is the formation of ribulose from sedoheptulose and triose. This reaction could result in the observed labeling in the following way:

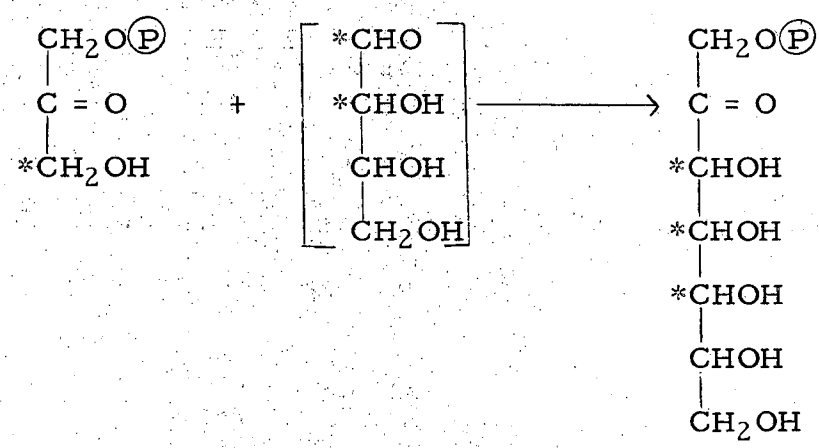


If the ribose-5-phosphate and ribulose-5-phosphate are then converted to RDP the resulting distribution of label would be that observed (carbon skeleton at right of reaction).

Origin of Sedoheptulose

The degradation data appear to eliminate the possibility of formation of sedoheptulose by a simple 6 + 1 or 5 + 2 addition, if we assume that no special reservoirs of pentose and hexose exist with distributions of radioactivity different from those measured. A reverse of the reactions proposed above for formation of RDP would require segregation of ribose and ribulose distributions as well as some other mechanism for labeling the ribose in the manner shown. It does seem likely that all the reactions involving rearrangements of sugars and perhaps those involving reduction of PGA as well are at least partially reversible in the time of these experiments. If all these compounds are intermediates in a cycle of carbon reduction, then during steady-state photosynthesis there will be a net "flow" of radiocarbon in the "forward" direction, but the possibility that the distribution of radiocarbon in later intermediates may reflect to some extent that of earlier intermediates cannot be entirely ignored.

The condensation of a triose with a C_4 fragment would give the observed distribution if the C_4 fragment is labeled in the carbon atoms 1 and 2:



Enzymatic evidence for this reaction and its reverse has been reported.[17, 20]

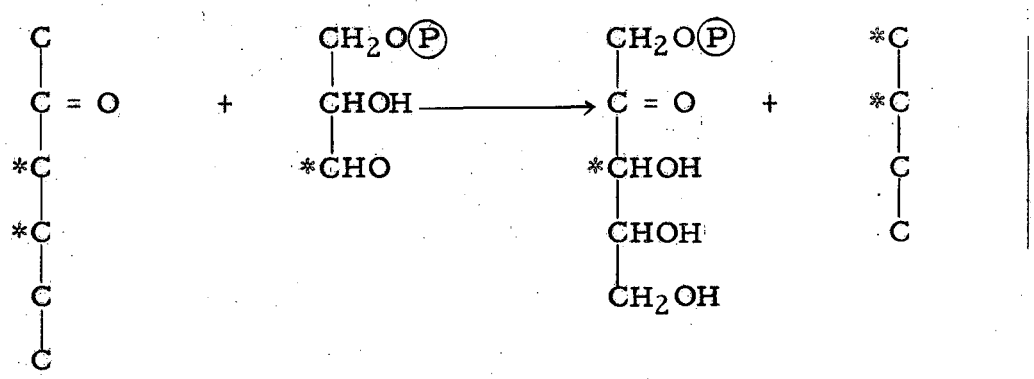
Origin of Four-Carbon Fragment

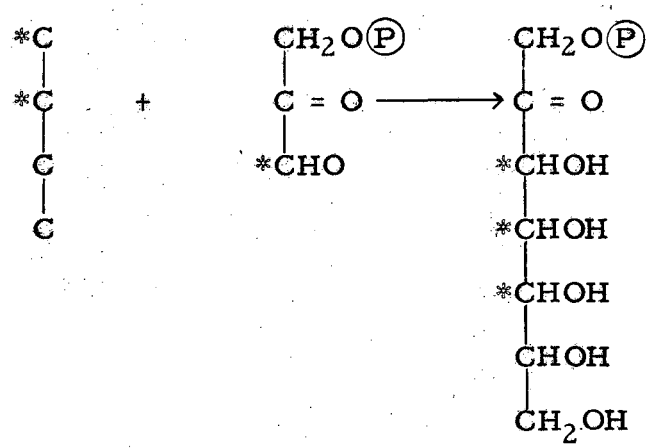
Two possible modes of formation of the four-carbon fragment with the above labeling are a $C_1 + C_3$ addition, and a $C_6 \rightarrow C_2 + C_4$ split. The $C_1 + C_3$ addition which leads to malic acid produces a C_4 fragment in the two terminal positions.[4] Therefore, the reduction of the dicarboxylic acid formed as a precursor to malic acid could not result in a C_4 fragment with the C^{14} distribution required for the formation of 3, 4, 5 C^{14} labeled sedoheptulose. There remains the possibility that the C_4 acid resulting from the second carboxylation might be reduced and split into C_2 fragments which could then condense with triose to give RDP, but there is yet no evidence for such a series of reactions although several isolable intermediates might be expected. The rapid introduction of radiocarbon into malic acid in earlier experiments[2] can be accounted for if it is assumed that the reservoir size of malic acid, depleted during the air flushing prior to the addition of $HC^{14}O_3^-$, was increasing after the addition of the radiocarbon due to the increase in total CO_2 pressure. Also, after the carboxyl group of PGA and phosphoenolpyruvic acid had become appreciably labeled, the malic acid is doubly labeled.

It is interesting to note that in the long-term "steady state" experiments in which the light was turned off,[7] the malic acid concentration dropped when the light was turned off rather than increasing as the PGA concentration increased. If malic acid were an indicator of a four-carbon intermediate in carbon reduction, the product of a second carboxylation, then one would expect its concentration to increase in the dark for two reasons. First, there no longer is reducing power that would reduce the carboxylation product to sugar if this product were an intermediate in CO_2 reduction. Second, the CO_2 concentration remains constant, while the concentration of phosphoenolpyruvic acid, which parallels the PGA concentration, increases. The decrease in malic acid concentration can be easily explained on the basis of the proposed light inhibition of pyruvic acid oxidation providing more acetyl-CoA, which can react with oxaloacetic acid derived from malic acid.

It is possible that there is another "second carboxylation" leading eventually to a four-carbon fragment which can react with triose to give sedoheptulose, but there seems to be no evidence whatever for such a reaction at present. Moreover, such a reaction should lead in short times to a four-carbon fragment somewhat more labeled in the terminal carbon position than in the second carbon position owing to dilution of the carbon introduced in the first carboxylation reaction by the PGA and triose reservoirs. This is not the case--in fact in the very shortest times the terminal carbon position of the hypothetical C_4 fragment (carbon four of sedoheptulose) is actually less labeled than the second position, at least in the soybean experiments.

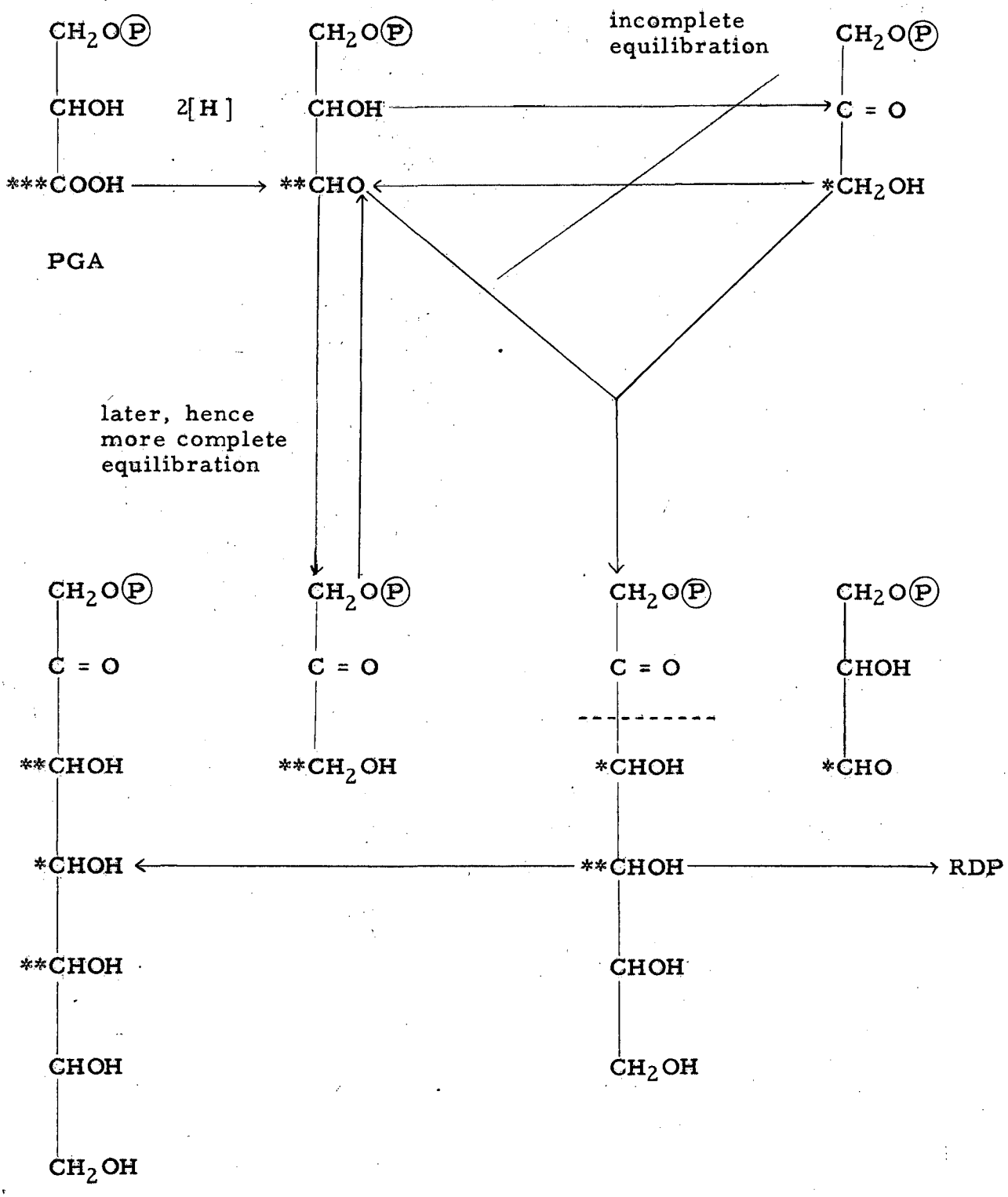
The most likely source of the C_4 fragment seems to be a $C_6 \rightarrow [C_4] + [C_2]$ split. Trioses could then react with $[C_4]$ and $[C_2]$ to give sedoheptulose and ribulose, respectively. One possible formulation of these reactions would be the following:





The reaction as written would be a transketolase reaction of the type reported by Racker, et al.[19] who found that this enzyme splits ribulose-5-phosphate, leaving a glyceraldehyde-3-phosphate and transferring the remaining two-carbon atoms to an acceptor aldehyde of 2, 3, or 5 carbons length. No mention was made of the effect of transketolase on ribose-5-phosphate with erythrose, which would result in the formation of fructose phosphate by a reaction which is just the reverse of the $C_6 \rightarrow C_2 + C_4$ split written above.

The carbon number 4 labeling observed in the case of the very short periods of photosynthesis with soybean leaves seem to cast some doubt on the $C_6 \rightarrow [C_2] + [C_4]$ split unless one can assume that the C_6 that splits is itself not symmetrically labeled at the shortest times, owing to different specific activities of the two trioses which react to give hexose:



Degradation of fructose from the 0.4- and 0.8-second experiments showed no significant difference between the two halves of fructose. It is quite possible, however, that the differences in denaturation rates of various enzymes mentioned earlier may influence the results in these short times.

Combining these reactions with others already proposed we have the following cyclic path of carbon reduction during photosynthesis. Sugars are indicated by the length of their carbon chains.

$$3\ C_5 + 3\ CO_2 \xrightarrow[12\ [H]]{} 6\ PGA$$

$$6\ PGA \longrightarrow 6\ C_3$$

$$2\ C_3 \longrightarrow C_6$$

$$C_6 + 2\ C_3 \longrightarrow C_5 + C_7$$

$$C_7 + C_3 \longrightarrow 2\ C_5$$

The net reaction for each turn of the cycle is

$$12\ [H] + 3\ CO_2 \longrightarrow C_3H_6O_3 + 3\ H_2O$$

Summary

Photosynthesizing plants have been exposed to $C^{14}O_2$ for short periods of time (0.4 to 15 sec.) and the products of carbon dioxide reduction analyzed by paper chromatography and radioautography.

Methods have been developed for the degradation of ribulose and sedoheptulose. These sugars, obtained as their phosphate esters from the above short $C^{14}O_2$ exposures and from other experiments, have been degraded and their distribution of radiocarbon determined.

The distribution of radiocarbon in these sugars, and other data, indicate that sedoheptulose phosphate and ribulose diphosphate are formed during photosynthesis from triose and hexose phosphates, the latter being synthesized in turn by the reduction of 3-phosphoglyceric acid.

Further evidence has been found for the previously proposed carboxylation of ribulose diphosphate to phosphoglyceric acid. Free-energy calculations indicate this step would proceed spontaneously if enzymatically catalyzed.

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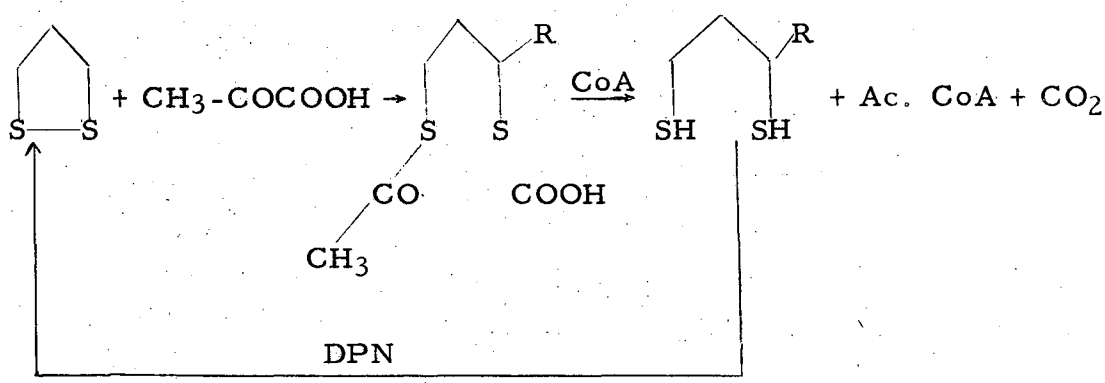
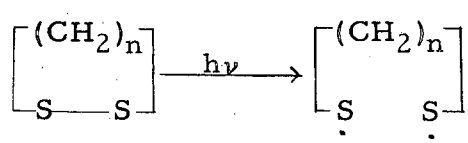
MECHANISM OF QUANTUM CONVERSION IN PHOTOSYNTHESIS

John A. Barltrop

Although plants that are allowed to photosynthesize in $C^{14}O_2$ rapidly assimilate labeled carbon into a series of compounds, very little of the C^{14} finds its way into the intermediates of the Krebs tricarboxylic acid cycle during illumination. If, after a period of photosynthesis, the light is turned off, the compounds of the Krebs cycle rapidly become labeled.[1] Thus there is a reaction path linking the photosynthesis and Krebs cycles which becomes blocked during illumination. With the discovery[2-6] that 6-thioctic acid is a coenzyme for the oxidative decarboxylation of pyruvate to active acetyl groups, which reaction is the only known method of feeding the Krebs cycle,[7-9] Calvin and Massini suggested that the reaction could be formulated thus:



Moreover, since the enzyme must be present in its oxidized form in order that the oxidation of pyruvic acid may proceed, these authors suggested that the reducing power formed in the presence of light shifted to the steady-state conditions of the enzyme toward its reduced (dithiol) form and thus prevented the intermediates of the photosynthetic cycle from entering the Krebs system. At this point, specimens of the isomeric 4-, 5-, and 6-thioctic acids became available to us through the courtesy of Dr. T. H. Jukes of Lederle Laboratories. The marked differences in their u. v. absorption spectra (Fig. 1), and in particular the displacement of the absorption peak to progressively longer wave lengths as the size of the disulfide ring diminished, suggested that this might be due to ring-strain. If one assumes that the absorption band is due to a transition:



then, since to a first approximation, the excited states of all the disulfides would have the same energy, the differences in λ_{max} would correspond to differences in the energies of the ground states - i.e., to ring strain. On the basis the shift from λ_{max} = 2500 for straight-chain aliphatic disulfide to λ_{max} = ca. 3300

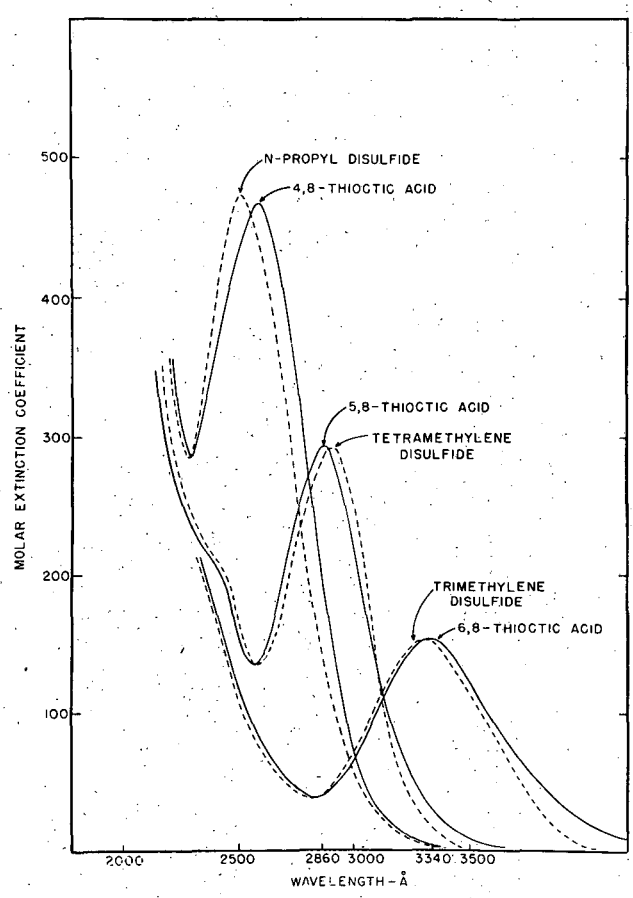
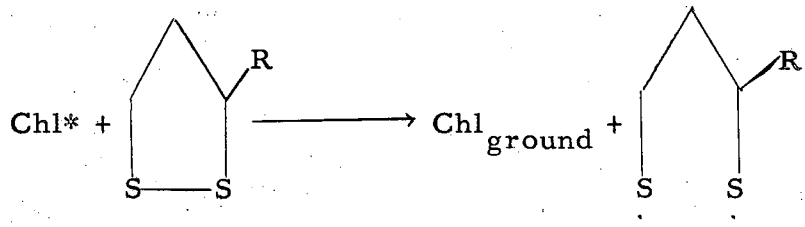

N-PROPYL DISULFIDE
4,8-THIOCTIC ACID
5,8-THIOCTIC ACID
TETRAMETHYLENE DISULFIDE
TRIMETHYLENE DISULFIDE
6,8-THIOCTIC ACID
MOLAR EXTINCTION COEFFICIENT
500
400
300
200
100
2000
2500
2860
3000
3340
3500
WAVELENGTH - Å

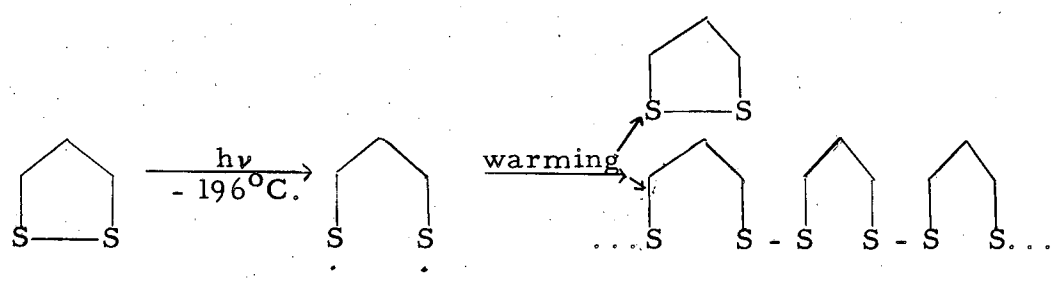
Fig. 1

for 6-thioctic acid implies that the latter experiences a strain of ca. 25-30 kcals., and that hence the dissociation energy of the -S-S- bond would be reduced by a like amount. The published values for D(R-S-S-R) vary from 50 to 70 kcals; thus the ring strain might well reduce the dissociation energy of the thioctic S-S- bond to ca. 30-40 kcals. There is evidence that light captured by the chlorophyll and associated pigments is passed on as quanta of 30 to 40 kcals. energy, and this led directly to the notion that 6-thioctic acid was itself the substance involved in the conversion of the electromagnetic energy of the quantum into chemical bond energy and that the process of quantum conversion in photosynthesis was the fission of the disulfide bond of 6-thioctic acid to give the dithiyl radical, i.e.,



Suggestions as to the subsequent transformations of the diradical have already been outlined in a preliminary communciation.[10] The purpose of the present paper is to amplify the results of the former.

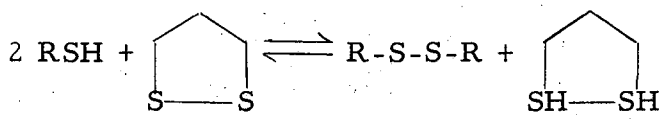
The suggestion as to the possible function of 6-thioctic acid in photosynthesis mentioned earlier required the photochemical fission of the -S-S- bond into thiyl radicals. The polymerization of trimethylene disulfide in light is consistent with the suggestion, but in order to obtain more definitive evidence trimethylene disulfide was photolyzed in an ether-pentane-alcohol glass at -196°C. Under these conditions, one could expect that the thiyl radicals produced by photolysis would be preserved from further reaction (a) because the rigid solvent would prevent their approaching each other and thus recombining and (b) because of the low temperature employed, the insufficiency of thermal activation energy would prevent their attacking the solvent. Hence it should be possible to build up a considerable concentration of thiyl radicals. When trimethylene disulfide was photolyzed under these conditions, a clear, pale salmon-colored glass resulted, which appeared to be stable in liquid nitrogen but which, on warming a few degrees to the point at which the glass lost its rigidity, rapidly became turbid with polymer. The clarified solution now contained >50% of the original trimethylene disulfide. These results were consistent with the interpretation:



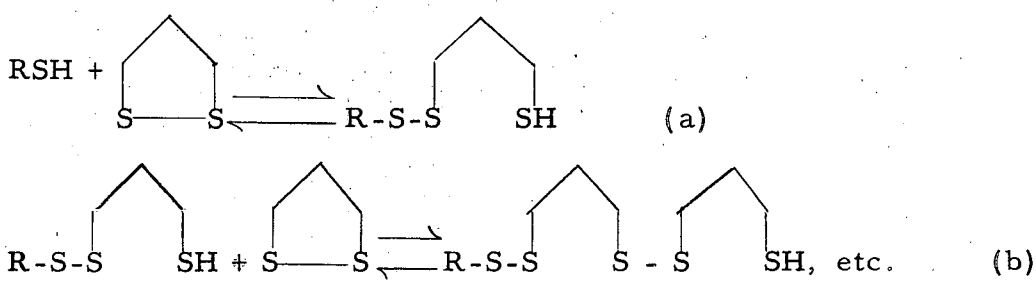
Confirmation of the production of free radicals by potolyzing trimethylene disulfied was sought by using diphenylpicrylhydrazyl which is itself a free radical, stable to air, and which has been used before for the detection of radicals.

Diphenylpicrylhydrazyl, with and without trimethylene disulfide solution, was both illuminated and kept in the dark. The color of the hydrazyl faded only when mixed with trimethylene disulfide and exposed to light. This result is also consistent with the notion that trimethylene disulfide on photolysis gives radicals, these then reacting with the hydrazyl.

The theory of quantum conversion outlined previously also requires that the S-S bond be weak enough to be ruptured by a quantum of ca. 30 to 40 kcals. The estimate of ring strain given earlier (ca, 25 to 30 kcals.) and derived from spectral data, is based on several assumptions of uncertain validity. In order to arrive at a more accurate value for the difference in this -S-S- dissociation energy between an aliphatic disulfide and trimethylene disulfied (i.e. ring strain) we exploited the observation that aliphatic thiols reacted in a reversible manner with trimethylene disulfide. After adding a thiol, the trimethylene disulfide was partly destroyed, the amount of destruction being correlated with the amount of added thiol; dilution of the solution caused a regeneration of trimethylene disulfide. The equilibrium being observed could be either:

(1) 

or

(2) 

The equilibrium constant calculated for reaction (1) does not fit the data obtained. The complex of reactions (2) can be given a simple mathematical formulation by noting that for each thiol group reacting with trimethylene disulfide a new thiol group is generated. If it is assumed that all the thiols in reaction (a) have the same equilibrium constant for the reaction in trimethylene disulfide, then the overall equilibrium constant can be represented by

$$K = \frac{[\text{aliphatic disulfide}]}{[\text{cyclic disulfide}] \times [\text{thiol}]}$$

Values of K calculated from the data by this equation are reasonably constant for the reactions of trimethylene disulfide with both benzyl mercaptan and β-mercaptoethanol.

For the reaction with benzyl mercaptan conducted at 24.1° and 35.35°C., the free energies of reaction are $\Delta F_{24.1}$ = -1.67 kcals. and $\Delta F_{35.9}$ = 1.74 kclas. Hence ΔH = -6.34 kcals. But ΔH, which is a measure of the

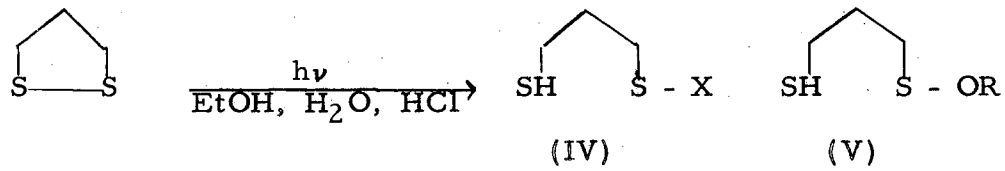
differences in bond energy between the cyclic disulfide and an aliphatic disulfide, is thus a measure also of the ring strain in trimethylene disulfide. It should be noted that although there is ca. 6 kcals. of heat energy available to drive the reaction to the right, this is opposed by an entropy term $T\Delta S$ of ca. 4.5 to 5 kcals., so that only ca. 1.5 to 2 kcals. of free energy is left.

Calvin and Massini's suggestion mentioned earlier,[9] that thioctic acid might be the coenzyme directly responsible for the oxidative decarboxylation of α-keto acids, led us to investigate the reactions between α-keto acids and trimethylene disulfide. When pyruvic acid or α-ketoglutaric acid was heated in alcoholic solution with trimethylene disulfide very little change occurred. Next, α-ketoglutaric acid and trimethylene disulfide were exposed to u.v. irradiation in a nitrogen atmosphere. It was found that whereas control tubes containing trimethylene disulfide rapidly became turbid owing to polymerization, the tubes containing trimethylene disulfide and α-glutaric acid remained clear although spectroscopic examination showed the complete destruction of the trimethylene disulfide. A series of similar experiments was performed with pyruvic, oxalic, and acetic acids. The following facts emerged: (1) With pyruvic and oxalic, as with α-ketoglutaric acid, the solution remained clear upon photolysis but with acetic acid polymerization of the trimethylene disulfide was only partly inhibited. (2) No detectable quantity of CO_2 was formed during the pyruvic and oxalic acid experiments. (3) The H^+ concentration was unchanged by photolysis. (4) Amperometric titration of thils against Ag^+ showed that rather less than one thiol group was liberated per molecule of trimethylene disulfide. (5) Iodine titration revealed the presence of reducing substances in the solution in addition to the thiols. Since the added acids were apparently unchanged by the photolysis and since acetic acid, which is a weaker acid than pyruvic and oxalic acids, was less effective as a polymerization inhibitor than the others, it appeared likely that these substances were inhibiting polymerization merely by virtue of their being acids and that mineral acids would exercise a similar effect. This was found to be the case. Photolysis of trimethylene disulfide in the presence of hydrochloric acid again caused no change in the acid titre. Also ca. one thiol group was liberated per molecule of trimethylene disulfide and excess reducing power was revealed by iodine oxidation as with organic acids. A small quantity of hydrogen sulfide was detected by a lead acetate paper.

The difference between the iodine and silver titrations posed the question as to which should be accepted as the index of thiol concentration. It was found in model titrations with other thils that with freshly prepared solutions the I_2 and Ag titres agreed but that with aged solution, partially oxidized by air, the iodine titre was much higher than the silver titre. This suggested that while both silver and iodine were equally effective for estimating pure thiols, the silver was probably the more specific reagent, since iodine was also reduced by the oxidation products of thiols.

The finding that approximately one thiol group was formed on photolysis from each molecule of trimethylene disulfide raised the question of whether one group was attached to each three-carbon fragment derived from the trimethylene disulfide or whether the photolysis produced a mixture containing some trimethylene dithiol and some three-carbon units without thiol groups, the average being one thiol for each C_3 unit.

Model experiments had shown that trimethylene dithiol could readily be oxidized--and in excellent yield--to trimethylene disulfide by iodine under acid conditions or by hydroxylamine and alkali. In spite of repeated experiments no trimethylene disulfide could be found in the photolysis solution after these treatments, thus demonstrating that the course of the photolysis could be represented schematically as:



The structure IV with X = OH would be a sulfenic acid. Little is known of their chemistry and only certain aromatic analogs are known to exist. From the nature of the reported dismutation products formed when synthesis of sulfenic acids is attempted one may attribute certain properties to a sulfenic acid or ester (R - SOR).

Thus, general considerations justify the conclusion that the primary photochemical tranformation occurring when trimethylene disulfide is photolyzed should lead to (V, R = OH, OEt, or Cl) and that the actual solution obtained at the end of photolysis should consist either of one or more of these entities or of some transformation product or products derived therefrom.

A large number of experiments was performed on the solution obtained by photolyzing trimethylene disulfide in the presence of acid. The facts which emerged are these:

On photolysis the 3300-Å band of trimethylene disulfide gradually disappeared and a more intense absorption built up at wavelength < 2500 Å, but even when all the trimethylene disulfide had been destroyed, there was a residual absorption in this 3300 Å region. However, thiols which have already been shown to be present in the solution show a similar absorption (λ_{max} = 2100 Å, $\epsilon_{max} \cong 200$) and it seemed possible that the thiol absorption was masking the bands of the groups present in the photolyzed solution. In order to neutralize as far as possible this masking thiol absorption, a solution of β-mercaptoethanol of concentration equal to the concentration of the initial trimethylene disulfide (silver titrations described earlier demonstrated the formation of ca. one thiol group per initial trimethylene disulfide molecule present at the commencement of photolysis) was introduced into the compensating cell of the spectrophotometer. In this way, the instrument automatically subtracts the absorption due to the thiols and the resultant spectrum should then represent the absorption of other groups present in the photolyzed solution. When this was done, a new band appeared (λ_{max} = 2320 Å). That this band is probably not an artifact is indicated by the fact that it remains in essentially the same position (λ_{max} = 2290 Å) when only one-half of the previous concentration of thiol is introduced into the compensating cell. That the photolysis solution contains entities sensitive to acid is demonstrated by the fact that while no change occurs in the spectrum if the solution is kept at -20° for 24 hours, keeping it at room temperature causes the 2320 Å peak to move to 2420 Å.

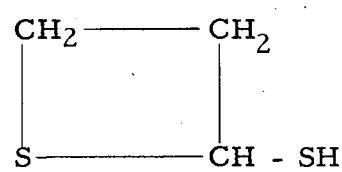
The photolysis solution is also sensitive to alkali, which causes instantaneous elimination of the 2320 Å peak, and the development of maxima at 2629 Å and more slowly at 3340 Å and 4100 Å and still more slowly at 2310 Å. On reacidifying, the bands at 3340 and 4100 Å are immediately destroyed and replaced by bands at 2820 Å and 2350 Å. Making alkaline again causes reappearance of the 3340 and 4100 Å maxima. With variations in degree, similar phenomena were encountered both with solutions of trimethylene disulfide photolyzed at low temperatures and in solutions photolyzed in the presence of sodium hydroxide.

Properties of Photolysis Product

(1) The photolyzed solution is markedly unstable, particularly with alkali.

(2) The solution possesses oxidizing power. This is demonstrated by the oxidation of trimethylene dithiol to trimethylene disulfide. Also the bands which appear at 3340 Å when the solution is made alkaline and at ca. 2900 Å when it is reacidified are strongly reminiscent of dithiocarboxylic acids. Hantsch and Bucerius[11] give for dithioacetic acid, λ_{max} = 2950 Å and for the ammonium salt, λ_{max} = 3330 Å. The formation of dithio acids from a substance which before photolysis was at the oxidation level of a disulfide must involve oxidation. Whether the tentative assignment of those bands to dithio acids is correct or not, the transformation of compounds of the photolysis solution into substances absorbing at such long wave lengths can only mean the development of multiple carbon or sulfur bonds (i.e. oxidation), for these bands can be developed in the absence of halogens.

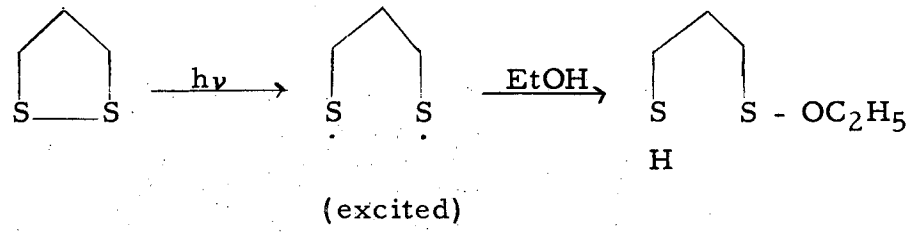
(3) The solution possesses reducing power in addition to that furnished by the liberated thiol groups, as shown by the value for the iodine titre. The arguments advanced earlier in this paper limited the structure of the primary photolytic product to (V, R = OH, OEt, Cl) or to (VI).



VI

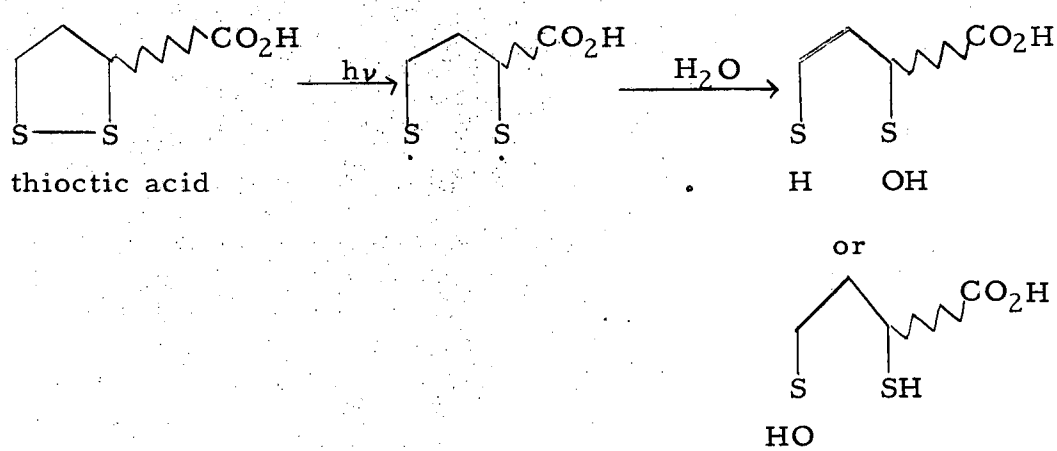
The fact that similar products appear to be formed when trimethylene disulfide is photolyzed under both acid and alkaline conditions eliminates structure (V, R = Cl). We can find no rationale by which to explain the diversity of products derivable from the photolyzed solution in terms of the structure (VI) and we are left only with (V, R = OH or OEt).

We can thus postulate with reasonable confidence the idea that when trimethylene disulfide is photolyzed in ethanol solution the sulfenic ester (V, R = OEt) is the primary product



and that the solution at the end of the photolysis contains its transformation products. The well-established instability of sulfenic acid derivatives, their extreme tendency to dismute (shown also by sulfur monoxide, the inorganic analogue), and their oxidizing power are completely in accord with the data obtained from photolyzed solutions of trimethylene disulfide.

If the suggestion that photolysis of trimethylene disulfide in ethanol solution leads to a sulfenic ester be correct, the equestion immediately arises as to what is its relevance to the process of photosynthesis. In an earlier communication, Calvin and Barltrop[10] advanced arguments in support of the idea that 6-thioctic acid was directly involved in the quantum conversion step of photosynthesis, but the steps by which water was oxidized to molecular oxygen were not specified. The above work with trimethylene disulfide leads us directly to the further suggestion that water itself is the primary hydrogen donor, i.e., that the primary photochemical act of photosynthesis is:

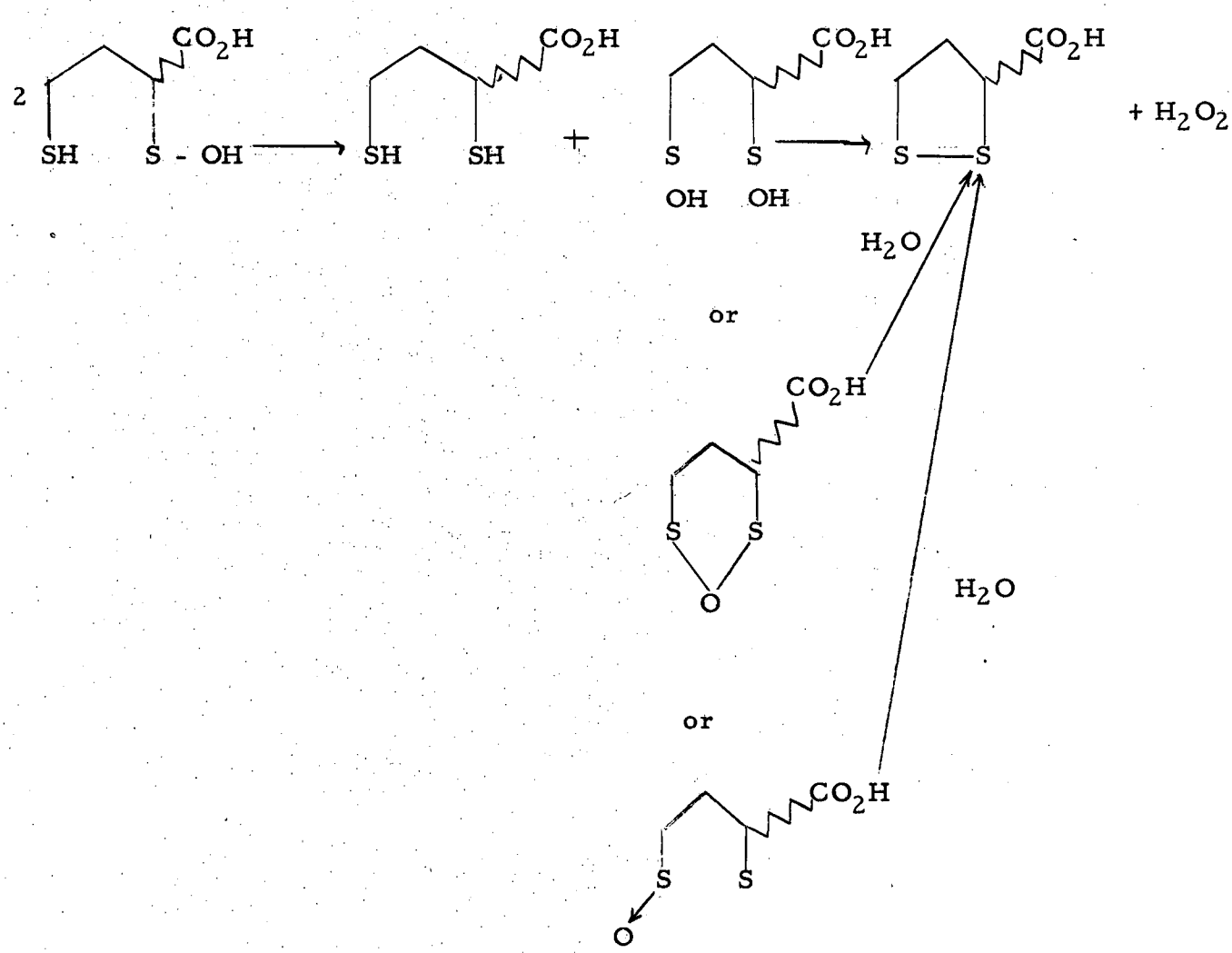


No thermodynamic measurements of the S-O link have been reported in the literature and there seems to be considerable diversity of opinion on the S-S bond. However, from the enhanced reactivity of sulfenic derivatives compared with disulfides there can be little doubt that S-S is the stronger bond, perhaps by as much as 5 kcals. From the known H-O and H-S bond energies, and assuming this 5 kcals. differential in the S-S and S-O bond energies, the ΔH for the above net reaction is about 35 kcals. If the size of the quantum passed on by the chlorophyll is 35-40 kcals., then this process would not only be possible but would represent a very efficient utilization of the quantum.

It should be noted that sulfenic acids are the sulfur analogues of alkyl hydroperoxides. Thus in one step, water has been raised to the peroxide level of oxidation.

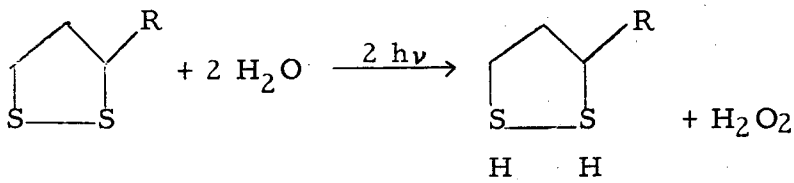
From the literature survey it is very clear that dismutation is characteristic of sulfenic acids. We thus feel justified in suggesting that the thioctic-

sulfenic acid, once formed, would dismute. In view of our limited understanding of the chemistry of sulfenic acids the nature of this dismutation is difficult to predict, but in order to crystallize the situation, we tentatively propose a dismutation into 6:8-dithiol-octanoic acid and a molecule at the energy level of hydrogen peroixde which then decomposes into hydrogen peroixde and 6-thioctic acid:



The dithiol can then reduce DPN and hence provide the reducing power required to convert carbon dioxide into sugars - the hydrogen peroixde leads through catalase to molecular oxygen.

Adding equations (A) and (B) gives the overall reaction:



(C)

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

and this leads, on purely stoichiometric grounds, to the requirement that four quanta be utilized for each molecule of oxygen evolved, in agreement with certain measurements of the quantum requirement of photosynthesis.

That two quanta can drive the reaction of equation (C) is suggested by its calculated free-energy requirement of + 69 kcals. Since this has to be provided by two quanta, the energy of the excitation passed on by chlorophyll should be not less than 35 kcals., a figure which is well within the bounds of possibility. These considerations of the energy relationships lend support to the proposed scheme for the quantum conversion process in photosynthesis.

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ISOLATION OF PERSEITOL AND MANNOHEPTULOSE

Arnold S. Nordal

Since the seven-carbon sugar, sedoheptulose, was found as an intermediate in photosynthesis,[a] naturally occurring seven-carbon compounds have commanded increasing interest. As a result of this interest it became necessary to obtain authentic samples of the seven-carbon alcohol perseitol and the seven-carbon sugar mannoheptulose for chemical identification and degradation of photosynthetic intermediates.

Perseitol was isolated for the first time from avocados by Muntz and Marcano[b] and mannoheptulose was isolated from the same source by La Forge.[c]

An initial attempt to isolate the two mentioned compounds from California avocados (variety "Thrift"), according to the method of E. M. Montgomery and C. S. Hudson,[d] gave a very poor yield. From 18 lbs. of fresh pulp about 5 g of pure crystalline perseitol and about 8 g crude mannoheptulose was isolated. Several other available varieties and brands of avocados which were analyzed also gave very low yields.

Montgomery and Hudson used the variety "Trapp" as a starting material. This variety, which grows in Florida, would not be available until September or October, and we decided, therefore, to try to find other sources for starting material. Paper chromatography of extracts showed that leaves from avocado trees grown in Berkeley contained considerable amounts of perseitol and mannoheptulose. The characteristic blue color produced by heptulose with orcinol reagent (Klevstrand and Nordal)[e] was used for the assay during the survey and isolation of mannoheptulose in the avocado leaves.

Experimental

The fresh leaves (35 lbs.) were ground in a Waring Blender with 3 parts of cold water and most of the plant tissue was removed by filtering under pressure through cheesecloth. The yellow-brown aqueous solution was heated to 80-90°C. for about one hour to destroy enzymes and to precipitate proteins. The extract was then clarified by centrifuging, evaporated under reduced pressure to 10 l., and run through one column of Dowex 50 and one column of Duolite A3 ion-exchange resins (both resins were of 80-100 mesh). During this procedure the color of the solution changed from dark brown to weak yellow. The deionized solution was then evaporated under reduced pressure to a thin syrup which was precipitated while hot by vigorous mixing with two volumes of ethanol at about 70°C. The yellow voluminous precipitate was filtered off and extracted twice with 1-1/2 l. boiling 60% ethanol in order to extract perseitol, which separated together with the slimy and pectic substances. The residue, which after drying amounted to about 115 g, was not more closely investigated.

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a A. A. Benson, J. A. Bassham, and M. Calvin, J. Am. Chem. Soc., 73, 2970 (1951).
b A. Muntz and V. Marcano, Ann. Chin. Phys., 63, 279 (1884).
c F. B. LaForge, J. Biol. Chem., 28, 511 (1916-1917).
d E. M. Montgomery and C. S. Hudson, J. Am. Chem. Soc., 61, 1654 (1953).
e Rolf Klevstrand and Arnold Nordal, Acta Chem. Scand., 4, 1320 (1950).

The combined alcoholic filtrates were evaporated under reduced pressure to a thick syrup (about 400 g). This was dissolved in 800 ml boiling methanol and put in cold storage overnight, whereby large amounts of perseitol, accompanied by small amounts of mannoheptulose, crystallized out and were filtered off. More perseitol separated during the next several days.

The crude perseitol was dissolved in the least possible volume of hot 80% methanol, decolorized with charcoal, and put aside for crystallization. After one more crystallization from 80% methanol the substance was pure. Yield: 55 g., m.p., 187-188°C.

Paper chromatographic examination of the mother liquor from the crude perseitol showed that the solution--besides small amounts of perseitol and large amount of mannoheptulose--contained considerable amounts of glucose, sucrose and fructose. To get ride of the hexoses and the sucrose, the mother liquor was evaporated to a thick syrup, and the residue dissolved in 2 l. of water and fermented with baker's yeast at 37°C for 24 hours. The yeast was filtered off and the alcohol and some water distilled away. The residue was again diluted with water to about 2 l. and the fermentation repeated with fresh yeast in the same way as mentioned above. The fermentation process was repeated three times in all until the fermentation residue no longer gave a positive test for fructose and sucrose when tested with the orcinol reagent.

The fermentation residue was evaporated under reduced pressure to a thick syrup which was dissolved in four volumes hot methanol and put in the cold room for several days. Almost pure mannoheptulose gradually crystallized and was recrystallized from about 85% methanol. The yield of pure sugar was about 150 g. From the mother liquor more crude sugar separated gradually.

THE METABOLISM OF FATTY ACIDS BY PANTOTHENIC ACID-DEFICIENT RATS

Martha R. Kirk and Ann M. Hughes

Exhalation of $C^{14}O_2$ after Injection of Labeled Heptanoate

Pantothenic acid-deficient (PAD) rats have been injected with $C^{14}H_3(CH_2)_5COONa$ and either (1) pantothenic acid or (2) mercaptoethylamine, using the techniques previously described (see Quarterly Report for March, April, and May, 1953). Compounds (1) and (2) are fragments of the Coenzyme A molecule, and were given in amounts equivalent to these present in the CoA given in the experiments described in the above report. Pantothenic acid did not change the rate or total output of exhaled $C^{14}O_2$ from that of PAD rats given $C^{14}H_3(CH_2)_5COONa$ alone. Mercaptoethylamine did not change the rate of $C^{14}O_2$ output, but increased the total by 10 percent over that of $C^{14}H_3(CH_2)_5COONa$ alone.

A short series of experiments on the metabolism of $C^{14}H_3(CH_2)_5COONa$ was conducted on a group of PAD rats from another laboratory. These rats were raised on a schedule in which the weight of food given to the controls is determined by the amount eaten by the deficient animals. Results differed greatly from those obtained with our own "ad lib." fed animals. Therefore, it is apparent that the nutritional state of the animals is of great importance in the study of fatty acid metabolism. Further studies are being made of the effect of various nutritional states on fatty acid metabolism.

Distribution of Activity in the Livers of Normal and Pantothenic Acid Deficient Rats after Injection of $C^{14}H_3COONa$

Differences in the rate at which normal and pantothenic acid-deficient (PAD) rats oxidize fatty acids to $C^{14}O_2$ and the effect of Coenzyme A on these rates have led to further investigation of the metabolic differences of these two types of animals. Particularly, an effort was made to discover which metabolic pathways seem most affected by pantothenic acid deficiency. To accomplish this, a study of the distribution of radioactivity in the livers has been carried out.

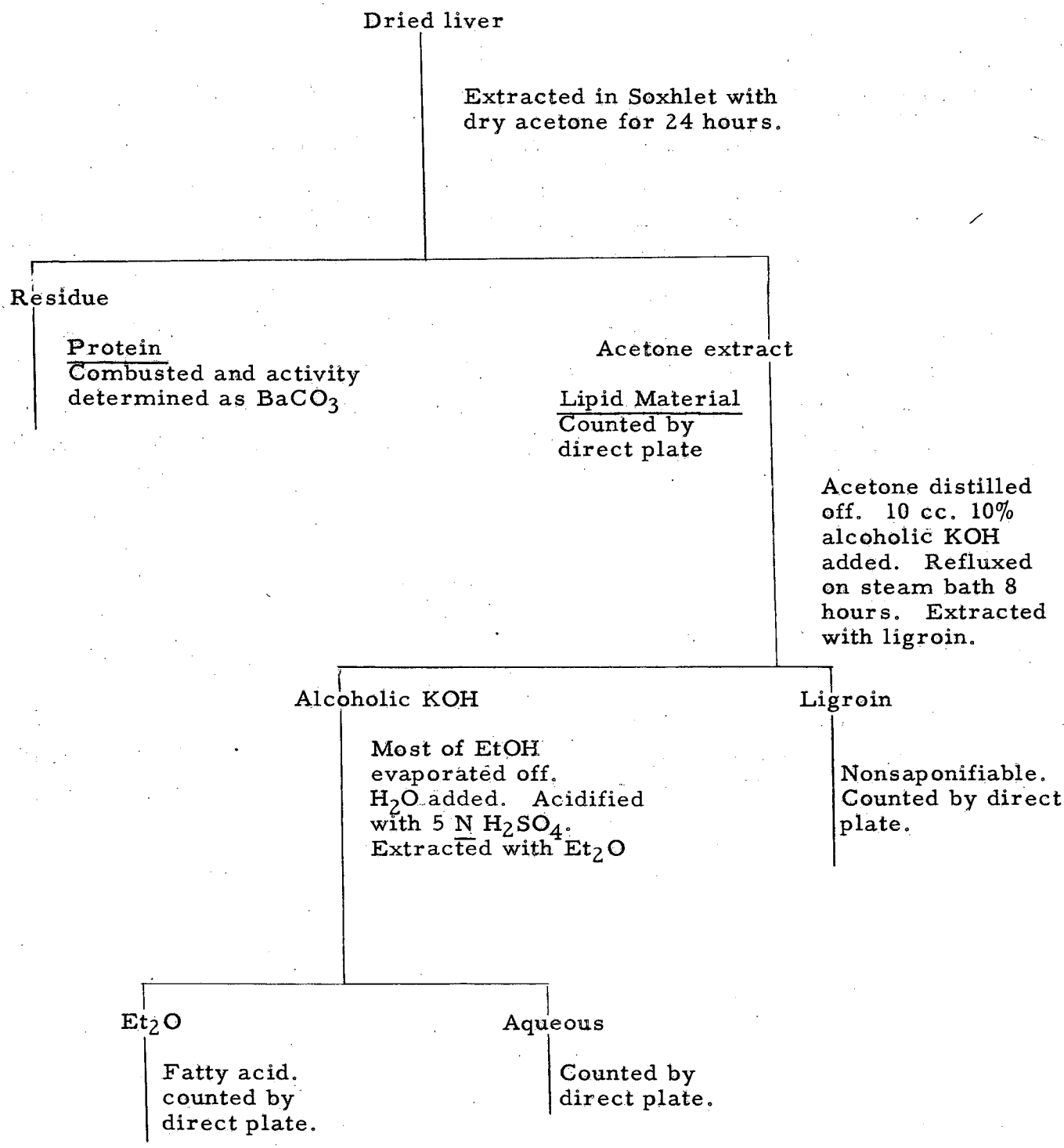
Experimental

PAD rats and normal rats were injected intraperitoneally with 2 mg (about 10 μc) of $C^{14}H_3COONa$. Some of these animals also received - about ten seconds prior to acetate administration - 6 mg of CoA (Pabst) by intravenous injection. At the end of one hour the animals were sacrificed. The livers were removed, rinsed in isotonic saline solution in order to wash off most of the blood, blotted gently with paper towels, immediately frozen with liquid nitrogen, and lyophilized.

A small portion of each liver was oxidized to CO_2 by the Van Slyke-Folch method, and radioactivity determined as $BaCO_3$.

CoA assays are being carried out on another portion according to the method of Stadtman (J. Biol. Chem., 191, 365 (1951)).

A larger aliquot portion of each was fractionated according to the following scheme:



Results

The results are summarized in the following tables.

Table I

PAD Rats	$C^{14}H_3COONa$ Only				$C^{14}H_3COONa$ + 6 mg CoA			
	Av. of 3		Rat B1		Rat C1		Av. of 4	
	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose
Liver		4.7		15.0		9.0		12.0
1. Protein	59.5	2.80	33.3	5.0	43.9	3.95	32.4	3.89
2. Lipid	37.3	1.75	60.8	9.12	51.9	4.67	65.6	7.87
a. Nonsapon	3.7	0.17	6.7	1.01	7.9	0.71	4.0	0.48
b. Fatty acid	22.2	1.04	52.9	7.94	38.6	3.47	56.9	6.83
c. Aqueous	10.5	0.49	1.4	0.21	2.9	0.29	5.1	0.61
Normal Rats	**Av. of 3**						**Av. of 2**	
Liver		4.5						3.8
1. Protein	58.9	2.7					59.0	2.24
2. Lipid	42.3	1.9					38.2	1.45
a. Nonsapon	6.4	0.3					3.7	0.14
b. Fatty acid	31.1	1.4					30.2	1.15
c. Aqueous	5.2	0.2					4.4	0.17

Rats B_1 and C_1 are reported individually because, although neither received CoA at the time of the last injection, B_1 had received several injections of CoA earlier for $C^{14}O_2$ studies, and C_1 had received one injection of pantothenic acid for the same purpose. The effect of these previous injections (increased percentage in liver; increased lipid and decreased protein percentages) is quite apparent from the results obtained.

Determinations were also made on a second group of six animals obtained from the Department of Animal Husbandry. These animals had all been made pantothenic acid-deficient by a slightly different schedule than that used in our laboratory, then fed in triads as follows:

No. 1 rat - no pantothenic acid, food "ad lib."

No. 2 rat - pantothenic acid, but only as much food as No. 1 would consume

No. 3 rat - pantothenic acid, but 15% less food than consumed by No. 1.

The rats used had been on the controlled food diet for approximately two weeks before use. The results are summarized in Table II.

Table II

"Controlled Food" Rats	$C^{14}H_3COONa$ Only					
Rat No.	1		2		3	
	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose
Liver		3.3		2.5		3.0
1. Protein	79.5	2.63	84.6	2.11	84.6	2.54
2. Lipid	18.5	0.61	14.5	0.36	15.3	0.46
a. Nonsaponifiable	3.4	0.12	2.0	0.05	2.0	0.06
b. Fatty acid	5.5	0.18	3.2	0.08	3.8	0.11
c. Aqueous	9.8	0.32	9.3	0.23	9.5	0.29

"Controlled Food" Rats	$C^{14}H_3COONa$ + 6 mg CoA					
Rat No.	1		2		3	
	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose	% Liver Act.	% Inj. Dose
Liver		5.3		7.1		6.9
1. Protein	49.1	2.60	35.9	2.55	38.2	2.63
2. Lipid	50.4	2.67	66.4	4.72	52.1	3.59
a. Nonsaponifiable	7.9	0.42	4.2	0.30	8.8	0.61
b. Fatty acid	27.7	1.47	44.0	3.12	39.2	2.70
c. Aqueous	12.9	0.68	17.1	1.21	4.0	0.27

Preliminary results indicate that livers of PAD rats contained much less CoA than normal rats, but that the CoA content of the liver of those which received CoA one hour prior to sacrifice was quite high in both PAD and normal animals.

Conclusions

1. Utilization of $C^{14}H_3COONa$ by the livers of PAD rats does not differ greatly from that of the normal rats, except that the normal rats synthesize fatty acids more readily.

2. Injection of a large amount (6 mg) of CoA makes very little change (after one hour) in the liver metabolism of normal rats.

3. Injection of a large amount (6 mg) of CoA into PAD rats not only more than doubles the amount of activity remaining in the liver after one hour, but increases that found in the fatty acid fraction by a still greater factor. One would conclude from this that protein synthesis has priority over fatty acid metabolism in the case of pantothenic acid deficiency. In addition, while protein synthesis remains more nearly normal, it is somewhat speeded up by CoA injections.

4. In the case of the "controlled food" rats, their entire metabolism rate seems considerably slowed, but the slowing down of fatty acid synthesis is still more dramatic. The general effect of administration of CoA is the same as that observed in other PAD rats and there seems to be no very significant difference between the three rats of each triad.

5. The increase in amount of activity in the nonsaponifiable fraction following injection of CoA is also significant.

INVESTIGATION OF THE GROWTH-INHIBITING PROPERTIES OF POLYGLYCOL 33-3

Ann M. Hughes

Polyglycol 33-3, supplied by Dow Chemical Company, is a surface-active agent which has been shown to inhibit the growing tips of young plants. Because of this property, investigations are being carried out here to study its pharmacological properties, particularly the possibility of a growth-inhibiting effect on cancer cells.

Mice were used in the first toxicity experiments, since mice are the animals most frequently used in cancer research. The effects of the compound, when injected intraperitoneally (i.p.), are similar to those of curare, i.e., flaccid muscular paralysis, first of the hind legs, gradually spreading forward. Death is apparently due to respiratory paralysis. When a sublethal dose is injected, the animal is completely paralyzed within five minutes, remains in this condition an additional ten minutes, and is completely recovered within twenty minutes of the time of injection. The same sublethal dose can be administered twice daily over a period of two months without producing cumulative effects or an increased threshold. The maximum tolerated dose in a single injection (i.p.) in mice is 5 to 7.5 μl/100 g body weight. The injection of 10 μl/100 g body weight is always fatal.

A few experiments with rats indicate that the therapeutic margin for these animals is even smaller. A dose of 6 μl/100 g body weight does not paralyze the animal, only making him sluggish. A dose of 7 to 8 μl/100 g is usually fatal.

Polyglycol 33-3 has been tested for antigrowth properties against two tumors to date, viz: Sarcoma 37 (in A-strain female mice) and Myeloid leukemia C1498 (in C57-strain female mice). Both of these tumors are subcutaneous, and can be transplanted directly. Polyglycol 33-3 showed no effect on the survival time of animals in which these tumors had been transplanted. Such a result might be expected in view of the fact that the polyglycol is obviously metabolized rapidly and probably never reaches the site of the tumor. It is planned to test the polyglycol against an ascites tumor which consists of cells growing free in the peritoneal fluid. With such a tumor, there will be more opportunity for the compound to come in contact with the tumor cells.

PREPARATION OF DIETHYL MALONATE-2-C^{14}

Doris Chin

Diethyl malonate is a well known synthetic intermediate, but relatively little work has been reported on the preparation of diethyl malonate-2-C^{14} for use in isotopic tracer studies. Ropp's preparation of diethyl malonate-2-C^{14} utilizes the condensation of ethyl acetate-2-C^{14} with ethyl oxalate and pyrolysis of the resulting ethyl oxalacetate.[1] Since this method gives only 19% diethyl malonate from sodium acetate-2-C^{14}, it is desirable to find another method which can increase the yield of the labeled compound.

Many methods are available for the synthesis of diethyl malonate,[2] but the best known is the formation of malonic acid from chloroacetic acid and subsequent esterification. Malonic acid-2-C^{14} has been prepared in good yield (76%, 84%)[1,3] and it seemed likely that direct esterification of the acid should be a successful way to form the labeled ester. Preliminary work with calcium malonate and ethanol in the presence of strong acid, following the method of Kao and Chen,[2] yielded about 30% crude product. The reaction between silver malonate and ethyl iodide proved to be a much better means of esterification, and a procedure was developed which yielded 60-70% redistilled ester from 20-25 millimols sodium acetate.

Experimental

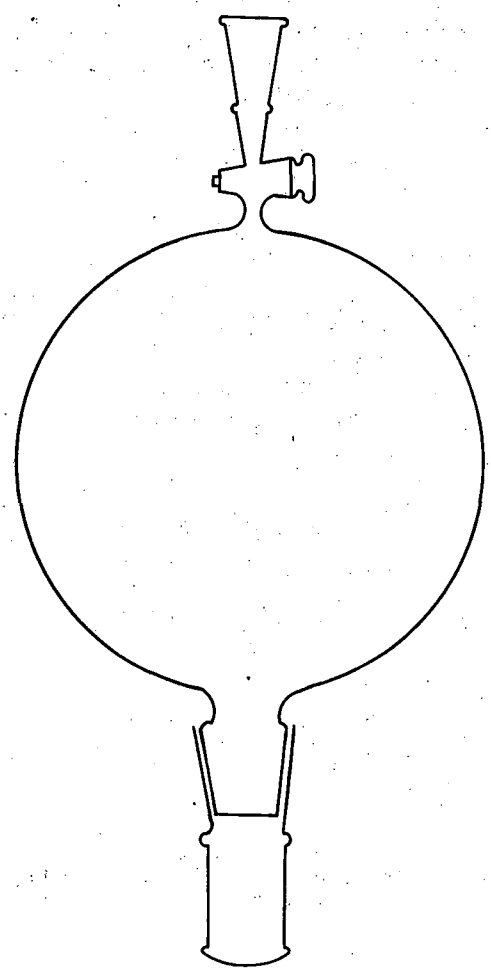
Diethyl malonate-2-C^{14} was prepared by the following series of reactions, using modifications of the methods of Hughes and Tolbert[4] and Weiner.[2]

$$\underset{\text{anhydrous}}{C^{14}H_3COONa} \xrightarrow{HCl(g)} \underset{\text{anhydrous}}{C^{14}H_3COOH} \xrightarrow[P,\ I_2]{Cl_2} ClC^{14}H_2COOH \xrightarrow[2)NaCN]{1)Na_2CO_3}$$

$$NCC^{14}H_2COONa \xrightarrow{NaOH} C^{14}H_2\,(COONa)_2 \xrightarrow{AgNO_3} \underline{C^{14}H_2\,(COOAg)_2} \xrightarrow{C_2H_5I}$$

$$C^{14}H_2\,(COOC_2H_5)_2$$

Anhydrous acetic acid was prepared by treating 2.0 g (24.5 mmols) of anhydrous sodium acetate-2-C^{14} with about 36 mmols of anhydrous hydrogen chloride. Sodium acetate was placed in the removable bottom of a gas-solid reactor (Fig. 1) and the apparatus reassembled with silicone lubricant and attached to the high-vacuum manifold. The last traces of water were removed by evacuation to a pressure of 50 microns. A 5-l storage bulb was evacuated and then filled with tank hydrogen chloride which had first been dried by passing through a concentrated sulfuric acid bubbler and a phosphorus pentoxide tower. About 36 mmols of this hydrogen chloride was admitted to the gas-solid reactor by carefully opening the stopcock of the reactor and measuring a pressure drop of 130 mm Hg in the 5-l bulb.

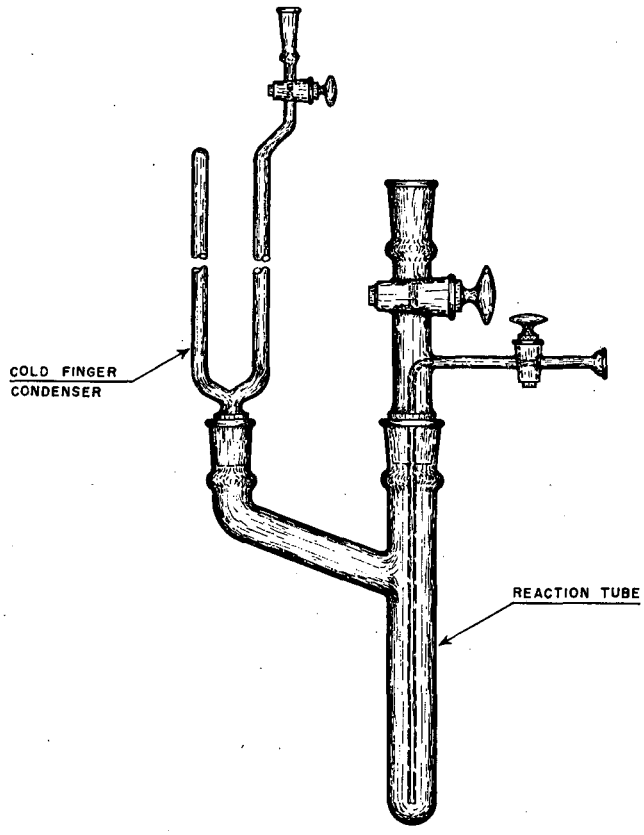


GAS SOLID REACTOR
(CAPACITY = 2 L)

MU 310

Fig. 1

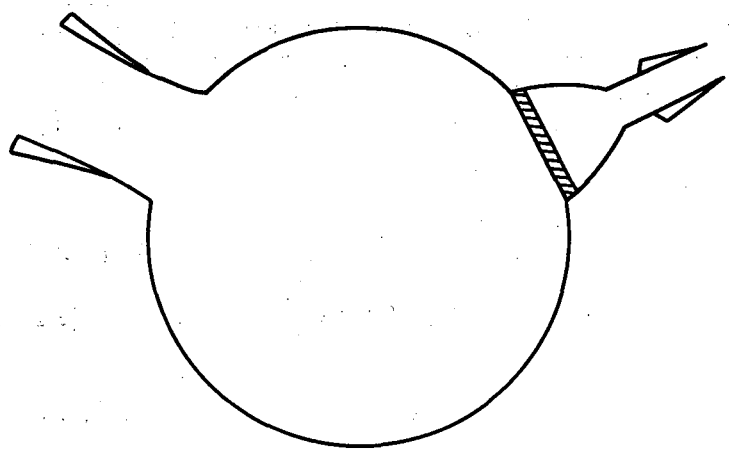
The gas-solid reactor was then removed from the vacuum manifold and the finger containing the solid was heated carefully over a Bunsen burner. The apparatus was held at such an angle that the acetic acid distilled into the bulb and did not drain back on the hot solid. The cake of solid was loosened by gently shaking, and heating continued until the solid became powdery grey. After cooling, the apparatus, was connected to the vacuum manifold and acetic acid distilled in vacuo into a liquid nitrogen-cooled trap. The trap was warmed with a dry ice-isopropanol bath and most of the excess hydrogen chloride removed by evacuation. Acetic acid was distilled in vacuo into the chlorination apparatus (Fig. 2) which contained 30 mg red phosphorus, 15 mg iodine and 0.2 ml acetyl chloride. The reaction mixture was heated to about 40° to allow any water present to react with the acetyl chloride. Suitable precautions were taken to accommodate any excess pressure. The bottom of the reaction tube was cooled in liquid nitrogen until all gaseous materials were collected. The liquid nitrogen was then replaced by a dry ice-isopropanol bath and excess hydrogen chloride removed by evacuation. The apparatus and its frozen contents were then transferred to a steam bath, and the condenser was filled with dry ice-isopropanol and opened to the atmosphere through a drying tube. Chlorine gas was passed through a concentrated sulfuric acid bubbler and a phosphorus pentoxide tower and then bubbled through the reaction mixture at 100° for two hours. The condenser required constant refilling with small chunks of dry ice to prevent loss of product. Toward the end of this period a yellow solid usually formed in the arm of the tube. The mixture was allowed to cool and solidify, the dry ice-isopropanol mixture was drained from the condenser, and most of the chlorine allowed to escape by evaporation. The condenser stopcock was closed and the reaction tube immediately immersed in liquid nitrogen to prevent pressure buildup. The chloroacetic acid product was distilled into the bottom of the tube by filling the cold finger of the condenser with boiling water and gently flaming other exposed parts of the apparatus. One milliliter of water was added, the mixture warmed to room temperature and residual chlorine allowed to escape through the stopcock. Immediately after chlorination, the product was transferred with 50 ml of water to a 200-ml round-bottomed flask, warmed to 50°C, neutralized with 1.7 g sodium carbonate and then cooled. Sodium cyanide (1.2 g, 24.4 mmols) dissolved in about 10 ml water was slowly added to the rapidly stirred solution, which was cooled if the reaction became too exothermic. The flask was heated on a steam bath for 1 hour to insure complete reaction. The solution was cooled and 1.0 g sodium hydroxide slowly added. The flask was heated on a steam bath in a hood and steam bubbled through the solution to aid the removal of ammonia. A stream of air was blown across the liquid surface to increase the rate of evaporation and thereby control the total volume of solution. Hydrolysis was considered complete when the vapor was neutral to moist litmus (about 3 to 4 hours). The clear yellow solution was filtered through sintered glass to remove a small amount of dark-brown precipitate and collected in a 200-ml round-bottomed flask containing a side arm fitted with a sintered glass filter (Fig. 3). Silver nitrate (8.5 g, 50 mmols) dissolved in about 25 ml water was slowly added to the rapidly stirred solution, and a copious grey precipitate formed. The supernatant liquid was filtered through the side arm and the filtrate tested with silver nitrate solution to insure the absence of soluble malonate. The precipitate was washed once with a small quantity of water and then dried by evacuation (overnight on an aspirator and then 4 to 8 hours on a high-vacuum line). Ethyl iodide (30 cc, 372 mmols) was poured over the dry silver precipitate, the flask fitted with a reflux condenser and the side arm capped. The mixture was refluxed and agitated with a glass-sealed magnetic stirrer for three hours and then stirred



CHLORINATION APPARATUS.

MU-4763

Fig. 7



ESTERIFICATION FLASK
(200 ml. capacity)

MU-6616

Fig. 3

overnight at room temperature. Malonic ester and excess ethyl iodide were filtered through the side arm into a 60-ml dropping funnel. The precipitate was washed 4 or 5 times with a total of 20 ml ethyl ether and the washings combined with the filtrate. The ether and ethyl iodide were flash distilled from a 30-ml 2-neck pear-shaped flask at atmospheric pressure. The apparatus was then assembled for vacuum distillation at about 30 mm Hg. Evacuation at room temperature for 1/2 hour removed the residual ether and ethyl iodide, and then the bulk of the product was distilled at 97-103°C. This distillate was collected in a 30 ml pear-shaped flask which was then assembled for another vacuum distillation at 30 mm Hg, and the fraction distilling at 105-106°C. was collected.

Table I

Analysis of Malonic Ester Products

	Literature and Theoretical Values	Inactive Preparation	Active Preparation
Yield from sodium acetate			
Crude		81.0%	85.2%
Redistilled		59.0%	68.3%
Boiling point at 30-31 mm Hg	101°C[5]	98-100°C	105-106°C
Refractive index	1.4143(20°C)[6]	1.4133(21°C)	1.4130(23°C)
Saponification equivalent	80.09	81.23	84.36
Chemical analysis			
C	52.50	52.44	52.47
H	7.50	7.76	7.81

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STUDIES ON ADENINE METABOLISM

E. L. Bennett and B. Krueckel

Turnover of Nucleotides and Nucleic Acids

During the period covered by this report, the rates of incorporation and turnover of adenine in the soluble nucleotides, desoxynucleic acid (DNA) and ribose nucleic acid (RNA), in C57 male mice have been studied. As previously reported, the extensive incorporation of adenine-4, 6-C^{14} into these fractions in the various tissues of the mouse has made it possible to determine the "half-life" or renewal rate of the adenine in them. Recently we have studied the incorporation and turnover using the same methods as previously described, but determining the specific activity of the soluble nucleotides (hydrolyzable by $Ca(OH)_2$ to 5-adenylic acid = "5-AMP") or adenine in the DNA or RNA in more tissues and at more frequent time intervals than previously. In addition, the "half-lives" of a rapidly and a more slowly metabolized fraction have been obtained from the turnover rate curves whenever possible.

A summary of the "half-life" of the adenine in the various fractions of the tissues is given in Table I. The Series I half-life data represent 5 C57 mice sacrificed at 2 hours, 1, 3, 7, and 16 days after injection of the adenine-4, 6-C^{14}, and have been presented in more detail in the previous quarterly report. However, the longer "half-lives" have been recalculated, primarily on the basis of 7-16 day data, and, in addition, the "half-lives" of a more rapidly metabolized component have been determined in a number of instances.

The Series II "half-life" data represent 7 C57 male mice sacrificed at 2 and 6 hours, 1, 2, 3, 7, and 16 days after injection of the adenine. When the data permitted, the turnover rates have been separated into 2 components. The 5-AMP data for this series represents duplicate analyses of the specific activity for each fraction, while the DNA and RNA data represent only a single analysis at each time interval and are subject to revision when the duplicate analyses are completed.

In general the results of the two series of experiments are in good agreement. The extensive incorporation of the adenine into the DNA of the carcass (including bone), large and small intestine, and stomach, and subsequent rapid turnover, have been confirmed. The incorporation of the adenine into the DNA of the liver, lungs, and kidney was very small and only very approximate half-lives could be obtained. The specific activity of the adenine in the heart was even less and no half-life could be determined. The "half-life" data for RNA and 5-AMP are similar to those previously reported.

Effect of X-Irradiation on Incorporation of Adenine

As discussed in the previous quarterly report, the effect of X-irradiation upon the incorporation of adenine into the acid-soluble 5-adenylic acid nucleotides, RNA and DNA has been investigated. The methods used have been described in the previous quarterly report.

The results obtained are presented in Table II. As previously described, the incorporation of adenine into DNA was markedly and rapidly changed

Table I

Half-Life of DNA and PNA Adenine and 5-Adenylic Acid in Various Tissues of C57 Male Mice

Tissues	Series I "Fast" Component	Series I "Slow" Component	Series II "Fast" Component	Series II "Slow" Component
		5-Adenylic Acid		
Stomach	12-17 hrs.	8 days	16 hrs.	6.5 days
Spleen and gonads	26 hrs.	11.7 days	32 hrs.	7 days
Small intestine	11 hrs.	4 days	20 hrs.	4.8 days
Large intestine			24 hrs.	5.3 days
Liver	19 hrs.	6 days	19 hrs.	6 days
Carcass	22 hrs.	13.5 days		12.3 days
Lungs			24 hrs	7 days
Kidney			17 hrs.	4.5 days
Heart			3.7 days?	12-19 days
		PNA Adenine		
Stomach				7.7 days
Spleen and gonads		14 days		9 days
Small intestine	19 hrs.	5.8 days	19 hrs.	20 days
Large intestine			20 hrs	5.3 days
Liver		9.3 days		7 days
Carcass		15.3 days		12.3 days
Lungs				---
Kidney				5 days
Heart				---
		DNA Adenine		
Stomach		9 days		6.7 days
Spleen and gonads		---		---
Small intestine	24 hrs.	8 days	24 hrs.	6.7 days
Large intestine			20 hrs.	4.7 days
Liver		18 days		20 days

Table I
(Continued)

	Series I		Series II	
Tissues	"Fast" Component	"Slow" Component	"Fast" Component	"Slow" Component
		DNA Adenine		
Carcass	26 hrs.	5.7 days	44 hrs.	6.3 days
Lungs		15 days		19 days
Kidney				30 days
Heart				---

Table II

Effect of X-Irradiation upon the Incorporation of Adenine-C^{14} into 5-Adenylic Acid, PNA and DNA of A-Strain Mice

	Tissue											
	Small Intestine						Large Intestine					
	5-Adenylic Acid Adenine		PNA Adenine		DNA Adenine		5-Adenylic Acid Adenine		PNA Adenine		DNA Adenine	
Time After X-Irradiation*	s.a.**	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control
Control	2670		530		119		2520		500		103	
	3010		385		185		3275		435		141	
1 hour	2760	107	550	104	92	77	2410	96	400	80	50	48
	2700	90	415	111	131	71	3050	93	325	75	47	33
6 hours	--	--	--	--	--	--	--	--	--	--	--	--
	3150	105	500	134	102	55	2800	86	385	89	48	34
24 hours	3050	119	540	102	113	95	2860	113	430	86	69	67
	3325	111	525	140	188	102	2710	83	360	83	57	40
48 hours	4260	166	1085	205	396	332	3200	127	725	145	140	136
	3600	120	660	176	375	200	3050	93	530	122	187	132

Table II

(Continued)

Time After X-Irradiation	Tissue: Carcass						Tissue: Internal Organs					
	5-Adenylic Acid Adenine		PNA Adenine		DNA Adenine		5-Adenylic Acid Adenine		PNA Adenine		DNA Adenine	
	s.a.	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control	s.a.	% Control
Control	175		163		132		700		77		40	
	173		122		134		785		77		51	
1 hour	156	89	100	61	31	24	665	95	59	77	17	42
	136	79	77	63	46	34	600	76	37	48	16	31
6 hours	--	--	--	--	--	--	--	--	--	--	--	--
	131	76	68	56	21	16	655	83	47	61	18	35
24 hours	120	69	88	54	8	6	755	108	60	78	4	10
	96	56	68	56	10.6	8	655	83	48	62	5.6	11
48 hours	113	65	69	42	5.3	4	680	97	62	80	2.2	5
	111	64	73	60	6.0	4	750	95	55	71	1.1	2

Table II

(Continued)

Time After X-Irradiation	Stomach: 5-Adenylic Acid Adenine s.a.	Stomach: 5-Adenylic Acid Adenine % Control	Stomach: PNA Adenine s.a.	Stomach: PNA Adenine % Control	Stomach: DNA Adenine s.a.	Stomach: DNA Adenine % Control	Liver: 5-Adenylic Acid Adenine s.a.	Liver: 5-Adenylic Acid Adenine % Control	Liver: PNA Adenine s.a.	Liver: PNA Adenine % Control	Liver: DNA Adenine s.a.	Liver: DNA Adenine % Control
Control	2600		320		40		1540		145		--	--
	2370		370		75		1930		160		5.1	
1 hour	2700	108	345	108	--	--	1565	102	171	118	--	--
	2150	91	280	76	31	42	1780	92	140	88	3.2	43
6 hours	--	--	--	--	--	--	--	--	--	--	--	--
	2415	102	225	61	15	20	2300	120	220	137	2.0	39
24 hours	2900	112	415	130	14	35	2420	157	260	180	--	--
	2970	125	430	116	22	29	2240	116	150	93	0.7	14
48 hours	2750	106	400	125	31	78	1775	116	215	148	--	--
	3455	145	400	108	35	47	1805	94	160	100	1.0	20

Table II

(Continued)

	Tissue					
	Kidney					
	5-Adenylic Acid Adenine		PNA Adenine		DNA Adenine	
Time After X-Irradiation	s.a.	% Control	s.a.	% Control	s.a.	% Control
Control	1810		230		--	
	1975		190		3.6	
1 hour	1880	104	234	102	--	
	1850	94	175	92	<1.0	<28
6 hours	--	--	--	--	--	--
	1845	93	204	107	1.0	28
24 hours	2800	155	320	140	--	--
	2100	106	204	107	<1.0	<28
48 hours	2240	124	264	115	--	--
	2440	124	246	130	<1.0	<28

*The X-irradiation with 800 r (LD_{50}) required approximately 1 hour. The time is calculated from the end of the irradiation to the time of injection; 2 hours later the animals were sacrificed. Two separate series of experiments are represented by the above data.

**The specific activity (s.a.) is recorded in dis/min/μg of adenine in the respective materials.

after X-irradiation, a large decrease was observed in all tissues after irradiation. The small and large intestines, however, showed a smaller decrease in incorporation of adenine into DNA and within 48 hours after X-irradiation the incorporation of adenine into DNA was at a higher than normal rate. These results are in agreement with those obtained with P^{32} by Kelly and Payne (Fed. Proc., 12, 76 (1953)).

The effect of X-irradiation upon the incorporation of adenine into the 5-AMP pool and RNA is considerably less than upon the incorporation of adenine into DNA. An increased specific activity of the 5-AMP in the small intestine, stomach, and liver was found, while a decreased specific activity of the 5-AMP in the carcass was noted. No significant change was observed in the specific activity of the 5-AMP of the spleen and gonads (internal organs) and large intestine.

The specific activity of the RNA adenine of the various tissues followed the same general pattern as the 5-AMP specific activity. An increase was observed in the specific activity of the RNA adenine of the small intestine, stomach, and liver (also large intestine at 48 hours), while a marked decrease was observed in the specific activity of the RNA adenine of the carcass, spleen, and gonads (and large intestine through 24 hours).

Δ^7-CHOLESTENOL CONTENT OF RABBIT SERUM

Richard M. Lemmon and Margaret Parsons

In the quarterly report from this group for the period of September, October, and November, 1951, it was reported that the quantity of a cholesterol isomer, Δ^7-cholestenol,[1] was unusually low in human blood serum containing a high content of S_f 10-20 lipoproteins (S_f = Svedberg units of flotation). It has been shown[2, 3] that lipoproteins in this particular S_f (or molecular weight) group are associated with the development of atherosclerosis. Although later analyses in this laboratory failed to show anything unusual about the Δ^7-cholestenol content of this particular serum (or of the isolated S_f 10-20 lipoproteins themselves), it was decided to investigate the possibility that this sterol might be of importance in the pathogenesis of atherosclerosis. Our interest in Δ^7-cholestenol was further increased by the reported evidence[4, 5] that this sterol is an intermediate in the biosynthesis of cholesterol. We therefore undertook a program to feed rabbits on a high Δ^7-cholestenol diet. During the feeding we examined the animals' blood sera for (a) the appearance of lipoproteins in various S_f groups, (b) changes in cholesterol concentration, and (c) appearance of Δ^7-cholestenol. The results of these experiments are given in the present report.

Experimental

Synthesis of Δ^7-Cholestenol

Approximately 510 g of Δ^7-cholestenol was synthesized as follows: Cholesteryl benzoate was converted (procedure of Bernstein, et al.)[6] to 7-dehydrocholesterol via N-bromosuccinimide bromination, dehydrobromination with dimethylamiline, and hydrolysis of the 7-dehydrocholesteryl benzoate to the free sterol. The 7-dehydrocholesterol was converted to Δ^7-cholestenol by dissolving 5 g of the acetone-recrystallized sterol in 50 ml of pure dioxane and hydrogenating[7] overnight in the presence of Raney nickel (prepared according to Adkins)[8] and at about 3 atmos. pressure. The completeness of the hydrogenation was followed both by the measured uptake of hydrogen and by the disappearance of the 282 mμ absorption maximum of 7-dehydrocholesterol. After the catalyst was removed by filtration, the crude Δ^7-cholestenol was precipitated on addition of water. The product was then twice recrystallized from acetone and the last traces of solvent were removed by drying under high vacuum.

The 510 g of Δ^7-cholestenol were obtained from about 5 kg of cholesterol -- overall yield from cholesterol was therefore about 10 percent. The conversions to 7-dehydrocholesterol were carried out on approximately 500-g quantities of cholesteryl benzoate. The melting points of the recrystallized Δ^7-cholestenol product were all in the range of 122-125°C, and the product was further identified by routine infrared spectra which showed the identity of our product with a sample of Δ^7-cholestenol kindly supplied to us by Professor Louis Fieser of Harvard University. Occasionally, the infrared spectra of batches of our product indicated the presence (up to 10 percent) of cholesterol. These small amounts of cholesterol were not removed.

Rabbit Feeding

Three groups of rabbits (New Zealand White strain) were used for the feeding program. There were three male and three female rabbits in each group. One group was maintained on a cholesterol (1 gram/rabbit/day) diet, the second group on a diet containing cholesterol and Δ^7-cholestenol (1 gram of each sterol/rabbit/day), and the third group was fed Δ^7-cholestenol (1 gram/rabbit/day). In all cases the feeding was maintained for two weeks. The sterols were dissolved in a small amount of Wesson oil and given to the rabbits mixed in with their standard diet of Alber's "rabbit chow".

Δ^7-Cholestenol Analyses

The Δ^7-cholestenol analyses were carried out on 2- to 5-ml portions of the serum. The red cells were removed by centrifugation and proteins were precipitated with ten volumes of boiling 1:1 acetone-alcohol. To the supernatant liquid was added 4 or 5 drops of 50 percent aqueous KOH and the solution was either refluxed for one hour or allowed to stand overnight at room temperature. The solution was concentrated to 5 to 10 cc., made slightly acid with dilute aqueous HCl, and the sterol digitonides precipitated by the addition of a few ml of a saturated solution of digitonin in ethanol. The digitonide was recovered by centrifugation, washed with 2:1 ether-acetone solution and then with ether, and dried under high vacuum.

Determination of the amounts of Δ^7-cholestenol present in the digitonide was made by a modification of the procedure of Moore and Baumann.[9] This procedure utilizes the great difference in the rates by which the two sterols produce color by reacting with the Libermann-Burchard reagent (9.5 ml Ac_2O + 0.5 ml conc. H_2SO_4). The digitonide to be analyzed was dissolved in glacial acetic acid (1 to 5 mg/ml). Exactly 1 ml of this solution was mixed with 2.0 ml of the above reagent, the mixture quickly transferred into a cell of a Beckman (Model DU) spectrophotometer, and the optical density of the solution at 625 mμ determined exactly one minute after the mixing of reagent and solution. (The material in the "solvent" cell of the spectrophotometer was 2 ml of the Liberman-Burchard reagent mixed with 1 ml of glacial acetic acid.) The Δ^7-cholestenol digitonide reaches its maximum color intensity under these conditions in about one minute; the color intensity of the cholesterol digitonide is less than 1 percent that of the Δ^7-cholestenol digitonide at this time. The values obtained for the optical densities at one minute of the two digitonides (both on the basis of 1 mg/ml in the mixture of solution + reagent) are as follows: Δ^7-cholestenol digitonide (infrared spectrum demonstrated the absence of cholesterol) showed values for D 625 mμ varying from 3.92 to 4.48; cholesterol digitonide (the cholesterol was purified of all Δ^7-cholestenol by preparation of the dibromide, recrystallization of the dibromide, and regeneration of cholesterol) showed values for D 625 mμ varying from 0.019 to 0.023. The variation in these values resulted from different temperatures which happened to prevail in the laboratory at the time, and from different sources of solvents. However, each time that a Δ^7-cholestenol analysis was made the above values were determined at the same time and under exactly the same conditions. From determinations made on known, weighed mixtures of cholesterol and Δ^7-cholestenol digitonides, it was estimated that percentages of Δ^7-cholestenol in cholesterol could be obtained with maximum errors in the Δ^7-cholestenol percentages of ± 0.4 percent.

Results

The amounts of Δ^7-cholestenol in the sera of cholesterol-, cholesterol- and-Δ^7-cholestenol-, and Δ^7-cholestenol-fed rabbits are recorded in Tables I, II, and III.

Table I

Δ^7-Cholestenol Content of the Digitonin-Precipitable Sterols from Sera of Rabbits Maintained on a Cholesterol (1 g/rabbit/day) Diet

	% Δ^7-Cholestenol		
Rabbit No.	Start	1 Week	2 Weeks
263	0	1.1	1.6
264	0	0	0.9
265	0	0.4	0.9
272	0	0.7	0.5
273	0	0.1	1.2
274	0	1.7	0.5

Table II

Δ^7-Cholestenol Content of the Digitonin-Precipitable Sterols from Sera of Rabbits Maintained on a Cholesterol and Δ^7-Cholestenol (1 gram of each sterol/rabbit/day) Diet

	% Δ^7-Cholestenol		
Rabbit No.	Start	1 Week	2 Weeks
266	0	6.6	2.2
267	0	2.9	0.8
268	0	5.7	0
278	0	3.5	1.1
279	0	6.4	0.9
281	0	1.4	0

Table III

Δ^7-Cholestenol Content of the Digitonin-Precipitable Sterols from Sera of Rabbits Maintained on a Δ^7-Cholestenol (1 g/rabbit/day) Diet

	% Δ^7-Cholestenol		
Rabbit No.	Start	1 Week	2 Weeks
320	0	12.0	12.1
321	0	8.8	8.3
322	0	4.8	8.4
323	0	9.3	8.9
324	0	12.5	10.5
325	0	10.0	10.0

In addition to the above results, ultracentrifugal analyses for the changes in relative amounts of lipoproteins in the various S_f groups have been determined by the Division of Medical Physics. These results (which will be described in an early paper) show that, insofar as the lipoprotein ultracentrifugal pattern is concerned, the effect of Δ^7-cholestenol in the diet of rabbits is very similar to that of cholesterol.

Cholesterol analyses have been carried out by the Division of Medical Physics on the rabbit sera described in this report. These analyses show that there is a great rise in serum cholesterol after feeding Δ^7-cholestenol.

Summary

As part of a program of investigation of the pathogenesis of atherosclerosis, a study has been made of the effects of feeding rabbits a diet high in the cholesterol isomer Δ^7-cholestenol. This study has shown that (a) Δ^7-cholestenol is readily absorbed through the intestinal wall, (b) this sterol produces the same pattern of serum lipoproteins as does cholesterol, and (c) feeding of Δ^7-cholestenol leads to a rise in serum cholesterol, thus further indicating that the former is a precursor in the biogenesis of cholesterol.

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EFFECT OF X-IRRADIATION OF MICE ON THE LIVER CONCENTRATION OF A NUMBER OF COENZYMES

G. Milhaud and M. Calvin

The strong prophylactic action of mercaptoethylamine (MEA) for animals receiving whole-body radiation;[1] the apparently special sensitivity to destruction by high-energy radiation of a number of sulfur-containing enzymes and compounds (particularly with -SH);[2] the structural relation between MEA and Coenzyme A, together with the central position among the reactions involved in the mobilization of energy within the cell by carbohydrate and fat metabolism of such compounds as glutathione[3] (glyceraldehyde hydrogenase), thioctic acid and thiamin[4] (pyruvic acid oxidase), and Coenzyme A (citric acid synthesis[5] and fatty acid metabolism[6]) focused our attention on these coenzymes as possible chemical sites or indicators for the early, if not primary, action of high-energy radiation on biological materials.

The demonstration by Maisin and co-workers[7] of a therapeutic action of MEA or glutathione in irradiated rats of a certain strain whose livers had been shielded from the action of the X-rays, as differentiated from animals in which other organs had been protected, suggested the liver as a possible organismic site for this action of the penetrating radiation.

Accordingly, we have investigated the effect of whole body X-irradiation of mice[8] on the liver concentration of the compounds glutathione,[9] Coenzyme A[10], thioctic acid,[11] and the sum of DPN and TPN.[12] Control analyses with authentic samples indicated the absence of interfering substances in the extracts from irradiated animals.

The result of one experiment at the 1000 R level involving 30 animals and a single time point is given in Table I. A separate experiment, also at 1000 R, involving another 10 animals showed no significant effect of irradiation on the DPN-TPN level, the mean values being, control -- 460 γ/g liver, and irradiated -- 470 γ/g liver. In another experiment involving 50 animals the dosage was 800 R and the levels of three of the coenzymes were followed daily over a period of seven days following the irradiation. The results are shown in Fig. 1.

Three rather straightforward conclusions emerge from these and related data:

(1) There is a rather pronounced reduction in the CoA level (to about 10 percent of control) induced by X-irradiation. This reduction seems rather specific and its onset comes earlier with higher doses. A similar, though smaller, effect is also apparent for thioctic acid.

(2) That this reduction is not due to direct attack by the primarily produced radicals upon these substances is indicated by the delay (one day) in the drop at 800 R and by independent tests upon dilute neutral aqueous solutions of these substances, as well as related ones.[13]

(3) The prior administration of MEA prevents the drop in both CoA and thioctic acid. In fact, there is additional evidence that the administration of MEA may raise appreciably the CoA level of liver in unirradiated animals.

These results are consistent with the notion that among the very early victims of the radiation are enzymes situated in the liver (controlling the synthesis of both CoA and thioctic acid), and that the normal turnover rate of CoA, at least, is rather large. If the further assumption is made that the synthesis of CoA requires metabolis energy which cannot arise without the CoA function, it becomes immediately apparent that if the rate of CoA synthesis is reduced below a critical minimum in the initial irradiation, its concentration will subsequently fall at an increasing rate. It would appear also that MEA may function in at least two different ways: (a) in general as a nontoxic, chain-breaking antioxidant to compete with critical substances for the radiation-produced radicals, and (b) as a more or less specific precursor and protector for Coenzyme A.

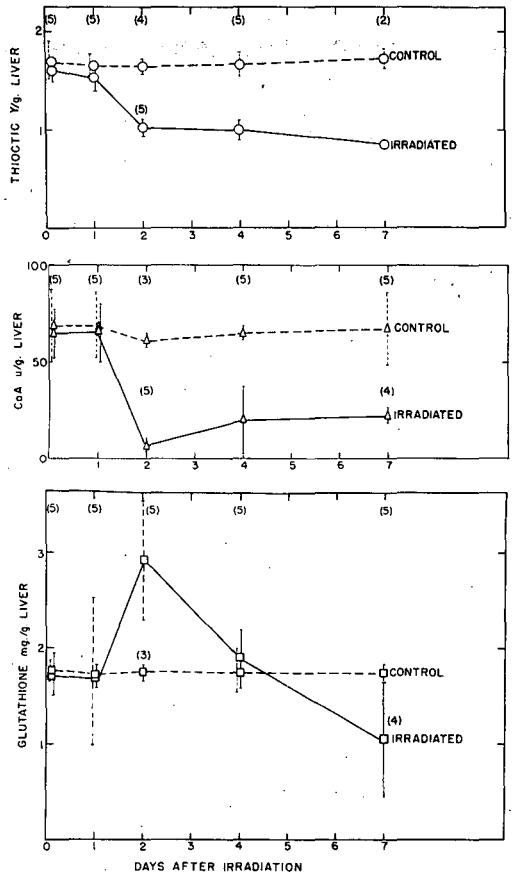
The implications of these ideas, together with a more general examination of the fate of other coenzymes and their fragments (vitamins), are under investigation.

Table I

Effect of Cysteineamine on Amounts of Coenzymes in Mice Livers After X-Irradiation

		Irradiated with 1000 R, 2-1/2 hours Prior to Analysis	
	Controls 10 Animals	4 mg Cysteineamine Prior to Irradiation 9 Animals	No Treatment 10 Animals
Glutathione mg/g liver	1.26 ± 0.21*	1.29 ± 0.20	1.99 ± 0.12
Thioctic acid mg/g liver	1.97 ± 0.29	1.97 ± 0.45	0.97 ± 0.18
CoA Units**/g liver	20.3 ± 2.2	23.8 ± 1.5	2.3 ± 1.8

*These are the average deviations from the mean.
**E. R. Stadtman and A. Kornberg, J. Biol. Chem., 203, 47 (1953).
1 μmol ≃ 316 units.



EFFECT OF 800 R IRRADIATION ON THE LIVER CONCENTRATION OF THREE SULFUR COMPOUNDS

(THE GLUTATHIONE AND CoA ANALYSES WERE DONE ON A SINGLE SET OF ANIMALS. THE THIOCTIC ANALYSES WERE DONE ON A SEPARATE SET OF ANIMALS)

THE PARENTHESES INDICATE THE NUMBER OF ANIMALS INVOLVED IN EACH POINT

MU-6358

Figure 1

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9. The method involves the use of glutathione reductase, for a sample of which we are indebted to Dr. E. Conn. See G. Milhaud, UCRL-____ (1953) and Arch. Biochem. Biophys., in press.

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GENERAL CHEMISTRY

W. M. Latimer, Director

METALS AND HIGH-TEMPERATURE THERMODYNAMICS

Leo Brewer, William Hicks, Richard Porter, Oscar Krikorian, and James Kane

Heats of Formation and Absolute Absorption Coefficients of High-Temperature Molecules

The new King furnace has been completed. Plans are being worked out for its use to determine the absolute absorption coefficients and heats of formation of important high-temperature molecules.

Reflection Coefficients of Molecular Beams

Apparatus has been constructed for the measurement of the reflection of molecular beams from solid surface and the determination of the important variables that control reflection.

Magnesium Oxide Gas

Study of the MgO spectrum in absorption and emission has confirmed early measurements that indicated that the generally accepted 1Σ ground state of MgO is higher in energy than a triplet state that is the true ground state. Thus MgO gas is more stable than previously believed on the basis of the old assignment of the ground state.

Refractory Silicides

Work on the ternary diagrams of systems containing Si, C, and refractory metals or Si, N, and refractory metals has been essentially completed and the regions of stability of the various phases determined.

Sodium Carbonate Vapor

Work is in progress on the study of Na_2CO_3 vapor. Results to date indicate that Na_2CO_3 forms a constant-boiling liquid composition close to Na_2CO_3 in composition. This constant-boiling liquid is in equilibrium with Na(g), O_2(g), and CO_2(g).

High-Temperature Halide Molecules

The study of CF and CF_2 has been completed. Work is in progress on the study of the effect of water on the volatility of molybdenum chlorides.

BASIC CHEMISTRY, INCLUDING METAL CHELATES

R. E. Connick, John Kury, Howard Mel, John Below,
Russell Sanborn, and A. E. Martell

Studies Involving Ammonia

(This investigation has been transferred to Livermore to be continued.)

Complexing of Scandium by Fluoride Ion

The extent of complexing of Si^{+3} by F^- in aqueous solution, at 25°C has been investigated using the method developed for In^{+++} (See UCRL-2202). The equilibrium quotients at an ionic strength of 0.50 are:

$$Sc^{+++} + HF = ScF^{++} + H^+ \qquad K_1 = 1700$$

$$ScF^{++} + HF = ScF_2^{\ +} + H^+ \qquad K_2 = 241$$

$$ScF_2^{\ +} + HF = ScF_3 + H^+ \qquad K_3 = 14.4$$

$$ScF_3 + HF = ScF_4^{\ -} + H^+ \qquad K_4 = 0.4$$

Thermodynamics of Thiosulfate

In an effort to clear up gross inconsistencies in the thermodynamic data for $S_2O_3^=$ ion, the equilibrium constant has been determined as a function of temperature for the new reaction:

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

A calorimetric heat of oxidation of $S_2O_3^=$ with Br_3^- has also been measured. From these sources, the thermodynamic properties have been calculated for $S_2O_3^=$ at 298.16°K with the results: ΔH_f^o = -154.0 kcal./mol, ΔF_f^o = -122.4 kcal./mol, S^o = 14.1 e.u. Discussion is given, particularly from the standpoint of the theory of aqueous entropies.

Discrepancies in thermodynamic data for BrO_3^- ion were felt to be primarily due to an inaccurate value for the heat of formation. This quantity has been redetermined from a calorimetric study of the reduction of BrO_3^- with I^- and the reduction with Br^-. Combining these heats with other heat and entropy data, we have the results for BrO_3^- at 298.16°K: ΔH_f^o = -18.3 kcal./mol and ΔF_f^o = 2.1 kcal./mol. These values have been discussed with respect to other experimental data. (See UCRL-2330).

Rare Earth Fluoride Complex Ions

See UCRL-2271.

Ferric Fluoride Complex Ions

See UCRL-2271.

Hydrolytic Polymerization of Zirconium

See UCRL-2268.

SPECTROPHOTOMETER STUDIES

The general problem has been to develop an instrument capable of spectrophotometrically following rapid reactions in solution. (By "rapid" is meant reactions having half-lives of 1 second or less.) In past months, a system had been conceived using the optical system and cell compartment of a Beckman model DU spectrophotometer, but using a photomultiplier as detector, and feeding its output to a dc oscilloscope with a slow sweep.

Under this contract, we have been engaged in the construction and testing of such an apparatus. We built a phototube compartment around an RCA 1P28 photomultiplier, to fit the Beckman, and obtained a 2000-volt power supply for the phototube voltage supply, and an RCA WO-56A oscilloscope as recorder. The equipment has been tested and found quite satisfactory. It is stable and reproducible to 2 percent at full-scale deflection on the cathode screen, and the displacement is proportional to extinction over the usable range of the screen. The inherent time lag in the apparatus is less than 1 millisecond for full-scale displacement. The apparatus is sufficiently sensitive to be used from 225 to 750 mμ.

Another problem attacked was that of rapidly mixing the reagents to initiate the reaction. Conventional mixing techniques require at least five seconds, which obviously would cancel the advantages of the recording instrument. A method was devised of adding one reagent by a hypodermic syringe to the other reagent already present in the absorption cell. To test this plan we constructed a special wide-mouth 2-cm pyrex cell with quartz windows, a special lid for the Beckman cell compartment on which a 5-ml hypodermic syringe is mounted, and a special cell holder, more flexible than the conventional Beckman holder. On the assumption that the rate of color change of an organic acid-base indicator is determined by the rate of mixing of indicator and acid (or base), this method of mixing was tested by injecting acid from the syringe into a mixture of base and bromcresol purple, the change in concentration of the base form of indicator being followed spectrophotometrically. The time required for 95 percent mixing has been found to be about 2/3 second. Current work is being devoted to increasing this rate by a factor of at least 10.

INFLUENCE OF FLUORINE SUBSTITUTION IN PROPERTIES OF COPPER (II) AND URANYL CHELATES

The following substances were prepared for study:

Bis-Acetylacetono Cu (II)
Bis-Trifluoroacetylacetono Cu (II)
Bis-Hexafluoroacetylacetono Cu (II)
Ethylenediimine bis-acetylacetone Cu (II)
Trimethylenediimine bis-acetylacetone Cu (II)
Ethylene diimine bis-trifluoroacetylacetone Cu (II)
Trimethylenediimine bis-trifluoroacetylacetone Cu (II)
Bis-Ethylacetoacetato Cu (II)
Bis-Ethyltrifluoroacetoacetato Cu (II)
Bis-Diethylacetoacetamido Cu (II)
Bis-Diethyltrifluoroacetoacetamido Cu (II)
Bis-acetylacetono UO_2 (II)
Bis-Trifluoroacetylacetono UO_2 (II)
Bis-Hexafluoroacetylacetono UO_2 (II)
Bis-Ethylacetoacetato UO_2 (II)
Bis-Ethyltrifluoroacetato UO_2 (II)
Hexafluoroacetylacetone
Bis-Acetylacetone ethylenediimine
Bis-Trifluoroacetylacetone ethylenediimine
Bis-Acetylacetone trimethylenediimine
Bis-Trifluoroacetylacetone ethylenediimine
Diethyltrifluoroacetoacetamide.

Note: Several of these are believed to be new compounds, although the literature survey is not yet complete.

Chemical behavior and solubility were studied on a qualitative basis. Extensive studies of the ultraviolet, visible, and infrared spectra were completed.

The final report will illustrate the principles and correlations deduced from the properties of related groups of the above compounds.

ABSORPTION OF TETRABORATE ION IN ANION EXCHANGE RESINS

Tetraborate ion is very weakly absorbed on Amberlite IRA-400 from a dilute ammonium chloride-ammonium hydroxide buffer of pH 10, while at pH 8 the absorption is about one-third as great. At a pH of 10, Dowex 1 does not absorb quite as well as does the Amberlite resin.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

L. A. Bromley, D. N. Hanson, C. R. Wilke, and T. Vermeulen

NOTES ON EXPERIMENTS IN PROGRESS

Preparation of Titanium Metal

A. W. Peterson, L. A. Bromley

Deposits of metallic titanium have been obtained using equipment previously described. Previous experiments have failed, due to pressure build-up in a closed system. Titanium will not deposit at high pressures. The deposits were formed when the pressure was maintained low with continuous evacuation; a high vacuum is unnecessary.

The first deposits obtained formed in globules on a 0.010"D Tungsten wire. The tendence of globule formation decreased when a 0.040"D tantalum wire was used.

Further experiment will be done with a 0.184"o.d. tantalum tube to determine minimum deposition temperature and to try to get a deposit of uniform thickness. The effect of pressure will be investigated.

Film Boiling from Subcooled Liquids

E. I. Motte, L. A. Bromley

Runs using ethyl alcohol are new being completed. The effect of increasing the energy of turbulence by screens is now being tested. It has been found that the transfer of heat from the vapor liquid interface into the bulk of the liquid is controlled by eddy diffusivity and not by molecular conduction. Liquids yet to be tested are benzene, hexane, and carbon tetrachloride.

Thermal Conductivity of Gases at High Temperatures

A. J. Rothman, L. A. Bromley, S. Whitaker

A total of 153 runs have been made, encompassing the conductivities of nitrogen, carbon dioxide, argon, helium, and air from 35°C to 780°C. The data are in the range of other investigators' data, where such are available. Conductivities for nitrogen and carbon dioxide are a few percent below those of Stops below 500°C, but they finally reach his values between 700 and 800°C. However, it is believed that these data are a few percent too high at 780°C. Experimental work on this first phase of the project is now complete and a report (UCRL-2339) is being written. Following the report, the cell will be redesigned to eliminate some of the experimental difficulties, viz., outgassing of the silver at high temperatures, corrosion of the stainless case by tin, etc.

Agitation of Liquid-Liquid System

J. L. Fick, T. Vermeulen

Studies of the agitation of immiscible liquid-liquid systems are being continued. A simple Weber number correlation as formulated earlier has now been found to apply to emulsion droplet sizes measured in the high-turbulence region of the mixing vessel. This correlation takes the form:

$$\frac{N^{1.2}\, d\, L^{.8}\, \bar{\rho}^{.6}}{\sigma^{.6}\, f\, \phi} = 0.084$$

where N is the paddle speed (RPS)
d is the mean droplet diameter of the disperse phase in the emulsion (cm)
L is the paddle diameter (cm)
$\bar{\rho}$ is an effective density of the bulk fluid defined as $0.4\ \rho_C + 0.6\ \rho_D$. (C refers to continuous phase, D to disperse)(g/cm^3)
σ is the interfacial tension between the two liquids (dynes/cm)
$f\ \phi$ is a function which represents the behavior of drop size with respect to volume fraction of disperse phase, i.e., the effect of increasing the volume fraction of disperse phase is to increase the drop diameter.

It has been shown also that results in a related study of gas-in-liquid dispersions can be correlated jointly with the liquid-liquid data by the use of a relation such as the following:

$$\frac{N^2\, d^{5/3}\, L^{4/3}\, \bar{\rho}/\sigma\, f\phi^{5/3}}{\left[1 + 5.4\text{x}10^{-6}\, d\, \sigma\, \rho_C\, \mu_C/f\, \phi\, \mu_D^3\right]^{1/3}} = 0.016$$

where the variables are the same as for the liquid-liquid case except for the addition of the viscosities of the disperse and continuous phases μ_C and μ_D in poises.

It has been found that the power required for mixing is proportional to W, the impeller width, while the emulsion droplet diameter is independent of W. Thus the smallest value of W that will give homogeneous mixing at a given speed should be selected whenever possible. Studies are therefore being made of the physical factors controlling the approach to bulk uniformity of mixing, i.e., a value of the mixing index of 100 percent.

STUDIES TO BE REPORTED

Solubility Studies — M. Cook, D. N. Hanson

This project is nearing completion and the results will be published shortly.

Thermal Diffusion in Liquids — J. Powers, C. R. Wilke

A modified glass-diaphragm diffusion cell has been constructed for measuring the Soret coefficient. A batch parallel-plate apparatus has also been built. The effect of angle of inclination of the pilot-plant scale thermo-gravitational equipment on the separation has been evaluated experimentally with a column that is eight feet long. A column four feet long has been constructed and operated to determine the effect of height on the separation. All work to date has been with the system ethyl alcohol-water.

Capacity of Perforated-Plate Liquid Vapor Contacting Columns — C. D. Hunt, D. N. Hanson, C. R. Wilke

The study of perforated-plate operating characteristics in a six-inch-diameter column has been completed.

The data have been used in the construction of design charts showing the operating limits of perforated plate columns.

The first draft of the report covering the work is written, and the final report will probably be issued during the next quarter.

Gas-Phase Mass Transfer Studies — E. J. Lynch, Loren Hov, C. R. Wilke

Major construction work on the 24" absorption tower has been completed. The tower has been packed with 1 1/2" carbon rings and should be in operation shortly. The 12" tower used for the study, UCRL-2057 Rev., has been repacked with larger rings and some data have been taken.

Construction has been completed on the fixed bed equipment and some preliminary data have been taken for vaporization of water into air.

Vacuum Flow through Annular Sections — Walter Dong, L. A. Bromley

This project consisted of measuring pressure drops of air, H_2, He, CO_2, and Freon 12 through four annular sections and a circular tube under vacuum. Measurements are made in the slip flow region, extending into either the molecular flow or streamline flow region as far as feasible.

As of June, 1953, data had been completed for two annular sections and data are now being taken for the circular tube. For each of the annular sections, all the gases can be correlated on a single plot. A spread was noticed with the circular tube but it may be due to a shift in the calibration of the McLeod gauge.

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10-7-53 bw