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Chemistry-Transuranic Elements

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CHEMISTRY DIVISION QUARTERLY REPORT

September, October, November, 1950

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I QUARTERLY PROGRESS REPORT. Project 48

G. T. Seaborg and I. Perlman

A. Chemistry of the Rare Earth and Actinide Elements

Vapor Pressure of Neptunium Metal

Merle E. Jones and B. B. Cunningham

During measurements of the vapor pressure of Pu and Np metals by effusion techniques, inconsistent values were obtained in different runs in which the same experimental setup and the same pellet of metal were used. A surface coating was suspected to be the cause of the deviations. This possibility was investigated experimentally with mercury, for which accurate vapor pressure data are available. A freshly cleaned Hg pellet and one which had been exposed to laboratory air for a period of three days were used. There was no visible coating on the latter and it appeared as bright as a freshly cleaned pellet but the measured vapor pressure was lower a factor of 8 to 10 than that of the fresh pellet. When a small piece of tantalum was placed in the effusion crucible with the apparently coated Hg drop and the crucible was vibrated during the measurements, the vapor pressure was found to be the same as that of the freshly cleaned drop.

The effect of a surface coating is thus apparent and methods for overcoming this difficulty in measurement of the vapor pressure of neptunium are being investigated.

The Behavior of Actinides and Lanthanides On Cation Exchange Columns with HCl as Eluting Agent

R. M. Diamond, K. Street Jr. and G. T. Seaborg

The experiments described in the previous quarterly report have been continued. As before the resin used was Dowex-50 spherical fines, hydrogen form, and the columns were 10 cm long by 1 mm or 1-1/2 mm in diameter. The flow rate was about 0.1 ml/min./cm^2. The results have been normalized to the 10 cm by 1 mm column and are given in Fig. 1 for Cm(III), Am(III), Pu(III), Y(III), Eu(III), Ce(III), La(III), Pu(IV), Sr(II) with 3, 6, 9 and 12 M HCl as eluting agent.

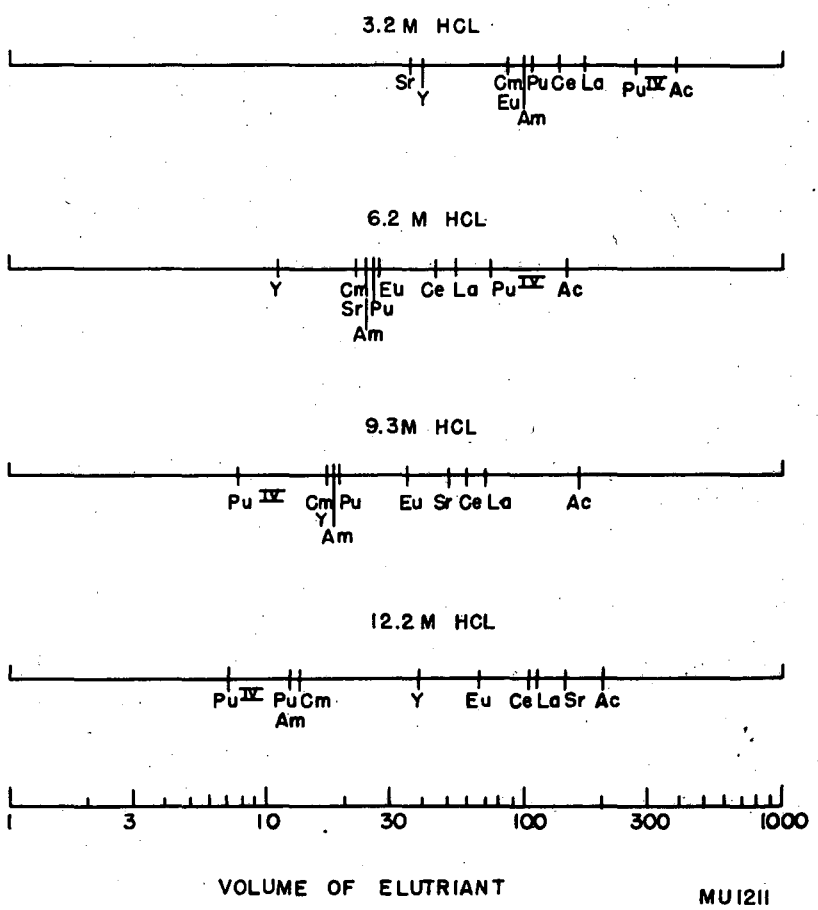
3.2 M HCL
Sr
Y
Cm
Eu
Pu
Am
Ce
La
Pu IV
Ac
6.2 M HCL
Y
Cm
Sr
Pu
Am
Eu
Ce
La
Pu IV
Ac
9.3M HCL
Pu IV
Cm
Y
Am
Pu
Eu
Sr
Ce
La
Ac
12.2 M HCL
Pu IV
Pu
Am
Cm
Y
Eu
Ce
La
Sr
Ac
1
3
10
30
100
300
1000
VOLUME OF ELUTRIANT
MU1211

Fig. 1

The Use of Tartaric Acid as an Eluting Agent for the Ion-Exchange Separations of Tracer Amounts of Americium and Curium

Richard A. Glass and K. Street, Jr.

Column separations of tracer quantities of Am, Cm, and several rare earths have been carried out on a 2 mm by 17 cm column packed with Dowex-50 resin. Both room temperature and high temperature runs, i.e., 60°C, 87°C, and 100°C, were made, using varying concentrations and pH's of tartaric acid-ammonium tartarate solution, to determine where the best separation is obtained.

The best room temperature run, using 0.1 M tartaric acid adjusted to pH 4.0 with NH_4OH, indicated about a 30 percent difference in the distribution coefficients (K_d) of Am and Cm. There was a decrease in total activity down to about 1 percent of the peak values in the region where the Am and Cm overlapped between the peaks. The disadvantages of these low temperature runs are the low rate of attaining equilibrium conditions, which limits the flow rate to about one 40 λ drop per 8 minutes; and the low solubility of ammonium acid tartrate at low temperatures, which prevents using a concentration of tartaric acid higher than about 0.1 M. The difficulty with both the low and high temperature separations, with regard to practical application, is that with anything larger than tracer quantities the peaks coalesce and the separation disappears.

The high temperature runs at 87°C, using 0.4 M tartaric at a pH of 3.3 and 0.8 M at a pH of 2.8, indicated about 20 percent difference in K_d with the activity dropping to 10 to 50 percent of the peak values between the peaks. The runs at 60°C and 100°C produced comparable results. Since equilibrium attainments are more rapid at high temperature, these columns were run at the flow rate of a drop about every 3 minutes. High temperature runs at lower concentrations and higher pH's than those indicated resulted in much smaller separation, which is a good indication that the singly charged tartrate ion is more efficient in producing a separation.

When the rare earths europium and promethium were put on the column with the Am and Cm, the Eu came off slightly ahead of the Cm and the Pm came off last, slightly behind the Am, at both high and low temperatures. At room temperature a 66 percent difference in K_d was obtained between Pm and Eu and at 100°C the difference was 45 percent.

Improvements in Microcalorimeters

S. R. Gunn and B. B. Cunningham

The semiadiabatic microcalorimeter described previously had thermal leakage modules of about 0.030 degrees per minute per degree thermal head. This rather large modulus led to considerable errors in the measurement of heat changes due to the error in extrapolation of rapid drift rates. By evacuation of the space between the calorimeter chamber and the submarine to a pressure on the order of 10^{-4} mm of mercury the heat exchanges due to air conduction and convection have been reduced until a leakage modulus of 0.010 is attained. It is hoped that the vacuum may be improved to lower this value still further and it is also planned to coat the calorimeter chamber with a reflecting material, such as platinum foil, in order to reduce heat exchanges due to radiation.

Some work has been done on the newer-vacuum-jacketed adiabatic calorimeter built here by E. F. Westrum, Jr. and LeRoy Eyring in 1949. The construction of the vacuum jacket has been found to be very unsatisfactory, and changes are being made to secure an effectively tight system.

Crystal Structure of OsO_4

Allan Zalkin and D. H. Templeton

X-ray diffraction investigations have been carried out on osmium tetroxide. Single crystal photographs of the substance were taken, and the compound was found to have a monoclinic cell. The following dimensions were obtained:

$$a = 9.40\ A^o$$
$$b = 4.52\ A^o$$
$$c = 4.75\ A^o$$
$$\beta = 125.5^o$$

The x-ray density was calculated to be 5.14 g/cm^2. The density reported in the Handbook for Chem. and Physics, 1947 edition is 4.906 g/cm^2.

The crystal was rotated about the b axis, (the needle axis) and the observed extinctions permit the three space groups C 2/m, C 2, and C m. The assumption was made that the OsO_4 molecule consisted of four tetrahedrally arranged oxygen atoms about each osmium; this arrangement necessitated just a 2 fold axis which was satisfied by the C 2 space group.

There are 2 molecules per unit cell. Actual final structure determination is still in progress, whereby the positions of the oxygens are being determined geometrically. The osmiums occupy the two fold position (000) (1/2 1/2 0).

Non-tetrahedral molecules were considered. Geometrical considerations, however, excluded all of those models other than tetrahedral.

There is considerable tendency towards twinning in this crystal. It was determined from layer line photographs that this occurred in all of our samples by reflection in the $20\bar{1}$ plane.

The Crystal Structure of $Na_2S_2O_3 \cdot 5H_2O$

Allan Zalkin and D. H. Templeton

The crystal structure of sodium thiosulfate pentahydrate is at present under investigation at this laboratory. X-ray diffraction data results in a monoclinic cell for the material. The space group being C_{2h}^5 - P $2_1/c$. The following values are the cell dimensions:

$a = 5.96 \pm 0.04$ A°
$b = 21.66 \pm 0.04$ A°
$c = 7.55 \pm 0.02$ A°
$\beta = 103° 58'$ (this value comes from the morphological investigations of de la Provostage[1])

a:b:c = 0.276 : 1 : 0.349 from x-ray data
a:b:c = 0.2745: 1 : 0.3508 from morphological investigations of de la Provostage[1]

The calculated x-ray density is 1.744 g/cm^2 as compared with 1.729 g/cm^2 the value given by the Handbook of Chemistry and Physics, 1947 edition.

There are four molecules in the unit cell. Our calculations indicate that there is insufficient room for the thiosulfate group to freely rotate, thus the thiosulfate group is apparently fixed in the crystal. Work is presently in progress to complete the structure determination with the evaluation of all of the atomic parameters.

---

[1] F. de la Provostage, Comp Rend 13 873 (1841)
Ann Chim Phys (3) 3 358 (1841).

The Crystal Structure of Thorium Nitrate

D. H. Templeton and Carol H. Dauben

The work done on this problem during the quarter has been reported in UCRL-1016.

Radiation Chemistry of Alcohols

A. S. Newton and W. R. McDonnell

Some results on the radiation chemistry of ethanol have been given in the previous Quarterly Report, UCRL-923. Preliminary results have now been obtained on several other alcohols and some generalizations can now be drawn from the data. The results are summarized in Table I.

At present even in favorable cases the oxidized products do not balance the reduced products so some oxidized products are apparently being missed. In the gas phase from every alcohol there is mass spectrometer evidence of several compounds in low yield which cannot be accounted for by the spectra of ordinary compounds. The only peroxide seen to date has been dimethyl peroxide and the only ether dimethyl ether, both from methanol. If peroxides and ethers are formed in higher alcohol bombardments their yields are small. This indicates a lack of reactivity of the H on the hydroxyl (except methanol case).

From the ratio of H_2 to methane it is seen that as the alcohol becomes more branched, the lower is the yield of H_2 and the higher than of methane. If we couple this with the glycol results in which non-adjacent glycols have not been observed, it follows that the hydrogen on the C to which the hydroxyl is attached must be especially susceptible to attack. In tertiary butanol no hydrogens are available at this point so the yield of methane is enhanced.

It is hoped that by careful distillation procedures some of the higher molecular weight materials can be identified. Even though the yield is small they will throw considerable light on the behavior of radicals in the liquid state. No evidence of large polymers has appeared and possibly these are not primary products in the bombardment of alcohols.

TABLE I

Yields of Products from Alcohols

(Yields in Molecules Product Formed/100 e.v.)

Product/Alcohol	Methanol	Ethanol	n-Propanol	Iso-Propanol	Tert-Butanol
Total gases	4.5	2.8	3.03	3.68	2.11
H_2	3.60	2.03	1.96	1.61	0.63
CO	0.34	0.081	0.28	0.051	<0.05
CH_4	0.22	0.27	0.125	0.78	0.72
C_2H_6	0.011	0.096	0.36	0.125	0.27
C_2H_4		0.086	0.12	0.031	0.06
C_2H_2		0.015	--	0.010	
C_3H_8			0.11	0.02	
C_3H_6			0.04	0.105	
"Butenes"				0.005	
Acetaldehyde				0.201	
Isobutane					0.14
Acetone?					0.04
Dimethyl ether	0.113				
Dimethyl peroxide	0.005				
Acid (H^+)	0.021	0.019	0.024	0.007	0.007
H_2O	0.97	0.94	N.D.	N.D.	N.D.
Formaldehyde	1.58	0.34	0.6	None	None
Higher aldehydes	None	1.26	0.7	0.75	None
Total carbonyl	None	1.67	1.4	1.85	1.4
Ethylene glycol	1.18				
Adjacent $-\overset{OH}{C}-\overset{OH}{C}-$	1.18	0.93	N.D.	0.27	
CH_2OH residue	2.36	0.25	N.D.	<0.03	N.D.
RCHOH residue	None	1.6	N.D.	0.16	N.D.
R_2COH residue	None	None	N.D.	0.33	N.D.
Non-adjacent glycols	None	Small	N.D.	Very Small	N.D.
Total glycols	1.18	0.93	N.D.	0.27	N.D.
Ketones	None	Small	Small	1.0	1.4

N.D. = Not Determined.

Glycol residues are shown by the aldehyde or ketone formed on periodic acid splitting. The yield is given for the residue in each case so the glycol would have half the yield if combined with a like residue.

B. Nuclear Properties and Transformations

Light Isotopes of Zirconium and Their Product Nuclei

G. D. O'Kelley and E. K. Hyde

90-Minute Zr^{87}. The end point of the Zr^{87} positron spectrum is 2.10 Mev (Fermi-Kurie plot). A careful study has been made of the product nuclei from this isotope. Zr^{87} emits no gamma-rays, but decays to an isomeric state of Y^{87}, which emits a very prominent 0.389 gamma-ray in addition to 8 gamma-rays from 1.07 to 1.76 Mev. The ground state of Y^{87} decays by pure electron capture to the isomer Sr^{87m}, having a single gamma-ray, which we find to be 0.394 Mev. Helmholz[1] previously measured this gamma-ray as 0.386 Mev.

Zr^{88}. Very little is known about this isotope except that it is a long-lived electron capture isotope, with two conversion lines observable on the beta-ray spectrometer corresponding to the K and L conversion of a 0.406 Mev gamma-ray. The half-life is about 200 days.

78-hour Zr^{89}. This isotope emits positrons of 0.910 Mev, and four gamma-rays. No electron capture is detected by absorption, and all Auger electrons can be accounted for by the internal conversion of the gamma-rays. The gamma-ray energies are 0.027 Mev, 0.396 Mev, 0.915 Mev, and 1.28 Mev. A tentative decay scheme is shown in Fig. 1.

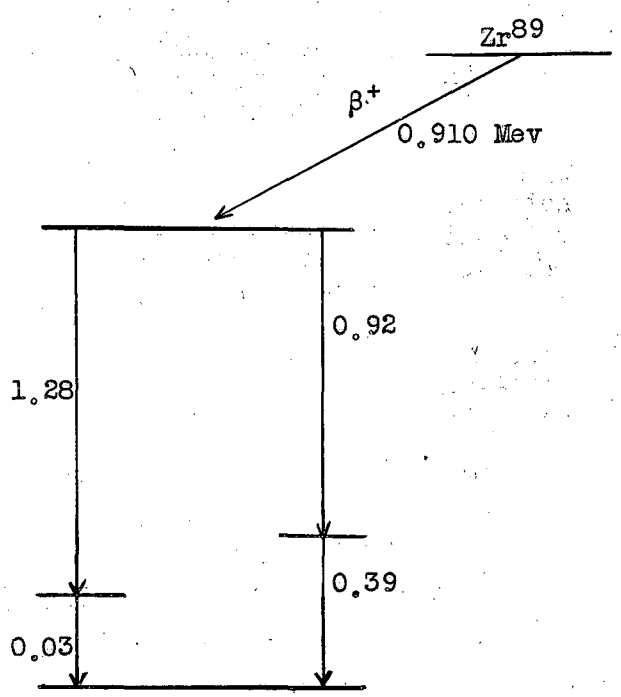

Fig. 1

Postulated Decay Scheme of Zr^{89}

Beta-Spectrum of P^{32}. As a check on the energy calibration of the beta-ray spectrometer, a sample of carrier-free P^{32} was run. The beta energy obtained from a Fermi-Kurie plot of the data was 1.685 ± 0.01 Mev. This value agrees very well with the recent values by Langer[2] (1.689 ± 0.01 Mev) and Caldwell, et.al.[3] (1.684 Mev), although lower than Siegbahn's value of 1.712 Mev. Two samples were run in our investigation; one source was only about 40 $\mu gm/cm^2$ thick, while the other was about 0.12 mg/cm^2 thick. Even after a correction for instrumental distortion was made according to the method of Primakoff and Owen, a distinctly non-linear Fermi-Kurie plot was obtained in the low energy region. The shape of the Fermi-Kurie plot is very similar to that obtained by Agnew[4], and the momentum distribution at low energies seems to indicate the same excess of electrons as that observed by Agnew and Warshaw, et.al.[5]

---

[1] Helmholtz, Phys. Rev. 60, 415 (1941).
[2] Langer, Phys. Rev. 76 641 (1949).
[3] Caldwell, et.al., ISC-56, August, 1949 .
[4] Agnew, Phys. Rev. 77, 655 (1950).
[5] Agnew and Warshaw, et.al., Phys. Rev. 80, 288 (1950).

Report on Investigation of Alpha Activity in the Rare Earth Elements

J. O. Rasmussen, S. G. Thompson, A. Ghiorso, and G. T. Seaborg

The long-lived artificial alpha activity previously found in gadolinium fractions has had assignment confirmed by further bombardments and chemical separations using a combination of sodium amalgam reduction separations and cation exchange column separations. Its alpha particle energy has been measured as 3.21 ± 0.03 Mev by the differential pulse analyzer. Since a sample of this activity has shown no detectable decay in one year, we can set a lower limit on the half-life of this isotope at about 25 years.

Fig. 1 shows a graph of relative yield of this 3.21 Mev alpha activity as a function of energy of alpha particles bombarding isotopic Sm^{147} (81.6 percent). The reaction threshold of about 30 Mev and steep rise must almost certainly correspond to an (α,3n) reaction. See, for example the (α,2n) and (α,3n) excitation curves for bismuth[1] and for indium[2]. An (α,3n) reaction on the main target material, Sm^{147}, yields Gd^{148}. From Sm^{144} (present to 1.10 percent in this sample) the (α,3n) reaction yields Gd^{145}. The fact that this 3.2 Mev alpha activity has been produced in good yield by bombardment of europium (stable masses 151, 153) with 50 Mev protons would seem to rule out the assignment to Gd^{145}, as the threshold of the $Eu^{151}(p,7n)Gd^{145}$ reaction must certainly be greater than 50 Mev. We have, therefore, assigned this 3.21 Mev alpha activity to Gd^{148}. This isotope has 84 neutrons, just two in excess of the stable shell of 82.

A small amount of alpha activity of 2.9 Mev energy has recently been observed in europium produced by bombardment of isotopic Sm^{147} by 10 Mev protons and chemically separated on a cation exchange column. The half-life of this activity has not yet been determined with certainty, but it is of the order of two months. The principal mode of decay of this activity must certainly be orbital electron capture. Mass assignment and determination of branching ratio between alpha decay and electron capture will be attempted.

The isotopically separated samarium with which these experiments were carried out was made available by the Oak Ridge Isotope Research and Production Division.

---

[1] E. L. Kelly and E. Segrè, "Some Excitation Functions of Bismuth," Phys. Rev. 75, 999 (1949)

[2] G. M. Temmer, "Excitation Functions for (α,n), (α,2n), and (α,3n) Reactions on Indium," Phys. Rev. 76 424 (1949).

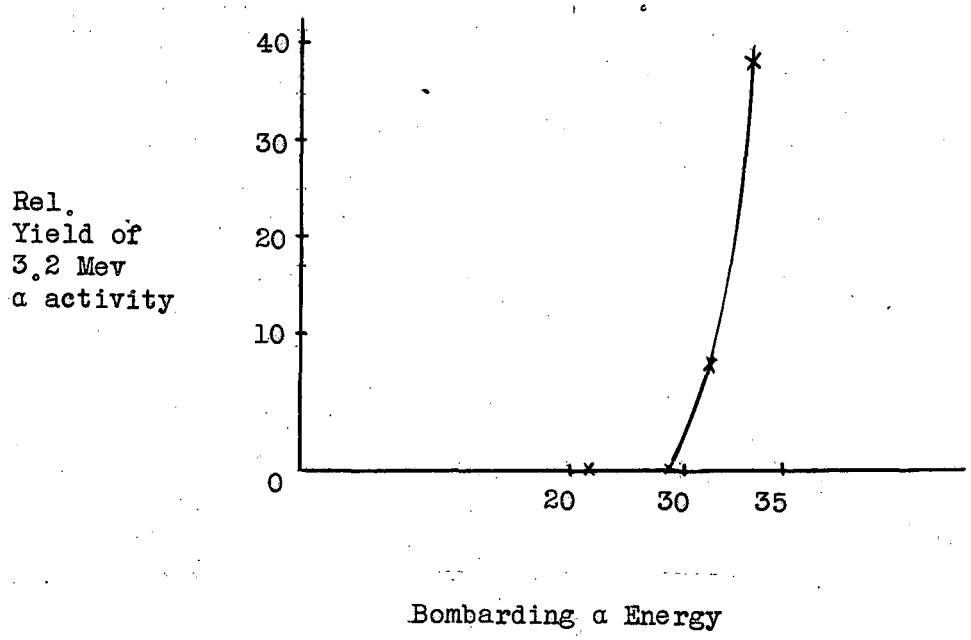
Rel.
Yield of
3.2 Mev
α activity
40
30
20
10
0
20
30
35
Bombarding α Energy

Fig. 1

Nuclear Spectroscopy: Decay Scheme of 21-hour Np^{236}

G. D. O'Kelley and D. A. Orth

It was possible to separate strong sources of Np^{236} from deuteron-irradiated U^{235} targets. The samples for use in the beta ray spectrometer were evaporated from a drop of concentrated hydrochloric acid solution on a Tygon film about 30 $\mu g/cm^2$ thick.

Auger electrons, internal conversion electrons, and beta particles were observed in the beta ray spectrometer. The beta spectrum is complex, and resolution of Fermi-Kurie plots of the data indicate beta energies of 0.51 and 0.36 Mev. A very prominent pair of conversion lines corresponding to the K and L conversion of a 0.150 Mev gamma ray are also seen. However, an estimate of the L-Auger intensities indicates far too many L-vacancies to be associated with the internal conversion of the 0.150 Mev gamma ray, even correcting for the 4.4 day Np^{234} electron capture isotope which was present in smaller amount. Careful absorption experiments are in agreement with the decay scheme shown in Fig. 1, in which the excess Auger electrons and x-rays are attributed to electron capture in Np^{236}. These data are in good agreement with the predictions that Np^{236} should electron capture as well as emit β^- particles.

Conversion lines from 4.4 day Np^{234} were also seen, and appear to fit a decay scheme as shown in Fig. 2. The K and L conversion lines from the 0.803 Mev gamma ray were the most prominent of the Np^{234} lines, while the others were very weak.

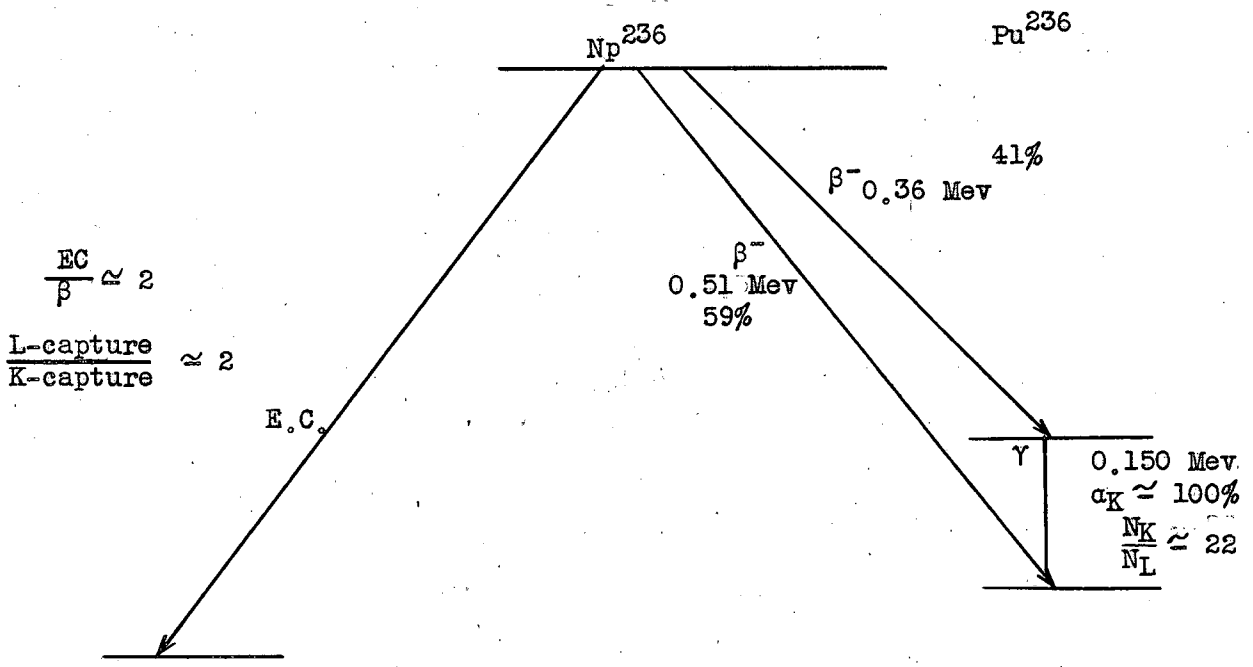

Fig. 1

Decay Scheme of Np^{236}

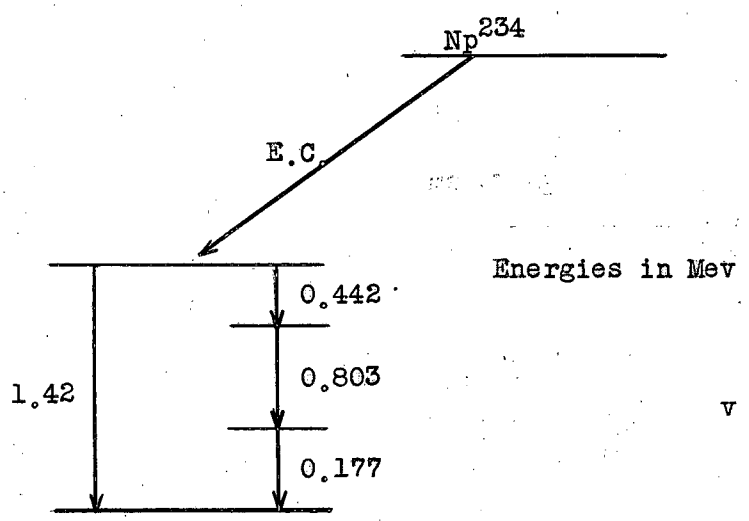

Fig. 2

Proposed Decay Scheme of Np^{234}

Specific Activity of U^{235}

E. H. Fleming, Jr., A. Ghiorso, and B. B. Cunningham

Known weights of enriched U^{235} have been electrodeposited quantitatively onto platinum and the samples counted, in order to determine accurately the specific activity of this nuclide.

In order to remove counting yield uncertainties involved in the use of the usual 2 π counters, two low geometry chambers have been constructed, such that only alpha particles emitted at an angle greater than 45° above the horizontal are counted. One chamber yields a counting rate of about 16 percent of that detected in a 2 π counter; the other, about 0.0026 percent.

The second chamber mentioned was designed for calibrating the first, by counting a sample containing about 4×10^5 alpha disintegrations in each. Uranium 234 is used for this purpose, along with U^{238} to make up sample mass. The geometry of the second chamber is calculated to within 0.1 percent, permitting an accurate calibration for the first.

A single set of counts to date indicates a specific activity for U^{235} of 4.68 disintegrations/minute/microgram, corresponding to a half-life of 7.21×10^8 yr. We believe the probable error of this determination to be less than $\pm$ 2 percent.

Naturally Occurring Plutonium

C. A. Levine and G. T. Seaborg

We have continued the investigation of naturally occurring plutonium. Seven minerals have been subjected to chemical treatments designed to isolate any plutonium present. The results from four of these minerals (the pitchblendes and Brazilian monazites) have been reported in previous Quarterly Reports. Table I gives a summary of the amounts of plutonium present in the ores.

No isotope of plutonium, other than Pu^{239}, was detectable in the plutonium fraction. It appears reasonable that the Pu^{239} was formed by (n,γ) reactions on U^{238}. Approximate calculations have been made which indicate that the concentration of plutonium found is reasonable if the total neutrons available from spontaneous fission, from (α,n) reactions taking place in the ore, and from cosmic rays are considered.

In the cases of the pitchblendes, neutrons made available by spontaneous fission of uranium and by the (α,n) reactions on light elements in the ore contribute the major portion of the neutrons. The number of neutrons contributed by cosmic rays seem relatively slight.

The fact that the Pu^{239} to uranium ratios in the monazite sands are approximately equal to the ratios in pitchblendes is probably fortuitous. Again, approximate calculations can be made which indicate the relative importance of each source of neutrons in forming Pu^{239}. In the cases of the monazite ores, (α,n) reactions caused by the alpha particles of the natural radioactive elements impinging on nuclei of the light elements are the most important source of neutrons. Spontaneous fission of the thorium and uranium present gives rise to a lesser amount of neutrons, and again, the cosmic ray contribution is relatively small.

A search was made for plutonium in fergusonite and carnotite in order to test the hypothesis that the Pu^{239} was formed by (n,γ) reactions on U^{238}. The potassium and vanadium in carnotite and the tantalum in fergusonite are elements with high neutron absorption cross sections. These elements would be expected to capture a large share of the neutrons available, thus decreasing the Pu^{239} to uranium ratio. No Pu^{239} was seen in either of the samples and only upper limits for the ratio could be set. In the case of the carnotite, this upper limit is a factor of ten to thirty below the ratios found in the pitchblendes and monazites. In the case of the fergusonite, due to background activity the upper limit for the Pu^{239}/uranium ratio is the same order of magnitude as the ratio in the pitchblendes and monazites investigated. With larger samples of fergusonite, a better limit could be set. A complete report on this investigation is being issued as UCRL-1025.

TABLE I

Summary of Results

	Pu^{239}/Ore	Pu^{239}/Uranium
Pitchblendes:		
Canadian (13.5%U)	9.1×10^{-13}	7.1×10^{-12}
Belgian Congo (38% U)	4.8×10^{-12}	12.0×10^{-12}
Colorado (50% U)	3.8×10^{-12}	7.7×10^{-12}
Monazites:		
Brazilian (0.24% U)	2.1×10^{-14}	8.3×10^{-12}
N. Carolina (1.64% U)	5.9×10^{-14}	3.6×10^{-12}
Fergusonite:		
Colorado (0.25% U)	$<1 \times 10^{-14}$	$<4 \times 10^{-12}$
Carnotite		
Colorado (10% U)	$<4 \times 10^{-14}$	$<0.4 \times 10^{-12}$

The Half-life and the Radiations from Cm^{241}

G. H. Higgins and K. Street Jr.

Several bombardments of Pu^{239} with helium ions of various energies have been made and a curium fraction chemically separated. Pulse analysis of the counting samples obtained when ~38 Mev bombarding particles were used showed the alpha energies of Cm^{240} (6.26 Mev), Cm^{242} (6.08 Mev), and a group at 5.89 ± 0.03 Mev. Bombardments at 26-28 Mev produced activities of Cm^{242} and the 5.89 alpha group. The half-life of this group as determined by subsequent pulse analyses was ~37 days.

The same samples were followed for decay on a windowless proportional counter which counted the Auger electrons and the half-lives of Cm^{242} (~162 days) Cm^{240} (26.8 days), and an activity of ~34 days were found. The samples bombarded at lower energies have not fully decayed but the initial half-life is >35 days and <50 days indicating little 27-day Cm^{240}.

On these bases the 35 ± 2 day activity is assigned to Cm^{241}. The EC/α branching ratio was determined to be approximately 20 from the assumed counting efficiency of 0.3 for the windowless proportional counter. Assumption of equal yield for the production of Cm^{242} and Cm^{241} at 26 Mev bombardment and comparison of the total alpha disintegration of each isotope yields the same ratio. The calculated alpha half-life is two years which is not unreasonable for a curium isotope emitting particles of this energy since the decay should be somewhat forbidden in view of the even-odd character of Cm^{241}. Since an energy of 6.0-6.2 Mev is expected for the ground state alpha transition of Cm^{241} on the basis of the systematics of alpha radioactivity, the 5.89 Mev alpha group is thought to be a fine structure group. There may be a second alpha group of 6.0-6.2 Mev which was not observed in these experiments but its abundance must be less than the 5.89 Mev group or its decay would have been noted in the Cm^{242} pulse analysis peak. It seems likely that the previously reported half-life (55 days) of Cm^{241} was in error.

Mass Spectrograph

F. L. Reynolds

During this period the mass spectrograph has been largely employed as shown in Table I for the determination of the abundance ratios of heavy element samples submitted by other members of the laboratory and by one outside research group.

In addition to the above the spectrograph was used to separate and collect small amounts (10^{-12} grams) of the Cm isotopes, Cm^{242}, Cm^{243} and Cm^{244}.

TABLE I

Sample	Mass						
	% 238	% 239	% 240	% 241	% 242	% 243	% 244
Special Canadian Pu	-	93.60	5.84	0.58	<.014	-	-
Chalk River	-	± .18	±.16	±.05			
Pu C. R. No. 1	62.9	11.7	1.60	.07	24.0	-	-
Am C. R. No. 1	-	-	-	95.1	1.09	3.76	-
Pu C. R. - L. A.	63.6	9.62	1.07	0.10	25.6	-	-
Cm C. R. - L. A.	-	-	-	-	95.75	2.15	2.02
Pu Crane No. 1	-	95.6	4.08	0.23	<.02	-	-
Pu Crane No. 3	-	98.55	1.37	0.09	-	-	-

C. Experimental and Service Chemical Work Related to the Physics Research Program.

Identification of Short Lived Products Produced by α's on Lead

R. F. Leininger and Noel Spiess

Three α activities have been observed following the bombardment of lead 208 by α's from the sixty inch cyclotron.[1] Chemical identification of these activities has been completed and more precise values of the half-lives determined.

1. The 0.52 second activity has been observed in the Po fraction when the Po^{211} daughter of At^{211} was quickly separated from the parent material. This is the first known direct determination of the half life of AcC' and indicates a factor of 100 error in Geiger-Nuttall for this activity.

Separation was achieved by flashing a dull red heat electrically to a Pt strip on which At had been deposited. Under these conditions At evaporates while Po remains on the Pt. Less than 1/2 second elapsed between separation and the start of counting.

2. The 25 second activity was shown to be a Po isotope by deposition onto silver from 2 molar HCl. The procedure used as adapted from one described by Meinke.[2] On the basis of excitation functions and bombardment of separated isotopes we have tentatively assigned the 25 second activity to Po^{211}.

3. The 2.17 minute activity has been identified as Bi^{211} (Ac C) and is formed presumably by an α,p reaction on Pb^{208}. The process used for chemical separation was as follows:

(a) 100 mg $PbCO_3$ was bombarded for thirty seconds by 40 Mev α's then dissolved in 2 drops of 8 M HNO_3 containing one mg of Bi, 6 drops of conc. HNO_3 were then added and the solution evaporated until lead nitrate precipitated.

(b) The supernatant from (a) was decanted in a 3 ml cone containing 4 drops of conc. ammonia and one ml of distilled water.

(c) To the above solution two drops of a one percent solution of "thionalid" were added to precipitate the bismuth.

(d) After centrifuging the tube was rinsed with 2 drops of acetone to remove excess water. The precipitate was then dissolved in two drops of hot acetone and transfered to a Pt plate, dried and then flamed.

This procedure was completed in four minutes and gave a yield in excess of fifty percent. The separation from lead was good but from Po was poor.

Since two of these activities have been assigned to the same isotope of polonium it is necessary to clarify the situation. We consider the 25 M

activity to be due to the ground state of Po^{211} and the 0.52 second activity to be an isomer.

The K capture branching which occurs in the decay scheme of Astatine 211 goes to Polonium 211. Branching between the two possible Po^{211} states might be expected and we have looked for it using the method of Section 1. Under the conditions of our experiment we observed no branching but could have resolved one part in one thousand.

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[1] N. Spiess, Physics Quarterly Report, UCRL-896

[2] W. Meinke, Chemical Procedures Used in Bombardment Work at Berkeley, UCRL-432.

Special Services

R. F. Leininger

The crystal program has been continued but has suffered minor setbacks due to impure stilbene.

D. Bio-Organic Chemistry Group

B. M. Tolbert

Synthetic and Experimental Chemistry

M. Calvin, B. Tolbert, P. Adams, R. Bartsch, R. Bonner, A. Fry, T. Geissman, S. Ikeda, D. Kritchevsky, R. Noller, R. Ostwald and R. Zeitschel.

Synthesis of Compounds. During the past quarter the following synthetic preparations have been undertaken. The radioactive scale of the reaction is indicated by the amount of starting activity and the yield, based on starting $BaC^{14}O_3$, if known yet, is also given. Most of the reactions were done on a 10-20 mmole scale.

High Specific Activity Preparations. Sodium bicarbonate in isotonic solution (10 mc., quantitative); sodium valerate-1-C^{14} (55 mc., 97 percent yield); sodium propionate-1-C^{14} (47 mc., 97 percent yield); methyl-C^{14} iodide (86 mc., 57-68 percent yield); propyl-1-C^{14} iodide (47 mc., 74 percent yield); isobutyl-1-C^{14} iodide (15 mc., 59 percent yield); amyl-1-C^{14} bromide (45 mc., 33 percent yield) sodium acetate-2-C^{14} (67 mc., 40-44 percent yield); sodium isovalerate-2-C^{14} (15 mc., 33 percent yield); sodium octanoate-1-C^{14} (2 mc., 94.4 percent yield); choline-methyl-C^{14} chloride (12 mc.); acetylcholine-methyl-C^{14} chloride (12 mc.); cyclohexanone-2-C^{14} (40 mc., 45 percent yield); valine-1-C^{14} (30 mc., 51 percent yield) succinic-2-C^{14} acid (10 mc., 30 percent yield); cuprous cyanide-C^{14} (10 mc., 65 percent yield); α-naphthoic-carboxyl-C^{14} acid (4 mc.); 1-naphthaleneacetic-α-C^{14} acid (2 mc.); glycol-1,2-C_2^{14} (0.1 mc., 24 percent yield, 0.2 mmole scale) benzoic-carboxyl-C^{14} acid (25 mc.)

Low Activity Preparations and Compounds Under Study. Valine-4,4'-C_2^{14}, norvaline-3-C^{14}, norleucine-3-C^{14}, leucine-3-C^{14}, n-butyl-1-C^{14} iodide, isopropyl-methyl-C_2^{14} iodide, isobutyl-1-C^{14} iodide, aspartic-γ-C^{14} acid, aspartic-β-C^{14} acid and vinylacetic-1-C^{14} acid.

Distribution of Labeled Compounds. The following list of compounds has been offered for distribution to the Isotopes Division. The approximate yields are indicated in parentheses: Butyl-1-C^{14} bromide (50 percent); amyl-1-C^{14} bromide (50 percent); isoamyl-1-C^{14} bromide (60 percent); isobutyl-1-C^{14} iodide (60 percent); isocarproic-2-C^{14} acid (30 percent); isobutyric-1-C^{14} acid (95 percent); isovaleric-1-C^{14} acid (94 percent); norleucine-3-C^{14} (40 percent); norvaline-3-C^{14} (40 percent); aspartic-γ-C^{14} acid (50 percent); aspartic-β-C^{14} acid (30 percent).

The following compounds have been scheduled for preparation in cooperation with the Division of Animal Husbandry of the University of California at Davis for the project of Prof. Max Kleiber on the studies of metabolism of cows: Sodium n-valerate-1-C^{14} (11 mc.); sodium n-caproate-1-C^{14} (11 mc.); sodium n-valerate-2-C^{14} (30 mc.); sodium n-caproate-2-C^{14} (30 mc); sodium isobutyrate-1-C^{14} (11 mc.); sodium isovalerate-1-C^{14} (11 mc.); aspartic-γ-C^{14} acid (20 mc.); aspartic-β-C^{14} acid (30 mc.); leucine-3-C^{14} (50 mc.); norleucine-3-C^{14} (25 mc.).

C^{14}-labeled Antihistaminic Drugs. The synthesis of several antispasmodic (antihistaminic) drugs has been undertaken for the study of the mechanism of physiological action and the fate of these compounds in the organism. The drugs being prepared are benadryl, pyribenzamine, and a representative of a group of structurally closely related drugs known to be inactive in pharmocological tests. Preparations of the necessary intermediates have been carried out, and the high activity preparation of benzoic acid labeled in the carboxyl group, which is the starting material for this work, is now being undertaken.

Synthesis of Glycol-1,2-C_2^{14}. A solution of 21.5 mg. of oxalic acid-1,2-C_2^{14} in 10.3 ml of dry ether was found to contain 3.0×10^7 dis./min. Excess diazomethane was added and the solution was concentrated to 5 ml. Excess freshly prepared lithium aluminum hydride solution was added and the reaction was continued in a sealed tube for 20 hours at 100°C. After cooling the solution was acidified with 1N sulfuric acid to pH 4.8 and the ether was evaporated. The insoluble residue was further extracted with water to give a volume of 17 ml. The extract was made alkaline and distilled in vacuo to give an aqueous solution of glycol-1,2-C_2^{14}.

The product and its activity was assayed as follows: To 1.0 ml of the distillate was added 5 mg of glycol carrier in 3 ml of water. Periodic acid (1N, 250 λ) was added. This is a 25 percent excess. After standing three hours at 20°C the solution was completely distilled and excess dimedon reagent added. The precipitate was recrystallized and its specific activity determined to be 105×10^4 dis/min/mg of formaldimedon. From this result it is calculated that 1.7×10^7 dis/min of glycol were obtained which represents a 57 percent yield.

Isopropyl-1,3-C_2^{14} Iodide. In previous quarterly reports we have described the attempted preparation of labeled isopropyl iodide by condensation of methylmagnesium iodide with acetaldehyde. Although this process is capable of giving fairly satisfactory yields the results are somewhat erratic and it has been found that the method is not satisfactory to use for high activity preparations.

As a consequence and since this product was still needed, a different method was developed for the preparation of this important synthetic intermediate, as follows:

$$C^{14}H_2CO_2Na \longrightarrow C^{14}H_3CO_2)_2Ba \xrightarrow{\Delta}$$

$$C^{14}H_3COC^{14}H_3 \xrightarrow[\text{Copper chromite}]{H_2} C^{14}H_3CHOHC^{14}H_3$$

$$\xrightarrow{P_4,\ I_2} C^{14}H_3CHIC^{14}H_3$$

The overall yield for this process is 75-80 percent based on the sodium acetate used.

In the procedure the barium acetate is converted to acetone by prolysis at 450°C. The acetone is then transferred in toto to a hydrogenation bomb and there reduced at 2500 psi of hydrogen and 150°C over copper chromite. The alcohol thus formed is, without further purification, treated with red phosphorus and iodine in the presence of excess water. An analysis of the product on a low activity run is underway to determine the exact purity.

Valine-4,4'-C_2^{14}. As was noted in the previous paragraph the isopropyl alcohol produced was converted to the iodide. This halide was chosen because the yields on this step are much higher than in the conversion of the alcohol to the bromide which was carried out previously. However, it was necessary to re-establish that the iodide was capable of giving satisfactory condensation with ethyl acetamidomalonate in the preparation of labeled valine. Preliminary runs on this reaction indicated that a condensation yield of 65-75 percent could be obtained.

Hydrolysis and decarboxylation of the product and purification of the resulting amino acid by a fractional elution from a Dowex 50 ion exchange resin column with 0.2 N hydrochlorid acid indicated that besides the valine and glycine (which comes from the excess acetamidomalonate) there were three other amino acids present, namely, an unknown, alanine and α-aminobutyric acid. Further investigation as to the source of these amino acids, either as contaminants from the halide or as by-products of the condensation, is underway. However, the isolation of pure valine by the chosen method of purification was satisfactory.

Norvaline-3-C^{14}, norleucine-3-C^{14} and leucine-3-C^{14}. In the last quarterly report a successful novel method for preparing alcohols and halides from fatty acids was described. Since these halides could be prepared in good yield the synthesis of a series of three-labeled amino acids from these intermediates has been investigated.

Norvaline-3-C^{14}. Low activity preparations of this amino acid were made by the condensation of n-propyl-1-C^{14} iodide with ethyl acetamidomalonic ester followed by hydrolysis and decarboxylation of the resulting condensation product by refluxing the material with 48 percent HBr for 72 hours. The purification of the mixture of amino acids formed in this process (valine, glycine, and traces of impurities) was carried out again by fractional elution of the materials from a Dowex 50 ion exchange resin column with 0.2 N hydrochloric acid. This purification was followed by a high vacuum sublimation The yield of pure norvaline in a preliminary warm run was approximately 60 percent based upon the halide used as starting material. A high specific activity run is in progress.

Norleucine-3-C^{14}. Preliminary runs on the synthesis of this amino acid by the condensation of n-butyl-1-C^{14} iodide and ethyl acetamidomalonic ester have shown that satisfactory condensation yields can be obtained. In this case, fractional elution from an ion exchange column is not necessary for purification since this amino acid is sufficiently insoluble in water that recrystallization gives good separation of the product from the accompanying glycine and small amounts of other amino acids.

Leucine-3-C^{14}. The synthesis of this material from isobutyl-1-C^{14} bromide is currently being studied.

1-Naphthaleneacetic-α-C^{14} Acid. About 4 mc of naphthoic-carboxyl-C^{14} acid was prepared by carbonating the Grignard reagent from 1-naphthyl bromide with an undetermined quantity of $C^{14}O_2$ recovered from some high specific activity preparations. After carbonation of the Grignard, the complex was decomposed with water and dilute acid and the ether solution separated. The aqueous solution was extracted twice with ether and the combined ether solution extracted with sodium carbonate solution. Acidification of the basic solution gave the desired acid, 562 mg. Of this material about 390 mg was reduced to the carbinol by dropping an ether solution of the acid into a solution of 0.8 g (0.021 mole) of lithium aluminum hydride in ether. The complex was decomposed with water and dilute acid and the carbinol extracted with ether. Bromination of the carbinol was accomplished by passing HBr into the ether solution of the carbinol. Two layers began to separate as soon as the HBr gas was added. The upper layer was separated, dried over anhydrous potassium carbonate, and the bulk of the ether distilled.

To the ether solution 10 ml of ethanol was added and the rest of the ether evaporated, then an additional 5 ml of alcohol, 5 ml of water and 425 mg (0.006 mole) of potassium cyanide were added, a crystal of potassium iodide was dropped into the solution and the material refluxed overnight. The solution was extracted with ether, the ether evaporated, and the residual material refluxed overnight with 1 g of potassium hydroxide in 10 ml of water. The reaction mixture turned brown, and tarry material was deposited on the sides of the flask (probably due to decarboxylation of the 1-naphthaleneacetic acid under these conditions).

Extraction with ether and re-extraction of the naphthaleneacetic acid from the ether with aqueous sodium carbonate gave the desired 1-naphthaleneacetic-α-C^{14} acid. The material was crystallized from hot water. Three fractions were obtained: (1) 24.6 mg., m.p. 130-131°; (2) 8.0 mg., m.p. 129.5-131°; (3) 91.7 mg., m.p. 128-129°, mother liquors, 13.9 mg. The first two fractions were obtained as light needles and the third as small, shiny platelets. The literature gives 131° and 129° as the melting point for this acid. Assay for specific activity of both the naphthoic-1-C^{14} acid and of the 1-naphthaleneacetic-α-C^{14} acid is in progress. It is expected that the final specific activity will be in the range 4-5 μc/mg.

Low Activity C^{14} Samples. In the last quarterly report the problem of the analysis of low activity C^{14} samples was introduced. It was pointed out that when 100 μc of a labeled compound, for instance glycine, is given to a human being a large part of the samples obtainable have a specific activity of 2 dis/min/mg of $BaC^{14}O_3$ or less. At present, these are being analyzed on an ionization chamber set up that was developed some years ago. This unit is now being improved and expanded. A modified and simplified series of vacuum lines for filling the chambers has been installed. Several modified styles of ionization chambers have been constructed and are as yet being tested.

We have found that glass chambers coated with gold on the inside are unsatisfactory for our work because of the high alpha background, presumably

from the glass. Brass chambers, which are in current use, are much better, although they still produce about 1-3 alphas per minute in a 100 cc chamber. Silver plating of these chambers does not materially reduce the alpha frequency. The most satisfactory chamber in this respect that we have thus far produced is an iron chamber which has one alpha for about every 5-10 minutes.

Even more serious than the alpha background in measuring low activity samples are the electrical characteristics of the insulator. Polystyrene and metal-to-glass seals are not satisfactory as the settling time for such chambers may be from a period of several hours to indefinitely. We have been using teflon insulators and have recently tried using a sapphire insulator chamber (courtesy of Applied Physics Corp., Pasadena). Our present data indicate that these two insulator materials are comparable as far as settling time is concerned, but more work is being done on this project.

To measure low activity breath samples a system has been set up which is capable of separating from the breath the CO_2 produced by the body and in purifying and filling an ionization chamber to a given pressure with this CO_2. At present, this series of steps is being carried out very laboriously by first collecting the breath in rubber balloons, absorbing the CO_2 in alkali, precipitating it as $BaCO_3$, drying, weighing, and then aliquoting the sample of this $BaCO_3$, converting it back to CO_2 and thence to fill an ionization chamber.

In the modified set up which has been built the breath is first passed through a water-cooled condenser which removes part of the water, and then the breath is passed through a spiral-sintered disk trap cooled with liquid nitrogen. This separates all the CO_2 and water in the breath from the remaining non-condensible gases. The water-CO_2 mixture is then passed through a spiral-sintered disk trap cooled in a Dry Ice-acetone bath. This removes the water from the CO_2 and the purified CO_2 is collected in a trap system from which it may be expanded to fill an ionization chamber. The ionization chambers being used for this work have a capacity of about 540 cc and this requires some 5-10 minutes of breathing.

Isotope Effect in the Decomposition of Oxalic Acid. The ionization chamber measurement of the barium carbonate activity produced from the CO_2 and CO from the decomposition of oxalic acid in sulfuric acid has not previously given satisfactory precision to enable significant conclusions to be drawn concerning the effect of changing the temperature of decomposition on the isotope effect. The ionization chamber measurements have been modified and standardized so as to obtain satisfactory precision in measurements made at a single temperature (100°C) and it is hoped that these same methods will make a satisfactory determination of the effect of temperature variation on isotope effect.

Isotope Effect in the Decarboxylation of Substituted Malonic Acids. The initial rapid CO_2 evolution upon acidification of sodium α-naphthylmalonate previously noted has been studied in more detail and found to be due to the presence of sodium carbonate produced in the hydrolysis of diethyl α-naphthylmalonate. This has led to a study of the hydrolysis of the ester

in an attempt to find conditions such that the basic carbonate split does not occur. In aqueous alcoholic sodium hydroxide the hydrolysis to the half ester proceeds rapidly followed by a slower hydrolysis of the second ester group. The hydrolysis of the second ester group fits the rate law d (half ester)/dt = - k (half ester) (OH^-) very satisfactorily. For hydrolysis at ~23°C in 82.5 percent alcohol, k = 2.35/moles hr. and for 44 percent alcohol k = 1.07/moles hr. In all cases of basic hydrolysis carbonate is formed, in amounts increasing with the concentration of base used and ranging up to 50 percent of theoretical. The hydrolysis becomes too slow to be practical at low enough base concentrations to decrease the carbonate split to a satisfactory value.

The "hydrolysis" of diethyl α-naphthylmalonate in absolute ethanol has also been studied in the hope that the ester would undergo the carbonate split exclusively, but to date only about 80 percent of the theoretical carbonate has been obtained. In this case, the reaction follows the rate law d(ester)/dt = -k (ester) with k = 0.070 hr^{-1}. Acid hydrolysis at low temperature (0°C) has proven to be too slow to be practical. Acid hydrolysis at higher temperatures is being studied, but the upper temperature is limited by the temperature at which decarboxylation of the free acid becomes important, and there is reason to believe that this is not far from room temperature.

Biological Chemistry

M. Calvin, E. Bennett, J. Campbell, L. Daus, S. Ikeda, M. Kirk, D. Kritchevsky, B. Krueckel, M. Lazarus and B. Tolbert

Metabolism of Labeled Purines. During the period covered by this report, studies have been made of the metabolism of guanine-4-C^{14} and 8-azaguanine-4-C^{14} in normal C-57 strain male mice. Experiments have also been made to determine possible effects 8-azaguanine may have on the metabolism of guanine-4-C^{14}.

8-Azaguanine-4-C^{14}. 8-Azaguanine was injected intraperitoneally in a 0.9 percent sodium bicarbonate solution and the mice were sacrificed 2 and 24 hours later. The liver, kidneys, spleen, stomach, and intestines, and the remainder of the carcass excluding hide and hair were removed, frozen immediately in liquid nitrogen and subsequently extracted with cold 10 percent TCA (TCA = trichloracetic acid), then hot 10 percent TCA, and finally dissolved in sodium hydroxide. After ether extraction of the TCA extracts, direct plates were made of the aqueous solutions to determine the radioactivity in the fractions obtained.

The carbon dioxide obtained from the breath after administration of 8-azaguanine-4-C^{14} did not contain sufficient activity to be determined with the Nucleometer, i.e., less than 2 dis/min/mg $BaCO_3$. It therefore can be concluded that an insignificant portion of the C-4 of azaguanine is metabolized to carbon dioxide or to compounds which in turn are readily converted to carbon dioxide. Essentially all of the activity injected was found in the urine (either that which had been excreted or that in the bladder at the time of sacrifice) even after as short a time as two hours.

After two hours, approximately 3 percent of the injected activity was found in the cold TCA extract of the stomach and intestines, 1 to 1-1/2 percent in the liver, and lesser amounts in the other organs (carcass value not obtained). The hot TCA extracts contained less than 10 percent of the activity of the cold TCA extracts. The sodium hydroxide extracts did not contain appreciable amounts of radioactivity.

After 24 hours, cold TCA extracts of the liver, stomach and intestines and carcass all contained 0.1 to 0.3 percent of the injected radioactivity. The hot TCA extracts contained comparable amounts of activity.

Guanine-4-C^{14}. Guanine-4-C^{14} has been injected as a suspension in saline and gum arabic. Some of this material remains unabsorbed after two hours. Therefore, in later experiments in which this compound has been used all organs were rinsed in 0.9 percent sodium chloride after removal from the animal in order to remove any particles of guanine on the surface of the organs. However, due to the nature of the guanine suspensions, more variability is to be expected in the experiments with guanine, particularly those of shorter duration. The percentages of the radioactivity from the injected guanine which was found in various organs from several experiments are shown in the Table I. No significant amount of radioactivity was found in the carbon dioxide of the breath.

TABLE I

Percentage of Injected Guanine Found in Various Fractions 2, 6 and 24 hours after Intraperitoneal Injection of 1.5 to 2.0 mg of Guanine Hydrochloride Hydrate (6 to 7 μc) as a Suspension in Gum Arabic-Saline.

Time Sacrificed After Injection (Hours)	2	6	24
Number of Experiments	4	3	4
Fraction			
Urine	50-70%	45-70%	75-90%
Blood	1-3	0.3-0.5	0.02-0.04
Cold TCA extracts of:			
Kidney	0.3-0.7	0.15-0.2	0.04-0.07
Liver	1-2	1-2	0.1-0.3
Stomach and Intestines	7-10	10-15	1.2-5
Carcass	10-20	10-20	2-10
Hot TCA extracts of:			
Kidney	0.01-0.03	0.01-0.02	0.01-0.03
Liver	0.2-0.3	0.3-0.4	0.2-0.5
Stomach and Intestines	1-2	0.3-0.4	0.5-0.6
Carcass	0.2-0.3	0.2-0.4	0.2-0.5

The urine contained the radioactivity almost entirely as allantoin (R_f = 0.30 in butanol-propionic acid-water; R_f guanine= 0.42) detected on paper via a mercuric sulfide test. Constant specific activity was also obtained with carrier recrystallization. A small amount of a second radioactive component with a R_f of 0.23 is also present. None of the guanine is eliminated unchanged.

Influence of 8-azaguanine on guanine-4-C^{14} metabolism. Experiments have been carried out to determine if 8-azaguanine has any effect on the metabolism of guanine-4-C^{14}. Guanine-4-C^{14} (1.5 to 2.0 mg) and inactive azaguanine (1.5 to 2.0 mg) were injected as a suspension in gum arabic. It is not believed that significant differences in the amount of radioactivity found in the various fractions could be demonstrated two or six hours after injection due to rather wide variability in the results. However, after 24 hours the results of two experiments which have been carried out indicate that 8-azaguanine may produce a significant difference in the amount of guanine-4-C^{14} incorporated into the hot TCA fraction (nucleic acid) of the liver, stomach and intestines and carcass of the C-57 mice used in these experiments. In the case of the liver, only 15-25 percent of the activity found present in the hot TCA extract of the controls was found in the similar fraction of the mice injected with both compounds. For the carcass and for the stomach and intestines the corresponding percentages were 40-60 percent. This apparent inhibition of incorporation of guanine in normal C-57 mice is being checked by further modified experiments.

The same excretion products are found in the urine of animals injected with guanine-4-C^{14} and inactive azaguanine as are found when only the former compound is injected; however, the amount of the minor component of R_f 0.23 may be slightly larger. Apparently no major inhibition exists in the elimination cycle leading to allantoin from guanine.

In Vivo Metabolism of Stilbamidine. In continuation of the studies which have been carried out with the Medical Physics Group on the metabolism of stilbamidine-amidine-C_2^{14} in normal and tumorous "A" strain mice, several experiments have been carried out using the centrifugal fractionation technique of Schneider and Hogeboom[1] to determine some properties of the stilbamidine (or radioactive compounds from it) which are found in the liver several days after injection.

Four days after administration of stilbamidine, the mice were sacrificed. The livers contained from 10-25 percent of the injected activity. The livers were homogenized in 0.88 M or 0.25 M sucrose and centrifuged at approximately 2000 RPM in an ordinary centrifuge to separate the "nuclear" fraction. After repeated homogenization and centriguation, the supernatants were centrifuged at 10,000 RPM for 15-45 minutes in a No. 40 rotor of a Spinco centrifuge to separate the "mitochondrial" fraction. The separation in 0.25 M sucrose was more satisfactory and will probably be used in the future. Approximately 75-85 percent of the radioactivity was found in the "mitochondrial" fraction, and most of the remainder of the activity was found in the "nuclear" fraction. Only a very small amount of the radioactivity was found in the supernatant from the "mitochondrial" fraction.

In one series of experiments (two mice) succinic oxidase determinations were made of the fractions obtained by the above procedure. This enzyme system is believed to be associated with mitochondria. It is felt that such determinations will give a measure of the proportion of the mitochondria in the "nuclear" fraction and thus enable one to determine if the major portion of the radioactivity remaining in this fraction is associated with the mitochondria or with another cellular component. Since the ratio of radioactivity/enzyme activity in each fraction was approximately the same, it is believed that the major portion of the radioactivity present in the livers is either associated with the mitochondria or present as a particle of similar physical characteristics. Further experiments are in progress to determine the nature of the radioactive compounds of the liver of stilbamidine-injected mice as well as studies of the radioactive compounds in the urine of such mice.

In Vitro Metabolism of Labeled Sodium Propionate. The study of the metabolism of labeled sodium propionate incubated with liver slices has been continued. As a final step an attempt is being made to identify the one remaining major unknown radioactive compound appearing on chromatograms of metabolic products.

A degradation of the unknown activity with chromic acid gave labeled CO_2 and an equivalent amount of labeled propionic acid and possibly some other labeled material. The propionic acid was identified as such by a comparison of titration curves and activity curves of a silicic acid elution chromatogram in which carrier valeric acid, butyric acid, propionic acid and acetic acid were present in carrier amounts. When the propionic acid had been identified, it was degraded by the Schmidt reaction to CO_2 and ethylamine which were counted for activity. Table II gives results of the degradations.

TABLE II

Compound Injected	CrO_4 Oxidation % of Activity in Unknown		Schmidt Degradation % of Activity in Propionic Acid	
	CO_2	Propionate	-COOH	$\alpha + \beta$
Sodium propionate-1-C^{14}	20%	65%	92%	2 ± 5%
Sodium propionate-2-C^{14}	6	77	0 ± 5	75

The above results suggested α-hydroxybutyric acid as a possible identity for the unknown. When the unknown activity was mixed with a carrier amount of this acid and chromatographed on silicic acid, the peak of the activity curve did not coincide with the peak of the titration curve and the ratio of activity to milliequalents was not nearly constant.

To test for adjacent hydroxy groups the unknown was treated with periodic acid. In preliminary experiments, labeled lactic acid was used in

trace amounts with appropriate carriers present to give CO_2, formic acid and an aldehyde. Rough rate determinations showed the trace of lactic acid was oxidized much slower than tartaric acid in macro amount, which, in turn, was oxidized slower than an α-glycol configuration would be. The unknown activity appeared to be oxidized even slower than trace amounts of lactic acid, thus eliminating the α-glycol structure as a possibility.

The degraded lactic acid in test runs gave quantitative activity balance with the carboxyl carbon going to CO_2, the α and β fragment going 30 percent to acetaldehyde and 66 percent to acetic acid. The aldehyde was characterized by chromatography as the p-nitrophenylhydrazone derivative on a silicic acid-celite column, the activity moving in a discreet band both with and without added carrier.

The unknown activity when degraded with periodic acid had at least 8 percent of the activity left in the reaction vessel after two days at room temperature, 9 percent was precipitated as $BaCO_3$, and 9 percent was present in a fraction containing p-nitrophenylhydrazine and carrier propionaldehyde derivative of p-nitrophenylhydrazone. Fifty-five percent of the activity was present as a steam distillable acid which chromatographed on silicic acid as propionic acid. The activity in the hydrazone fraction, when chromatographed on silicic acid-celite was the derivative of neither acetaldehyde nor propionaldehyde, being more strongly adsorbed than either of these.

Work is continuing on the identification of this unknown compound or compounds. A further short investigation of the rate of oxidation of lactic acid in trace and macro amounts is also projected.

Chicken Feeding Experiments. The method for isolating the various labeled components from eggs of a hen fed sodium acetate-1-C^{14} has been worked out in some detail; separation of unsaturated and saturated acids is still being studied. Extraction of the dried yolk with alcohol-ether 3:1 leaves behind the extracted yolk protein. The solvent is distilled and the residue taken up in petroleum ether; addition of acetone plus a few drops of alcoholic magnesium chloride solution precipitates the phospholipids. The supernatant is concentrated, dissolved in alcohol and saponified by five hour reflux with 15 percent aqueous potassium hydroxide. The solution is then acidified and the acids and cholesterol taken up in ether.

Efforts to separate the cholesterol from acids by chromatographic methods (using alumina or IRA-400 resin) showed that some esters were still present. This was shown by a carbonyl absorption band which was present in the infra-red spectrum of some of the eluate. Inasmuch as more drastic methods are inadvisable at this stage of the separation, the idea of more vigorous saponification at the initial stage was abandoned. The cholesterol can be precipitated from the original solution with digitonin, and the acids remaining will again be passed over a column of IRA-400 resin. The neutral material will then be saponified more completely.

From the aqueous portion of the saponification mixture the glycerol can be obtained by distilling the solution to dryness, extracting the salts with pyridine and treating the pyridine solution with benzoyl chloride. This

should give glycerol tribenzoate.

For separation of the acids, chromatographic methods will be investigated.

To date, all yolks have been extracted, and protein phospholipid and cholesterol (in varying amounts) have been obtained from each. None of these fractions has been assayed for radioactivity.

Paper Chromatography of Steroids. The question has arisen whether the Quilon solution currently being used (2 percent Quilon and 2 percent neutralizing solution) is the most efficacious for paper chromatography experiments. To test this, a sheet of paper was prepared where a 4 percent solution of both Quilon and neutralizer was used, and another using 2 percent Quilon with no neutralizer. Tritiated cholesterol was chromatographed on these papers as well as on paper prepared in the usual fashion. The solvent was methanol. The R_f found in the case of the 2 percent and the 4 percent papers was 0.56 (as reported before); with the paper which had been prepared without neutralizer the R_f was 0.61. In addition, a green zone was observed about three-quarters of the way down the paper, as if the Quilon had been washed down. It thus appears that the neutralizer is necessary.

A number of steroids have been obtained from various sources and experiments on the chromatography of some of these have begun. In all cases the compounds were detected with a spray consisting of a saturated solution of iodine in petroleum ether. R_f values for the materials in various solution on 2 percent Quilon treated paper are listed in the following Table III.

TABLE III

Paper Chromatography of Steroids

Compound	$EtOH/H_2O$ 8:2	$EtOH/H_2O$ 7:3	Dioxane	EtOH	CH_3OH
1	0.96	1	0.99	0.97	0.72;0.76;0.76
2	0.93	0.95	0.98	1	0.72;0.76
3.	1	0.87	1	1	0.74;0.80
4	0.97	0.95	0.96	1	0.82;0.78

Compounds: (1) Dehydroepiandrosterone; (2) drhydroepiandrosterone acetate; (3) testosterone; (4) testosterone acetate.

Incorporation of Radioactivity from Sodium Isobutyrate-3-C^{14} in the Rat. In order to obtain some unequivocal data concerning the incorporation of the radioactivity into steroids from rats fed sodium isobutyrate-3-C^{14} the homogenized

bodies of the three rats fed this material were extracted with alcohol-ether 3:1. The separate fractions were saponified with potassium hydroxide and the cholesterol separated as the digitonide. The digitonide was dissolved in pyridine and plated. The results are tabulated below in Table IV:

TABLE IV

Rat	Digitonide (gm)	Mg plated	Dis/min/mg Digitonide
1	0.0482	0.9	160
2	1.1086	1.02	185
3	0.1548	0.47	360

The cholesterol will be separated from each of these and characterized before any effort is made to dilute it for subsequent work.

Other Projects. In connection with various other groups of the University and the Radiation Laboratory a number of biological problems are being studied on a cooperative basis. These are listed below:

1. The half-life of the red blood cell in man and in rats (with Donner Laboratory of Medical Physics).

2. The rate of body turnover of carbon when administered as glycine-2-C^{14} (with Donner Laboratory of Medical Physics).

3. The incorporation of radioactivity from labeled lactic acid in virus (Department of Biochemistry and Virus Laboratory).

4. The incorporation of labeled fatty acids into milk by cows (with Division of Animal Husbandry, Davis, California).

5. The turnover of carbon in cows (with Division of Animal Husbandry, Davis, California).

6. The incorporation of simple radioactive compounds in sugar and the metabolic pathway of sugar formation in diabetic and normal dogs (with Division of Physiology).

7. The localization of stilbamidine in normal and neoplastic mice (with Donner Laboratory of Medical Physics).

8. The chemical role of cholesterol in heart disease and body metabolism (with Donner Laboratory of Medical Physics).

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[1] Schneider and Hogeboom, J. Biol. Chem., 172, 619 (1948).

Photosynthesis Chemistry

M. Calvin, A. Benson, J. Bassham, M. Goodman
V. Lynch, W. Stepka, H. Lew and C. Ouellet

Introduction. The chemical processes involved in the reduction of carbon dioxide to the numerous constituents of a green plant are amenable to investigation with radioactive carbon dioxide. In view of the extremely high sensitivity of the methods for detection of C^{14} compared to those involving gas pressure measurements the use of radiocarbon has greatly accelerated our understanding of the reactions involved in photosynthesis.

The prime requisite for such an investigation is a method for separation, identification and determination of the heretofore unidentified compounds involved in this sequence of reactions. The use of filter paper chromatography combined with radio chromatography has fulfilled these requirements to date.

The study of the plant metabolism carried out in this laboratory may be divided into two catagories. The most important is the study of the reactions involved during steady state photosynthesis. In such experiments a normal photosynthesizing plant is given $C^{14}O_2$ so that the conditions are not changed during the transition from normal isotope to radioisotope. The second type of experiment involves abnormal conditions of photosynthesis such as that occurring with partially poisoned plants or under nonsteady state photosynthesis. Typical examples of the last type of experiments are those where large changes in carbon dioxide partial pressure occur or where illumination is changed or ceased during or prior to the administration of $C^{14}O_2$. Correlations of these experiments on abnormal systems has greatly increased our understanding of the normal processes.

Chromatography of Phosphorylated Compounds. The intermediates of carbon reduction as involved in the conversion of carbon dioxide to free sucrose have been shown to be phosphorylated compounds. Whether any of the intermediates are unphosphorylated is open to question.

The two methods applied in this laboratory for the separation of phosphate esters are paper chromatography and strong base anion-exchange resin chromatography. The two methods are complimentary in that separation on filter paper is applicable for the separation of complex mixtures of compounds only in very small amounts. The uni-dimensional exchange resin separation may be applied to almost unlimited amounts of compounds. It has been possible to separate known mixtures of phosphate esters quantitatively on exchange resins while in filter paper chromatography the adsorption on the paper gave rise to serious losses of radioactivity products. This irreversible adsorption has now been partially corrected by improved techniques.

Experimental Procedure. The advantages of acidic solvents in the chromatography of acidic compounds such as phosphate esters have been previously demonstrated in this laboratory as well as in others. However, in solvents containing an alcohol and strongly acidic carboxylic acids the rapid esterification becomes serious. To effect the rapid movement of phosphate compounds, it has been found that filter paper washed with acidic solutions such as 0.1 percent

oxalic acid or 0.1 percent oxalic acid plus KH_2PO_4, followed by thorough water washing gives a paper with improved properties. The prewashing apparently saturates the impurities in the paper and renders it somewhat acidic. Alkaline areas in the paper are neutralized so that the acids being chromatographed are not ionized and hence disassociated. The R_f values of acidic compounds are pH dependent since paper chromatographic properties of a substance parallel its distribution constants. Hence, the acidic compounds, notably phosphoglyceric acid and phosphoglycolic acid are found to move relatively farther on acidic paper than on neutral paper.

By using such prewashed paper it has been possible to separate phosphate esters much better than previously done and without the generally observed streaking caused by adsorption. This technique has been applied to the separation of P^{32}-labeled compounds of the alga, Scenedesmus. In this case many compounds not involved in sucrose synthesis are observed.

Exchange Resin Chromatography. Separation of phosphoglyceric acid, inorganic phosphate, fructose diphosphate and hexose monophosphates has been reported from this laboratory. Phosphoglycolic acid ($CH_2OPO_3H_2 - CO_2H$) and phosphoglyeric acid ($CH_2OPO_3H_2 - CHOH - CO_2H$), because of their similar chemical and physical properties, are more difficult to separate by conventional methods. Elution of this pair of compounds has been studied from Dowex A-2 resins and although the results are not yet certain it is felt that a separation will be possible. Natural phosphoglycolic acid isolated by paper chromatography from plant extracts after photosynthesis with $C^{14}O_2$ and radiophosphate is being used for correlation with the properties of the authentic materials.

Hydrolysis of Phosphate Compounds. Although inorganic phosphate determinations have been widely used to determine the rate of hydrolysis of phosphate esters, the results are not descriptive of the identity or yields of the organic products. The organic products of acid and enzymatic hydrolysis of the early phosphorylated intermediates of photosynthesis are being studied in this labroatory, primarily for the purpose of the identification of unknowns and the determination of the sequence of their synthesis.

The phosphate esters separated or partially separated by chromatography on paper are eluted and heated in acid or incubated with a phosphatase preparation prepared from sprouted barley seedlings. Since the phosphatase preparation contains all the constituents of barley sprouts, sufficient amounts of glucose, fructose and the commonly occurring amino acids are readily detectable on resultant chromatograms and aid in identification of the hydrolysis products. No enzymatic activity other than hydrolysis has been apparent.

When the phosphate-containing compounds from chromatograms of extracts of $C^{14}O_2$-fed algae, barley, sugar beet, soy bean or Rhodospirillum rubrum (photosynthetic bacterium) were subjected to such hydrolysis five major products are observed. The phosphates from 60 second photosynthesis by soy bean leaves showed over fifty different compounds in small amounts. While some of these may be breakdown products, it is likely that most of them represent the organic moieties of phosphate esters. The major products of such hydrolysis are the same in all plants studied.

Identification and Sequence of Compounds Formed in Steady State Photosynthesis. Earlier work of this laboratory reported that phosphoglyceric acid was followed by triose and hexose monophosphates which were identified by co-chromatography with authentic materials. These compounds have now been systematically identified by phosphatase hydrolysis. Five phosphorylated compounds have now been sufficiently separated by paper chromatography to give single hydrolysis products. These are phosphoglyceric acid, phosphoglycolic acid, two different glucose monophosphates and an unknown diphosphate of a sugar of lower molecular weight than hexose. Fructose monophosphate has been separated from all the other radioactive plant phosphates except one which appently is not that of a common hexose. Identification of these unknown compounds is in progress. It has been shown that the phosphorylated compounds of very short photosynthetic experiments (2 second and 4 second photosynthesis by barley leaves) include little or no radioactive glucose phosphates. As the time of photosynthesis increases, the fraction of activity in glucose phosphates increases and finally exceeds that in other monophosphates by several fold.

C^{14}-P^{32} Experiments. *Scenedesmus* suspensions have been allowed to grow 20 hours in radiophosphate and $C^{12}O_2$. They were then washed and given $C^{14}O_2$ for one, three and five minutes. The high specific activities obtainable in this way makes it possible to prepare radiograms rapidly and supply phosphates of known chromatographic properties for hydrolysis, resin separations and co-chromatography with authentic materials.

Coincidence of radioactive areas on P^{32} radiograms and C^{14} radiograms (obtained after P^{32} decay) has been observed for those compounds with chromatographic coordinates characteristic of phosphate esters. Enzymatic hydrolysis has given free sugars or acids and a corresponding amount of inorganic radiophosphate.

A primary purpose for such doubly-labeled experiments, particularly those where the intermediates are saturated with both C^{14} and P^{32} is the identification of unknown phosphates. Since the P/C ratio in such compounds is a simple number, it is expected that such measurements will give added information regarding the nature of phosphates of unknown compounds as well as that of unknown phosphates of known compounds. Preliminary experiments have been inconclusive since phosphorylated compounds, other than photosynthetic intermediates, were not sufficiently well separated from the compounds under investigation.

Periodate Degradation of Fructose. Radioactive fructose, obtained by paper chromatography from a soy bean leaf which had been allowed to photosynthesize for 60 seconds with $C^{14}O_2$ gas according to previously described procedures, was degraded with periodic acid. Less than 0.5 mg fructose containing 17,000 cts/min was eluted from paper chromatograms and added to 50 mg (0.4 millimoles) unlabeled calcium glycerate and the mixture dissolved in 0.5 ml H_2O. A solution of 1.0 N periodic acid (0.8 ml) was added and the flask stoppered. After thirty minutes the contents of the flask were neutralized and the volatile contents distilled into a solution of dimethyldihydroresorcinol at pH 7 from which upon acidification, a precipitate of formaldimedon was obtained which was recrystallized and counted to give the radioactivity in formaldehyde fragments (carbon atoms one and six of fructose and the beta carbon from glyceric acid).

The residue from the distillation was treated with excess (4 ml) periodic acid in a closed system and, after several hours, the volatile contents of the reaction flask were distilled into a solution of a carbonate-free NaOH. Barium carbonate was precipitated from this solution and the supernate acidified and steam-distilled, neutralized, and concentrated to give barium formate. The radioactivity in the barium carbonate is a measure of the labeling of carbon number two while the barium formate radioactivity is derived from C_3, C_4 and C_5. The results are listed in Table V.

TABLE V

		Theoretical Yield	Specific Activity	Total Activity	% of Total
$C_1 + C_6$	formaldimedon	117 mg	37.1	4,350	23.0
C_2	barium carbonate	83.7 (actual yield)	36.1	3,010	15.5
C_3, C_4, C_5	barium formate	45.4	26.1	11,800	61.5
				19,160	
starting activity 19,500 cts/min					

It can be assumed from work in this laboratory and others that the two halves of the hexose molecule are labeled symmetrically. The C^{14} i distribution is then: $C_1 = C_6 = 11.5$ percent; $C_2 = C_5 = 15.5$ percent; and $C_3 = C_4 = 23$ percent.

Identification of Phosphoglycolic Acid. This hitherto unreported compound has been observed in radiograms of $C^{14}O_2$ photosynthesis by Scenedesmus, sugar beet leaves, barley leaves and soy bean leaves. Its chromatographic properties are almost identical to those of its homologue phosphoglyceric acid. Its R_f in phenol is the same as that of the latter while its R_f in butanol-acetic or propionic acid solvents is only slightly greater.

This compound has been hydrolyzed to give C^{14}-labeled glycolic acid which was identified by co-chromatography with authentic glycolic acid. Since the amounts of natural phosphoglycolic acid isolated are small (ca. 10^{-5} M in plant tissue), it has been found advisable to add carrier glycolic acid to prevent its volatilization during chromatography. Identification using ion exchange resin elution with synthetic phosphoglycolic acid is in progress.

Radio Materials Feeding Experiments. Ethanol-2-C^{14} Feeding Experiments. One possible explanation for the large accumulation of labeled glycolic acid when photosynthesis is performed in 5 percent ethanol solutions as described below, is preferential metabolism of ethanol as a C_2 substrate. This was tested by allowing Scenedesmus to photosynthesize in solutions containing 5 percent

ethanol-2-C^{14}. In two experiments with ethanol containing 0.1 μc/mg the amount of C^{14} fixed in insoluble or non-volatile products was negligible compared to the rate of photosynthesis. The radiograms were observed to show fixation in several compounds including malic acid.

Glycol-1,2-C^{14} Feeding Experiments. Since a symmetrical C_2 intermediate has been postulated as a precursor to the C_2 carbon dioxide acceptor, glycol-1,2-C^{14} was tested. Radioactive glycol was not appreciably assimilated when Scenedesmus suspensions were illuminated for periods as long as 30 minutes in the presence or absence of carbon dioxide.

Influence of Ethanol, Temperature and pH on Photosynthesis. Effect of Ethanol. Two minutes of photosynthesis at 10,000 foot candles, Scenedesmus.

Ethanol, already diluted, was added to the algae and allowed one minute to penetrate into the cells before injection of $NaHC^{14}O_3$.

The fixation, total and soluble, and the fixation in glycolic and glyceric acid are given in Table VI. The activity in the other compounds showed no striking effects. The relatively enormous increase in glycolic and glyceric may result from the blocking of an enzyme or from participation of the ethanol in the metabolism, or both.

TABLE VI

Effect of Ethanol on Photosynthesis: Scenedesmus, 2 min., at 10,000 foot candle light

% Ethanol	Counts/min/gram cells			
$C^{14}O_2$ assimilated	0	5	10	20
Total	1,200,000	800,000	175,000	75,000
Solubles	700,000	430,000	90,000	-
Glycolic acid	3,300	22,000	10,000	-
Glyceric acid	5,000	8,000	1,400	-

Effect of Temperature. Scenedesmus, Two minute Photosynthesis at 1200 foot candles.

In order to avoid adaptation phenomena, the algae, preilluminated at 25°C, were brought up to the temperature of the experiment in about one minute and at once given $NaHC^{14}O_3$ for a two-minute photosynthesis period. The temperature was kept constant within ± 0.5°C during photosynthesis. The main results are shown in Table VII and in Fig. 1.

TABLE VII

Effect on Temperature on Photosynthesis:
Scenedesmus; 2 min. at 1200 foot candles

Temperature, °C	25	32	37	40	42	44
Total	1870	2550	2200	1050	570	250
Solubles	1500	2060	1580	860	550	250

The maximum for total assimilation is at 32°C; the chromatograms would place it nearer 37°C. At any rate, the CO_2 atmosphere was not controlled and that throws doubt upon the significance of the exact position of that maximum.

Fig. 1 shows the absolute activities in the various compounds over that temperature range. Before the assimilation dies out at about 45°C, there is an enormous increase in the sucrose and some in the aspartic. It is felt that these effects may be due to a piling up of these intermediates behind heat denatured enzymes connected with the synthesis of polysaccharides and proteins. Surprisingly, if one plots the logarithms of the activities against the inverse absolute temperatures, one obtains straight lines. It may be possible to interpret some of the processes by a study of their temperature coefficients. The slopes correspond to the following activation energies:

Sucrose (25 - 37°C)	E = + 55 k cal/mole
Sucrose (40 - 44°C)	E = - 150 k cal/mole
Phosphates (25 - 37°C)	E = - 6 k cal/mole
Phosphates (40 - 44°C)	E = - 100 k cal/mole
Aspartic/malic (32 - 44°C)	E = + 28 k cal/mole

The ones above 100 k cal may be associated with the energies of activation of the heat denaturation of enzymes, which would account for the negative signs. The + 55 and + 28 could be either activation energies or heats of reactions corresponding to shifts of equilibria in the more or less steady state.

pH Effects. Further study of the pH effects indicates that they are strongly influenced by the light intensity. Unfortunately, a third, still uncontrolled factor is the CO_2 concentration during photosynthesis. Some idea of the interconnection between pH and light intensity can be had from examination of Fig. 2.

The maximum seems to shift from low to high pH as the intensity increases. The one general trend that shows up clearly under all conditions is that the fixation into free sucrose is always favored by acid media and fixation into malic acid by alkaline media. The data indicates that malic has a secondary optimum at pH 4. That would agree with Ochoa's latest results[1] that the

enzyme catalyzing pyruvate + CO_2 + TPN $\underset{red}{\rightleftharpoons}$ Malate + TPN_{ox} works above pH 7, whereas that which catalyzes the assimilation through oxalacetate works best at pH 4.5.

Localization of the Radioactive Products in the Cell. Conditions: Spinach, 2 min. Photosynthesis 15,000 f.c.

The spinach was frozen and ground in liquid nitrogen. It was felt that separation of the fractions through centrifugation allowed too much time for a possible redistribution of the products according to their solubilities. Instead, they were separated in about 30 seconds by simultaneous filtration with water in series through 8 layers of cheese cloth and a paper filter. The cheese cloth contained the empty broken cells and some unbroken ones, the paper filter mostly the chloroplasts, and the filtrate was a reasonably colorless sap. Another half of that powder was kept whole and extracted as usual to serve as a control.

Most of the water-soluble compounds were found in all three fractions, although in different percentages. The fats were found exclusively in the chloroplasts and the phosphates exclusively in the cell fragments retained by the cheese cloth, from where the water had failed to extract them, possibly because they were retained by the cell walls. This suggests that the failure of mixtures of chloroplasts and sap to fix $C^{14}O_2$ may be due to the fact that in such experiments, the cell walls are usually discarded as mere empty bags. Table VIII gives an estimate of the compositions of the three fractions.

TABLE VIII

Spinach, 2 Min. Photosynthesis

Labeled Compound	Chloroplasts	Sap	Walls
Phosphates	0%	0%	12%
Fats	38	0	0
Sucrose	43	40	60
Others	19	60	28

[1] Ochoa, Nature, 165, 675 (1950).

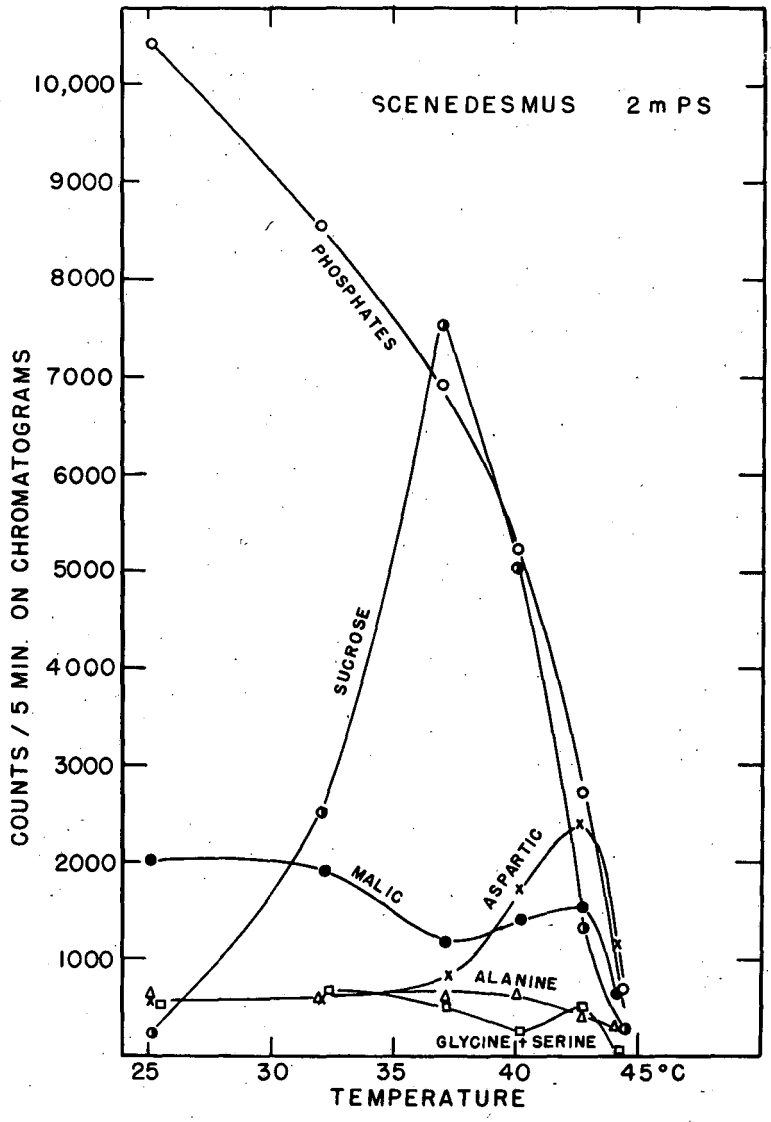
SCENEDESMUS 2 m PS
COUNTS / 5 MIN. ON CHROMATOGRAMS
10,000
9000
8000
7000
6000
5000
4000
3000
2000
1000
PHOSPHATES
SUCROSE
MALIC
ASPARTIC
ALANINE
GLYCINE + SERINE
25
30
35
40
45°C
TEMPERATURE

FIG. 1 MU1197

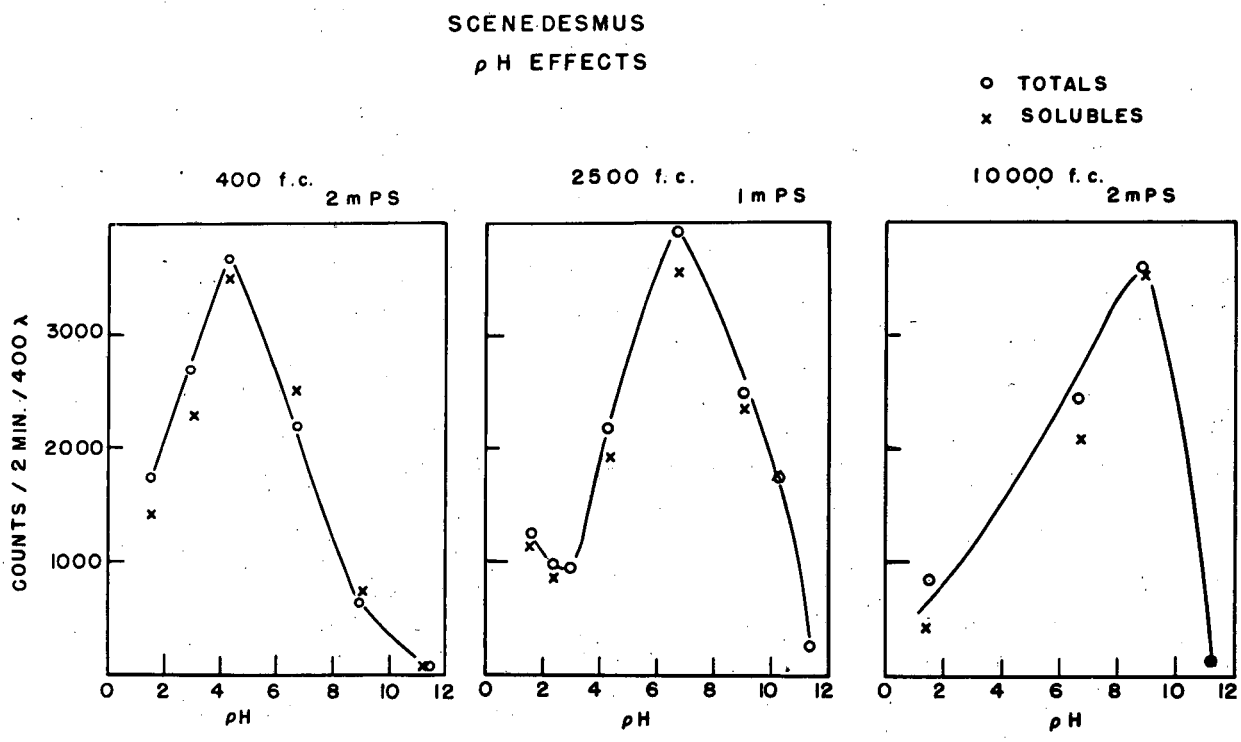
SCENE DESMUS
ρH EFFECTS
o TOTALS
x SOLUBLES
400 f.c.
2 m PS
2500 f.c.
1 m PS
10000 f.c.
2 mPS
COUNTS / 2 MIN. / 400 λ
3000
2000
1000
0
2
4
6
8
10
12
ρH

FIG. 2

MU 1198

II QUARTERLY PROGRESS REPORT. Project 48B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics

L. A. Bromley, G. Elliott, R. Edwards
Norman Leroy, James Robbers, Donald Hanson

Heat Transfer in Forced Convection Film Boiling. The equipment has been modified by the exchange of the carbon tube for a graphite tube, which is used as a resistance heater. The graphite tube provides a more uniform temperature distribution around the tube than the carbon tube. The data has been retaken for the heat transfer coefficients for the film boiling of ethyl alcohol with liquid velocities up to 10 ft./sec. A theory has been proposed to explain the heat transfer coefficients in this type of boiling. It includes a development for determining the correct temperature at which each of the physical properties of the film should be evaluated.

Liquid Metal Systems. The high temperature x-ray investigation of the Na-U-Bi ternary system has shown that solid phase in equilibrium with the liquid at the composition $Na_{.246}U_{.041}Bi_{.713}$ and at 381°C was the same as the unidentified phase reported in the previous Quarterly Report UCRL-923. This phase has been identified as the intermetallic coumpound UBi_2. The essentially pure phase has been prepared by reacting the required proportions of 200 mesh UBi and Bi in a sealed degassed quartz capillary. The reaction time was about 4 days at about 500°C in order to reach completion. Good x-ray films for the UBi_2 have been obtained and should enable the structure of UBi_2 to be further elucidated.

Report UCRL-687 has been issued. This report described an apparatus for maintaining inert gas "dry-box" working chambers free from oxygen and water vapors. The dry-box gas is circulated over streams of liquid sodium, which are supplied by a pump, and which pass over a contacting tray. Included are calculations to show that with this apparatus an atmosphere may be obtained and maintained whose reactivity is less than the best vacuums obtainable in which similar manipulations may be accomplished.

Gaseous Hydroxides Species of Mo and W. No conclusive results have been obtained. This work has continued at a reduced rate due to the need for repairs on laboratory equipment.

B. Basic Chemistry, including Metal Chelates

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Germanium Chemistry. The free energy, heat and entropy of sublimation of GeI_4 as functions of temperature have been determined.

For $GeI_4(s) = GeI_4(g)$ (25°C - 144°C)

$$\Delta H^o = 22,700 - 10\ T$$
$$\Delta F^o = 22,700 + 10\ T \ln T - 103\ T$$
$$\Delta S^o = 93 - 23 \log T$$

Work is now in progress on measurements of the equilibrium:

$$2\ GeI_2(s) = Ge(s) + GeI_4(g).$$

Also, the heat of solution of GeI_4 in water is being measured.

When complete, the above data are to be used, along with other data, in evaluating the aqueous oxidation potentials of germanium.

The Polymerization of Aqueous Zirconium. This is a new investigation that will continue the work initiated by Connick and Reas (AECD-2491) on the polymer formation of Zr(IV) polymers in $HClO_4$ solutions. However this study will use as its principal tool the aqueous equilibrium between Zr(IV) and its first TTA complex:

$$Zr^{+4} + HK = ZrK^{+3} + H^+$$

The previous work employed a benzene-water system and a study of the extraction coefficient of Zr(IV) and its TTA chelate. It is hoped that the method to be employed in the present investigation will yield more accurate results.

Experimental work to date has been limited to purification and preparation of reagents. Crude $ZrOCl_2$ was purified and obtained as $ZrOCl_2 \cdot 8H_2O$ by a series of crystallizations from HCl solutions. This was converted into a $Zr(ClO_4)_4$ solution by fuming with concentrated $HClO_4$. Crude TTA has been purified by crystallizations from n-hexane solutions, but the product so obtained is still not of the desired purity as indicated by unsatisfactory melting points.

Thermodynamics of Rhenium. The cell $Hg/HgO/Ba(OH)_2 \cdot 8H_2O$(sat'd)$/BaMnO_4/MnO_2/Pt$ has been measured using various preparations of MnO_2(pyrolusite) and $BaMnO_4$. The potentials were measured over a period of several weeks and in general achieved constancy for a given cell after the first week. The results of different cells are erratic, however, and lead to a value of the $MnO_2 - MnO_4^=$ potential, E_B^o of -0.66 v., this is surely too negative and indicates that $MnO_4^=$ would be extremely unstable with respect to disproportionation into MnO_2 and MnO_4^- in basic solution. This is, of course, in contrast to the observed behavior.

It is felt that the explanation for the erroneous potential probably is that the MnO_2 is undergoing a transition to a form more stable than our reference form, pyrolusite. A report of this work is being prepared.

Measurements of the cells, $Hg/HgO/KOH(m)$, $KReO_4(m)/ReO_2/Pt$ are nearing completion and a report of this work will be made shortly.

Pure ReO_3 has been prepared by the reaction of CO and Re_2O_7 and by the reaction of CO_2 and ReO_2. The preparations are of a purity suitable for use in determining the heat of the $ReO_3 - ReO_4$ - couple.

Electron Exchange Rate Between Fe^{2+} and Fe^{3+}. The ion-exchange resin method of effecting a change in the ratio, $(Fe^{3+})/(Fe^{2+}) = R$, has been applied at two lower iron concentrations, but maintaining $(HClO_4)$ constant at 0.4 M as before.

For approximately equal ferric and ferrous concentrations of 5×10^{-4}M, and 10 seconds exposure to the resin, R changed from 1.1 to 0.26. This result together with the measured change in specific radioactivity of the solution, was used to calculate α, the fraction of complete exchange occurring in the interval of time t between introduction of the active Fe^{3+} and exposure to the resin. For $t = 35 \pm 10$ secs, $\alpha = 0.8$. Assuming the half-time for complete exchange is about 45 seconds, as reported by Dodson, this corresponds to about 20 percent induced exchange in the resin method.

In order to decrease the relative uncertainty in the time, the experiment was repeated with a two-fold dilution in the iron concentration. The change in R was very small, only from 1.3 to 0.76, and for $t = 45 \pm 10$ secs, $\alpha = 1.24$. This error in this result is greater than that predicted by statistical error theory. A possible explanation is that partial oxidation of Fe^{2+} is occurring in the filtering procedure. An experimental check showed that, indeed, upwards of 10 percent of the Fe^{2+} was oxidized in this step.

A new filtering apparatus has been designed and built to eliminate this difficulty, by isolating the solution from contact with stopcock grease, and allowing the process to be carried out under an atmosphere of nitrogen. In addition, the sintered glass filter itself is being extensively treated to remove any possible oxidizing agents which might be causing the difficulty.

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