UCRL-769

UNIVERSITY OF CALIFORNIA — BERKELEY

RADIATION LABORATORY

DISCLAIMER

UNIVERSITY OF CALIFORNIA
Radiation Laboratory

Cover Sheet
Do not remove

DECLASSIFIED

INDEX NO. UCRL 769
This document contains 25 pages
This is copy 66 of 70 Series A

~~CONFIDENTIAL~~ ~~SECRET~~ Issued to Santa Fe

Classification

Each person who receives this document must sign the cover sheet in the space below

Route to	Noted by	Date	Route to	Noted by	Date
K. NIELSEN	[signature]	9-19-50			
SC	SC	12/6/51			

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

CHEMISTRY DIVISION QUARTERLY REPORT

March, April and May, 1950

June 30, 1950

****

Berkeley, California

STANDARD DISTRIBUTION: Series A	Copy Numbers
Argonne National Laboratory	1-10
Atomic Energy Commission, Washington	11-12
Brookhaven National Laboratory	13-16
Carbide and Carbon Chemicals Division (K-25 Plant)	17-18
Carbide and Carbon Chemicals Division (Y-12 Plant)	19
General Electric Company, Richland	20-25
Hanford Operations Office	26
Iowa State College	27
Kellex Corporation	28
Knolls Atomic Power Laboratory	29-32
Los Alamos	33-35
Mound Laboratory	36-38
Naval Radiological Defense Laboratory	39
NEPA Project	40
New York Operations Office	41-42
Oak Ridge National Laboratory	43-48
Patent Branch, Washington	49
Technical Information Division, ORE	50-64
UCLA Medical Research Laboratory (Warren)	65
University of California Radiation Laboratory	66-68
University of Rochester	69-70

INFORMATION DIVISION
Radiation Laboratory
Univ. of California
Berkeley, California

TABLE OF CONTENTS

Table of Contents (Continued) Page

Table of Contents (Continued) Page

I QUARTERLY PROGRESS REPORT. Project 48

G. T. Seaborg and I. Perlman

A. Chemistry of the Rare Earth and Actinide Elements

Production of Curium Metal

W. W. Crane, J. C. Wallmann, and B. B. Cunningham

Until the present time, curium is the only actinide element which has not been produced in the metallic form. Measurement of the densities of U, Np, Pu, and Am has indicated possible correlations of density with the metallic state valence; a measurement of the density of Cm, as well as the melting point and other physical properties, would be extremely helpful and for that reason Cm metal production was attempted.

Several micrograms of Cm was purified by means of a cation exchange column and CmF_3 precipitated. This precipitate was dried at ~120°C and loaded in a sintered BeO crucible. Ba metal reductant was placed under the sintered crucible in a soft BeO outer crucible. The capped double crucible system was fired at 1275°C for 1 min. in a high vacuum line. In each of two runs Cm metal was produced: the first run giving a pellet of <1 μgm size and the second a thin film of metal on the bottom of the sintered BeO crucible. The metal was silvery in appearance and apparently was strongly magnetic, though the presence of iron is a possibility. Further metal productions will be undertaken when additional Cm becomes available and on these pieces a density measurement will be obtained.

The Vapor Phase Hydrolysis of Rare Earth Trichlorides

A. Broido and B. B. Cunningham

The flow method previously described (UCRL-460) has been used to determine the thermodynamics of the reaction:

$$AmCl_3 + H_2O \rightleftharpoons AmOCl + 2HCl$$

Values obtained for the equilibrium constants for the hydrolysis reaction of lanthanum, praseodymium, and samarium trichlorides have been reported previously. (UCRL 550 and UCRL 635)

Briefly the method used consists of passing N_2 -H_2O-HCl gas mixtures of known composition over a sample of the rare earth compound mounted on a quartz fiber cantilever-type microbalance which is contained in a pyrex tube. The sample is maintained at the desired temperature by an electrically heated tube furnace. If the $(HCl)^2/(H_2O)$ ratio exceeds the value of the equilibrium constant at a particular temperature, the sample is converted to the trichloride; if not, the oxychloride results.

The values obtained for the $AmCl_3$ hydrolysis are given in Table I. In addition, all of the results obtained to date on the various hydrolysis reactions, together with calculations of entropies and heats of reaction, have been summarized in UCRL-666. It had been intended to check the results determined using this technique by obtained calorimetrically the heat of formation of a rare earth trichloride and oxychloride. Unfortunately, in the short time that was available in which to attempt this experiment, it was found impossible to prepare a sample of lanthanum or praseodymium oxychloride which could be dissolved rapidly enough to make this determination possible with the apparatus available.

TABLE I

Equilibrium Constant for the Reaction

$$AmCl_3 + H_2O \rightleftharpoons AmOCl + 2HCl$$

Experiment Number	T (°K)	K
1	846	68.1
2	828	57.1
3	818	46.5
4	809	32.4
5	786	22.7
6	756	13.4
7	741	11.7
8	720	7.1
9	700	4.9
10	679	2.5

The Crystal Structure of $NaYF_4$ and $NaGDF_4$

D. H. Templeton and C. H. Dauben

In a recent paper Hund[1] gives the crystal structure of β-$NaYF_4$, the high temperature form which is obtained by heating YF_3 and NaF and quenching at about 500°C. This phase is face-centered cubic with

$$a = 5.448 \pm 0.001 \text{ Å}$$

In this paper a plot is also included of the intensities of the lines vs. θ for Cobalt Kα radiation for α-$NaYF_4$, which apparently the authors were unable to index. These lines correspond to a hexagonal lattice with

$$a = 5.9 \text{ Å}, \quad c = 3.5 \text{ Å}$$

The observed density according to Hund is ~4.23 and the calculated density from x-ray data is = 4.4.

Zachariasen[2] reports the structures of the following compounds:

$NaLaF_4$ hexagonal $a = 6.167$ kX, $c = 3.819$ kX

$NaPuF_4$ hexagonal $a = 6.12$ kX, $c = 3.75$ kX

$KCeF_4$ f.c. cubic $a = 5.894$ kX

α-$KLaF_4$ hexagonal $a = 6.526$ kX, $c = 3.787$ kX

β-$KLaF_4$ f.c. cubic $a = 5.931$ kX

In addition we have a sample prepared by J. Wallmann in August, 1949 in an experiment in which he attempted to make GdF_2 by reduction of GdF_3 with Na. The reflections could be separated into two groups; one set of lines corresponding to a face-centered cubic lattice;

$$a = 5.29 \text{ Å}$$

the other lines corresponding to a hexagonal lattice;

$$a = 6.05 \text{ Å}, \quad c = 3.59 \text{ Å}$$

On the basis of the above data it is now assumed that these phases correspond to two forms of $NaGdF_4$ as suggested by Zachariasen[3].

The structure of the cubic form is of the fluorite type with complete intermixture of the sodium and rare earth atoms. According to Zachariasen the structure of the hexagonal form is a disordered structure Space Group C $6_3/m$ (C_{6h}^2) Z = 3/2 with

1.5 M + .5 Na in ± (1/3, 2/3, 1/4)
1 Na in (0,0,0; 0,0,1/2)
6 F in ± (x,y,1/4; y-x,$\bar{x}$,1/4; $\bar{y}$,x-y,1/4)

(with x = 0.385, y = 0.286 for $NaLaF_4$)
only half the sites 0,0,0; 0,0,1/2 are occupied by Na and only alternate sites along the same 6-fold axis. The structure is closely related to UCl_3 type structure (see ref. 2).

It is hoped to prepare the α-$NaYF_4$ soon and also to prepare α and β-$NaGdF_4$ again to check these observations.

---

1 F. Hund, Z. für anorg. Chem. 261, 106 (1950).
2 W. H. Zachariasen, AECD-2162, MDDC-1283, CC-3401.
3 W. H. Zachariasen, private communication.

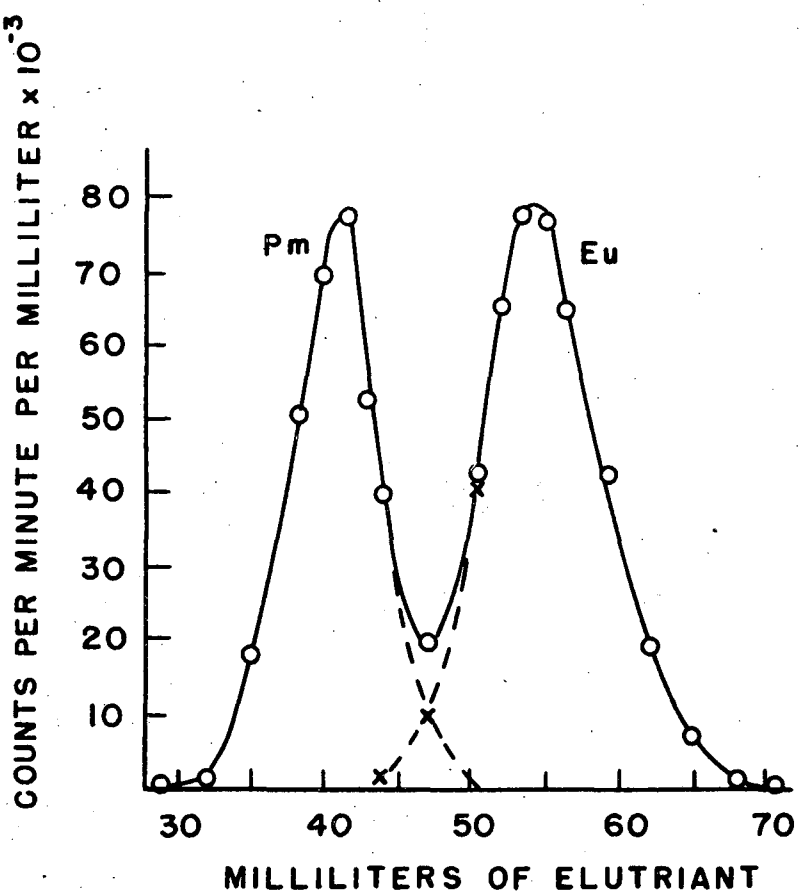
Anion Exchange Separation of Rare Earths and Actinide Elements

E. H. Huffman and R. L. Oswalt

The citrate complexes of rare earth elements at low pH have been found to adsorb on an anion exchange resin and to give a separation of the elements when eluted with citrate solution at low pH. The adsorption of these anions is very strong at pH values higher than about 3.0 and it is necessary to make the eluting solution more acidic in order to move them noticeably on a column.

A solution of the tracers Pm^{147} and Eu^{154} in 0.25 ml of 0.0125M citric acid, adjusted to pH 2.1 with hydrochloric acid (final chloride concentration about 0.003M) was put on a column of 250-500 mesh Dowex A-1 resin 14.9 cm long and 0.08 sq. cm cross section. This column had been prepared by treating the original chloride form of the resin with a citrate solution of the same concentration and pH. Elution at the rate of 1.5 ml per hour with the same citrate solution gave the results shown in Fig. 1. The solid curve shows the tracer count without absorber, and the broken line extension of the europium section represents the count taken with an absorber of 39.3 mg per sq. cm which cuts out the Pm^{147} radiation. The count with absorber is multiplied by 3.7 to correct for the partial absorption of the Eu^{154} activity. The extension of the promethium section was obtained by difference. The order of elution of these two elements is the reverse of that which has been obtained by cation exchange, a fact which, of itself, may be useful. At lower pH's the elution rate is greater.

A similar separation has been attempted with americium and curium at pH 2.3 and 0.0125 M citrate. Although a definite difference in the elution peaks was shown, the degree of separation was not very marked. The results are shown in Fig. 2. The solid curve represents the total alpha count and shows no break. The broken curves are based on the differential alpha pulse analysis made by A. Ghiorso, for Am^{241} (5.48 Mev) and Cm^{242} (6.08 Mev).



MU 448

FIG. 1

Fig. 1

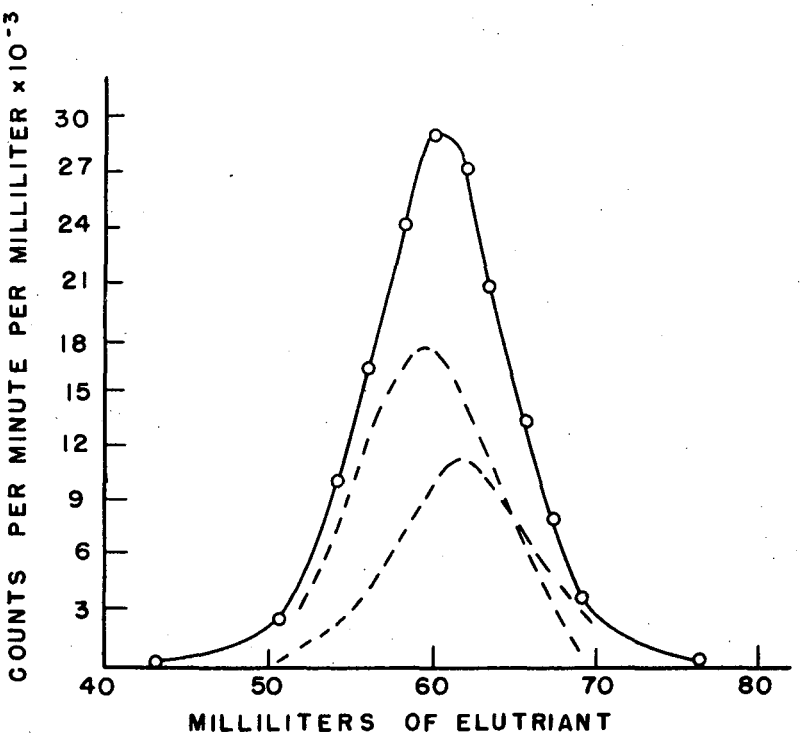


FIG. 2 MU 449

Fig. 2

Capacity of Dowex A-1 for U(VI) in HCl Solutions

Donald A. Orth and K. Street, Jr.

The behavior of UO_2^{++} in HCl solutions with respect to Dowex A-1 resin has been investigated. In the last quarterly report, the distribution coefficients of tracer solutions were given. The capacity of the resin for UO_2^{++} has been measured and may be partially interpreted.

The chloride capacity of a small bed of resin in a sintered glass filter funnel was measured by passing through HCl solutions, removing excess HCl with air and eluting with water and then with $NaHCO_3$ solution. The water fraction, analyzed for chloride, showed an amount of chloride present which was directly proportional to the concentration of the original HCl solution with which the resin was saturated, over concentrations ranging from 1M to 10M HCl. The carbonate fraction always yielded the same value, which should correspond to the fixed number of quaternary amine groups. (Fig. 1)

Various uranyl chloride solutions were made up with HCl to yield 10M chloride and 0.01M to 4M UO_2^{++}. These were passed through the bed until the resin was saturated, and then the UO_2^{++} on the resin was measured. With increasing UO_2^{++} concentrations, the uranium capacity of the resin approached the fixed number of chlorides as determined by the carbonate elution. If it is assumed that only these fixed positions can bind the uranyl chloride complex, this would suggest that the complex has a single negative charge: i.e., $UO_2Cl^-_3$, $UO_2HCl^-_4$, etc.

To determine the role of H+, the capacity was measured with two solutions: (a) 1M UO_2^{++}, 8M H+, 10M Cl- and (b) 1M UO_2^{++}, 0.5M H+, 7.5M Li+, 10M Cl-. The UO_2^{++} on the resin was the same in both cases, showing no hydrogen ion effect.

Some transference experiments were performed in order to test the correlation between the distribution coefficients from the equilibrations, and the charge on the complex. A stack of filter paper disks were moistened with various HCl solutions, U^{233} tracer was placed on the center disk, and voltage was applied to give 100 milliamperes current through the paper. The tracer on the paper disks was then counted to determine the distribution. Above 3M HCl the activity drifted to the anode and below, to the cathode. This corresponds to the equilibration evidence, which shows uranium beginning to be absorbed by the resin in HCl solutions between 2M and 3M.

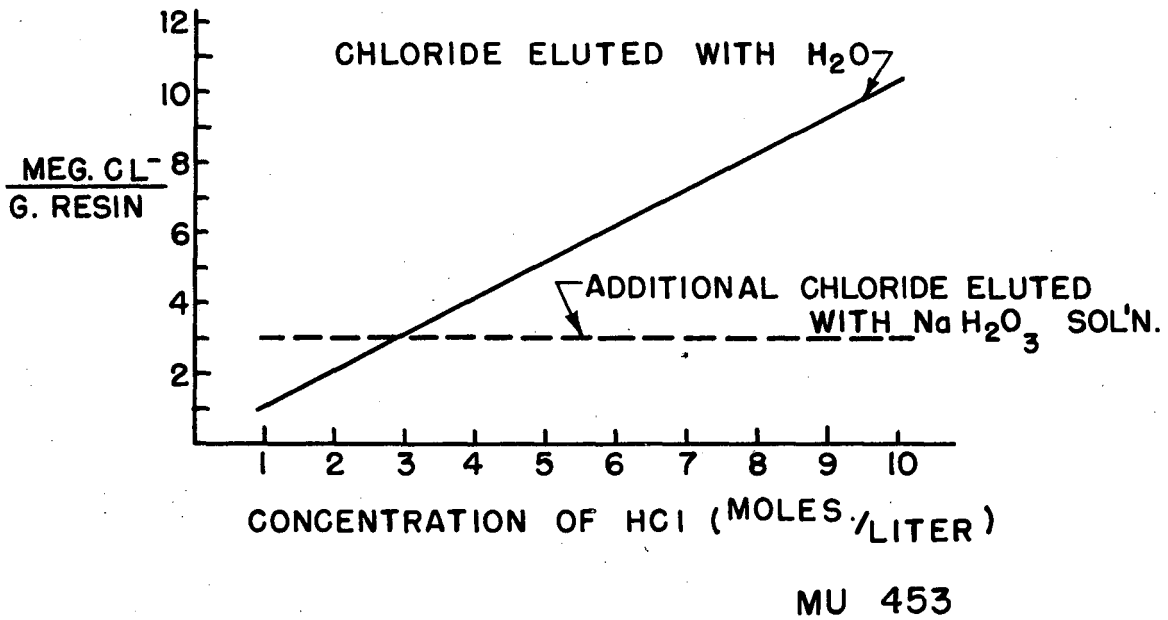
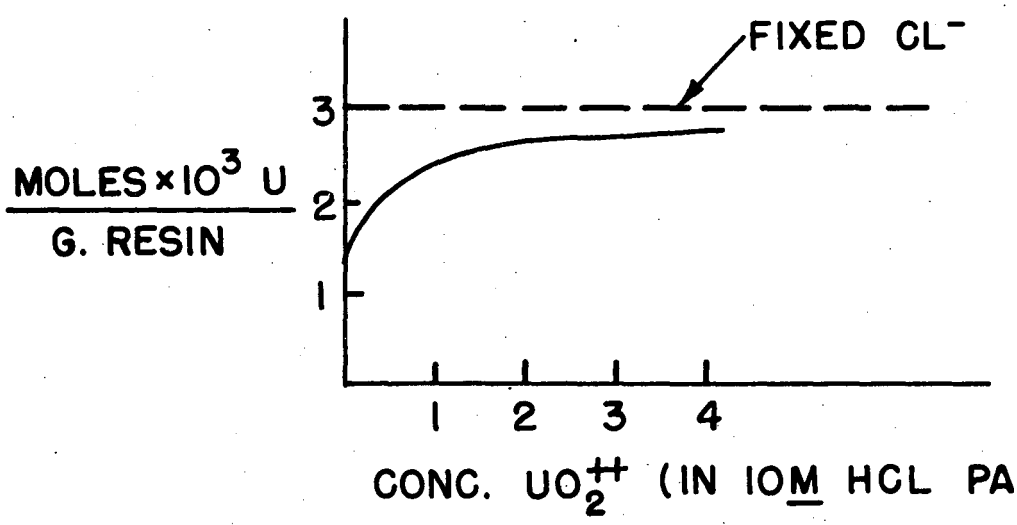

12
10
8
6
4
2
MEG. CL⁻
G. RESIN
CHLORIDE ELUTED WITH H2O
ADDITIONAL CHLORIDE ELUTED
WITH Na H2O3 SOL'N.
1 2 3 4 5 6 7 8 9 10
CONCENTRATION OF HCl (MOLES/LITER)
MU 453

FIG. 1

Fig. 1



MU 454

FIG. 2

Fig. 2

Separation of Zirconium and Hafnium by Extraction with Trifluoro-beta-diketones

E. H. Huffman and G. M. Iddings

Further studies have been made on the chelation-extraction separation of zirconium and hafnium, with the view to increasing the concentrations which can be dealt with. Data for thenoyltrifluoroacetone (TTA) and benzoyltrifluoroacetone (BTA) extractions have been obtained.

The TTA extractions from 4.0M perchloric acid solutions which were 0.04M in zirconium and hafnium have given equilibrium constants of about 1.2×10^7 for zirconium and about 5.8×10^5 for hafnium, where these constants are expressed by

$$\log K = \log R - 4 \log (TTA).$$

Zirconium is monomeric in the aqueous phase at the concentration used. The degree of separation is thus seen to be essentially the same as previously obtained with 2.0M perchloric acid solutions and more than a ten fold increase in concentration of the zirconium (hafnium) can be used. Attempts to use 0.1M zirconium in 5M perchloric acid gave precipitates at the interface of the aqueous and organic layers.

Similar extractions with benzene solutions of BTA gave values for the equilibrium constants of about 3.0×10^5 for zirconium and about 1.5×10^4 for hafnium. It is thus seen that the distribution into the organic phase for BTA is not as favorable as it is for TTA, and the degree of separation of zirconium and hafnium is the same. The solubility of the BTA chelates in benzene also was no higher than for the TTA chelates, contrary to expectations.

Radiation Chemistry

A. S. Newton and W. R. McDonell

Results of preliminary analysis of chemical products produced by irradiation of 40 ml of pure ethanol with 28 Mev alpha-particles integrated to 3.53 μ a.h. are shown in Table I.

TABLE I

Gaseous Components

Product	Moles	$\frac{\text{e.v.}}{\text{molecule}}$	$\frac{\text{molecules}}{\text{100 e.v.}}$
Total Gases	0.0417	44.2	2.27
H_2	.0314	58.6	1.61
CH_4	.0039	475	0.21
C_2H_4	.0014	1300	0.077
CO	.0013	1425	0.070
Others (estimated)	.0022		

Several components present in small yield are as yet unaccounted for. The yield of CO_2 is negligibly small.

TABLE II

Liquid Products (determined chemically)

Product	Moles	$\frac{\text{e.v.}}{\text{molecule}}$	$\frac{\text{molecules}}{\text{100 e.v.}}$
H_2O	$12.4 \pm .3$	149	0.67
H+	$0.3 \pm .05$	6000	0.02
Formaldehyde	$4.2 \pm .2$	438	0.23
"Acetaldehyde"	$11.5 \pm .5$	160	~ 0.63
Total Carbonyl	$18.3 \pm .2$	100	1.00
"Ketone"	2 - 3	900 - 600	~ 0.14
"Glycol"	10 - 13	180 - 140	~ 0.60
			~ 3.2 total

The determination of "acetaldehyde" as shown in Table II includes all higher aldehydes calculated as acetaldehyde. The "ketone" data is the difference between the total carbonyl and aldehyde determinations, and is rather uncertain. "Glycol" indicates only glycols with OH on adjacent carbons and is calculated as ethylene glycol. Anticipated major products not yet determined include ethers and other alcohols. Mass spectroscopic analysis of the liquid fraction indicates several other unidentified components and interpretation of this data is proceeding.

The mole equivalents of reduced products equal approximately the mole equivalents of oxidized products.

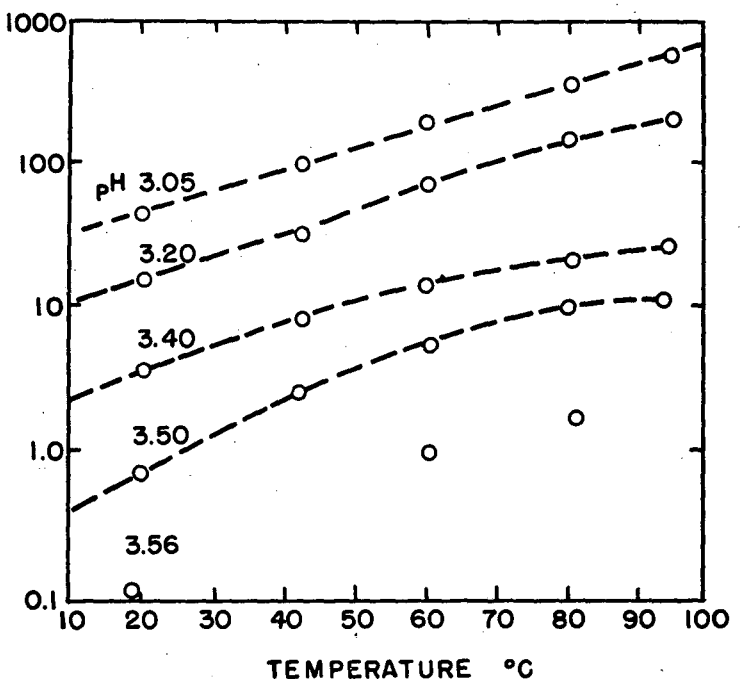
Distribution of Am^{+++} between Dowex 50 and Ammonium Citrate Solutions

G. H. Higgins and K. Street, Jr.

The distribution of Am^{+++} between ionex ion exchange resin and 5 percent ammonium citrate solutions has been studied at different temperatures and acid concentrations. The results of this study are shown in Fig. 1 where

$$Kd = \frac{\text{counts Am in resin}}{\text{counts Am in soln.}} \times \frac{\text{vol of soln. in cc}}{\text{wt of oven-dried resin in grams.}}$$

Promethium tracer was checked against americium at room temperature and at 90°C and the ratio of their Kd's was found to be constant.



LOG KD. VS. TEMP. FOR Am IN AMMONIUM CITRATE SOLUTIONS AT DIFFERENT P.H.'S.

MU 450

FIG. 1

Fig. 1

B. Nuclear Properties and Transformations

"Fission" of Medium Weight Elements

Roger E. Batzel and Glenn T. Seaborg

The fission reaction has been observed with high energy accelerator projectiles for elements as light as tantalum[1] but has not been reported for medium weight elements. The present note presents evidence for the occurrence of reactions which are probably most properly described by the term "fission" and which seem to occur with very small yield throughout the region where this type of reaction is only slightly exoergic or even endoergic with respect to mass balance.

In the course of detailed investigation of the spallation of copper and the variation of the product yields with energy of the bombarding particle the threshold for formation of radioactive Cl^{38} (38 minute half-life) from elemental copper was studied. The energetically most economical way in which Cl^{38} might be formed by spallation reactions is by emission from the bombarded copper nucleus of nucleons in groups such as alpha-particles instead of single nucleons. The energetic requirements for the reaction $Cu^{63}(p,pn6\alpha)Cl^{38}$, in which the maximum number of alpha-particles are emitted, include (1) the mass difference between the reactants and the products and (2) the excitation energy which the alpha-particles must have in order to pass over the coulombic barrier. Since the reaction is endoergic with respect to atomic masses, about 50 Mev must be supplied by the impinging proton to make up the mass difference. If the alpha-particles are considered as coming out consecutively, a value of about 50 Mev can be obtained for the coulombic requirement and thus the threshold for this spallation reaction is roughly 100 Mev.

The production of Cl^{38} was definitely observed at proton bombardment energies beginning at about 60-70 Mev with a cross section of some 10^{-32} cm^2 which increased smoothly with energy to about 10^{-31} cm^2 at 100 Mev. The identification was made in all cases through chemical separation, measurement of half-life with a Geiger counter and observation of the sign of the beta-particles with a simple beta-ray spectrometer. The possibility that the observed activity might be due to impurities in the copper was eliminated through analysis by radioactivation methods for all possible impurities which might account for the observed yield of Cl^{38}. In order to explain the low threshold it must be assumed that substantially larger particles than alpha-particles are emitted and the reactions are therefore of a type which might more properly be termed fission. As an example, the extreme reaction $Cu^{63} + p \longrightarrow Cl^{38} + Al^{25} + n$, which is energetically most economical but still endoergic, has a threshold of about 50 Mev as calculated by adding the amount of energy necessary to make up the mass difference to the excitation energy required for the potential barrier assuming that the fragments are spherical and tangent at the nuclear radii (taken as $1.48 \times 10^{-13} A^{1/3}$ cm). This calculated threshold is admittedly very rough, but illustrates that the threshold for this type of reaction is definitely lower than for the above described spallation reaction. The cross section is calculated on the basis of the single isotope observed and does not correspond to a total fission cross section, in the sense that the term is ordinarily used, where the yield of all the fission products is summed.

This result made it seem worthwhile to investigate other elements in the

middle portion of the periodic system in order to see whether analogous reactions might occur as a general rule. Bromine was bombarded and the variation with energy of the yield of radioactive Sc^{44} (3.9 hr. isomer) was studied. The threshold for the reaction $Br^{79}(p,p7n7\alpha)Sc^{44}$ is about 180 Mev and that for $Br^{79} + p \longrightarrow Sc^{44} + P^{34} + 2n$ is about 80 Mev calculated as above. The Sc^{44} was observed with proton energies of 125 and 140 Mev with cross sections of about 10^{-32} cm^2, with the value at the higher energy several times that at the lower energy, and the yield was too small to measure at 70 Mev. Special purity ammonium bromide was synthesized and used in all bombardments.

The comparable reactions for silver, $Ag^{107}(p,pn10\alpha)Co^{61}$ and $Ag^{107} + p \longrightarrow Co^{61} + Sc^{45} + 2n$, have calculated thresholds of about 210 and 60 Mev, respectively. In this case the fission reaction is exoergic by about 10 Mev. The isotope Co^{61} (1.8 hours) was observed at an energy of 180 Mev with an approximate cross section of 10^{-32} cm^2 based on elemental silver, but the results must be termed borderline due to the difficulty in resolving the Co^{61} due to the silver from that formed by a small amount of copper impurity. Radioactivation methods were used to determine the amount of copper present. Unfortunately, the yield of Co^{61} from the copper impurity was about as much as that from the silver itself.

The formation of gallium isotopes from tin bombardment was also studied, and isolation of the gallium fraction showed the characteristic activities of Ga^{66} (about 9 hours) and Ga^{72} (about 14 hours). The calculated threshold for $Sn^{118}(p,7n10\alpha)Ga^{72}$ is about 230 Mev and that for the reaction $Sn^{118} + p \longrightarrow Ga^{72} + Ca^{45} + 2n$ is about 70 Mev; the fission reaction is exoergic by about 10 Mev. The Ga^{66} and Ga^{72} were observed at energies of 150 and 180 Mev with cross sections based on elemental tin of about 10^{-32} cm^2 and with the values at the higher several times those at the lower energy; the yield at 100 Mev was too small to identify and no yield was detectable at 80 Mev.

Apparently, when the energy threshold requirements are met, large fragments are emitted among the competitive products of nuclear reactions throughout the entire range of atomic numbers of the elements. This is certainly not surprising and the measured yields reported here seem to be quite reasonable. It seems certain that the size of the fragments varies continuously from those (neutrons, protons, deuterons, alpha-particles, etc.) which accompany what we for convenience call spallation reactions, through intermediate sizes (for example,[2,3] Li^8, etc.), and on up to sizes such that the nucleus is split essentially into several pieces of comparable weight. Apparently a number of reactions in which there occurs the latter type of nuclear splitting have been observed in the present investigation and perhaps the term "fission" is as proper a name as any to apply to this process.

It should be emphasized again that the above cross sections are calculated on the basis of the single isotope observed and do not represent the total fission cross sections for the given elements in the sense that this term is ordinarily used. It should also be pointed out that the number of cases in which similar reactions can be studied at present is very limited due to the strict requirements on purity of the bombarded substance, the high factor of radioactive purification necessary, and the sensitivity needed in detecting the product isotope.

The bombardments were carried out on the 184-in. cyclotron, and the required energy was obtained by adjusting the distance of the target from the periphery of the tank.

References.

[1] Perlman, Goeckermann, Templeton, and Howland, Phys. Rev. 72, 352 (1947).
[2] S. Wright, Phys. Rev. 77, 742 (1950) (A).
[3] L. Marquez and I. Perlman, unpublished work (1950).

Tin Isotopes

A. S. Newton and W. R. McDonell

An attempt to verify a previously reported Sn activity, 41-min., 2.5 Mev β^-, 0.27 Mev γ assigned to Sn^{121} (UCRL 395) has been unsuccessful. It is concluded that this activity, which was originally present in rather low yield, represented an impurity not eliminated in the chemical separations.

Bi^{207} and Related Activities

H. M. Neumann and I. Perlman

The work relating to Bi^{207}, previously reported in UCRL 635, has been continued. The experiment, previously reported, wherein Bi^{206} and Bi^{207} were found as alpha-decay products of At^{210} and At^{211}, has been repeated and the yields of the daughters verified. The values derived from these yields are: (1) The half-life of Bi^{207} is 10 years if its counting efficiency is 10 percent, 100 years if counting efficiency is 100 percent; (2) The α-branching of At^{210} is 1 percent if the Bi^{206} counting efficiency is 10 percent, 0.1 percent branching if counting efficiency is 100 percent.

A sample of At^{210} and At^{211} was carefully examined with the pulse analyzer in hopes of detecting the alpha-particles from At^{210}. A sample of pure At^{211} was also prepared for purposes of comparison with the mixture. No alphas from At^{210} were detected, and as a result we may set the following limits on the alpha-branching: the branching is less than 0.3 percent if the alpha-particles are obscured by the Po^{210}(5.30 Mev) alphas; it is less than 0.1 percent if the At^{210} alphas have any other energy. Obviously, the Bi^{206} counting efficiency is greater than 30 percent.

The pulse analyses revealed to new alpha-particle peaks of low abundance at 6.60 Mev and 6.90 Mev. These are attributed to Po^{211}, and the abundance of its alpha-groups are: 7.43 Mev, 98.9 percent; 6.90 Mev, 0.6 percent; 6.60 Mev, 0.5 percent. The indicated branching of At^{211} decay from these experiments is 40.8 percent by alpha-emission and 59.2 percent by electron capture.

The Bi^{207} prepared by At^{211} decay has been compared with the long-lived Bi prepared by deuteron bombardment of lead, and which is believed to be a Bi^{207}-Bi^{208} mixture. Neutron binding energy measurements and known decay energies allow us to calculate the decay energy of Bi^{208} to Pb^{208} as 2.5 ± 0.3 Mev. The lowest known excited state of Pb^{208}, and probably actually the lowest excited state, is at 2.62 Mev. It is thus likely that the radiations from Bi^{208} would consist only of x-rays, plus possibly the 2.62 Mev γ-ray. The electrons from the mixture give an absorption curve identical to that of pure Bi^{207}, as would then be expected.

Examination of the mixture with a crude β-ray spectrometer showed a complex spectrum with two distinguishable lines at 450 and 860 Kev, corresponding to γ-rays of 540 and 950 Kev. The higher energy line is in such intensity to suggest it is a transition to the ground state of Pb^{207}. These data, in conjunction with the observed alpha-groups from Po^{211}, gives the following excited levels of the Pb^{207} nucleus: 530, 830, and 950 Kev. Evidence for the 530 Kev level comes from both Po^{211} and Bi^{207} decay. Harvey[1], from his study of the (d,p) reaction on Pb^{206}, reports a double level at 0.9 Mev, corresponding to the 830 and 950 Kev levels, but does not report a 0.5 Mev level.

Since the 530 Kev level might correspond to the 1.6 min. activity reported by Waldman and Collins[2] to be formed by excitation of lead with 650 Kev x-rays, lead was quickly removed from its parent and examined for presence of the short activity. A definitely negative result was obtained.

---

[1] Harvey, Bull. Am. Phys. Soc., No. 3, April, 1950.
[2] Waldman and Collins, Phys. Rev. 57, 338 (1940).

Neutron Defficient Polonium Isotopes

D. G. Karraker and D. H. Templeton

Bombardments of bismuth with protons and deuterons from the 184-inch cyclotron have resulted in the identification of two short alpha-decay activities of polonium, one of 11 minutes and one of 17 minutes half-life. The polonium was chemically separated, and half-lives and energies were obtained through the use of the pulse-analyzer. Two groups of alpha-particles were found to decay with an 11-minute half-life; one group of 5.84 Mev and a second of 5.76 Mev. The 5.84 Mev group is $\sim$9 times as abundant as the 5.76 Mev group. It has not been proved that there is one 11-mimute activity with two alpha-groups, but this possibility is favored at the present. The 17-minute alpha has an energy of 5.67 Mev.

Astatine Isotopes

G. W. Barton, A. Ghiorso, and I. Perlman

The study of the astatine isotopes has been continued with the assignment of some mass numbers by chemical and radioactive means. The parent-daughter relationship in these cases was shown by timed milkings of the daughter activities. The parent activity could be identified by the change in yield as a function of time, while the daughter activity was identified by its radiations and half-life. An up-to-date summary of the data indicated is shown in Table I.

TABLE I

Isotope	Half-life	Mode of Decay	Alpha-energy	Identified by Milking
At^{209}	5.5 hr.	α, electron capture	5.65 Mev	200 yr. Po^{209} 14 d. Bi^{205}
At^{208}	6.3 hr.	e.c.	not observed	3 yr. Po^{208}
At^{207}	1.9 hr.	α, e.c.	5.75 Mev	5.7 hr. Po^{207} 52 hr. Pb^{203}
At^{206}	2.6 hr.	e.c.	not observed	9 da. Po^{206}
At^{205}	23 min.	α, e.c.	5.90 Mev	14 da. Bi^{205}
At^{204}	24 min.	e.c.	not observed	12 hr. Bi^{204}

Additional isotopes have been observed using the vacuum carrier system from the 184-inch cyclotron. The mass numbers of these have not been identified.

7 min.	α	6.10 Mev
1.7 min.	α	6.35 Mev
43 sec.	α	6.45 Mev

Beta-decay Energy of Ac^{226}

Lynn Hall and D. H. Templeton

In order to extend the energy surface in the heavy region by closing α-β cycles, some α-energies must be estimated from a plot of α-disintegration energy v. mass number. By combining these estimations with experimentally determined values, one may calculate β-decay energies in those cases in which no β-transition has been observed. Thus, in the 4n + 2 series the α-systematics are extended from Pb^{210} to Cm^{238} as shown. (The numbers are decay energies in Mev; e stands for estimated, c means calculated by closing cycles.)

Pb^{210}	← 7.83	Po^{214}	← 7.25	Em^{218}	← 6.64	Ra^{222}	← 6.41	Th^{226}	← 5.96	U^{230}	← 6.26	Pu^{234}	← 6.61	Cm^{238}
↑ 5.37^c		↑ 3.15		↑ 2.73^c		↑ 2.09^c		↑ 1.05^c		↑ 0.54^c		↓ 0.42^c		↓ 0.98^c
Tl^{210}	← 5.61	Bi^{214}	← 6.83	At^{218}	← 6.00^e	Fr^{222}	← 5.37^e	Ac^{226}	← 5.45^e	Pa^{230}	← 5.40^e	Np^{234}	← 6.05^e	Am^{238}

To lend more credence to the estimated and calculated values in the region of Cm^{238}, an experimental check on one of the values can be made. Ac^{226} was chosen for this purpose.

Th foil was bombarded with 350 Mev protons to produce Ac^{226}. The foil was then dissolved in conc. HCl containing a trace of F^-. The bulk of the Th was removed by extraction into 1M TTA in benzene at a pH of 1.0-2.0. The rare earths and actinium were then extracted into the benzene phase at pH of 6.0-7.0, and finally into 0.1M HCl. Two methods were employed to separate the actinium from the rare earths. In the first method, the rare earths were extracted away using 3M TTA at pH of 3.0-3.2 and Ac subsequently extracted into 0.15M TTA at a pH of 6.0-7.0. The second method consisted of transferring the activities onto a resin column of Dowex-50 (NH_4^+ form) and eluting with "5 percent citrate" at a pH of 4.0. The rare earth fraction was located by its β-activity, and the actinium by the α-peak.

Al absorption curves were taken at various times, so that the decay could be followed through each absorber. From each of these decay curves was resolved a 10-day line (Bi^{213} in equilibrium with Ac^{225}). The resulting Ac^{226} line varied in slope from 24 to 39 hours, (averaging 32 hours) fitting in also the 6-hour Ac^{228} line. The activity of the Ac^{226} was then read at a certain time on all decay curves, and these activities were plotted as an Al absorption curve. The resulting curves, together with their Feather analyses are shown for two experiments in Fig. 1. Two lines of limiting slope were drawn through the absorption points of each experiment, and Feather analyses made accordingly. The end points give an average of 1.17 Mev within the limits of 1.10 and 1.24 Mev.

As a further check on the β^- energy, a column-separated sample was run on the bender, and a crude Kurie plot made. The end point determined in this manner fell within the above limits.

If it is assumed that there is no γ-ray (none has been observed), the decay energy is assigned as 1.17 ± 0.10 Mev, and this value is seen to compare closely to the calculated value of 1.05 Mev.

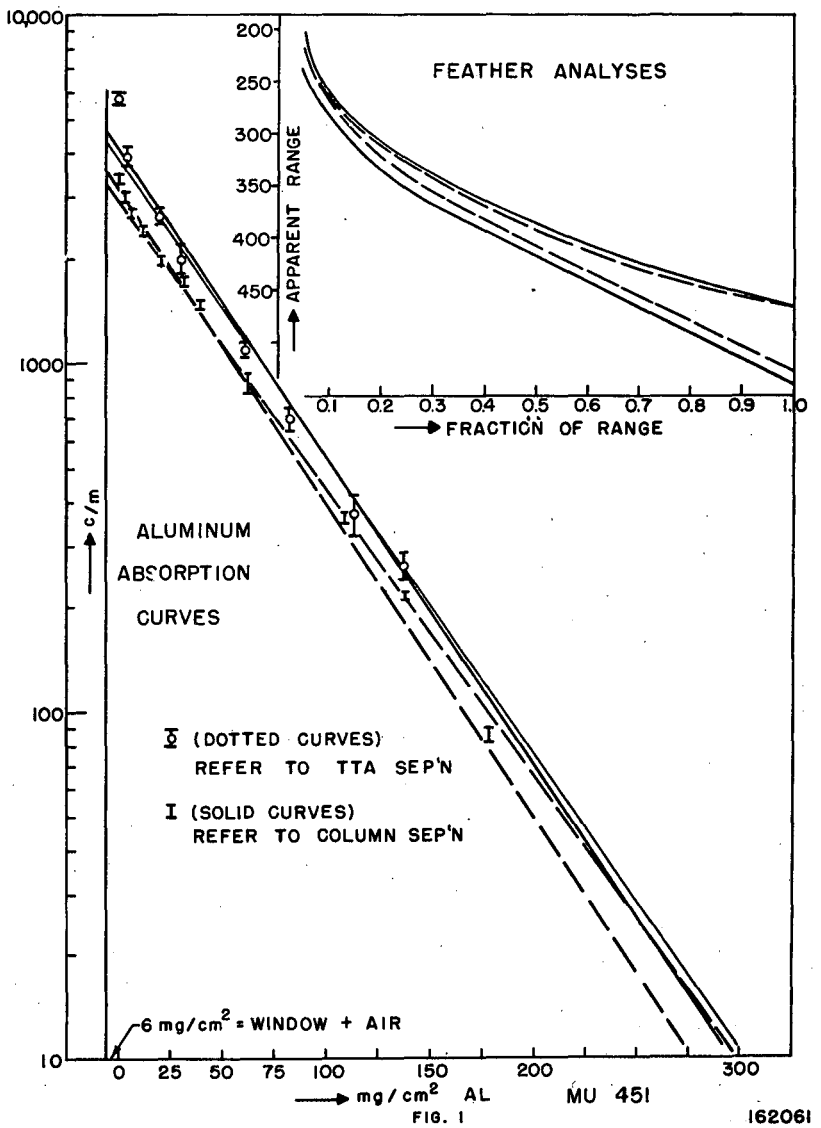

10,000
1000
100
10
c/m
ALUMINUM
ABSORPTION
CURVES
(DOTTED CURVES)
REFER TO TTA SEP'N
(SOLID CURVES)
REFER TO COLUMN SEP'N
6 mg/cm² = WINDOW + AIR
0 25 50 75 100 150 200 300
mg/cm² AL
MU 451
FEATHER ANALYSES
200 250 300 350 400 450
APPARENT RANGE
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
FRACTION OF RANGE
FIG. 1
162061

Fig. 1

Specific Alpha-Activity of U^{235}

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

Last quarter, it was reported that samples of U^{235} were prepared for counting by subliming the acetyl acetonate of U^{235} onto very thin Formvar films. This procedure has been abandoned temporarily. A new method, consisting of electrodeposition of uranium from an ammonium oxalate solution, is now being used. This procedure is identical with that described by Hufford and Scott in National Nuclear Energy Series IV, 14B, except for the type of electrodeposition cell. The cell being used consists of a textolite plastic cylinder, cut to accomodate a two inch platinum disc, 5 mils thick. The disk rests on a monel steel cylindrical block and is separated from the cylinder by a neoprene gasket. The cylinder is cut with an outside shoulder, so that a brass collar, screwed to a brass base, holds the cylinder firmly against the neoprene gasket, establishing a water-tight seal against the platinum disc. Very uniform films have been prepared with an average thickness of $\sim 100\ \mu gm/cm^2$.

After counting, the uranium is removed from a platinum disk with hot, one normal sulfuric acid and is titrated in the same manner as described before. This consists of reducing the uranyl ion with metallic cadmium at $\sim 60^{\circ}C.$, oxidizing the uranic ion with an excess of standard ceric ion, and back-titrating with ferrous ion. Ferrous orthophenanthroline is used as the indicator.

Some trouble has been encountered in this volumetric procedure. Inconsistent blanks have resulted of the order of from 1/2 to 10 percent. Through a systematic search, these blanks have been attributed to minute amounts of cadmium metal remaining in the solution after removing the cadmium reductor. By heating this solution in an atmosphere of argon, the excess cadmium is oxidized. However, since it is difficult to keep the atmosphere entirely free from air from 1-1/2 to 2 percent of the uranic ion is reoxidized.

The preliminary values obtained to date for the half-life of U^{235} are:

7.07×10^8 yr.
7.45×10^8 yr.
7.26×10^8 yr.
7.05×10^8 yr.

Search for Plutonium in Nature

C. A. Levine, A. Ghiorso, and G. T. Seaborg

Pitchblende Ores. We have continued the investigation of naturally occurring plutonium. In UCRL 635, the amount of Pu^{239} found in pitchblende obtained from Colorado, from Canada, and from the Belgian Congo was reported. Since it is postulated that the Pu^{239} is formed from neutron capture by U^{238}, the figure of interest would be the amount of Pu^{239} present per part of uranium.

Analyses of the ores for uranium gave 13.5 percent uranium in the Canadian ore, and 38 percent uranium in the Belgian Congo ore. Not enough Colorado ore was saved to run an analysis, but it is estimated that the Colorado ore contains about 50 percent uranium. The abundance of plutonium in relation to the uranium, then, is

Belgian Congo ore: (38 percent U): 1 part Pu^{239} per 8.9 (10^{10}) uranium
Canada ore: (13.5 percent U): 1 part Pu^{239} per 1.7 (10^{11}) uranium
Colorado ore: (50 percent U): 1 part Pu^{239} per 1.5 (10^{11}) uranium

Approximate calculations were made in order to find out if this amount of plutonium could come from the uranium present assuming the mode of production to be

$$U^{238} + \text{neutron} \longrightarrow U^{239} \xrightarrow{\beta^-} Np^{239} \xrightarrow{\beta^-} Pu^{239}$$

The neutrons were assumed to have come from three sources: (1) neutrons from cosmic rays, (2) neutrons emitted during spontaneous fission of uranium, (3) neutrons from (α,n) reactions on surrounding light elements.

From these three sources, enough neutrons are formed, according to our approximations, so that all the plutonium could be accounted for.

Monazite Sands. From the alpha-decay systematics and from considerations of the energy surface in the heavy element region, it was thought that Pu^{244} might be stable with respect to beta-decay and possibly have a long enough half-life (10^8 yrs.) to exist in nature. Since no alpha-particles of energy comparable to what one would predict for Pu^{244} were seen in the pitchblende experiments, it was decided to look in monazite sands. This ore was chosen as a likely location for any naturally existing plutonium since Pu^{3+} and Pu^{4+} follow rare earth and thorium chemistry. Monazite is a mixture of thorium, rare earths, and uranium.

The monazite investigated was from Brazilian sources and had a composition as follows: U_3O_8, 0.24 percent; ThO_2, 6.7 percent; $(R.E.)_2O_3$, 59.7 percent; P_2O_5, 29.3 percent; TiO_2, 2.4 percent, Fe_2O_3, 1.8 percent.

The plutonium was extracted from the ore as follows: One kilogram of the ore was digested at 200°C for 3 hrs. with 1300 ml of conc. H_2SO_4. This leaves only a few grams of unchanged material. A tracer amount of Pu^{238} equal to 80 counts a minute was added to the ore during digestion. The digested mass was cooled and added to the ore during digestion. The digested mass was cooled and added to 8.5 liters of conc. HCl and stirred for 12 hours. This ensured that the plutonium, uranium, etc. would not be trapped in the insoluble chlorides which precipitated.

The supernatant and washings from the chloride precipitate were made 11M in HCl by adding HCl gas and put through an ion-exchange column packed with pre-washed Dowex A-1 anion exchange resin. The uranium, plutonium, and iron are adsorbed on the resin while the thorium, rare earths, phosphate, etc. wash through. The resin was then washed with four column volumes of conc. HCl, and then the uranium, plutonum and iron eluted from the resin with 0.2N HCl. To this solution containing uranium, plutonium, and iron was added a trace of HNO_3 and SO_2 gas to reduce plutonium to the lower oxidation state. Lanthanum carrier was added to the solution and the solution made 2N in HF. This separated plutonium from the bulk of the uranium and iron. The fluoride precipitate was washed and treated with KOH to form the hydroxide. This was dissolved in HNO_3, oxidized with $KBrO_3$, and saturated with NH_4NO_3. The plutonium and remaining uranium were extracted by mixing with three separate portions of pre-oxidized diethyl ether. The plutonium and uranium were then re-extracted back into some SO_2- containing water. The volume of this water solution was reduced by evaporation, 0.2 mg of lanthanum per milliliter of solution added, the solution made 0.5N in HNO_3 and 2N in HF. This fluoride precipitate was washed, metathisized to hydroxide with KOH, dissolved in HNO_3, oxidized with $KBrO_3$ to oxidize any plutonium to Pu^{+6}, and the lanthanum carrier re-precipitated with HF. To the supernatant was added SO_2 to reduce plutonium to a lower oxidation state again, and 0.2 mg of lanthanum per milliliter of solution was added slowly and the solution allowed to digest for two hours. The plutonium and its lanthanum carrier (which now amounted to about 1.0 mg La) were separated from the solution, metathisized to the hydroxide, dissolved in three drops of conc. HNO_3, and oxidized with $KBrO_3$. The Pu^{+6} was extracted with three portions of pre-oxidized diethyl ether, and the ether allowed to evaporate on a platinum plate. The sample deposited on the plate was essentially weightless.

This sample was placed in the pulse analyzer and the energies of the emitted alpha-particles were examined.

There were 20 counts per minute which could be attributed to the Pu^{238} tracer, indicating an overall chemical yield of 25 percent for plutonium.

There were 0.36 counts per minute of alpha-particles corresponding to Pu^{239} energy.

There were less than 0.05 counts per minute of alphas which would be at the predicted range of Pu^{244}.

The amount of Pu^{239} present corresponds to one part Pu^{239} per 1.16 (10^{11}) parts uranium, which is in the same range as the results from pitchblende. This is somewhat surprising, since the smaller uranium content means capture by the uranium of a much smaller percentage of the neutron flux in the ore. This is probably at least partially balanced by the increased neutron flux from α,n reactions due to the presence of thorium.

Beta-Ray Spectrometer

W. W. Crane, G. D. O'Kelley and I. Perlman

Two recent irradiations of Am^{241} in the Hanford Pile have yielded large quantities of Am^{242}. It has been possible to obtain information of the beta and gamma spectra of 16-hr. Am^{242m} and 100-yr. Am^{242}.

The first sample of the mixture of Am^{242m} and Am^{242} was mounted on a backing of 0.4 mg/cm^2 of nylon + 0.1 mg/cm^2 of Al leaf to render the source conducting. Conversion lines corresponding to a γ-ray of 50 Kev were seen, which decayed with a 16-hr. half-life, indicating that they were from Am^{242m}, and not from some contamination such as Cm^{242}. The continuous spectrum was run automatically by a method previously described, from which the β^- energy was found to be 0.632 Mev. The abundance of 50 Kev γ-rays is thought to be about 20 percent of the β^- particles. The abundance of the conversion electrons is obtained in the beta-ray spectrometer is about 5 percent of the β^- spectrum. Thus, the 50 Kev γ is about 20 percent converted. Because of the low abundance of the γ-ray referred to the 16 hr. beta-spectrum, it would seem that the γ-ray is from the isomeric transition.

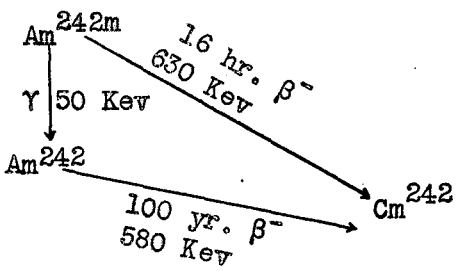
Although the sample was predominantly 16-hr. Am^{242m}, sufficient 100-yr. Am^{242} remained after decay of the short-lived activity for a determination of the energy of the long-lived isomer. This was found to be about 580 Kev.

In this work it was found that a very thin (~20 μg) acid resistant film can be made by dropping clear tygon paint on tap water. These films are resistant to all acids tested as well as salt solutions. They can be made conducting by evaporating gold on the surface in a vacuum.

A second sample mounted on 0.03 mg/cm^2 tygon was investigated. The 16-hr. Am^{242m} β^- energy was measured as 628 Kev taking the mean of the two measurements a value of 630 $\pm$ 5 Kev is obtained. The Fermi-Kurie plots are included in Fig. 1.

The value of 0.630 $\pm$ 0.005 Mev for the Am^{242m} β^- is in excellent agreement with the recent value of 0.63 $\pm$ 0.01 Mev from Al absorption reported by Grunland, etal. (Phys. Rev. 78, 69 (1950))

Coincidence measurements will also be made. Tentatively, we propose the following scheme, both from the standpoint of γ-ray abundance and the energy values.



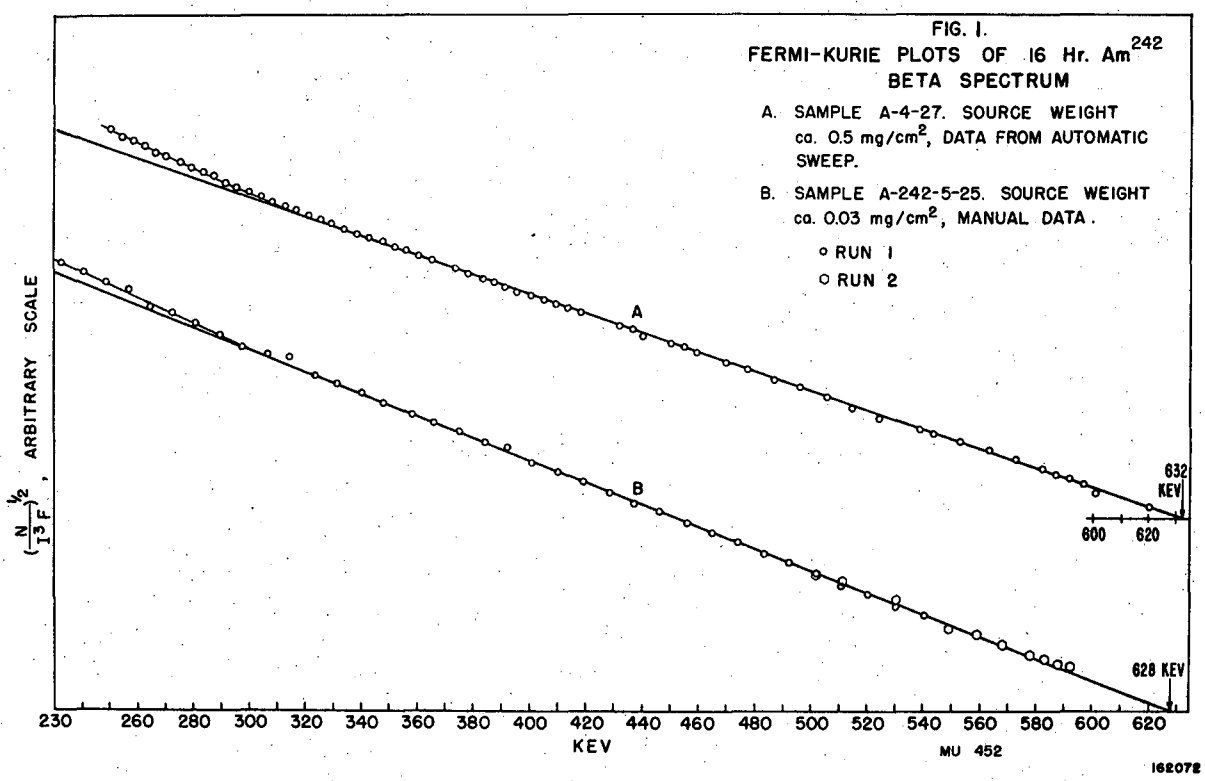


FIG. I.
FERMI-KURIE PLOTS OF 16 Hr. Am242
BETA SPECTRUM
A. SAMPLE A-4-27. SOURCE WEIGHT ca. 0.5 mg/cm2, DATA FROM AUTOMATIC SWEEP.
B. SAMPLE A-242-5-25. SOURCE WEIGHT ca. 0.03 mg/cm2, MANUAL DATA.
o RUN 1
o RUN 2
A
B
632 KEV
600
620
628 KEV
230
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
KEV
MU 452
162072

Fig. 1

Limit on K-capture Branching of Cm^{240}

G. H. Higgins, K. Street Jr.

An upper limit for the K/α branching ratio of Cm^{240} has been set at 0.5 percent. Cm^{240} was produced by an α,3n reaction on Pu^{239} and isolated from the target material and other activities by repeated separations on cation ion exchange columns. Isotopic assay of the curium fraction was made by means of the pulse analyzer and the activity allowed to decay for ~7 days. Ion exchange separation of the americium fraction was effected and the americium purified by a second column separation. Analysis of the decay curve showed no observable Am^{240}(50 hr. K capture) when the samples were counted in a windowless counter. Alpha pulse analysis showed largely Am^{241} and some Cm^{240} and Cm^{242}.

A limit was set on the number of counts of Am^{240} which would not have been observed and the counting efficiency of the Auger electrons in the nucleometer was assumed to be 100 percent. The chemical yield was calculated from fraction of the total elution curve which was mounted for counting.

Mass Spectrograph

F. L. Reynolds

Most of this period has been spent in studies on the behavior of curium samples in the spectrograph. The curium was evaporated from nitrate solutions on either a wolfram or tantalum filament and converted to the oxide. Ions of Cm^{+} and CmO^{+} were obtained from these surface ion sources and the resulting spectra collected on a photographic plate or recorded electronically.

It is interesting that a photographic plate that has been exposed to a Cm^{242} beam retains a considerable amount of curium activity after development. This is not true of most elements, the developing process removes generally all of the active deposit. Alpha track sensitive transfer places can thus detect the position of the Cm^{242} isotope on a plate before or after the development process. For example, many of our exposures showed two lines four mass units apart, which proved to be Cm^{242} and Pu^{238}. The Pu^{238} being present because of the alpha-decay from Cm^{242}. The mass position of Cm^{242} was verified by the above mentioned alpha-transfer technique and also by adding pure Pu^{239} to this mixture.

During the course of this work we have detected the presence of curium isotopes of mass 243 and 244. The curium was separated from a Hanford neutron bombarded Am^{241} sample and introduced in the spectrograph using the above outlined technique. For details, see UCRL 758.

Attempts were made to collect sufficient amounts of Cm^{244} for pulse analysis. Scattering of the Cm^{242} isotope was considerable during this collection run resulting in a rather poor separation for the pulse analysis of mass 244 position. This work will be repeated at a later date.

An enhanced sample of strontium obtained from Oak Ridge gave the following isotopic ratio.

Mass	Percent
84	65.7
86	11.2
87	2.2
88	20.8

Work is in progress to get in operation a G.E. 60° sector spectrograph purchased during the war years. This instrument will be used for more accurate abundance ratio runs on the heavy isotopes.

Shop work is just about completed on the new 75 centimenter radius spectrograph and assembly work is starting on this instrument.

Spontaneous Fission of Cm^{242}

R. L. Shuey

The energy distribution of the fragments resulting from spontaneous fission of Cm^{242} is being studied with the equipment shown in Fig. 1. As indicated, the equipment consists of a double ionization chamber and its associated electronics.

The data has been taken and is in the process of being analyzed. Complete details of equipment and results will be given in a later report. The data analyzed to date indicates to a first approximation no difference between spontaneous and slow neutron fission.

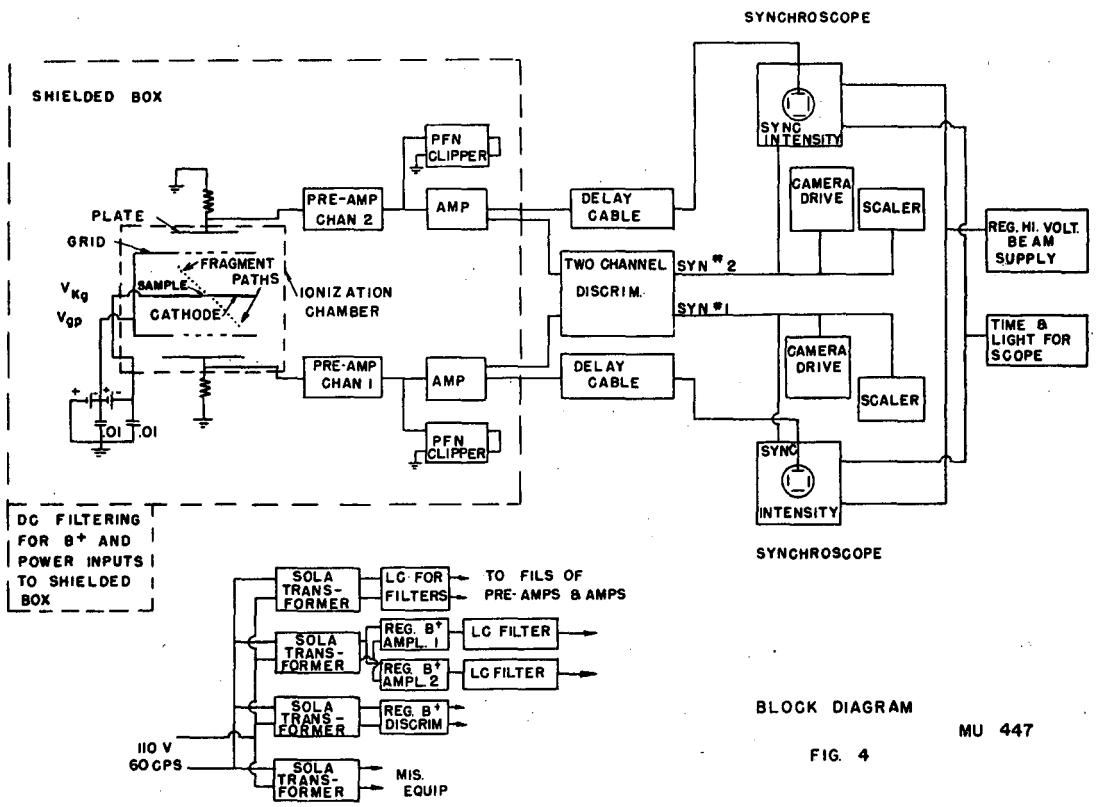

SYNCHROSCOPE
SHIELDED BOX
PFN CLIPPER
PRE-AMP CHAN 2
AMP
DELAY CABLE
SYNC INTENSITY
CAMERA DRIVE
SCALER
REG. HI. VOLT. BEAM SUPPLY
PLATE
GRID
FRAGMENT PATHS
SAMPLE
CATHODE
IONIZATION CHAMBER
V_{Kg}
V_{gp}
TWO CHANNEL DISCRIM.
SYN #2
SYN #1
TIME & LIGHT FOR SCOPE
PRE-AMP CHAN 1
AMP
DELAY CABLE
CAMERA DRIVE
SCALER
.01
.01
PFN CLIPPER
SYNC INTENSITY
DC FILTERING FOR B+ AND POWER INPUTS TO SHIELDED BOX
SYNCHROSCOPE
SOLA TRANS-FORMER
LC FOR FILTERS
TO FILS OF PRE-AMPS & AMPS
SOLA TRANS-FORMER
REG. B+ AMPL. 1
LC FILTER
REG. B+ AMPL. 2
LC FILTER
SOLA TRANS-FORMER
REG. B+ DISCRIM
110 V 60 CPS
SOLA TRANS-FORMER
MIS. EQUIP
BLOCK DIAGRAM
MU 447
FIG. 4

Fig. 1

C. Preparation of Certain Compounds and Related Work

E. Segrè

Astatine

R. F. Leininger

For some time now experiments have been in progress in an attempt to measure the partition ratio of astatine between an aqueous solution of known composition and an organic phase (carbon tetrachloride, benzene, or N-heptane).

The aqueous solution used was 0.1 Molar in sulfuric acid and 0.1 Molar in ferrous sulfate and in ferric sulfate. The function of the iron salts was to establish in the solution a definite oxidation-reduction potential to help override the effects of trace impurities in the reagent grade chemicals used and in the distilled water.

Experiments were made under one set of conditions in an effort to show that a reproducible equilibrium was set up. The results were inconclusive since the partition ratios in a given experiment would be consistent among themselves but would not be reproducible from day to day. The difficulties are the same as those encountered in the chemistry of tracer amounts. To date, partition experiments have been valuable as a tool in qualitative experiments, but have failed to yield useful quantitative data.

Crystal Program

R. F. Leininger

During the three month period, 10 crystals of stilbene weighing 110 grams each have been grown and distributed to various departments of the project.

Plastic Counters

R. F. Leininger

Polystyrene plastics containing from 0-10 percent terphenyl have been prepared and tested as scintillation counters. The 5 percent plastic had a counting rate 100 times less than stilbene under identical conditions of grain, background etc. Further experiments are contemplated.

Lithium Absorber

O. Chamberlain and C. Wiegand

A set of metallic lithium absorbers has been prepared to degrade the 184-inch cyclotron proton beam. The lithium was melted in argon and poured into brass containers with thin stainless steel (0.005 in.) windows at one end and 0.005 in. copper at the other. The set consists of 5 absorbers with a total length of ~110 cm of lithium or~60 g/cm^2.

Differential Chambers

O. Chamberlain and C. Wiegand

Changes have been made in the physical set up of the differential chambers. The electrical system is the same as previously reported. The chambers have been thermostated ($\pm$ 0.1°C) and provision has been made for sensitive measurement and control of the gas pressure in the chambers.

D. Bio-Organic Chemistry Group

M. Calvin and B. M. Tolbert

Synthetic and Experimental Chemistry

M. Calvin, B. Tolbert, P. Adams, R. Bartsch, E. Bennett, R. Bonner, A. Fry, I. Gray, S. Ikeda, E. Jorgensen, B. Neivelt, R. Ostwald and R. Selff

Production Synthesis of Isotopically Labeled Compounds. The following high specific activity production syntheses of labeled compounds for various purposes have been undertaken in the last three months:

1. For the Isotopes Division of the Atomic Energy Commission (the percent yields are estimated values for the particular run)

Compound	Scale of Reaction (millicuries)	Yield, %
Cyclohexanone-2-C^{14}	10	35-48
Oxalic acid-1-2-C^{14}_2	10	30
Cuprous cyanide-C^{14}	10	70
Choline Chloride-methyl-C^{14}	10	50
Acetylcholine chloride-methyl-C^{14}	10	40
Butyric acid-2-C^{14}	10	35-40
Succinic acid-1-C^{14}	10	50
Succinic acid-2-C^{14}	10	30
Isopropyl bromide-methyl-C^{14}	10	30-40
Vinyl acetic acid-1-C^{14}	10	50

2. For the College of Agriculture, University of California Davis. (Prof. Max Kleiber), AEC supported project on the metabolism of C^{14} compounds in cows: Sodium propionate-1-C^{14}, Scale of reaction, 11 mc; sodium propionate-2-C^{14}, 30 mc; sodium butyrate-1-C^{14}, 11 mc; sodium butyrate-2-C^{14}, 30 mc; sodium bicarbonate-C^{14}, 10 mc.

3. For the Division of Medical Physics, Radiation Laboratory. Cholesterol-T^3, 1.3 curies (Dr. John Gofman); sodium bicarbonate-C^{14}, 1.61 mc. (Drs. Sand and Tobias)

Preparation of Alcohols and Halides from Fatty Acids. The preparation of labeled alcohols and, in particular halides, from fatty acids is an important synthetic step in the preparation of labeled compounds. This reaction has been investigated by numerous people and we ourselves have used several methods including lithium aluminum hydride reduction, hydrogenation of similar esters (methyl formate, ethyl acetate, etc.) and the reduction of mixed esters (desired acid plus high molecular weight alcohol). Each of these procedures has a range of molecular weight alcohols for which it is useful, but none of them has been completely satisfactory for the molecules ranging from C_1 to C_5.

In a recent series of patents, direct reduction of mixed heavy metal salts (cadmium, nickel, lead, etc.,) of the high molecular weight fatty acids by high pressure hydrogenation has been described. Since the simplicity of the procedure makes it an excellent possibility for small scale radioactive preparations, the extension and improvement of this method to the lower molecular weight compounds has been investigated.

On the basis of the patent data a mixture of 90 percent cadmium and 10 percent nickel salts by weight was used, and since propyl bromide was a compound of immediate importance the reduction of propionic acid salt of these metals was studied most. Since the fatty acid is normally stored as the sodium salt, this material was first treated with an excess of ion exchange resin to convert it to the free acid, and then an equivalent quantity of fresh precipitated cadmium-nickel hydroxide was added to the aqueous solution and the desired salt obtained by evaporating the solution to dryness.

It was found that at high hydrogen pressure and temperatures the acid was largely converted to propyl propionate. As a novel addition, one-two grams of copper chromite catalyst were added to the salt mixture in the hope that this would permit the reduction of the ester without interfering with the conversion of cadmium-nickel salt to the ester. The results were satisfactory in every case.

Following these preliminary experiments a series of reactions was carried out at varying temperatures, pressures and times to determine the optimum conditions for the preparation. When the reaction was carried out at temperatures from 300-350°C and hydrogen pressure from 2000-2500 psi and a time of one-half to three hours, the hydrogenation product contained from 12-70 percent hydrocarbons, presumably propane; these conditions are similar to those described in the patent. In the temperature range of 250-300°C with a hydrogen pressure of 2500 psi and a heating time ranging up to nine hours, hydrocarbons were still present in the product and the highest yield of propanol obtained was 50 percent.

In an attempt to increase the yield of propanol and, at the same time, keep down the formation of propane, lower temperatures and higher pressures were investigated. It was here that optimum conditions were found for this reduction, namely, an initial hydrogen pressure (cold) of 3500 psi, presence of one-two grams of copper chromite catalyst and a bomb temperature of 225-240°C for a time of nine hours.

The data for a radioactivity preparation are as follows: Sodium propionate-1-C^{14} (1.44 g., 5.5×10^4 dis./min/mg) was converted to the cadmium-nickel salt and mixed with 1.5 g. copper chromite in a high pressure reaction vessel. The vessel was pressured to 3500 psi with hydrogen and heated at 225°C for nine hours with

shaking. The product consisted of 1.14 g. of a solution of 74 percent propanol and water, or a 94 percent yield. This sample was combusted to barium carbonate. The calculated specific activity was 8.9×10^3 dis./min./mg; observed activity, 8.5×10^3 dis./min./mg. A sample of propanol prepared in an identical manner was converted to propyl bromide by treatment with phosphorus tribromide in a yield of 67 percent based on sodium propionate.

By the same method and with the same conditions, except that the temperature of the bomb was raised to 240°C, reductions have been carried out on sodium formate, sodium acetate, sodium butyrate and sodium valerate. The data for these preparations are summarized in Table I.

Halides were prepared from these alcohols by the use of either phosphorus and iodine or phosphorus tribromide. The yields are as follows: methyl iodide, 78-85 percent from sodium acetate; ethyl iodide, 54-72 percent from sodium acetate; propyl iodide, 80 percent from sodium propionate; butyl bromide, 58 percent from sodium butyrate; amyl bromide, 58 percent from sodium valerate. Work is underway to establish the purity of these compounds.

It is felt that the yields of butyl and amyl alcohol from the corresponding fatty acid by this method of preparation could be increased if more time were spent on investigation of the optimum hydrogenation conditions. At present, however, we are only in need of the first three alcohols, so only trial runs were made on the latter two to determine if there was satisfactory reduction. Index of refraction measurements on the halides have shown that isomerization does not occur during the process of this reaction.

Synthesis of Purines Labeled with C^{14}. During the period covered by this report the syntheses of high specific activity guanine-4-C^{14}, 8-aza-guanine-4-C^{14}, adenine-4,6-C^{14} and 8-aza-adenine-4,6-C^{14} have been essentially completed. The synthetic routes used were those described in previous quarterly reports. Adenine and 8-aza-adenine were obtained in approximately 20 percent yield from cyanide, 8-aza-guanine in 50 percent yield and guanine in 40 percent yield.

Using the Kramer-Kistiakowsky method as modified by Loftfield 40 mc of $BaC^{14}O_3$ (1258 mg, 31.8 μc/mg) was converted to aqueous sodium cyanide in 80-85 percent yield. The residue from the generation of cyanide contained approximately 4 mc of a compound which is non-volatile from either acid or base but which can be converted to carbon dioxide by basic hydrolysis.

Thirty-four mmoles of inactive sodium cyanide and 44 mmoles of sodium chloroacetate were added to the sodium cyanide solution and the resulting cyanoacetic acid-3-C^{14} was extracted with ether and esterified with diazoethane. Approximately 1 mc of $C^{14}O_2$ was evolved during the extraction. The ether solution of ethyl cyanoacetate-3-C^{14} was divided in two equal portions, each containing an estimated 17 mmoles (15 mc) of ethyl cyanoacetate. After distillation of the ether and subsequent benzene extraction to remove water, guanine and 8-aza-guanine were prepared from one portion and adenine and 8-aza-adenine from the other as follows:

1. Preparation of Adenine Sulfate-4,6-C^{14} and 8-aza-adenine-4,6-C^{14}: - Ethyl cyanoacetate-3-C^{14} was converted in 75 percent yield to cyanoacetamide by the

TABLE I

Starting Material	Specific Activity	Alcohol Product	Yield of Alcohol from RCO_2Na	Obs. Activity of $BaC^{14}O_3$ from Combustion of Alcohol	Calculated Activity of $BaC^{14}O_3$
$HC^{14}O_2Na$	2.33×10^4 dis/min/mg	$C^{14}H_3OH$	83-88%	7.19×10^3 dis/min/mg	7.93×10^3 dis/min/mg
$CH_3C^{14}O_2Na$	2.2×10^4	$CH_3C^{14}H_2OH$	70-75%	4.36×10^3	4.57×10^3
$CH_3CH_2C^{14}O_2Na$	5.5×10^4	$CH_3CH_2C^{14}H_2OH$	94%	8.5×10^3	8.9×10^3
$CH_3CH_2CH_2C^{14}O_2Na$	2.00×10^4	$CH_3CH_2CH_2C^{14}H_2OH$	55-70%	2.54×10^3	2.7×10^3
$CH_3(CH_2)_3C^{14}O_2Na$	1.37×10^4	$CH_3(CH_2)_3C^{14}H_2OH$	55-65%	1.68×10^3	1.72×10^3

action of aqueous concentrated ammonium hydroxide; the resulting cyanoacetamide was treated with phosphorus pentachloride to yield malononitrile-1-C^{14} which was subsequently reacted with benzenediazonium chloride in an acetic acid-sodium acetate buffer to yield 1.70 g. (67 percent) of phenylazomalononitrile-1-C^{14}. The reaction of this product with formamidine in sodium butoxide-butanol solution produced 1.60 g. of 4,6-diamino-5-phenylazopyrimidine, 80-85 percent pure (70 percent yield corrected for purity).

The crude 4,6-diamino-5-phenylazopyrimidine was produced by the action of powdered zinc in glacial acetic acid to 4,5,6-triaminopyrimidine and isolated as the sulfate; 1.23 g. (80 percent) was obtained. The triaminopyrimidine sulfate (608 mg) was converted by the action of sodium nitrite in an acetic acid-sodium acetate buffer to 8-aza-adenine-4,6-C^{14}. After decolorization and recrystallization, 270 mg of 8-aza-adenine was obtained. Yield: 80 percent or 20 percent from cyanide. Specific activity: approximately 6.2 μc/mg. The ultraviolet adsorption spectrum of the compound at pH 2 agrees with that of an analytically pure sample (E_{max} 10,200 at 264. E_{min} 3000 at 230), and chromatography on paper using butanol-propionic acid-water indicates only one compound visible by means of ultraviolet light and only one spot visible on a radiogram.

The 4,5,6-triaminopyrimidine sulfate (612 mg) was heated at 160°C with formamide and formic acid to prepare adenine. After removal of the formamide under reduced pressure, 340 mg (69 percent) of adenine sulfate-4,6-C^{14} (4.4 μc/mg) was obtained. By addition of inactive adenine, 84 mg additional adenine sulfate-4,6-C^{14} (2.6 μc/mg) was isolated. The total yield was 80 percent or 20 percent from cyanide. The ultraviolet adsorption spectra of the fractions agree with the literature and authentic samples, and only one spot visible by ultraviolet light is obtained upon filter paper chromatography using butanol-propionic acid-water as the solvent system. A radiogram also indicates the presence of only one radioactive compound.

2. Preparation of Guanine-4-C^{14} and 8-aza-guanine-4-C^{14}: - The second portion of ethyl cyanoacetate-3-C^{14} was condensed with guanidine in sodium methoxide-methanol solution. The methanol was removed and the syrup remaining was dissolved in a sodium acetate-hydrochloric acid solution and diazotized with sodium nitrite. The rose-red precipitate of 2,4-diamino-5-isonitroso-6-hydroxypyrimidine was filtered and reduced directly by the action of sodium hydrosulfite in an approximately neutral solution. The 2,4,5-triamino-6-hydroxypyrimidine was isolated as the sulfate, 2.65 g., 50 percent yield from cyanide. By the addition of inactive 2,4,5-triamino-6-hydroxypyrimidine sulfate to the filtrate, 338 mg of additional 2,4,5-triamino-6-hydroxypyrimidine sulfate (1.9 μc/mg) was isolated.

2,4,5-triamino-6-hydroxypyrimidine sulfate (1.36 g., specific activity 3.2 μc/mg) was converted to 8-aza-guanine-4-C^{14} by the action of sodium nitrite in a sodium acetate solution. After treatment with Norite and recrystallization, 750 mg (93 percent) of 8-aza-guanine-4-C^{14} was obtained. From 338 mg of 2,4,5-triamino-6-hydroxypyrimidine sulfate (1.9 μc/mg), 173 mg of 8-aza-guanine-4-C^{14} was obtained. The total overall yield of 8-aza-guanine from cyanide was 52 percent. The ultraviolet adsorption spectra of all fractions indicate that they are essentially pure and they all exhibit only one visible fluorescent zone after chromatography on paper with either butanol-propionic acid-water or butanol-diethylene glycol-water in an ammonia atmosphere. Radiograms indicate that less than 3 percent of the activity remained at the origin as an impurity.

2,4,5-Triamino-6-hydroxy-pyrimidine sulfate (1.29 g., specific activity 3.2 μc/mg) was refluxed with formic acid and sodium formate, and after removal of the formic acid at reduced pressure the product was recrystallized from dilute hydrochloric acid to yield 924 mg (82 percent) of guanine hydrochloride dihydrate (3.6 μc/mg). The ultraviolet spectrum obtained indicates that it is essentially pure. Paper chromatograms have not yet been completed.

Synthesis of Sodium Formate-C^{14} and Methyl Iodide-C^{14}. In an earlier section of this report the conversion of sodium formate-C^{14} to methyl iodide-C^{14} in 78-85 percent yields was described. Since sodium formate can be made in good yields from $C^{14}O_2$ by the high pressure reduction of potassium bicarbonate in the presence of a palladium-charcoal catalyst, the possibility of use of this method as a good route to the formation of pure methyl iodide has been investigated.

A simplified procedure was tried as follows: A solution of a known amount of potassium hydroxide was added to the catalyst in the stainless steel bomb. The bomb was then closed, connected to the vacuum line and a quantity of $C^{14}O_2$ equivalent to that of the potassium hydroxide distilled into it. Various catalyst preparations were used with yields ranging from 32-84 percent; the highest yield was obtained with commercial 30 percent palladium-charcoal. By adjusting the pH of the solution after the absorption of the $C^{14}O_2$ this yield could be increased by about 5 percent. This would correspond to an overall yield of 70-76 percent of methyl iodide based on $C^{14}O_2$. This is as good as can be done by the use of lithium aluminum hydride and the yields are much more consistent than those obtained by high pressure reduction of $C^{14}O_2$ to methanol. Since the product should be of an unambiguous nature, that is, no solvents enter into the reaction which are capable of introducing spurious ethyl or butyl iodides, it is felt that this may prove to be a very satisfactory procedure.

Synthesis of Stilbamidine-amidine-C^{14}. A high specific activity preparation of stilbamidine has been started to make available more material for further multiple myeloma experiments on human patients. As the initial phase of this reaction 25 mc of $BaC^{14}O_3$ have been converted to potassium cyanide via the method of Kramer and Kistiakowsky as modified by Loftfield, and this product has then been converted to cuprous cyanide in an overall yield of 63 percent based on $C^{14}O_2$. This material has been sent to Dr. J. C. Reid, National Cancer Institute, Bethesda, Maryland, for the next phase of the preparatory work.

Synthesis of Cyclohexanone-2-C^{14}. The synthesis of this compound by the Tiffeneau ring expansion has been described in previous quarterly reports. Although the 2,4-dinitrophenylhydrazone derivative of this compound had been obtained, the purity of the product was not certain. Index of refraction measurements showed it to be contaminated with an impurity, probably ether.

Therefore, another synthesis was completed using low activity C^{14}. The product was obtained in 48.5 percent yield (One ml with an index of refraction, n_D^{20}, 1.444; commercial material n_D^{20}, 1.4520). By a group of indices of refraction the product appeared to be 92 percent cyclohexanone and 8 percent diethyl ether.

In an attempt to prove this, isotope dilutions were made on the product and the 2,4-dinitrophenylhydrazone derivative of each mixture obtained and analyzed. The derivatives were combusted to $C^{14}O_2$ and counted as $BaC^{14}O_3$. The purity was

determined by this method to be 91 percent, which checks well with the results from the index of refraction measurements.

As an intermediate for the synthesis of ring-labeled toluene some of the cyclohexanone was converted to 1-methyl-cyclohexanol-1. The product was obtained in 81 percent yield by the reaction of the ketone with methylmagnesium iodide. This compound can now be dehydrated and dehydrogenated to toluene. However, because of the low overall yield from $BaC^{14}O_3$ to toluene, further work on this project has been temporarily discontinued.

Synthesis of Vinyl Acetic Acid-1-C^{14}. The synthesis of this compound via allyl cyanide has been described in the last quarterly report in approximately 37 percent yield. The high specific activity preparation of this material proved unsuccessful; a quantity of oily polymer only was obtained. It was felt a better plan to try some alternate methods of preparation of this compound rather than work further with the cyanide method.

Carbonation of the allyl Grignard reagent made from the allyl chloride by Gilman's technique has been described in the literature with very poor yields. However, this reaction was tried with greatly modified reagent ratios and the results proved surprisingly satisfactory. Yields of 75-80 percent of vinyl acetic acid from $C^{14}O_2$ on a 1-3 mmole basis were obtained. In this procedure 1 mmole $C^{14}O_2$ is distilled into 1.1 mmole Grignard reagent. The mixture is then warmed to Dry Ice-isopropyl alcohol temperature and occasionally shaken over the period of one hour. The acid may be recovered either by extraction into ether or chloroform with subsequent re-extraction into an equivalent amount of 1 N sodium hydroxide, or by steam distillation.

Samples from warm runs when chromatographed in one dimension with a solvent consisting of 100 cc 95 percent ethanol plus 1 cc concentrated ammonia, produced but one spot as detected by spray tests. The product is probably, however, contaminated with a small amount of crotonic acid.

Cyanhydrin Sugar Synthesis. The synthesis of glucose labeled with C^{14} has been an important problem but the reaction has not been very successful, particularly in small-scale preparations. As a new modification a number of techniques have been combined to make a workable sugar synthesis starting with labeled potassium cyanide on a 1 mmole scale. The procedure is as follows:

One mmole of potassium cyanide in 5 cc of water is mixed with 1 mmole arabinose in 5 cc of 0.07 N acetic acid and left overnight in a stoppered flask. The mixture is dried at low temperatures and 5 cc of methanol and dry benzene plus 100 λ concentrated sulfuric acid are added and the mixture refluxed in a Soxhlet extractor containing anhydrous calcium sulfate for an hour to simultaneously hydrolyze the nitrile and esterify the sugar acids. Most of the solvent is next boiled off, 2 cc of water added and the mixture transferred to the reduction flask; the latter consists of a 10 cc Erlenmeyer flask equipped with two side wells in which micro pH electrodes are placed. The whole is immersed in an ice bath on a magnetic stirrer. About 3 mmoles of 2 percent sodium amalgam are next added and with continuous stirring the pH is maintained at 3-3.5 by the addition of 1 N sulfuric acid. After the reduction is complete the solution is deionized by passage through ion exchange resins and concentrated at low temperatures. On the basis of paper chromatography the radiocyanide went only to mannose and glucose.

Preliminary yield data are not yet complete, and work is now in progress on a satisfactory method for the separation and recovery of the starting sugar and the labeled mannose and glucose. It is planned to use paper columns.

Carboxyl-labeled Amino Acids. The preparation of all the amino acids labeled in the carboxyl group is of some interest, particularly for analytical purposes. Therefore, the possibility of using the Strecker synthesis on a number of readily available aldehydes with labeled cyanide has been investigated.

An exploratory run of alanine was made using acetaldehyde, ammonium chloride and hydrogen cyanide. A yield of 10 percent was obtained, and a second experimental synthesis of valine was undertaken starting with isobutyraldehyde. Some refinements have been made in the purification procedures, but final yield data are not yet available.

Synthesis of Valine-Methyl-C^{14}. A high specific activity synthesis of valine labeled in the methyl group was undertaken by the reaction of labeled isopropyl bromide with acetamidomalonate, as described in the last quarterly report. The preparation was unsatisfactory, apparently due to impurities and questionable yields of the isopropyl bromide. A small amount of material with a specific activity of 0.25 μc/mg was obtained, however. This preparation is now awaiting a better synthesis of the halide.

Synthesis of Diethylmalonate-2-C^{14}. A low activity run of this compound described in the last quarterly report has been completed as well as a check low activity preparation. The yields were determined by saponifying a weight sample of the product before all the low boiling material was removed by vacuum distillation. Yields of 49 percent and 42 percent based on sodium acetate-2-C^{14} were obtained, or 25-30 percent based on the $C^{14}O_2$ used to begin the synthesis. After removal of the low boiling ether, the following data were obtained. Specific activity: observed, 438 dis/min/mg; calculated, 434 dis/min/mg. Anal: Observed: C, 50.61 percent; H, 7.37 percent. Calculated: C, 52.49 percent; H, 7.55 percent.

Synthesis of Myristic Acid-1-C^{14}. The standard method of Grignard carbonation has been followed in the preparation of this C^{14} fatty acid. Six mmoles of the Grignard were reacted with 3 mmoles of $C^{14}O_2$. The carbonation was allowed to proceed for one and one-half hours. The yield based on titration was 98.8 percent and that based on radioactivity was 92 percent.

Synthesis of Labeled Dicarboxylic Plant Acids. The high specific activity preparation of malic acid-1-C^{14} and succinic acid-1-C^{14} in progress at the time of the last quarterly report was apparently attacked by microorganisms, for a chromatogram of the non-crystallizing oil showed almost a dozen unidentifiable spots. The ordinary methods of distribution between water and ether failed to separate the malic and succinic acids and would not effect a purification to the point of crystallization. This is not the first time we have had difficulty with microorganisms attacking small amounts of organic materials, although amino acids usually are more susceptible. It indicates again the extreme care needed with small-scale syntheses in many unusual aspects.

A repeat of this run is now in progress and has shown the following yields: Sodium acetate-1-C^{14}, 98 percent and ethyl acetate-1-C^{14}, 98 percent. Seventy-five percent of the theoretical uptake of hydrogen has been observed and the product is now being separated by fractional extraction.

To produce a high yield of succinic acid and avoid the tedious separations of malic and succinic acids, a low activity run is in progress attempting the hydrogenation of ethyloxalacetate completely to succinic acid.

Experimental Chemistry.

1. Decomposition of Oxalic Acid. High specific activity oxalic acid-1,2-C_2^{14} has been prepared in 50.8 percent yield with 15 percent CO_2 recovery by the reaction of CO_2 with potassium in the presence of sand at 360°C. A portion of this $H_2C_2O_4$ is to be used in a study of the isotope effect in the decomposition of $H_2C_2O_4$ with sulfuric acid. Using unlabeled $H_2C_2O_4$ the decomposition has been carried out at temperatures ranging from 35-105°C in 100 percent sulfuric acid. The reaction is rapid enough so that it goes nearly to completion in four days at 35°C and is extremely rapid at 100°C. The rate of decomposition is very sensitive to the sulfuric acid concentration. Attempts are in progress to obtain a satisfactory carbon material balance. So far, the amount of carbon monoxide recovered has not been quantitative, but it is hoped that a better combustion procedure will eliminate this difficulty.

2. Isotope Effect in the Decarboxylation of Labeled Malonic Acids. All attempts to prepare diphenylmalonic acid and α-naphthylmalonic acid by the replacement of halogen by cyanide in the corresponding acetic acids have so far ended in failure. However, a new approach seems to offer promise of success. Both diethyl diphenylmalonate and diethyl α-naphthylmalonate have been prepared in good yields by condensation of the corresponding ethyl acetates with diethyl oxalate and subsequent decarboxylation. The hydrolysis and isolation of the substituted malonic acids is to be attempted in the near future.

3. The Dakin-West Reaction. A study of the Mechanism of the Base Catalyzed Formation of Acetylated Amino Ketones from Acetic, Anhydride and Amino Acids. The reaction of amino acids with acetic anhydride in the presence of pyridine or sodium acetate results in the fomation of an acetylated amino ketone and the evolution of carbon dioxide.

$$RCHNH_2CO_2H \xrightarrow[\text{pyridine}]{\text{Acetic anhydride}} R\text{-}\underset{\displaystyle NH\text{-}C\text{-}CH_3}{\overset{\displaystyle O}{\overset{|}{C}}} - \overset{\displaystyle O}{\overset{\|}{C}} - CH_3 + CO_2$$

There is some question in the literature as to whether the evolved carbon dioxide comes from the carboxyl group of the amino acid or of the acetic anhydride.

Carboxyl-labeled phenylalanine has been subjected to this reaction and the resulting carbon dioxide and acetylated amino ketone have been analyzed for radioactivity, and within the experimental errors the acetylated amino ketone is completely inactive and the carbon dioxide has the same specific activity as the carboxyl carbon of the phenylalanine. More refined measurements are in progress, and the reaction is also to be carried out using carboxyl-labeled alanine.

Biological Chemistry

M. Calvin, E. Bennett, J. Campbell, L. Daus, I. Gray, M. Gordon, S. Ikeda, M. Kirk, D. Kritchevsky, J. Krone, B. Krueckel, M. Lazarus and J. Weaver

Synthesis of Potential Cancer-Inhibiting Agents: 2,6-Diamino- [3',2'-h] -Thiazolinopurines. Various investigators have reported that the concentration of nucleic acids in tumor-bearing animals is greater than in normal animals. From these results it has been postulated that purine inhibitor, specifically adenine and guanine, could be found which would readily retard tumor growth. The adenine inhibition of 2,6-diaminopurine, which was originally postulated by Brown to be an intermediate in the biosynthesis of guanine, has been described. Kidder has shown the antimetabolite action of 8-aza-guanine (guanazolo), and from which results it was postulated that all tumor cells may differ from ordinary cells in that the former metabolize guanine and the latter do not. More recent work as well as the earlier studies show that guanine utilization is subject to species specificity.

In our work it was thought to be of interest to prepare a series of purine analogs in which ribylation at the 7 and 9 position is blocked and in which ribylation might take place at other places in the molecule in order to find compounds of greater cancer inhibiting action than those described above.

The synthesis of the purine and purine analogs which has been undertaken is detailed in previous quarterly reports, but a summary of this work is presented as follows:

1. The following new derivatives of 2,6-diaminopurine have been prepared: 8-mercapto-,8-acetonylmercapto-, 8-carboxymethyl-mercapto-, and 8-carbethoxymethylmercapto-2,6-diaminopurine.

2. The synthesis of 2,6-diamino-4'-methyl- [3',2'-h] - thiazolinopurine has been described and a route to 2,6-diamino- [3',2'-h] - thiazolinopurine has been indicated.

3. 2,6-Diamino-8-hydroxypurine has been prepared by an improved procedure.

4. Ultraviolet spectra of the above compounds have been measured in acid, neutral and alkaline solution.

Amino Acid Content of Serum Protein from Normal and Atherosclerotic Patients. In an experiment to see whether any correlation could be found between the blood serum, amino acid content and the lipoprotein S13 content (see Medical Physics reports, Dr. John Gofman, etal) a series of about fifty paper chromatograms were run.

The serum protein was precipitated with 80 percent alcohol, the supernatant evaporated and extracted with ether to remove the lipids, the residue made acid and the amino acids dissolved out in 95 percent alcohol. The amino acid extract was then analyzed by two-dimensional paper chromatography using phenol-water and butanol-propionic acid-water mixtures. The resultant ninhydrin reaction spots were compared with a standard amino acid chromatogram. The expected amino acids were found in both S13 positive and S13 negative samples and no noticeable difference

in quantity of any individual amino acid was detectable.

The Metabolism of Simple Fatty Acids by in vitro Liver Slices. The studies of the metabolism of labeled sodium propionate, acetate and bicarbonate when incubated with mouse liver slices have continued.

In an attempt to increase CO_2 fixation when bicarbonate is incubated with liver slices an alternate medium containing glucose was tried. Less $C^{14}O_2$ was fixed, however, and the same products as observed in a previous experiment were found, namely, alanine, aspartic and glutamic acids and glucose. The major product from these bicarbonate incubations has now been identified and shown to be urea.

In the incubation of labeled propionate and acetate with liver slices a considerable amount of activity is found in a series of unknown spots which are tentatively identified by paper chromatography as hydroxy acids. Since the position of these molecules does not correspond to any of the known α-hydroxy acids, a number of the β-hydroxy acids and related compounds have been synthesized and chromatographed to check the identity of the unknown products. These compounds include β-hydroxybutyric, β-hydroxypropionic, β-hydroxycaproic, pyrrolidone carboxylic and pyrotartaric acids.

Using data from this series of compounds it has been found that the major acid product from acetate metabolism corresponded on a two-dimensional paper chromatogram to β-hydroxybutyric acid. However, none of the major products from the propionate incubation appeared to coincide with this material or the other synthesized substances.

Adequate separation of a number of the liver metabolites in paper chromatography has not been achieved with the solvents mentioned above. Therefore, the solvent system propanol-ammonia-water has been investigated in an attempt to achieve greater spread of the radioactive spots. A series of unlabeled acidic compounds was run in this solvent system, and it was found that dicarboxylic acids have R_F values of less than two-thirds their corresponding R_F values in an acid solvent while monocarboxylic acids have much the same R_F values in both systems. This enables one to obtain an excellent separation of the mono- and dicarboxylic acids, particularly lactic and succinic, which are difficult to separate. Other advantages include sharper chromatograms (less streaking) and decreased loss of volatile acids.

Among other phases of this project being worked on at present are attempts to prepare derivatives by acetylation and benzoylation of several suspected hydroxy acids.

Detection of Tritiated Compounds in Paper Chromatography. Location of tritiated compounds in paper chromatography has posed a problem because of the difficulties involved in the detection of the active spots. Because of the low energy of its emitted β-rays, the assay of tritium has usually been done in the gaseous phase; this procedure does not lend itself to the location of tritiated compounds on paper. A possible method, which was tried in this laboratory, involves the cutting of the paper into squares and counting each square in a windowless counter such as the "Nucleometer". This method is disadvantageous since it renders the paper useless

for further work. A counting tube has been developed which will measure the radiation from the spots in question easily and accurately.

The counting apparatus consists of a windowless, bell-shaped GM tube of about 1 inch in diameter, a gas flow regulator and a source of helium, saturated with alcohol at 0°C. The tube is wrapped in lead foil to minimize background radiation and to add ballast so a constant resistance to gas flow through the paper is maintained.

A rate of gas flow of 300 ml/min. was maintained. This rate insured a reproducible counting rate. At this pressure the system showed the following characteristics:

Starting voltage	1125 volts
Threshold voltage	1200 volts
Plateau	200 volts
Optimum operating voltage	1350 volts.

For scanning, the tube is allowed to rest on the paper and is slowly moved over the paper in a definite pattern. The location of activity is recorded on a scaler. Where a particular area is to be counted the tube is flushed for at least thirty seconds, after which the scaler is turned on and the activity determined.

This apparatus has been used to scan and count papers holding either C^{14} or tritium containing compounds. Table II summarizes the results obtained using various types of counters. For counting in the "Nucleometer", papers were cut into appropriately sized pieces.

Once the active spots have been located on the paper, it is desirable to make a radiogram of the paper in order to have an accurate outline of the active area. The low energy of tritium radiation (0.015 Mev) and the high self-absorption make this difficult. We have found, however, that a satisfactory image of an area containing a tritiated compound is obtained on Eastman "No-Screen" x-ray film in 24 hours, if the area has an activity of 15,000 counts/min/cm^2 as recorded by the windowless tube. (C^{14}, 0.16 Mev, requires a recorded activity of about 200 counts/min/cm^2 in order to give a satisfactory image in 24 hours.)

Paper Chromatography of Fatty Acids. The straight chain fatty acids from C_1 to C_7 have been separated by paper chromatography using two different solvents. These are 60 parts propanol, 30 parts concentrated ammonia and 10 parts water, or 60 parts pyridine, 20 parts concentrated ammonia and 10 parts water. The C_1 to C_5 acids can be separated from each other completely, but in the case of the C_5 to C_7 acids only partial separation has been accomplished.

Many solvents and solvent systems have been tried in an attempt to separate the higher fatty acids, in particular palmitic and stearic acids. In cases where separation was accomplished, streaking of the spots interfered with the separation, and in cases where there was no streaking no separation occurred. The following solvent systems were tried, all in varying mixtures: pyridine-ethyl acetate-water; water-ammonia-ethanol; and water-concentrated ammonia with each of the following organic materials: butanol, propanol, amyl alcohol, ethanol, lutidine, piperidine,

TABLE II

Sample	Windowless Tube (All data as counts per minute x 64)	"Nucleometer" (All data as counts per minute x 64)
Tritiated cholesterol on paper	191;193;193	173;201;133[a]
Tritiated cholesterol on glass plate	118;119;115	210;191;172[a]
Tritiated cholesterol on aluminum plate	47;47;48	93;94;93
Stearic acid-1-C^{14} on paper	95;93;97	164;168;173

a. Very erratic and unsteady counting.

methylamine, dimethylamine, trimethylamine and tetramethyl ammonium hydroxide.

Quilon treated Whatman No. 1 filter paper and a silica gel treated Whatman filter paper gave no better results than ordinary filter paper.

Utilization of the Branched Chain of Isobutyric Acid Studied with C^{14}. In a previous quarterly report the synthesis of sodium isobutyrate-1-C^{14} and sodium isobutyrate-3-C^{14} has been described. In a series of biological experiments with these materials about 0.075 mg/g body weight of the sodium salt has been injected into a 200 g Curtis-Dunning rat. The breath samples were collected at intervals and the urine and feces were collected after five hours when the animal was sacrificed. The liver was removed, ground in a mortar and fractionated to obtain the total lipid, amino acid, protein and glycogen fractions.

In the case of the carboxyl-labeled material 80-85 percent of the injected activity was excreted in the breath as $C^{14}O_2$. In the case of the methyl-labeled isobutyrate 45-50 percent of the activity was excreted in the breath. Both of these were over the period of five hours. Based on the specific activity of the material injected, the ratio of the specific activities of the C^{14} excreted should be 1.82; experimentally it was 1.76. However, the initial rate of excretion of $C^{14}O_2$ from the animal given the sodium isobutyrate-1-C^{14} was greater than that of the animal given the sodium isobutyrate-3-C^{14}.

The difference in these rates together with the amount of radioactivity incorporated in the various fractions of tissues (See Table III) indicates that isobutyrate is degraded to CO_2 and a three-carbon fragment. This may proceed in either or both of two ways: (1) The direct decarboxylation of the isobutyrate to CO_2 and acetone; (2) by β-oxidation of the acid to a substituted malonic acid followed by decarboxylation to give CO_2 and propionic acid.

Based on the study of the intermediates together with a study of the results listed in Table III, the following scheme is postulated:

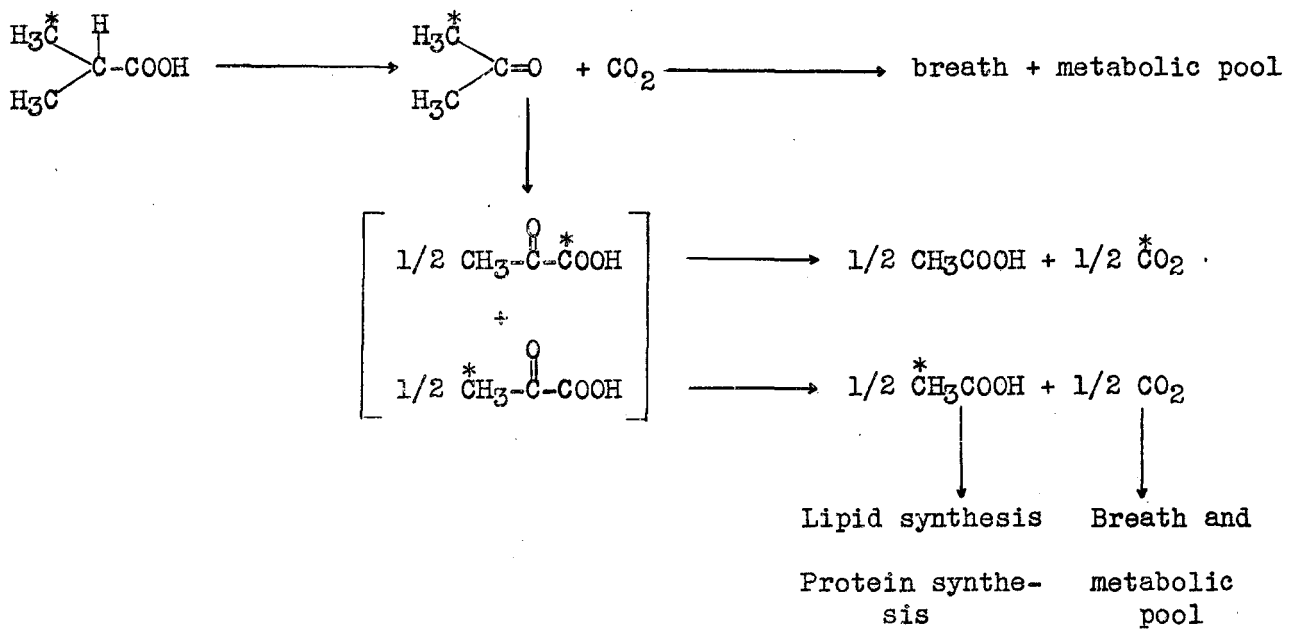


TABLE III

C^{14} Content of Tissues and Liver Fractions of Rats Injected with Sodium Isobutyrate-1-C^{14} and Sodium Isobutyrate-3-C^{14} Compound Injected **

Fraction	Sodium Isobutyrate-1-C^{14}		Sodium Isobutyrate-3-C^{14}	
	Specific Activity dis/min/mg dry Tissue	% of Injected Dose	Specific Activity dis/min/mg dry Tissue	% of Injected Dose
Breath	3700.	87.	2100.	47.
Urine	10170.	1.48	3350.	0.25
Total lipid of liver	186.	0.11	960.	0.4
Total fatty acids of liver	--*	--*	650.	0.3
Non-saponifiable	--*	--*	650.	0.03
Liver Protein	110.	0.32	562.	1.41
Liver glycogen	926.	0.65	300.	0.07

* The specific activity was so low as to make these values insignificant.

** These results are the average of three rats for each compound. Individuals did not vary more than 5 percent from the mean.

Paper Chromatography of Cholesterol. In the last quarterly report preliminary experiments on the paper chromatography of cholesterol were mentioned. This work has now been extended as follows:

Using tritiated cholesterol, the active spots on paper were determined by cutting the paper into appropriately sized squares and counting each in a "Nucleometer". In later experiments direct counting was used. In butanol-propionic acid-water mixtures the cholesterol moved with the front in every case. Using alumina impregnated paper and solvents such as petroleum ether and benzene, it was noted that the material had a tendency to streak and that the results were not duplicable. In general, the most active spots were near the solvent front. Using propylene glycol saturated paper and a solvent consisting of toluene saturated with propylene glycol the activity was all found at the front.

The most promising experiments have been carried out with paper which has been treated with a waterproofing agent ("Quilon", a DuPont product . . . stearato chromic chloride). The solvents used have been aqueous alcohols of various dilutions. With ethanol, the solutions which were 60 percent alcohol or less did not move the material from the origin. Using 70 percent and 80 percent ethanol, the active spots were roughly half way between the origin and the front, but the counts showed streaking. Using 80 percent, 85 percent, 87-1/2 percent and 90 percent propanol, the streaking seemed to be cut down, with the activity closer to the front. In one experiment, the propanol was diluted with water which had first been saturated with benzene. In this case the streaking was cut still further, but the spots moved even closer to the front. This indicates a procedure for future experiments.

Incorporation of Radioactivity from Sodium Isobutyrate-3-C^{14} in Cholesterol. The isolation of labeled cholesterol from the carcass of a rat fed sodium isobutyrate-3-C^{14} has been attempted. The sodium salt was injected intraperitoneally into a rat and after keeping the animal in a respiration cage for five hours it was sacrificed, certain organs extirpated and the carcass homogenized and lyophilized. An aliquot of the carcass was thoroughly extracted with ether and the ether aliquot separated into lipids and free fats. The lipids were separated into saponifiable and non-saponifiable fractions. From the latter fraction, there was recovered 44 mg of cholesterol, m.p. 144-5°, identical with known material as shown by infra red spectrum. The specific activity of the cholesterol is 0.03 μc/mg. About 10 percent of the total activity seems to be in the non-saponifiable fraction.

An attempt is being made to partially degrade the cholesterol by the method of chromic oxide oxidation to give oxidation of the side chain. A total of one g. of material was used. The yields of oxidation products are very poor (Ruzicka obtained a yield of about 1 percent of the androsterone derivative, as did Fernholz and Wallis). The oxidation is still under way and no results are available at present.

Incorporation of C^{14} in Eggs from a Chicken Fed Labeled Acetate. In order to prepare a number of biologically synthesized compounds an exploratory experiment on the feeding of a chicken large quantities of radioactivity in the form of sodium acetate-1-C^{14} has been undertaken.

In this experiment a chicken was fed 9.55 mc of sodium acetate-1-C^{14} continuously with its feed over a period of ten days and the eggs laid during this

period were collected. The chicken was then sacrificed and the ovary and its contents obtained. Two large yolks, No. 8 and No. 9, were obtained, and some smaller ova were cut out and combined and the remainder of the ovary kept as such. The eggs were all separated, lyophilized and stored, the shells were dried and pulverized, the ova were homogenized and lyophilized as was the ovary.

For counting, the separate portions were burned, the carbon dioxide precipitated as barium carbonate and plated. The data are summarized in Table IV.

The totals of recovered radioactivity are as follows:

Yolks and ova	495.	μc	or 5.2 percent
Whites	43.	μc	0.45 percent
Shells	116.	μc	1.21 percent
Ovary	10.5	μc	0.11 percent

The total recovered activity was 665. μc, corresponding to 6.9 percent.

The yolks will be worked up to recover the cholesterol and phospholipids. The other portions of the egg will be stored temporarily.

TABLE IV

Date	Egg No.	Yolk		White		Shell	
		gm	μc	gm	μc	gm	μc
4/29	1	8.56	1.2	3.35	0.2	6.50	4.8
4/30	2	8.61	32.25	3.27	3.25	6.22	16.9
5/2	3	8.81	32.9	4.40	8.30	6.95	16.0
5/3	4	8.48	75.35	4.08	6.75	6.13	33.4
5/5	5	8.30	24.45	4.30	11.25	6.35	20.5
5/6	6	8.37	110.8	3.54	8.1	6.11	13.7
5/7	7	9.33	113.45	4.21	5.25	5.00	10.7
	8	5.85	64.8	Eggs from ovary, no whites or shells			
	9	3.25	30.45				
	Ova	1.43	9.36				
	Ovary	2.44	10.50				

Photosynthesis Chemistry

M. Calvin, A. Benson, J. Bassham, E. Bissell, M. Goodman, C. Ouellett
L. Schou, V. Lynch, W. Stepka and N. Tolbert

The identity, amounts and rates of formation of the intermediates of CO_2 reduction during photosynthesis are being determined as a part of a general program of study of this fundamental energy transfer process. The initial work of the group was directed towards the proof that CO_2 fixation and reduction by plants does not itself involve photochemical processes. Since this has been demonstrated, attention has been directed toward the next step in the overall photosynthetic process, namely the utilization of the light produced reducing energy to reduce the fixed CO_2 to carbohydrates.

The study of the reduction processes of photosynthesis should be considered a general biochemical problem. The identity and sequence of intermediates in photosynthesis have been found to be similar in all plants, monocotyledons and dicotolydons, algae, and photosynthesizing bacteria so far studied. In each case the nature of the primary carboxylation process, as well as that of subsequent intermediates, appears to be comparable if not identical.

The Intermediates of Sucrose Synthesis. It was observed that sucrose is the first free sugar to appear in plants during timed $C^{14}O_2$ fixation experiments and that glucose and starch appear subsequent to it. Also, it has been previously demonstrated that the first stable product of $C^{14}O_2$ fixation is phosphoglyceric acid. The intermediate compounds in sucrose synthesis during photosynthesis are not yet known and are being investigated. Since phosphoglyceric acid is the first product, the compounds of the established glycolytic sequence were investigated as possible intermediates. These intermediates are largely present as their phosphates in plants and they have been partially separated by two-dimensional paper chromatography. The separated phosphates have been eluted with water from the paper chromatogram and hydrolyzed by a crude malt phosphatase preparation to give free sugars for rechromatography and identification. No free fructose or trioses have been observed except as a result of hydrolysis during analysis or as a result of apparent phosphatase action during the killing process.

The results of phosphatase hydrolysis of the phosphate intermediates of photosynthesis have been in agreement with identifications previously reported from this laboratory. Hydrolysis of the triose phosphates has given free trioses; hydrolysis of hexose monophosphates gives a mixture of fructose, glucose and an unidentified sugar. In the previous report, this unknown was identified as mannose, but this has been shown to be in error. It has been suspected to be D-sorbose but an authentic specimen for comparison is not yet available.

The importance of this unknown sugar is suggested by it predominance in the hydrolysis products of very short photosynthesis. When barley leaves are exposed to one second of steady state photosynthesis in $C^{14}O_2$, 85 percent of the C^{14} fixed is found in glyceric acid or its phosphates. Of the remaining phosphates, about 60 percent of the radioactivity is unknown, 10 percent in fructose, 20 percent in trioses and a negligible amount in glucose phosphates. When the time of steady state photosynthesis with $C^{14}O_2$ is increased to one minute and the hexose monophosphates are hydrolyzed, the percent C^{14} in the sugars is in the reverse order from

that of very short periods. Then glucose contains more C^{14} than fructose and fructose contains more than the unknown. The identification of this unknown sugar is under investigation.

In order to visualize the mechanism of synthesis of the hexose molecule, the distribution of radiocarbon within the hexose has been examined in several experiments and found to correspond to that found in the C_3 compounds, phosphoglyceric acid. A collection of the pertinent degradation results to date is given in Table V.

Further Studies on Phosphorylated Intermediates. Differences in products have been observed to be dependent on the manner of stopping a photosynthesis experiment. Thus, the possibilities of enzymatic dephosphorylation occurring during the killing process must be considered. With leaves of higher plants, a considerable amount (sometimes major) of free glyceric acid has been observed. In these cases appreciable hydrolysis products of triose and hexose phosphates have been observed. The correlation between killing method and results was recognized and has been tested in the following experiment.

Four one-gram bundles of barley leaves were allowed 60 seconds of steady state photosynthesis in $C^{14}O_2$ and were each instantly killed in a different fashion. The first (A) was killed as has been the practice in this laboratory for the past year. The leaves were forced rapidly into liquid nitrogen, ground under liquid nitrogen in a mortar and the cold powder was poured into boiling ethanol and re-extracted in 20 percent ethanol. Such a procedure gives rapid and efficient extraction of the radioactive products.

The second bath of leaves (B) was killed by forcing them into boiling absolute alcohol and re-extracted in 20 percent ethanol as was done in this laboratory prior to the development of the liquid nitrogen method. In the third (C) method, leaves were killed in boiling water and extracted with 80 percent and 20 percent ethanol, and in the fourth (D) the leaves were killed in boiling 85 percent n-butanol and extracted in absolute, 80 percent and 20 percent ethanol.

The results as observed in the radiograms of methods (A), (B), (C) and (D) were identical and showed no hydrolysis of sugar phosphate and relatively little free glyceric acid. The radiograms previously obtained from liquid nitrogen-killed leaves usually, but not invariably, showed free trioses and hexoses.

It is known that the destruction of the cellular structure by freezing destroys photosynthetic activity but not respiratory activity. The latter process involves glycolysis and phosphatase activity is closely associated with glycolysis and known to exist in plant tissue. It is possible that the phosphorylated compounds are very susceptible to hydrolysis. This hydrolysis may be even more rapid in the absence of the photosynthetic reducing power which is most probably utilized for the formation of high energy phosphate linkages. Since the killing process of the powdered frozen leaves requires only an instant, the hydrolysis must be rapid indeed.

The Source of the α and β Carbon Atoms of Phosphoglyceric Acid. Degradation of phosphoglyceric acid, the earliest observed intermediate in photosynthesis, has shown that this material in very short photosynthesis experiments was largely

TABLE V

C^{14} Distribution in Photosynthetic Products of Barley and Scenedesmus

Conditions[aa]	Glyceric Acid			Glycolic Acid		Sucrose		
	-COOH	-CHOH	$-CH_2OH$	-COOH	$-CH_2OH$	C3,4	C2,5	C1,6
Barley								
Preillum: 2 min. in Dark	96.	2.6	1.7					
4 sec. PS	87.	6.5	6.8	48.5	51.5			
15 sec. PS	56.	21.	23.	50±5	50±5			
15 sec. PS	49.	25.	26.			52.	25.	24.
30 sec. PS				48.	52.			
30 sec. PS[a]	75.	6.	9.					
40 sec. PS				47.	53.			
60 sec. PS	44.[b]	30.	25.			37.	34.	32.
Scenedesmus								
5 sec. PS	95.[c]	2.5	1.2					
30 sec. PS						87.[d]	7.	6.
30 sec. PS[e]	81.	7.	10.					
30 sec. PS[e]MI[f]	73.	12.	15.					
60 sec. PS[e]	51.	24.	25.					
60 sec. PS[e]MI	48.	24.	28.					
60 sec. PS[e]	44.	25.	31.					
60 sec. PS[e]MI	43.	27.	30.					

(aa) Experiments are steady state photosynthesis at 10,000 f.c. unless other stated.
(a) 1,000 f.c.
(b) Alanine obtained from this extract was 48 percent carboxyl-labeled.
(c) Under the same conditions, chlorella produced phosphoglycerate labeled 95, 3 and 2 percent resp.
(d) In this extract malic acid was labeled 6.5 percent and aspartic acid 4 percent in the non-carboxyl carbons.
(e) 3,000 f.c.
(f) Malonate inhibited.

carboxyl-labeled and the α and β carbon atoms have little radioactivity. A two-carbon molecule should then be the desired precursor, but a C_1 molecule may be involved. Thus far, glycolic acid and glycine and small amounts of acetaldehyde are the only labeled two-carbon molecules found in plants.

Carrier isolations of formaldehyde and acetaldehyde have allowed a calculation of their concentration in the alga Scenedesmus during photosynthesis. They are present in extremely low concentration, 10^{-6} to 10^{-7} M, and this fact throws serious doubts on their possible participation. Such amounts might be expected as breakdown products of other compounds present such as pyruvic acid or pyruvic aldehyde.

It was observed that glycolic acid accumulates in the light in the absence of CO_2. Since under these conditions no photosynthesis was possible and thus the C_2 acceptor and its precursors build up, it was suggested that this molecule is related to the C_2 carbon dioxide acceptor. Glycolic acid was isolated by paper chromatography from the products of 15 second steady state photosynthesis with barley leaves and it was then degraded by periodate oxidation. Both carbon atoms of the glycolic acid were found to be equally labeled.

A one-second experiment with barley leaves gave labeled glyceric acid, phosphoglyceric acid and a small amount of active triose phosphates as the only products, but less than 1 percent glycolic acid was observed. The same experiment, but with four seconds photosynthesis in $C^{14}O_2$ showed a small but significant amount of glycolic acid. This was degraded as well as the glyceric acid from the same experiment. The distribution of C^{14} in the glyceric acid was: carboxyl position, 87 percent; α-carbon, 6.5 percent; β-carbon, 6.8 percent. Or, in terms of total activity in the latter two carbons alone, α-carbon, 49 percent and β-carbon 51 percent. Distribution of C^{14} in the glycolic acid from the same experiment was found to be: Carboxyl-carbon, 48.5 percent; α-carbon, 51.5 percent. In Table V, additional information is presented.

These results suggest a close relationship between the two carbon compounds, glycine and glycolic acid, and the α and β carbon atoms of phosphoglyceric acid. The symmetrical labeling of the glycolic acid has stimulated a search for other possible two carbon intermediates.

Effect of pH on Photosynthesis. A number of chemical and physical factors exerting an influence on photosynthesis have been investigated. One of the most important of these is the effect of hydrogen ion concentration. The purpose of this phase of investigation is to determine any possible separation of reactions by adjusting conditions of a steady state photosynthesis experiment.

In a series of experiments, 0.2 g. of the alga Scenedesmus were suspended in 20 cc M/300 phosphate buffer and exposed to light during ten minutes (while air was bubbled through gently). At the end of that period, 5 μc of $NaHC^{14}O_3$ was injected and the algae were allowed to photosynthesize for one or two minutes and were then killed in boiling ethanol.

The shape of the curve showing the total amount of C^{14} fixed in a given time against the pH of the medium depends upon the light intensity and also apparently upon the less easily controllable concentration of CO_2 in the medium. While the

maximum assimilation took place at about pH 7 in light of ~5000 f.c., a few experiments indicate that it is shifted to pH 5 at 400 f.c. and to pH ~9 at 20,000 f.c. The products obtained at these various light intensities are being analyzed by radiochromatography. Some analytical data are available for four experiments at 10,000 f.c. and indicate that the trend is the same as that previously reported for a series of 1500 f.c. experiments. As the pH increases there is a decrease in the percentage of sucrose, glycine + serine, and also alanine while there is a marked increase in the percentage of malic acid and possibly also in aspartic and phosphopyruvic acids. Other compounds show minor, or seemingly erratic, variations. The following set of condensed data (Table VI) shows the most important pH effects at 10,000 f.c.

The large increase in sucrose at pH 1.6 is accompanied by a corresponding decrease in the phosphates and may not be significant. The increase of malic in alkaline media may be due to a selective effect of pH upon certain enzymes. Various investigators have reported that the incorporation of $C^{14}O_2$ into malic acid is negligible at pH 4 but proceeds rapidly at pH 2.7. However, one should not overlook the possibility, that if the concentration of CO_2 is greater in the alkaline media, the ratio $h\nu/CO_2$ will be smaller and the carbon will tend to be incorporated into low-energy compounds rather than into sugars.

Effect of Ethanol on Photosynthesis. A few experiments in the presence of ethanol have shown that the assimilation of C^{14} by Scenedesmus in two minutes photosynthesis at 15,000 f.c. decreases from unity in water to 0.65, 0.15 and 0.5 in 5 percent, 10 percent and 20 percent ethanol, respectively. The effect of ethanol on the percentages of some of the radioactive products is shown in Table VII.

Effect of High Temperature on Photosynthesis. In two minutes photosynthesis by Scenedesmus (D-3) at various temperatures it was found that the rate of fixation of C^{14} increases up to about 32°C and then decreases gradually. The limit above which no assimilation takes place is about 45°C for algae which have been heated from 25-45°C in about two minutes. The products obtained at six temperatures in that interval are being analyzed. The products formed in a preliminary experiment at 40°C are compared in Table VII to those obtained in an identical experiment at 25°C and 1200 f.c.

The addition of ethanol seems to inhibit the formation of malic and aspartic acids and favors the intensities of two spots which, from their location, seem to be glycolic and glyoxylic acids. The rise in temperature from 25-40°C favors free sucrose at the expense of the phosphates, possibly by a simple acceleration of a hydrolytic process.

Location of the Radioactive Products in the Cell. In discussing the processes involved in photosynthesis it would be helpful to know where each process takes place in the cell. Efforts are being made to analyze the radioactive compounds located in the chloroplasts, the cell sap or the cell walls.

In a typical experiment a washed spinach leaf is exposed to light of 10,000 f.c. in air during 20 minutes and then allowed to assimilate the $C^{14}O_2$ under the same conditions in two minutes of photosynthesis. The cell is then put into liquid nitrogen and ground in a mortar to a fine powder. This powder is washed with water on eight layers of cheesecloth, which retains most of the intact cells and cell

TABLE VI

pH Effects for 2 min. PS at 10,000 f.c. Light Intensity

pH	1.6	6.7	9.0	11.4
Total Assimilation (Counts/ min./100 mg cells)	7600	45,000	79,400	13,400
Percent of total assimilation found in:				
Phosphates	14.5%	46%	58%	46%
Malic Acid	6	23	25	37
Phenyl pyruvic acid	< 1	3.7	3.3	2.5
Aspartic acid	6	7	5	10
Alanine	6	3	1.7	1.5
Glycine + Serine	26	6.8	4.4	< 1
Sucrose	26	9.4	1.6	< 1

TABLE VII

Effects of Ethanol and High Temperature

Percent of Total C^{14} Assimilation Found in:	0% EtOH	10% EtOH	25°C	40°C
Phosphates	49%	57%	60%	13%
Malic Acid	19	11	13	15
Aspartic Acid	17	2	14	12
Alanine	4	5	5	5
Glycine + Serine	7	4	6	7
Sucrose	1	0	2	47
Indefinite Spots	1.5	8		

fragments, allowing the chloroplasts and sap to go through. These can be separated by centrifugation, but more rapidly by immediate aspiration through a filter which retains the chloroplasts. In such an experiment 85 percent of the activity was found in the sap, only 2 percent in the chloroplasts and the rest in the cheesecloth, which probably contained some intact cells.

Chromatography of the products indicates that all the fats and all the substances insoluble in 80 percent ethanol were in the chloroplast fraction. The chloroplasts were relatively richer in sugars and the sap in phosphate esters. The acids were found in comparable amounts in both fractions.

It is clear that a fundamental difficulty in experiments of this type is to prevent a redistribution of the compounds according to their solubilities during the fractionation. For instance, much of the water-soluble contents of the chloroplasts may be extracted during the filtration.

Carbon Dioxide Fixation by Microorganisms. As mentioned earlier in this report the identity and sequence of intermediates in photosynthesis has been investigated and found similar in a number of organisms. The details of some of the current experiments are outlined below.

Washed cell suspensions of Tetrahymena geleii, a protozoa, were exposed to $C^{14}O_2$ for 40 minutes. The radioactive products were separated by paper chromotography and located by radioautography. The majority of the activity was found in succinic acid. (See Van Niel, etal, Proc. Nat. Acad. Sci., 28, 157 (1942)). The percent of total fixed activity found in each compound is shown in Table VIII.

The radioactive compounds formed by mycelia of two water molds, Allomyces arbuscula and Blastocladia Pringsheimii, when exposed to $C^{14}O_2$ for 40 minutes were determined. Aspartic, fumaric, succinic, malic and glutamic acids were found to contain radioactivity.

When cells of Clostridium kluyveri were exposed to radioactive carbon dioxide for 40 minutes, over 90 percent of the fixed activity was found in alanine. Glutamic, aspartic and malic acids were also radioactive.

Cell suspensions of Lactobacillus casei fixed very little radioactivity during a 40-min. exposure to $C^{14}O_2$. Therefore, the organisms were grown in the presence of radioactive carbon dioxide for 16 hours. The cells were then separated from the growth medium. The majority of activity was found in the washed cells. The radioactive compounds of both fractions are now being determined.

TABLE VIII

Compound	Atmospheric Medium: CO_2 +	
	Air	Nitrogen
Succinic acid	65%	78%
Fumaric acid	1	1
Malic acid	8	12
Alanine	4	2
Glycine	2	1
Glutamic acid	13	6
Aspartic acid	4	2

II QUARTERLY PROGRESS REPORT. Project 48B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics

L. Brewer, L. A. Bromley, Raleigh McKisson, G. Elliott, Russell Edwards
L. Templeton, Alexander Lindsay

Gaseous Hydroxides Species of Mo and W. This work is continuing.

Liquid Metal Systems. Report UCRL 671 on the "Heats of Formation of Sodium Potassium Alloys", has been issued. Report UCRL 688 on the new high temperature calcrimeter will be issued shortly. Report UCRL 689 which summarizes the heats of formations of the compounds in the sodium-tin systems measured in the new high temperature calorimeter described in UCRL 688 is nearly complete.

A high temperature camera is in operation for study of ternary metal systems.

Thermal Conductivity of Gases. The optical system necessary to measure the thermal conductivity of gases has been designed and is being built. Flow meters have been designed, built and calibrated to allow preparation of various mixtures of gases.

Heat Transfer in Forced Convention Film Boiling. The equipment is still under construction.

All other problems have been completed and fully reported by the following papers: (Summary of papers and reports of Metals and High Temperature Thermodynamics group, July 1, 1949 to June 30, 1950.)

National Nuclear Energy Series, Plutonium Project Record, Div. IV

Vol. 14B (1949).

Paper 6.40 - The Thermodynamic Properties and Equilibria at High Temperatures of the Compounds of Plutonium, by L. Brewer, L. Bromley, P. W. Gilles, and N. L. Lofgren, pgs. 861-886.

Paper 15.7 - The Halides of Neptunium by L. Brewer, L. Bromley, P. W. Gilles, and N. L. Lofgren, pgs. 1111-1118.

Vol. 19B (1950)

Paper 1 - Investigations in the Liquid-Solid Equilibria of the Two-component Systems Composed of the Bromides and Iodides of Strontium and Barium, by E. D. Eastman, N. C. Melchior, and A. E. Stickland, pgs. 1-5.

Paper 2 - Temperature-Composition Diagrams of Metal-Metal Halide Systems by E. D. Eastman, D. D. Cubicciotti, and C. D. Thurmond, pgs. 6-12.

Paper 3 - The Thermodynamic and Physical Properties of the Elements, by Leo Brewer, pgs. 13-39.

Paper 4 - Thermodynamic and Physical Properties of Nitrides, Carbides, Sulfides, Silicides, and Phosphides, by L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, pgs. 40-59.

Paper 5 - The Thermodynamic Properties of Common Gases, by Leo Brewer, pgs. 60-75.

Paper 6 - The Thermodynamic Properties of the Halides, by L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, pgs. 76-192.

Paper 7 - The Fusion and Vaporization Data of the Halides, by Leo Brewer, pgs. 193-275.

Paper 8 - The Thermodynamic Properties of Molybdenum and Tungsten Halides and the Use of These Metals as Refractories, by L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, pgs. 276-311.

Paper 9 - The Heats of Formation of CeS, Ce_3S_4, and Ce_2S_3 at 25°C. by M. W. Evans, pgs. 312-320.

Paper 10 - The Heat of Reaction of the Cerous-Ceric Couple in 0.5 Molal Perchloric Acid at 25°C, by B. J. Fontana, pgs. 321-329.

Vol. 12B (1950)

The Thermodynamic Properties and Equilibria at High Temperatures of Uranium Halides, Oxides, Nitrides, and Carbides, by L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren.

Journal Papers.

Utilization of Equilibrium Vapor Pressure Data by L. Brewer and A. W. Searcy, Journal of Chemical Education, 26, 548-552 (1949).

Preparation and Properties of Refractory Cerium Sulfides by E. D. Eastman, L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, Journal of the American Chemical Society, 72, 2248-2250 (1950).

Preparation and Properties of the Sulfides of Thorium and Uranium by E. D. Eastman, L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, Journal of the American Chemical Society, June 1950.

The Thermodynamics of Gaseous Cuprous Chloride, Monomer and Trimer, by L. Brewer, and N. L. Lofgren, Journal of the American Chemical Society, June, 1950.

Mixed Oxide-Sulfides of Cerium and Thorium, by E. D. Eastman, L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, submitted for publication to Journal of the American Chemical Society.

The Heats of Formation of CN, N_2, and NO by L. Brewer and L. K. Templeton, submitted for publication to Journal of the American Chemical Society.

High-Melting Silicides by L. Brewer, A. W. Searcy, D. H. Templeton, and C. H. Dauben, accepted for publication by Journal of the American Ceramic Society.

Preparation and Tests of Refractory Sulfide Crucibles, by E. D. Eastman, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, submitted for publication to Journal of the American Ceramic Society.

Heterogeneous Equilibria and Phase Diagrams by L. Brewer, accepted for publication by Annual Reviews of Chemistry, 1950.

Declassified Project Reports, not duplicating above papers.

The Crystal Structure of UBI by L. Brewer, R. K. Edwards, and D. H. Templeton, AECD-2730, Aug. 31, 1949.

The Stability of Gaseous Nickel Oxide by L. Brewer and D. Mastick, UCRL-532, Nov. 1949.

Theoretical Calculations of the Stability of Solid and Gaseous Species in the Alkali and Alkaline Earth Systems, by L. Brewer and D. Mastick, UCRL-533, Nov. 1949.

The Stability of Gaseous Ferrous Oxide, by L. Brewer and D. Mastick, UCRL-534, Nov. 30, 1949.

The Gaseous Species of the Aluminum-Aluminum Oxide System, by L. Brewer and A. W. Searcy, UCRL-552, Aug. 31, 1949.

The Stability of Gaseous Manganous Oxide by L. Brewer and D. Mastick, UCRL-570, Nov. 1949.

The Stability of Gaseous SnO, ZnO, CdO, CuO, PbO, GeO, SiO and TiO, UCRL-571, Nov. 1949, by L. Brewer and D. F. Mastick.

The Sticking Coefficients of Carbon and Iron Atomic Beams, by L. Brewer and D. F. Mastick, Nov. 1949.

Borides of Uranium and Thorium, by L. Brewer, D. Sawyer, D. Templeton and C. H. Dauben, 14 Feb. 1950. UCRL-603.

A Study of the Refractory Borides, by L. Brewer, D. Sawyer, D. H. Templeton and C. Dauben, 24 Feb. 1950. UCRL-610.

The Higher Fluorides of Plutonium, by L. Brewer, UCRL-633.

Paper in Progress

The Choice of the Proper Refractories for the Casting of High Melting Electropositive Metals, by L. Brewer.

B. Basic Chemistry, including Metal Chelates

Z. Z. Hugus, R. E. Connick, K. Mattern

Lanthanum Chemistry. The study of ionic strength effect on the acid constant for TTA resulted in the calculation of the equilibrium constant for the reaction: $Li^+ + HK = LiK + H^+$. The constant is approximately 1.6×10^{-6}. Further investigation of the TTA continued to show a factor of two discrepancy with the value of the ionization constant for TTA, as calculated by E. Zebroski (BC-63) to be $K_o = 6.2 \times 10^{-7}$. Our present values, as calculated from spectrophotometric measurements, are $K_o = 2.8 \times 10^{-7}$ and $K_{\mu=1} = 6.1 \times 10^{-7}$.

The exact value of the molar extinction coefficient for enolate ion was desired for this work. Two approaches were used in calculating it. (1) The addition of $TTA_{aq.}$ solution to a high-pH buffer, and (2) the solution of $TTA_{(s)}$ in dilute base. To take into consideration the decomposition of the TTA under these conditions, samples were acidified and extracted into benzene before and after reading the absorption for the enolate ion peak. From these extracted samples, the concentration of total TTA at the time of the spectrophotometric observation was determined. A molar extinction coefficient of 21,400 at 3380Å was determined. (17,000 ± 2,500 at 3470Å were calculated from various data in BC-63).

The constants for the complex-ion formation of La^{+3} with various inorganic ions are now being determined. A rough constant for the sulfate reaction is

$$La^{+3} + SO_4^{=} = LaSO_4^{+} \qquad K = 20 \ (\mu=1)$$

Electron Exchange Rate Between Fe^{3+} and Fe^{2+}. A procedure for determining the exchange rate in $HClO_4$ solution has been developed, involving the use of radioactive Fe^{59} and the hydrogen-form Dowex-50 ion-exchange resin. Preliminary experiments have been performed to fix the optimum concentrations for maximum sensitivity to wide variation in exchange rate, and indicate that, providing the resin does not catalyze the exchange, even very rapid exchange rates may be determined.

Carrier free Fe^{59} prepared by cyclotron bombardment, will be available shortly for continuation of the investigation.

Thermodynamics of Rhenium. The $ReCl_6^{=}$ - ReO_4^{-} heat is being investigated at present. The difficulty experienced in freeing K_2ReCl_6 from $K_2ReCl_5(OH)$ has been overcome and it is hoped to obtain sufficient amounts of the latter to permit x-ray analysis and possibly a value for the heat of oxidation to ReO_4^{-}.

Re_2O_7 and K_2ReBr_6 have been synthesized and made available for x-ray analysis. This Re_2O_7 will be used in ReO_3 synthesis.

$BaMnO_4$ samples made by various methods have too much impurity for calorimetric purposes. Further synthetic methods and techniques are being employed to overcome this difficulty.

C. Ore Reduction

E. F. Orlemann, S. A. Sorenson, L. W. Tregillus

Phosphate Complexes of U(VI). Changes in personnel have resulted in a new approach to the subject. The TTA chelate extraction method with fluorimetric measurement of the uranyl concentration has been discarded in favor of the direct spectrophotometric determination of the complex species. The absorption in one molar phosphate solutions has been found to be proportional to the concentration of the complex at 240 mu, permitting a study of the pH and phosphate dependence for the equilibrium between the complex and the precipitate. The composition of the precipitate (UO_2NaPO_4) is being verified by titrimetric, colorimetric, spectrographic, and x-ray diffraction methods.

LMB/7-7-50
Information Division