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Chemistry-Transuranic
Elements

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CHEMISTRY DIVISION QUARTERLY REPORT

June, July and August, 1951

October 23, 1951

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I QUARTERLY PROGRESS REPORT. Project 48

G. T. Seaborg and I. Perlman

A. Chemistry of the Rare Earth and Actinide Elements

α- Particle Spectrograph

Frank Asaro

Construction. The new vaporizing system for spectrograph sources is nearly completed.

Plans have been completed and assembly started on an α, γ coincidence circuit capable of counting coincidences between gamma rays of a given energy and particular alpha rays in the complex alpha spectrum.

Spectrograph Geometry. By removing all slits in the source system, the geometry of the spectrograph was increased to 4×10^{-5} with a peak half thickness of 10 kev for a 4.8 Mev alpha particle.

Spectrograph Runs.

Am^{241}: The absolute alpha energy of the most prominent group of Am^{241} was determined against an Em^{222} standard to be 5.475 Mev ± 2 kev. It was similarly determined against Po^{210} to be 5.473 Mev ± 2 kev.

Another low energy peak and a high energy peak were found in the complex structure of Am^{241}. Also the highest energy peak was split into two alpha groups. The resulting complex structure of Am^{241} is shown in Table I.

Table I

Abundance of α group (percent)	Absolute particle energy (Mev)	Energy levels relative to highest energy transition (kev)	Particle energies corresponding to transitions (kev)
0.15		0	5.543
0.15		11.2	5.532
0.10		42.8	5.501
84	5.474	70.3	5.474
14		112.9	5.432
1.3		166.5	5.380

U^{234}: Two alpha groups were observed separated by 48 kev. The abundance of the lowest energy group was 29 percent.

Pu^{238}: Two alpha groups were observed separated by 44 kev. The abundance of the lowest energy group was 29 percent.

Calibration of Spectrograph. The spectrograph was calibrated against the alpha complex structure in Ra^{226} and the difference in energy between the main group in Ra^{226} and Em^{222}. Both experiments indicate previous energy differences were 4 percent too low. All values have been corrected.

Ion-Exchange Elutions of Actinides and Lanthanides with Hydrochloric Acid Solutions and Dowex-50 Resin

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

The previous work has been continued. The elution behavior of curium(III), americium(III), plutonium(III), actinium(III), plutonium(IV), neptunium(IV), uranium(IV), thorium(IV), plutonium(VI), neptunium(V), uranium(VI), lanthanum (III), cerium(III), europium(III), ytterbium(III), yttrium(III), strontium(II), barium(II), radium(II), cesium(I) from Dowex-50 cation exchange resin columns has been studied with 3.2 M, 6.2 M, 9.3 M, and 12.2 M HCl solutions. These elutions show that in high concentrations of hydrochloric acid the actinides form complex ions with chloride ion to a much greater extent than the lanthanides. To explain these results, a partial covalent character must be ascribed to the bonding in the transuranium complex ions. A reasonable structure for such covalent bonding may involve hybridization of the 5f orbitals in the actinide elements. A summary of the elution results is given in Fig. 1

Microcalorimeter

S. R. Gunn and B. B. Cunningham

Heat of Solution of $NaUO_2Ac_3$ and $NaPuO_2Ac_3$. In connection with the investigation of the americium(IV) - americium(III) couple it was necessary to determine the heat of solution of $NaAmO_2Ac_3$ in 0.5 M $HClO_4$. Since it was expected that this value would be nearly the same as that for the corresponding uranium and plutonium salts, these were prepared by the usual methods and the heats of solution measured in the microcalorimeter previously described. Three samples of from 11 to 17 mg of $NaUO_2Ac_3$ from two preparations gave a value of $\Delta H = -0.65 \pm 0.15$ kcal/mole and two $NaPuO_2Ac_3$ samples of 9 and 12 mg gave a value of $\Delta H = -0.55 \pm 0.20$ kcal/mole. These values have been corrected for the heat of association of acetic acid and represent the heat of the reaction

$$NaMO_2Ac_{3(c)} \rightleftharpoons (Na^+ + MO_2^{++} + 3Ac^-)\ (0.5\ N\ HClO_4)$$

Solubility measurements on $NaPuO_2Ac_3$ in water[1] have indicated that the solubility increases with increasing temperature; the calorimetric data indicate a value for the heat solution of approximately + 2 kcal/mole.

---

[1] J. W. Hamaker and G. E. Sheline, Manhattan Project Metallurgical Laboratory Report CN-654.

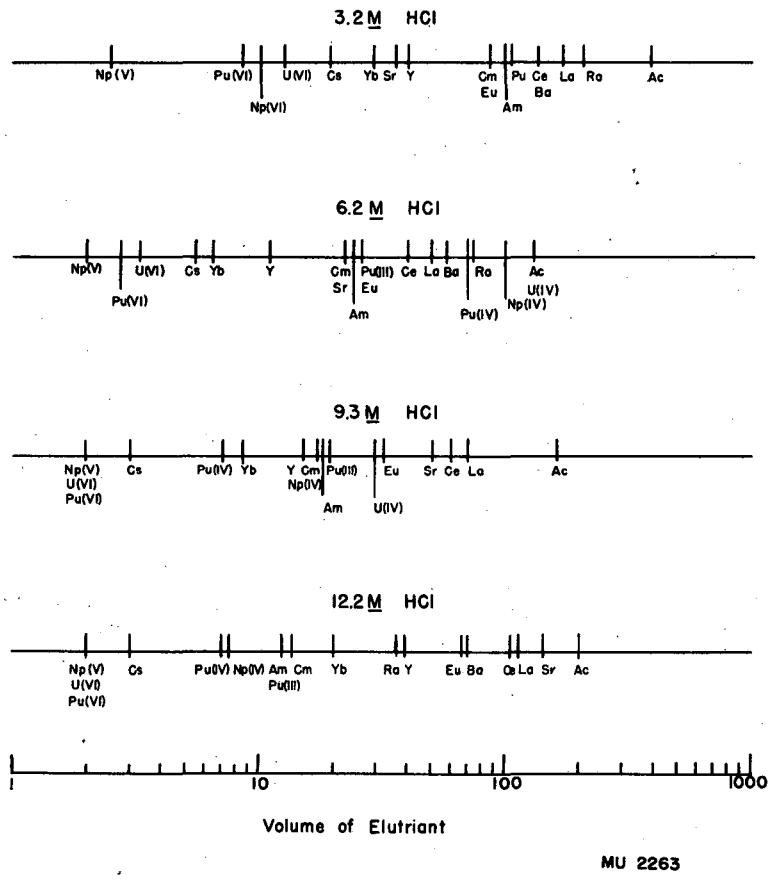

3.2 M HCl
Np(V)
Pu(VI)
Np(VI)
U(VI)
Cs
Yb
Sr
Y
Cm
Eu
Pu
Am
Ce
Ba
La
Ra
Ac
6.2 M HCl
Np(V)
Pu(VI)
U(VI)
Cs
Yb
Y
Cm
Sr
Pu(III)
Eu
Am
Ce
La
Ba
Ra
Pu(IV)
Ac
U(IV)
Np(IV)
9.3 M HCl
Np(V)
U(VI)
Pu(VI)
Cs
Pu(IV)
Yb
Y
Cm
Np(IV)
Pu(III)
Am
Eu
U(IV)
Sr
Ce
La
Ac
12.2 M HCl
Np(V)
U(VI)
Pu(VI)
Cs
Pu(IV)
Np(IV)
Am
Cm
Pu(III)
Yb
Ra
Y
Eu
Ba
Ce
La
Sr
Ac
1
10
100
1000
Volume of Elutriant
MU 2263

Fig. 1

While the lack of precision in the proceding values is in part due to the very small heats being measured, the operation of the calorimeter during the measurements was not satisfactory. The circuits were rebuilt and the accuracy and reproducibility of the instrument was investigated by making the usual measurements of the heat of solution of magnesium metal, which has been used in the past as a calorimetric standard in this laboratory. The heat of solution of magnesium in 1.5 M HCl has been accurately determined on the macro scale to be -111.322± 0.041 kcal/mole.[2] Five runs with samples of from 220 to 290 micrograms weight gave a value of -110.2±1.3 kcal/mole for the heat of solution in 1.5 M HCl. Part of the variation in these values may arise from variations in the amount of hydrogen dissolved in the solution. The calorimeter solution is saturated with hydrogen before starting a run and a correction is applied to the magnesium heats on the assumption that all hydrogen produced in the reaction is saturated with water vapor and none is dissolved in the calorimeter solution. This correction amounts to 0.317 kcal/mole of hydrogen at 25° C. However, it is probable that during the period (2 - 3 hours) required to pump the kinetically vacuum jacketed calorimeter down to operating pressure, some of the hydrogen diffuses out of the solution and reaction chamber and that part of the hydrogen produced in the reaction is dissolved in the solution, thereby liberating heat. It has also been observed that magnesium samples of this size sometimes become attached to hydrogen bubbles and float on the surface of the acid while dissolving; thus the gas evolved might not become saturated with water. To avoid these uncertainties, it was decided to investigate the use of the heat of dilution of sulfuric acid as a calorimetric standard. These heats of dilution have been accurately determined on the macro scale by Kunzler and Giauque.[3] Preliminary experiments indicated that the reaction in the calorimeter was essentially complete within 0.5 minute. Constant boiling sulfuric acid, the composition of which has been recently very accurately determined as a function of pressure,[4] was prepared in an all Pyrex apparatus. The heat of dilution of this concentrated acid to a dilute sulfuric acid solution of $H_2O:H_2SO_4$ mole ratio = 50 is -0.1655 calories per milligram. The first lot of acid was loaded into the calorimeter bulbs which were exposed to the room atmosphere. Seven samples of from 4 to 17 milligrams gave a value of ΔH= -0.1477 ± 0.0025 cal/mg. Evidently the acid had absorbed a considerable amount of moisture during its relatively brief exposure to the atmosphere. A second lot of constant boiling sulfuric acid was loaded into the bulbs in the controlled atmosphere of a nitrogen dry box. Three samples loaded under these conditions gave a value of 0.1644 ± 0.0010 cal/mg; two others differed by fifty and twenty-five percent. It is probable that these last two values are to be attributed to the difficulty of loading the sample bulbs and the possibility of wetting the necks.

At best, however, the results were still unsatisfactory and the difficulty appeared to lie in the operation of the calorimeter itself. The galvanometer used in balancing the bridge containing the resistance thermometer was unstable and fluctuated greatly. Frequently the drifts were roughly in the shape of a sine wave of a few minutes period. These difficulties probably are due to thermal or leakage currents originating in the circuit. The resistance thermometer and its bridge have been rebuilt with an improved design in an effort to improve the accuracy of the instrument.

---

[2] C. H. Shomate and E. H. Huffman, J. Am. Chem. Soc. 65, 1625 (1943)
[3] J. E. Kunzler, Ph. D. Thesis, University of California, 1950
[4] J. E. Kunzler and W. F. Giauque, unpublished work.

B. Nuclear Properties and Transformations

Some Nuclear Properties of the Neutron Deficient Americium Isotopes

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

Americium isotopes of mass less than 241 were produced by bombarding Pu^{239} with protons and deuterons of various energies. The americium was chemically separated and samples were counted with a windowless methane counter, xenon geiger counter, and gamma ray scintillation counter. The decay was followed and alpha particle energies were measured on the 48 channel pulse analyzer.

When the bombarding particles were 100 Mev protons the 12 hour Am^{239} was seen accompanied by 5.75 Mev alpha particles, and K and L x-rays. The partial alpha half-life was 40 years. Another electron capture component which decayed with a 1.3 hour half-life was resolved and alpha particles of 6.01 Mev decaying with nearly the same half-life were noted.

The procedure was repeated with 17.5 Mev deuterons as bombarding particles. The 53 hour Am^{240} was seen with its known radiations, the characteristics of Am^{239} noted above were verified and a 2 hour electron capture activity accompanied by a 0.7 Mev gamma ray was seen. This activity was assigned to Am^{238} on the basis of the bombardment energetics.

Since the 17.5 Mev deuteron bombardment did not produce the 6.01 Mev alpha particle emitter produced by the 100 Mev proton bombardment, it was concluded that this alpha particle was the result of the decay of Am^{237}. It was further concluded that the primary mode of decay of this nuclide is by electron capture decaying with half-life between 1 and 1.5 hours with a partial alpha half-life of three to four years.

Beta Activity in Naturally Occurring Neodymium Isotopes

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

Samples of mass separated Nd^{145} and Nd^{150} were examined in a windowless proportional counter which had been checked for sensitivity and counting efficiency with electrons of around 10 kev energy. Considering corrections for these factors and self absorption the lower limit set on the half-life of each is 2×10^{12} years.

X-Ray Spectroscopy of Radioactive Elements

C. I. Browne and I. Perlman

Am^{241}. The bent-crystal x-ray spectrometer, described earlier by Barton, Robinson and Perlman,[1] has been modified by the use of a larger crystal of topaz, rather than quartz, and the replacement of the proportional counter by a scintillation counter using NaI(Tl) crystals and an RCA 5819 photomultiplier tube. The gross effect of these changes was to increase the sensitivity of the instrument by a factor of approximately 150, and thereby bring into observational range nuclides of longer half-life and rarer x-rays.

The study in the spectrometer of a sample of approximately 2 mg of Am^{241} resulted in the observation of the following spectrum of lines:

Table 1

	E_{here} (kev)	E_{Barton} (kev)	E_{extrap} (kev)	Line Assignment	Relative Intensity (here)
1.	13.85 ± 0.05	--	13.79	$NpL\alpha_2$	9
2.	14.07 ± 0.05	13.98 ± 0.03	13.98	$NpL\alpha_1$	44
3.	17.82 ± 0.05	17.79 ± 0.03	17.78	$NpL\beta_1$	100
4.	20.91 ± 0.07	--	20.81	$NpL\gamma_1$	20
5.	26.51 ± 0.07	--	--	gamma	20
6.	59.8 ± 0.3	--	--	gamma	360

Column 2 of Table 1 refers to the values reported by Barton,[1] who observed the $L\beta_1$ and $L\alpha_1$ lines in such low intensity that he was unable to report relative intensities to any degree of accuracy. Column 3 refers to the values calculated by Barton[1] on the basis of a Moseley extrapolation using Seigbahn's data on thorium and uranium.

The intensities reported in column 5 represent the results of correcting the data for absorption of the radiation between sample surface and detector (no self-absorption corrections were made) and for the reflectivity of the crystal. We are in process of measuring the dependence of the integrated reflectivity coefficient on energy for a bent topaz crystal; pending the results of this experiment, the Am^{241} data were calculated for both a $1/E$ and $1/E^2$ reflectivity dependence.[2] The latter calculation leads to a conclusion inconsisten

[1] Barton, Perlman, and H. P. Robinson, Phys. Rev. 81, 208 (1951)
[2] Lind, West, and Dumond, Phys. Rev. 77, 475 (1950)

with the results obtained by Martin,[3] so that the values reported in Table 1 are based upon the assumption of a 1/E dependence.

It is noteworthy that the intensity ratio of $L_{\beta 1}$ to $L_{\gamma 1}$, both of which arise from an L_{II} vacancy, and that of $L_{\alpha 2}$ to $L_{\alpha 1}$, both of which arise from an L_{III} vacancy, are in essential agreement with the values reported by Allison[4] in electron bombarded uranium.

It is further noteworthy that no x-rays arising from L_I vacancies were observed; an upper limit can be placed on $L_{\beta 3}$ x-rays present, and from this estimate, together with Allison's[4] data on relative abundance of x-rays following a given shell vacancy, and Kinsey's[5] calculated values of fluorescent yields in the L shells, the formation of L-shell vacancies in the transition can be expressed as 26 percent L_I, 43 percent L_{II}, and 31 percent L_{III}, keeping in mind that the L_I figure is an upper limit. These results are generally similar to those obtained by Barton[1] with Cm^{242} decay, where he observed 20 percent L_I, 28 percent L_{II}, and 52 percent L_{III} vacancies (L_I an upper limit) in that they contrast sharply with Kinsey's[5] assumption of ratios of excitation in L shells in internal conversion of 100:10:1 for the L_I, L_{II}, and L_{III} respectively.

On this basis, the ratio of intensity of the 59.8 kev gamma ray to total L vacancies is 1 ± 0.2, a figure in agreement, as noted above, with the results obtained by Martin on his scintillation crystal gamma spectrometer. If a $1/E^2$ dependency of the integrated relectivity coefficient is used, the ratio of L vacancies to the 59.8 kev gamma ray is 1/3.6, which is inconsistent with the results of Martin and Crane and Ghiorso,[6] who observed the radiations on a 48 channel pulse analyzer using a proportional counter as detector.

The figure of 59.8 ± 3 kev for the higher energy gamma ray compares very favorably with that of 59.4 ± 1.0 obtained by O'Kelley[7] in a double focussing beta ray spectrometer.

The observed gamma rays are also to be compared with the alpha particle spectrum observed by Asaro and Perlman,[8] who find levels in Np^{237} at 10 ± 1, 41.8 ± 0.6, 68.3 ± 1, 111 ± 2, and 165 ± 10 kev, with 85 percent of the alpha transitions resulting in the formation of the 68.3 ± 1 kev state. On the basis of energy differences alone, a gamma transition from the 68.3 ± 1 state to the 10 ± 1 state would have energy of 58.3 ± 2 kev, while a transition from the 68.3 ± 1 state to the 41.8 ± 0.6 state would have energy of 26.5 ± 1.5 kev. The agreement of these figures with the observed gamma energies offers a tentative partial decay scheme, as well as a confirmation of both observations.

<u>Nd^{140}</u>. (J. O. Rasmussen, C. I. Browne, J. Surls, and D. Martin) The nuclide Nd^{140} has been previously reported[9] as a 3.3 day K capture, and its daughter, Pr^{140}, as a 3.4 minute K capture and β^+ emitter. We have studied this decay chain in the bent crystal x-ray spectrometer, the double focussing beta ray

---

[3] D. M. Martin, private communication
[4] Compton and Allison, X-Rays in Theory and Experiment, 2nd Ed., p 645
[5] Kinsey, Can. J. Res. 26A, 404 (1948)
[6] W. W. T. Crane, and A. Ghiorso, unpublished data.
[7] G. D. O'Kelley, UCRL-1243
[8] F. Asaro and I. Perlman, to be published
[9] Wilkinson and Hicks, Phys. Rev. 75, 1687 (1949)

spectrometer, and the scintillation gamma spectrometer. We find the positron spectrum to have an allowed shape, with an endpoint energy of 2.23 ± 0.02 Mev, which leads to an (ft) value for the transition of 2 x 10^4, and the conclusion that the transition is allowed. There is meager evidence for a low abundance lower energy component of approximately 1 Mev. The electron spectrum shows up to 1 Mev only a line at 28.9 ± 0.4 kev, which we attribute to L Auger electrons in cerium, or praseodymium, or both.

The gamma spectrum shows clearly only K x-rays and annihilation radiation, although the possibility of a very low abundance gamma ray of energy 1.0 - 1.2 Mev cannot be excluded.

The K x-ray spectrum shows the unresolved $K_{\alpha 1 \alpha 2}$ doublet of both praseodymium and cerium, as well as the $K_{\beta 1}$ lines of both these elements. The intensities are: $PrK_{\alpha 1 \alpha 2}$:$CeK_{\alpha 1 \alpha 2}$:$PrK_{\beta 1}$:$CeK_{\beta 1}$ = 100:50:28:11. The absence of other means of producing K x-rays leads us to attribute all observed x-rays to K capture, from which we conclude that the K/β^+ ratio in Pr^{140} is 1.0 ± 0.1. This value is to be compared with the theoretical fk/f+, as obtained from the plots of Feenberg and Trigg,[10] of 1.0. If the praseodymium-cerium transition is allowed, the neodymium-praseodymium must be allowed also, and assuming an ft≈ 2 x 10^4, the decay energy may be roughly calculated as 120 ± 40 kev.

Ce^{140}, an even-even nucleus, may be considered as being in an S_0 state; application of Gamow-Teller and Nordheim[11] selection rules for an allowed transition then place Pr^{140} in an S_1 state. Both nucleons lie in the shell $50 < N \leq 82$, with possible states from the Mayer model, of $g_{7/2}$, $d_{5/2}$, $d_{3/2}$, $S_{1/2}$, and $h_{11/2}$. Application of Nordheim's rule 9 leads[11] to the conclusion that the intrinsic spin and angular momentum of the neutron and proton are parallel in one case and antiparallel in the other. If the spin of Pr^{141} (5/2) and Ba^{137} (3/2) are taken to apply to the odd nucleons in Pr^{140}, the states of the odd nucleons may be taken as $d_{5/2}$ for the proton and $d_{3/2}$ for the neutron. Antiparallel coupling of the angular momenta would then lead to an S over-all state as required from considerations above.

This entire discussion has neglected L capture. A self-consistent calculation based upon our data, Rose's theoretical L/K ratio of 0.14 for infinite decay energy, and a theoretical (fk) versus W_0 plot leads to a conclusion that Nd^{140} decays 26 percent by L capture and 79 percent by K, and that Pr^{140} decays 58 percent by β^+, 37 percent by K capture, and 5 percent by L capture. The decay energy of Nd^{140} is calculated to be 105± 40 kev, and the β^+/K ratio in Pr^{140} is calculated as 1.5.

We have also integrated the β^+ spectrum and the Auger spectrum, obtaining a ratio of β^+ to e^- of ~320 to 1. This results in a K/e^- ratio of ~640/1, or an average fluorescence yield of K x-rays of 0.99^+. Even in view of the high errors involved in the method, the fluorescence yield must be at least 0.9.

---

[10] Feenberg and Trigg, Revs. Modern Phys. 22, 399 (1950)
[11] Nordheim, Phys. Rev. 78, 294 (1950)

Investigation of the Heavy Isotopes of Radium and Actinium

William A. Jenkins and Glenn T. Seaborg

Ra^{230} and Ac^{230}. The bombardment of thorium metal with 150 Mev deuterons in the 184-inch cyclotron followed by extensive chemistry pursuant to the isolation of radium has demonstrated the presence of a new radium isotope. The final chemical step consisted of a high temperature resin exchange column (citric acid, pH 7.5) which served to separate from the rare earths and barium and also definitely established the chemical identity by virture of the presence of other radium activities which could be identified by their characteristic alpha particle energies. This isotope proved to decay by beta emission with a half-life of one hour. The energy of the beta emission was measured by a Feather analysis of an aluminum absorption curve, resolution of a Fermi plot from an analysis on a low resolution beta ray spectrometer and by visual endpoint observation from an analysis on a high resolution beta ray spectrometer.

Analysis	Beta particle energy (Mev)	
Feather analysis	2.1	
Fermi analysis	2.2	1.2
Visual observation	2.2	1.2

From the predicted disintegration energies, it seemed most probable that the 1.2 Mev beta particle was associated with the one hour decay of Ra^{230} while the 2.2 Mev beta particle was associated with the daughter activity Ac^{230}. Attempts to milk the actinium activity have been singularly unsuccessful and as a consequence the half-life is limited to <1 minute. These reactions may be written thus:

$$Th^{232}(d,d2p)Ra^{230} \xrightarrow[1\ hr]{\beta^-} Ac^{230} \xrightarrow[<1\ min]{\beta^-} Th^{230}.$$

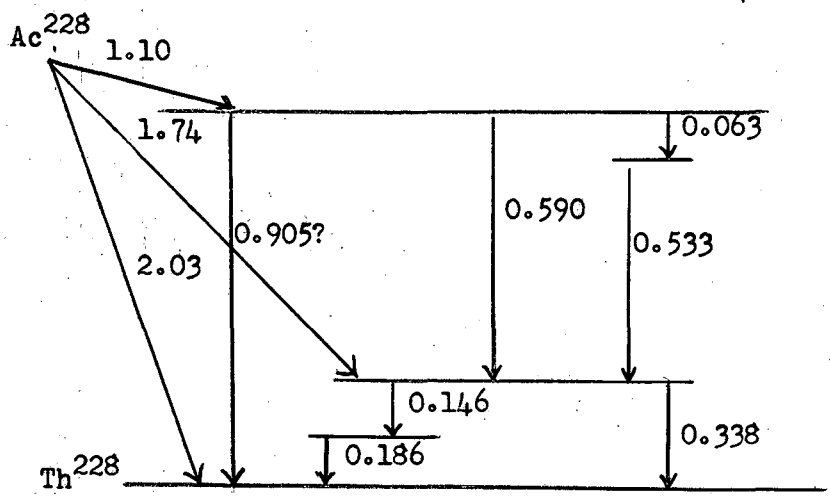
Unfortunately, not enough Th^{230} can be produced by this method to definitely establish the mass of this isotope but the energy systematics are unique sufficiently to definitely rule out all possibilities with the possible exception of

$$Th^{232}(d,d2pn)Ra^{229} \xrightarrow{\beta^-} Ac^{229} \xrightarrow{\beta^-} Th^{229}$$

The predicted half-life of Ac^{229} is, however, of the order of hours. Preliminary experiments have been made on the reaction $Ra^{226}(\alpha,p)Ac^{229}$ to clear up this point.

$MsTh_2(Ac^{228})$. Experiments to determine the disintegration energy of Ac^{228} on the high resolution beta ray spectrometer have definitely established the presence of three components in the beta spectrum and preliminary data indicate the presence of seven gamma rays. The following decay scheme is

consistent with these data but is probably not the unique answer:



The high energy gamma is only slightly converted and evidence for its existence is sketchy.

On An Isomeric State Of RaE

Harris B. Levy and I. Perlman

This work is a continuation of the work of Neumann, Howland, and Perlman as reported in Phys. Rev. 77, 720 (1950).

Bismuth was irradiated in the pile under high neutron flux. The principal reaction was $Bi^{209}(n,\gamma)Bi^{210}$. The bismuth was allowed to stand until all the RaE had decayed out. It was then rigorously purified of all other alpha activities (especially Po^{210}) and extraneous beta and gamma activities.

A residual activity remained in the bismuth after the rigorous purification, and finally reached a constant specific activity of 1.9 ± 0.1 alpha disintegrations per minute per milligram and 2.0 ± 0.2 electrons or beta particles.

The alpha activity was assigned to an isomeric state of Bi^{210} on the basis of experiments in which four minute Tl^{206} was milked from the bismuth. The beta activity was assigned to the Tl^{206} daughter.

The energy of the alpha particle was determined on the differential pulse analyzer to be 5.03 ± 0.05 Mev. (A later unpublished value was reported to be 5.00 Mev).

This was the status quo as of June, 1951.

Some of the bismuth containing the residual alpha activity was electromagnetically separated and the mass fractions 208,209, and 210 collected.

The specific activity of the bismuth in each mass fraction was determined to be as follows:

210

Sample 1 - 64.2 c/m per mg of Bi
Sample 2 - 71.3 c/m per mg

209

Sample 1 - No detectable activity.
Sample 2 - 0.3 c/m per mg of Bi

208

Sample 1 - 0.6 c/m per mg of Bi
Sample 2 - 0.9 c/m per mg

All of the above specific activities are measured alpha activities. On pulse analysis only ~ 5.0 Mev (Bi^{210}) alphas were found. No correction was made for counter geometry in the above figures.

These specific activities are of the order of magnitude calculated to result from the separation based on the assumption that the alpha activity was due to Bi^{210}. (Why the specific activity of the 208 fraction should be higher than that of the 209 fraction is not known, but it does not affect the conclusion that the activity is due to Bi^{210}.)

The bismuth samples were prepared by electrodepositing metallic bismuth from a solution ~1.5 N in H_2SO_4 using a current of 0.01 amp and a voltage of 2.0 - 2.5 V.

Some of the isotopically unseparated bismuth (in the form of BiOCl) was dissolved and bismuth plated out to recheck the specific activity. The following results were obtained:

alpha activity

Sample 1 - ~2.6 c/m/mg = 5.0 dis./min per mg) assuming 52 percent
Sample 2 - ~2.1 c/m/mg = 4.0 dis./min per mg) geometry

beta activity

Sample 1 - ~2.0 c/m/mg = 6.7 dis./min per mg) assuming 30 percent
Sample 2 - ~1.25 c/m/mg = 4.2 dis./min per mg) geometry

Sample 2 is the more reliable of the two runs.

A thin sample of bismuth was plated from the enriched 210 fraction, and the sample was run several times on the differential pulse analyzer. The energy of the alpha particle was now found to be 4.93 ± 0.02 Mev. From this new value of the alpha particle energy the disintegration energy of long-lived

Bi^{210} is calculated to be ca. 5.03 Mev. Recent work by Alburger and Friedlander (Phys. Rev. 82, 977 (1951)) has lowered the disintegration energy of Tl^{206} to 1.51 Mev. Since the disintegration energy of RaE is contingent on the disintegration energy of Tl^{206}, it has been recalculated to be ca. 5.06 Mev. As a consequence, doubt exists as to whether or not the decay of RaE represents the ground state transition. In fact, if these data are taken at face value the obvious conclusion is that RaE represents a higher isomeric state.

Approximately 50 gms of the isotopically unseparated BiOCl was dissolved in HCl and rigorously purified of Po^{210}. It has been set aside and will be examined at a later date to see if any Po^{210} has grown in.

Neutron-Deficient Europium and Gadolinium Isotopes

R. W. Hoff, J. O. Rasmussen, and S. G. Thompson

A study has been made of the light europium and gadolinium isotopes produced by bombardment of samarium and europium oxides with energetic protons, deuterons, and helium ions. In addition to the natural oxides, electromagnetically concentrated isotopes of samarium obtained from ORNL were used as target meterials. Separations of europium and gadolinium from samarium were made using a cation exchange resin column (Dowex-50, pH of citrate = 3.3 - 3.5) at elevated temperatures. Separations of europium and gadolinium were made using a sodium amalgam reduction method to overcome the difficulty of their separation in a cation exchange column. The conversion electron energies were determined from aluminum and beryllium absorption data. A search for positron emission in most of the nuclides studied was made using a 180° beta ray spectrometer of low resolution. Gamma ray energies were determined using a pulse analyzer employing a scintillation counter. Table I shows the results of this survey in the light europium and gadolinium region. Included are two isotopes of europium, Eu^{147} and Eu^{148}, which have been previously mentioned in a Quarterly Report (UCRL-1196).

Table I

Isotope	Type of Radiation	Half-life	Radiation Characteristics	Produced by	Bombarding Particle Energy	Chemical Separation
Eu^{144}	β^+	18 ± 2 min.	2.5 Mev β^+	$Sm^{144}(p,n)Eu^{144}$	8.5 Mev	none
Eu^{145}	EC, e^-	5 ± 1 days	0.2 Mev e^-	recoil nuclei from α decay of Tb^{149}	--	--
				$Sm^{147}(p,3n)Eu^{145}$	50 Mev	ion exchange column
Eu^{146}	EC, e^-	38 ± 3 hrs.	0.4 Mev e^-	$Sm^{147}(d,3n)Eu^{146}$	19 Mev	none
				$Sm^{144}(\alpha,2n)Gd^{146}$	25 Mev	none
				$(\alpha,pn)Eu^{146}$		
Eu^{147}	EC, e^- no β^+	24 ± 2 days	2.88 ± 0.1 Mev α 0.2 Mev e^- $\alpha/EC \sim 10^{-5}$	$Sm^{147}(p,n)Eu^{147}$	8.5 Mev	ion exchange column
Eu^{148}	EC, e^-, γ no β^+	50 ± 2 days	0.38 Mev e^- 0.6 Mev γ	$Sm^{148}(p,n)Eu^{148}$	8.5 Mev	none
Eu^{149}	--	>50 days	--	$Sm^{149}(p,n)Eu^{149}$	8.5 Mev	none
Gd^{149}	EC, e^-, α, γ	9 ± 1 day	3.0 ± 0.5 Mev α 0.35 Mev e^- $\alpha/EC \sim 10^{-5}$	$Eu^{nat}(p,3n)Gd^{149}$	28 - 32 Mev	sodium amalgam
				$Sm^{147}(\alpha,2n)Gd^{149}$	28 - 30 Mev	none
				$Sm^{nat}(\alpha,xn)Gd^{149}$	36 Mev	sodium amalgam

High Energy Zinc Spallation

William J. Worthington and Glenn T. Seaborg

The spallation products of zinc when bombarded with 340 Mev protons are under investigation and the following nuclides have been tentatively identified: $Cu^{61,64,67}$; $Ni^{57,65}$; $Co^{55,56,58m,58,61}$; $Fe^{52,59}$; $Mn^{51,52,56}$; $Cs^{49,51}$; $U^{47,48}$, Ti^{45}, $Ca^{45,57}$; $Ga^{66,67}$, and Zn^{62}. A partial list (Table I) of the cross sections for the formation of these nuclides has been compiled showing the variation of results between independent determinations.

Table I

Nuclide	σ (millibarns)
Ga^{66}	0.69, 0.60
Ga^{67}	1.6, 1.2
Zn^{62}	7.2, 6.6
Cu^{61}	34, 32
Cu^{64}	23, 23
Cu^{67}	4.5, 4.7, 4.4
Ni^{57}	2.3, 1.9
Ni^{65}	0.43, 0.32
Co^{55}	1.4
Co^{56}	1.8
Co^{58}	47 (includes independent yield of Co^{58m})
Co^{61}	1.6
Fe^{52}	0.20, 0.19
Fe^{59}	0.64, 0.65

The Co^{56} and Co^{58}, having the same half-life were distinguished by means of an aluminum absorption curve. The Co^{58m} was in evidence in the decay, but, inasmuch as its counting efficiency is unknown, its independent yield could not be calculated. The high observed count would seem to indicate that the greater contribution of the Co^{58} as reported actually derives from Co^{58m}. In calculating the above cross sections the observed counting rates were corrected for back scattering from the mount, self scattering, and self absorption in addition to the usual corrections. The cyclotron beam intensity was monitered by observing the formation of Na^{24} in an aluminum foil bombarded concurrently with the zinc.

High Energy Bismuth Fission

W. Biller

In the study of the fission of bismuth by 340 Mev protons the following yield data have been obtained: Cu^{61} 3.9; Cu^{64} 23; Cu^{67} 7.6; Se^{73} 0.28; Se^{81} 23; Rb^{86} 150; Sr^{89} 103; Sr^{91} 59; Mo^{99} 100; Ag^{111} 34; I^{124} 3.1; I^{125} 10; I^{126} 1.7. The yields are relative to Mo^{99}.

Other bombardments for which the yield values have not yet been calculated resulted in the identification of the isotopes: Co^{60}, $Ni^{65,66}$, $Br^{80,82,83}$, $Zr^{95,97}$, $Nb^{95,96}$, $Pd^{109,111,112}$, $Ag^{112,113}$, I^{123}.

An activity which decayed with a 36 hour half-life was observed in the cobalt fraction. The 37 hour Ni^{57} is an improbable contamination since it was undetected in the nickel fraction. It is hoped that improved chemical techniques will aid in the definite assignment of this activity.

Specific Activity Determinations

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

U^{235}. The electronic circuits used in conjunction with the medium geometry alpha counting chamber described in previous quarterly reports were eventually restored to satisfactory working order after being plagued with a series of subtle troubles. The U^{235} depositions are under observation in this chamber at this time.

U^{236}. About 20 mg. of highly enriched U^{236} was obtained as the oxide from the Y-12 plant at ORNL. It was found that the material was chemically impure to the order of 3 percent. The oxide was dissolved in hot concentrated HNO_3, made neutral with concentrated NH_4OH, and the impurities, chiefly iron, were precipitated as hydroxides in the presence of carbonate. After centrifugation, the supernate was made acid and all the carbon dioxide was boiled off. The pure uranyl ion was precipitated as the oxalate, dried, and baked to U_3O_8 in an open platinum crucible. Spectrographic and x-ray analysis revealed the resulting material to be 99.7 percent pure U_3O_8.

Five uniform samples were prepared by quantitative electrodeposition. Each sample was weighed as the oxide with the aid of a Ryerson quartz-fiber torsion balance. The samples varied in weight from 0.4520 to 1.4076 mg and were deposited over an area of approximately 11 sq. cm.

The samples were counted in the medium geometry chamber previously mentioned. The results are cited in Table I.

Table I

Sample	Weight (mg)	c/m (low geom.)	c/m/mg
1	0.7755	8891	11465
2	0.4520	5211	11529
3	1.0586	12234	11557
4	1.0301	11884	11537
5	1.4076	16295	11576

The calibration of the counter with a U^{234} sample of the same diameter as the U^{236} samples, and an accurate pulse analysis of the U^{236} are being undertaken at the present time. The final results will be reported in the next Quarterly Report.

The Self-scattering and Self-absorption of Beta Particles

W. E. Nervik

The technique of measuring the self-scattering and self-absorption correction factors for beta particles in active sample thicknesses from 1 to 20 mg/cm^2 has been reported in the Quarterly Report, UCRL-1365, for March, 1951.

This technique was utilized to measure the correction factors for six different beta active isotopes, each of which had a single beta particle and no gammas in its decay scheme. Correction factors versus sample thickness curves were obtained for each of the carrier-free isotopes mixed with known amounts of NaCl, RbCl, and $Pb(NO_3)_2$. These salts were selected because they seemed to meet the necessary requirements for solubility and temperature stability needed in the atomizer and deposit collecting apparatus used to prepare the samples. In certain cases the activity and the inactive cation formed insoluble compounds (i.e. $Pb(NO_3)2$ - W^{185}) or had a marked tendency to adhere to the walls of the glass apparatus (i.e. $Pb(NO_3)_2$ - P^{32}). Wherever possible a similar inactive salt was substituted, i.e. $TlNO_3$ substituted for $Pb(NO_3)_2$ in the P^{32} run. Pertinent data on the isotopes used are summarized in Table I.

Table I
Salts and Beta-active isotopes used for measuring self-scattering and self-absorption

Isotope	Half-life	Max- Beta Energy	Salts for which data were obtained
S^{35}	87d	0.167 Mev	NaCl, $Pb(NO_3)_2$
Pm^{147}	4y	0.223 Mev	NaCl, $Pb(NO_3)_2$, RbCl
W^{185}	73d	0.43 Mev	NaCl, RbCl, $Pb(NO_3)_2$
Pr^{143}	13.8d	0.92 Mev	NaCl, RbCl, $Pb(NO_3)_2$
P^{32}	14.3d	1.7 Mev	NaCl, RbCl, $Tl(NO_3)$
Y^{90}	65h	2.2 Mev	NaCl, RbCl, $Pb(NO_3)_2$

Curves of correction factor versus sample thickness for the three salts were quite similar in characteristics and appeared to differ only in degree. The curves for all except the softest beta particles showed a rather sharp rise up to a maximum value at a sample thickness of approximately 4 mg/cm^2, then a gradual decrease for thicker samples. Curves for NaCl are included in Fig. 1. In order to facilitate interpolation, it was considered desirable that curves be drawn for more even beta energy intervals. To do this, data on correction factor (f_{ssa}) versus beta energy were taken from Fig. 1 for sample thicknesses of 3, 5, 10, 15, and 20 mg/cm^2 and plotted on Fig. 2. These data gave points for reasonably smooth curves, from which f_{ssa} versus sample thickness values were taken for beta energies of 0.15, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 Mev and plotted on Fig. 3. A similar procedure was used for $Pb(NO_3)_2$ and the final working curve is included in Fig. 4.

When the self-scattering work was begun an attempt was made to find a mathematical equation which would fit the data. To date this has been unsuccessful. It is recommended, therefore, that the curves be used directly to obtain emperical correction factors for self-scattering and self-absorption.

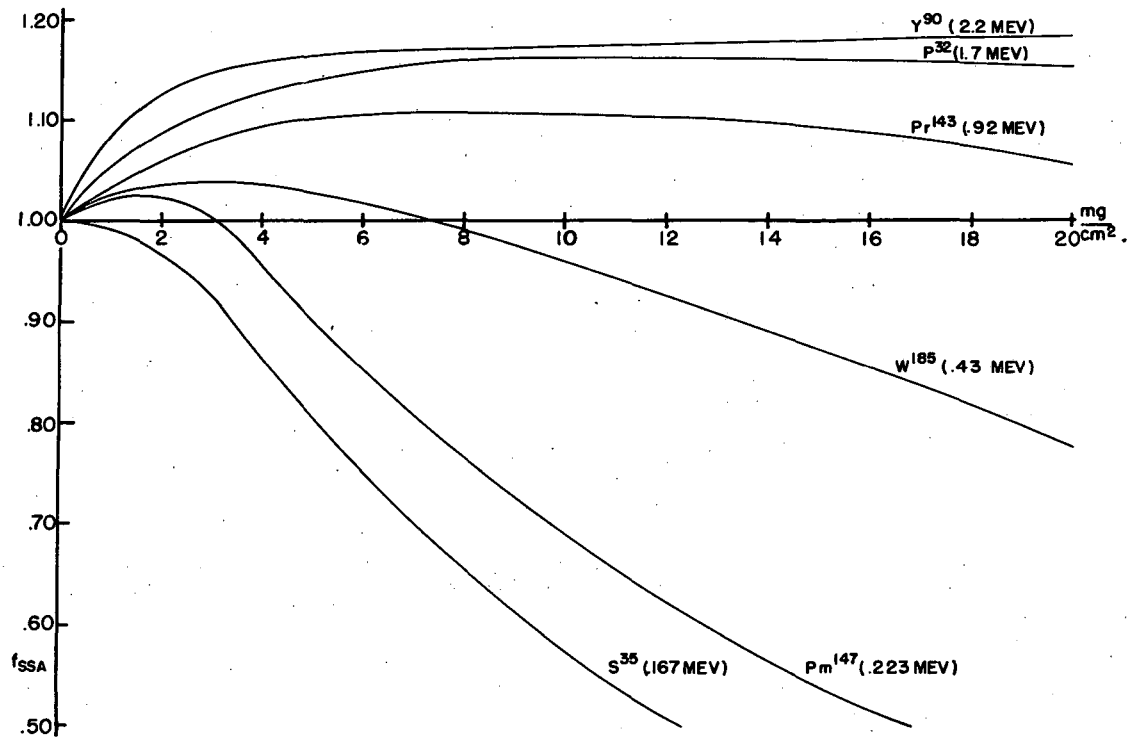


FIG. 1 $\left[f_{SSA} \text{ vs. } \frac{mg}{cm^2} \text{ Na Cl}\right]$

MU 2695

Fig. 1

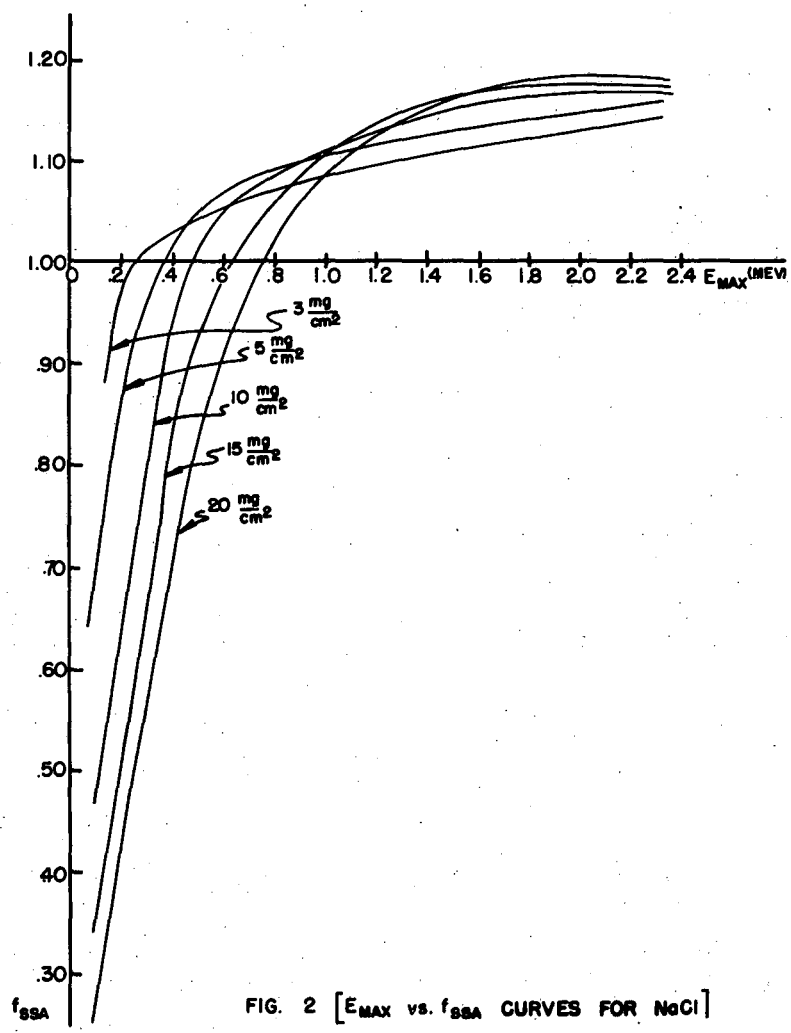


FIG. 2 [E_{MAX} vs. f_{SSA} CURVES FOR NaCl]

MU 2696

Fig. 2

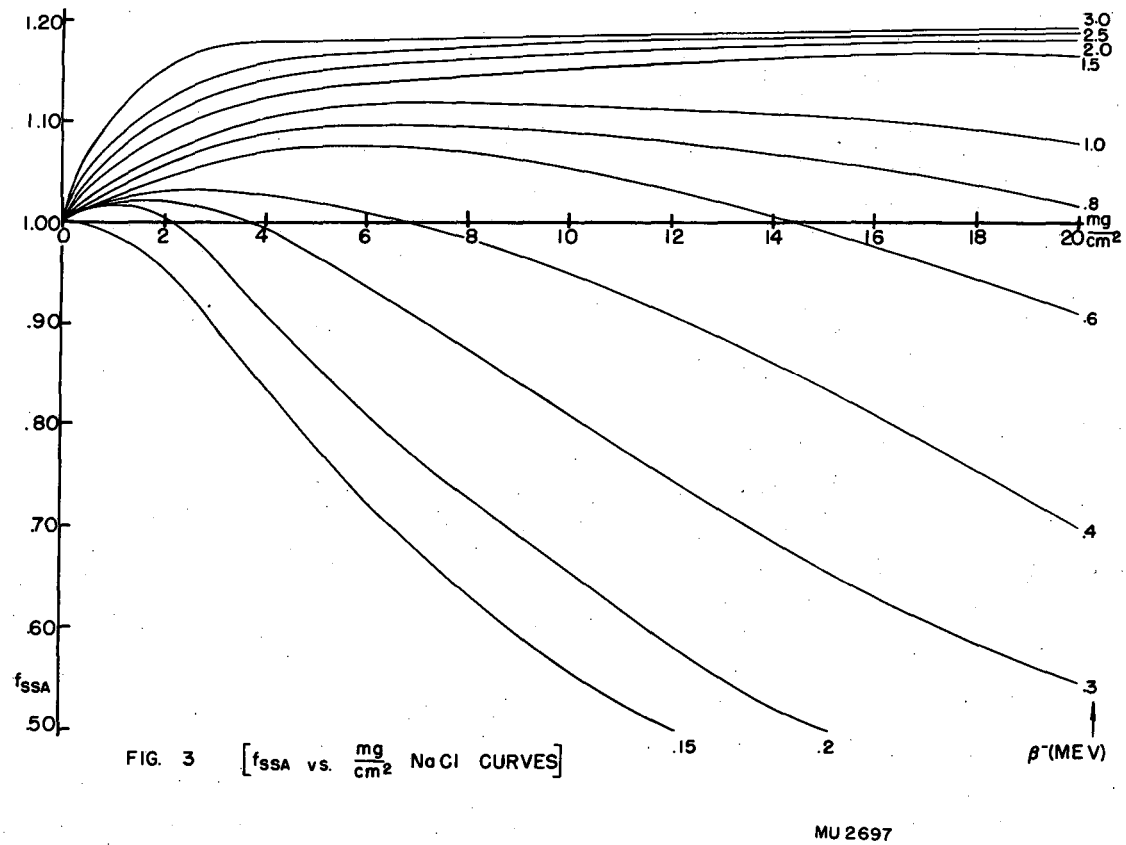


FIG. 3 [f_{SSA} vs. $\frac{mg}{cm^2}$ NaCl CURVES]

Fig. 3

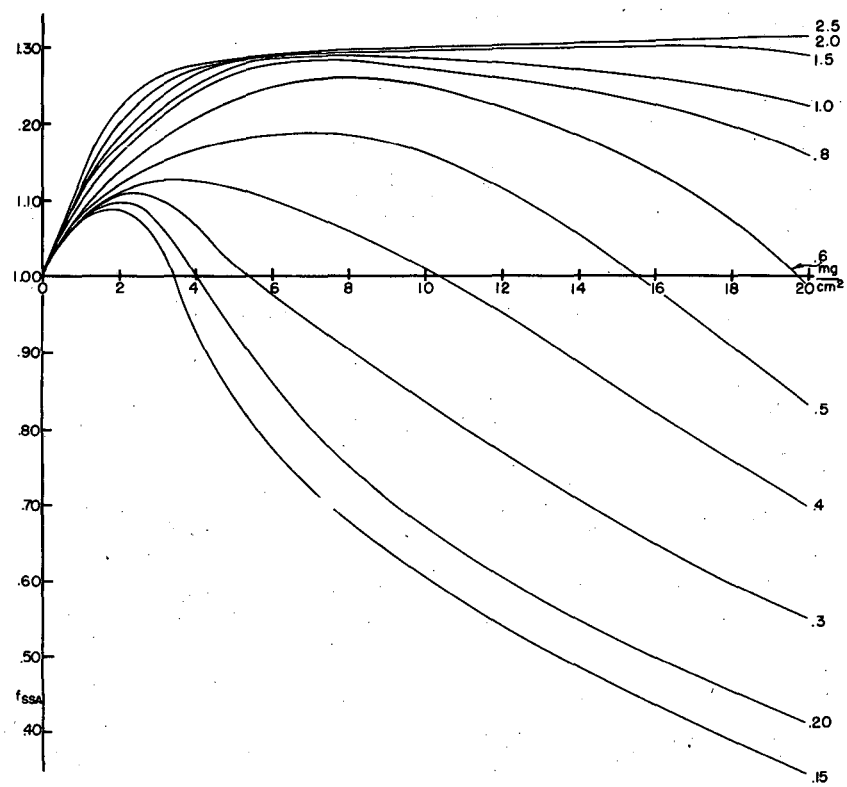


FIG. 4 $\left[f_{SSA} \text{ vs } \frac{mg}{cm^2} \text{ Pb } (NO_3)\right]$

MU 2698

Fig. 4

Modification and Use of the Mass Spectrograph

F. L. Reynolds

Mass Spectrograph II. An improved pumping system consisting of two 260 ℓ/sec DPI pumps with straight line flow has proven far superior to the single 260 previously installed with longer pumping length. It is now possible to pump the instrument down to 5×10^{-6} mm Hg in less than sixty minutes, while the previous pumping equipment required about five hours to reach a vacuum of 1×10^{-5} mm Hg. It is hoped that shorter lived radioactive isotopic masses may thus be ascertained in the machine.

Two attempts to establish the mass assignments of promethium activities from cyclotron bombardments of neodymium have not proved successful. No attempt was made to separate promethium from the normal neodymium, which was about 95 percent Nd^{150} in these runs. This lead to insufficient activity on the collector plate to make radioactive mass assignments. The experiment will be repeated, attempting to separate the promethium by a resin column separation before submitting the sample to the mass spectrograph.

Design work has started on the project of building an electron multiplier for the detection of positive ions in the spectrograph.

The bombardment source built for MS II is yet untried due to the lack of electronic units, namely an emission regulator unit. A more flexible high voltage supply is also needed to successfully operate a bombardment source in this machine.

Mass Spectrometer I. This instrument is now in operation, the bombardment source has been revamped to take solid samples and to volaltilize these samples into the bombardment chamber at temperatures as high as 2500° C. Detection on this instrument is by electrometer means only.

Other Instruments. Considerable time has been spent considering the possibilities of a new type of electrostatic alpha particle spectrometer and a low energy beta particle spectrometer using electrostatic fields. These instruments have not reached the stage where a detailed report is in order.

C. Bio-Organic Chemistry Group

Edited by B. M. Tolbert

Synthetic and Experimental Chemistry

M. Calvin, B. Tolbert, P. Adams, R. Bartsch, P. Chiado, D. Kritchevsky, R. Noller, R. Ostwald, R. Zeitschel and K. Booman

Preparation of Labeled Intermediates and Compounds. During the past quarter the following high-specific activity preparations have been carried out: Sodium acetate-2-C^{14}, 195 mc., 75-80 percent yield from CH_3I; methyl-C^{14} iodide, 150 mc., 55-90 percent yield from formate-C^{14}; isopropyl-2,3-C^{14} iodide, 35 mc., 70 percent from sodium acetate-2-C^{14}; sodium formate C^{14}, 200 mc., 83-90 percent yield from barium carbonate; glycine-2-C^{14}, 87 mc.; several preparations of cholesterol-T, 0.5-0.8 mc. per gram, yield approximately 40 percent; chloroacetic-1-C^{14} acid, 43 mc.; chloroacetic-2-C^{14} acid, 38 mc.

Sodium Acetate-2-C^{14}. A study was made to determine if any advantage could be gained by a variation in the manner of preparing labeled methyl grignard during the synthesis of sodium acetate-2-C^{14}. It was thought that perhaps the slow addition of the halide to the ether-magnesium mixture might result in fewer side-reaction products than the procedure currently in use in this laboratory. (Tolbert, J. Biol. Chem. 173, 205 (1948).) It has been shown in a previous report that distillation of an entire sample of amyl bromide into ether and magnesium, followed by carbonation on the vacuum line resulted in yields of caproic acid varying from 20-35 percent. In this same reaction slow addition of the halide followed by carbonation with a slow stream of tank carbon dioxide raised the yield to 70-75 percent.

The methyl-iodide reaction does not exhibit these differences. In three experiments using the slow halide addition and slow carbonation technique, yields varied from 70-78 percent. Average yields obtained from the routine vacuum line synthesis are 70-85 percent.

Reduction of Fatty Acids to Alcohols. The problem of preparing methanol and methyl iodide in good yields from $C^{14}O_2$ has been reopened using lithium aluminum hydride. Several methods have been used in the past for the preparation of methyl iodide from carbon dioxide; each has suffered some major defects. Thus, direct high pressure hydrogenation of CO_2 requires a special catalyst that may be difficult to prepare. Reduction of CO_2 with lithium aluminum hydride on a micro scale using diethyl carbitol as solvent results in an impure product due to the splitting of the solvent. The two-step procedure of the high-pressure hydrogenation of potassium bicarbonate to formate over a palladium catalyst and a second high-pressure hydrogenation of the cadmium-nickel salts of formic acid over a copper chromite catalyst yields a pure product in good yields. However, this method requires many steps.

Therefore the reduction of CO_2 with lithium aluminum hydride has been reinvestigated using the solvent and decomposing alcohol described by Cox, et al. (J. Chem. Soc., 3167 (1950).) i.e., tetrahydrofurfuroxy tetrahydropyran and tetrahydrofurfuryl alcohol, respectively.

Because of the similarity in the problem of reducing carbon dioxide and acetic acid using lithium aluminum hydride and because of the greater ease of handling acetic acid, the problem has been studied using acetic acid rather than carbon dioxide. Ethyl iodide has been prepared from acetic acid on an eighteen millimole scale according to the following reactions:

$$CH_3COOH \xrightarrow[\text{2. Alcoholysis}]{\text{1. } LiAlH_4} C_2H_5OH \xrightarrow{HI} C_2H_5I$$

The liberated alcohol was converted to the iodide in a gas-sweep system modified after the one described by Melville, et. al. (J. Biol. Chem. 169, 419 (1947)) for the conversion of methyl formate to methyl iodide. After the ethyl iodide was washed with sodium carbonate solution and dried with phosphorus pentoxide, it was weighed and analyzed. The yield was 85-90 percent based on the acetic acid taken and the product of excellent purity as judged by the refractive index (n_D^{20}= 1.5123). The conversion of carbon dioxide and some other fatty acids to the corresponding iodide or bromide will now be tried. The products will then be checked by mass spectrographic analysis for trace contaminants.

Valine-4',4-C_2^{14}. Tracer-level preparations of valine-4,4'-C_2^{14} have been completed and a 35 mc. preparation is in progress. This amino acid is synthesized by the condensation of isopropyl-2,3-C_2^{14} iodide with excess ethyl acetamidomalonate in potassium tertiary butoxide. The crude hydrolysis product contains glycine, valine and three other amino acids, at least one of which is radioactive as shown by paper chromatographic analysis.

A study is being made of the purification of this mixture through fractional elution from ion exchange resins. The resins under investigation are Dowex-50, a cation exchanger, and Dowex-2, an anion exchanger.

Attempts at displacement development with a slow flow of dilute ammonium hydroxide through Dowex-50 were unsuccessful. All of the amino acids were displaced in a single, concentrated band. Fractional elution with a very slow flow of dilute HCl through Dowex-50 separates valine satisfactorily from the other amino acids and in one run has yielded a sample of valine-4,4'-C_2^{14} hydrochloride containing 41 percent of the activity introduced as isopropyl iodide. There is, however, a trace impurity which is radioactive and is not an α-amino acid.

Experiments are underway to see if an equally satisfactory fractionation can be obtained by elution with ammonium hydroxide from Dowex-2. This method, if successful, would have two advantages over the HCl elution. The valine would be isolated directly instead of being obtained as the hydrochloride, and the large volumes of solution and long period of elution necessary with Dowex 50 might be shortened with use of the anion exchanger.

Labeled Di- and Tri-peptides. It was found that phthalylglycine can be prepared directly from the crude synthetic glycine mixture which contains an equimolal amount of ammonium chloride, thus obviating the necessity of purifying the glycine.

The yield of glycine-1-C^{14} from 42.7 mc. of barium carbonate was heated at 180-185° for eight minutes with 3.077 g. of phthalic anhydride. The mixture was cooled and recrystallized from 10 percent alcohol to give 3.532 g. of phthalylglycine, m.p. 187-192°. The specific activity as determined by plating and counting was found to be 10μc/mg., and thus there was obtained 35 mc. of phthalylglycine.

The preparation of glycyl-1-phenylalanine from phthalylglycine was reported previously. Glycylglycine was also prepared from this intermediate.

The acid chloride of phthalylglycine was prepared by warming 1.766 g. of phthalylglycine with 1.83 g. of phosphorus pentachloride in 6 ml. of benzene for one hour. The solvent was removed in vacuo and 18 ml. of dioxane was added. One half of this solution was added to a mixture of 324 mg. glycine and 228 mg. magnesium oxide in 6 ml. of water. The addition was made over the course of 1/2 hour, and the solution was stirred for an additional two hours. Hydrochloric acid was added to dissolve the excess magnesium oxide, and the solution was extracted six times with ethyl acetate. The organic solution was filtered and evaporated to leave a solid which was recrystallized from ethanol. There was obtained 563 mg., m.p. 225-227°. The specific activity of this sample was determined to be 8 μc./mg. The procedure for removing the phthalyl group from phthalyl dipeptides has been described previously.

Vinylacetic-1-C^{14} Acid. The preparation of labeled vinyl acetic acid was undertaken for a study of the oxidation of this compound to acetic and acetyl phosphate and its reduction to butyric acid as catalyzed by extracts of the bacterium, Clostridium kluyveri. The difficult problem in this synthesis is to obtain a product free of crotonic acid. Carbonation of the allyl-grignard reagent proved a satisfactory method to prepare the carboxyl labeled acid. The reagent was formed by slowly adding 20 mm. allyl chloride dissolved in 50 ml. ethyl ether to 25 ml. rapidly stirred ether containing 60 mm. magnesium turning. After 1 hour, 25 ml. toluene was added which partially dissolved the thick slurry. About 50 ml. of the solution containing 11-12 mm. reagent were transferred to a 250 ml. flask fitted with a stopcock and joint, the contents frozen in liquid nitrogen and the system attached to the vacuum line. Then 9.5 mm. $C^{14}O_2$ was frozen into the flask which was removed from the line and slowly rotated in an alcohol-Dry ice bath for 1/2 hour to effect the carbonation at minimum temperature. Finally a solution of 20 mm. conc. H_2SO_4 in 4 ml. ether was added to decompose the reaction mixture at -70°. The reaction was completed by warming the solution to 0° and adding 5 ml. water. The vinyl acetic acid was recovered by immediately decanting the ether layer into a two-tube extraction train containing 50 ml. water in each tube. About 500 ml. ether which was used to wash the nearly dry residue in the reaction flask was used to extract the product away from water soluble materials. The effluent solution was titrated with 0.200 N NaOH to a phenol red end point (about pH 7). The aqueous solution was immediately separated from the ether and lyophilized. The maximum yield obtained in a warm run (~1 μc/mm.) was 86 percent (titration, weight, and activity). Absorption spectra measurements showed an E_{max} at 210 mμ of 565. The material gave a single radioactive spot on a paper chromatogram developed with 1 percent conc. NH_4OH in methyl cellosolve. A higher activity run (0.5 mc./mm.) gave 81 percent yield and a single chromatogram spot but E_{max}-210 mμ = 1242 indicating about 7 percent contamination with crotonic acid.

Because other means of distinguishing the isomeric acids were inconclusive the absorption spectra of crotonic and vinyl acetic acids were measured. The absorption of sodium crotonate (E_{max}-210 mμ = 10,070, 0.010 mg./ml. water) and of sodium vinyl acetate (E_{max}-210 mμ = 540, 0.100 mg./ml. water) both begin about 250 mμ and rise continuously to the 200 mμ limit of the Cary spectrophotometer. Since the absorption peak lies beyond instrumental limits, the E_{max}-210 mμ value was arbitrarily chosen for comparison purposes. A product prepared by carbonation of the grignard reagent product gave the lowest value observed for vinylacetate, E_{max}-210 = 540, while a redistilled sample of material prepared by hydrolysis of allyl nitril gave E_{max}-210 = 875. Although the absorption of the former sample may represent more contribution from crotonic than from vinyl acetic acid, it is apparent that the product is of a quality equal to or better than that prepared from the nitrile.

Guanine-8-C^{14}. Guanine-8-C^{14} was prepared for use in a bacterial fermentation study. The synthesis involved formylation of 1 mm. 2,3,4-triaminopyrimidine hydrochloride with 1 mm. formate-C^{14} (3.02 mc.) and subsequent cyclization of the formylpyrimidine in N-formyl morpholine at 200° (Balis, et. al., J. Biol. Chem. 188, 217 (1951).) The overall radioactive yield was 9 percent if the 0.5 mm. recovered formate is disregarded. The weight yield was 72 percent. The radioactive yield of intermediate formylpyrimidine was 47 percent. Some preliminary experiments indicated use of a cyclization solvent such as diethyl or dibutyl carbitol would give a product with an activity yield as high as 9 percent. A more suitable solvent (N-ethylmorpholine perhaps) might be found if it were necessary to improve the yield.

Glucose and Mannose-1-C^{14}. Progress has been made in developing a preparative chromatographic separation of glucose and mannose by a modification of the ion exchange separation described by J. X. Khym and L. P. Zill, (J. Am. Chem. Soc. 73, 2399 (1951)). Ninety mg. (0.5 mm) of each sugar and 62 mg. H_3BO_3 were dissolved in 1.5 ml. H_2O and added to a 6.5 milliequivalent Dowex-2-borate column in a 5 x 25 mm. tube, with another 2 ml. water as rinse. A 3 percent solution of tartaric acid containing an equivalent amount of H_3BO_3 was then applied as a displacement developer and the composition of the effluent solution followed with a recording differential refractometer. In the first 70 ml. of eluate the recorder trace showed 3 steps, the first corresponding to mannose, the second to glucose and the third to the developer. This type of displacement development offers the great advantage over elution development of high loading on relatively small amounts of adsorbent and the consequent reduction in effluent solution volume, but the products usually must be segregated and rechromatographed by elution development if it is necessary to effect quantitative separation in a single batch.

Carbon 14 labeled Aspartic Acid. In the last Quarterly Report, UCRL-1365, work was reported on the separation of aspartic acid and glycine with amberlite IR-4B followed by precipitation of the aspartic acid with alcohol at pH 2.9. This proved to be unsatisfactory as a dark material is precipitated.

Paper chromatographs which had not been completed at the time showed that there were at least two radioactive impurities in the aspartic acid fraction and that there was still an appreciable amount of aspartic acid left with the glycine. Both fractions were combined and dried with several portions of conc.

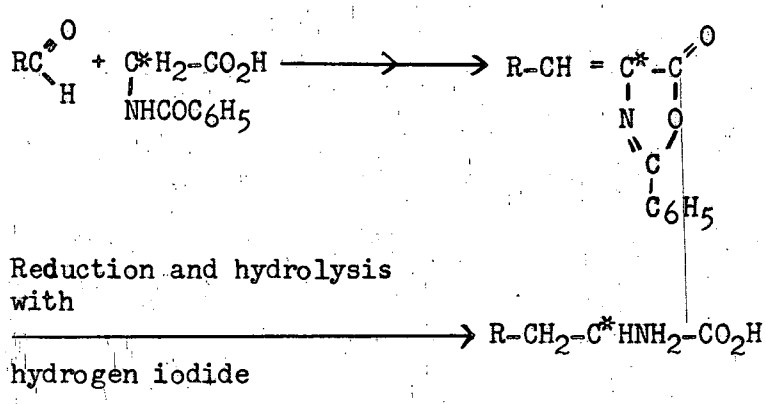
HCl to remove all alcohol. The mixture was placed on a Dowex 5-cation exchange column, washed with water and then eluted with 1 N HCl at a linear flow rate of 0.08 cm. per min.

The water wash contained a fair percentage of the starting activity. Chromatographic analysis indicated that there was no aspartic acid in this fraction. Paper chromatographic analysis of the subsequent fractions indicated that there were probably seven radioactive impurities, most of which were not ninhydrin active.

The aspartic acid was recovered (45 percent of starting activity of $BaCO_3$) without any other radioactive or ninhydrine active impurities. Sodium chloride which came off of the Dowex 50 column with the aspartic acid is now being removed by passing the mixture through a Dowex 2 anion exchange column.

Azlactone Preparation of α-amino Acids. This synthesis is being investigated as a means of preparing a number of labeled amino acids.

1) Condensation in acetic anhydride



(R is generally an aromatic group)

Azlactones of the following aromatic aldehydes have been prepared; benzaldehyde, anisaldehyde, o- and p-chlorobenzaldehyde, 2,3-dimethoxybenzaldehyde, p-dimethylaminobenzaldehyde, 3,4-diethoxybenzaldehyde, m-nitrobenzaldehyde, o-methoxybenzaldehyde, salicylaldehyde and veratraldehyde. All of these compounds readily form azlacetones when heated with hippuric acid on a hot plate in acetic anhydride solution with freshly fused sodium acetate. Heating is continued until solution is complete after which the reaction mixture is warmed on a steam bath for about an hour. The crystals of the azlactone generally begin to appear early in the latter stage and are yellow to orange in color.

A small portion (5-7 ml) of ethanol (95 percent) is added to the reaction mixture, and the mass of crystals is broken up; after standing overnight, the crystals are filtered off and washed with several small portions of ice-cold alcohol followed by a small amount (1 ml) of boiling water. The compound thus obtained is found to be pure enough to give satisfactory results in the reduction. Recrystallizations from benzene of several of the products were carried out, but the azlactones so purified seemed to give no better yields in the reduction step than did these crude compounds. Yields for the azlactone

preparations have been running 60-65 percent. When much higher yields have been obtained, the products were too impure for further use without recrystallization.

The azlactone of α-benzoylaminocinnamic acid (i.e. product from the condensation of benzaldehyde and hippuric acid) was the one on which most of the investigation was carried out. The reduction was carried out using a hydrogen iodide-red phosphorus mixture in acetic anhydride in a 3-neck flask fitted with a dropping funnel and condenser.

The HI is added to the reaction mixture over a period of about 20 minutes after which the solution is refluxed on a steam bath. Initially a time of 3-4 hours was being allowed for the refluxing, and 47 percent HI was being used. Results were very poor, however, More concentrated HI (55-58 percent, sp. gr., 1.7) and reflux times of 12-15 hours have improved the yields greatly.

After this period of reflux, the reaction mixture is allowed to cool and the phosphorus is filtered from the solution. The filter is washed with small amounts of glacial acetic acid. The solution is evaporated to dryness under reduced pressure, a small portion of water (5 ml) is added to the residue, and the evaporation to dryness is repeated.

To the residue in the flask are added equal portions of ether and water (5-8 ml each). Separation of the two layers is carried out after complete solution of the residue is obtained. The ether layer is discarded while the water layer is extracted three more times with small portions of ether. Some decolorizing charcoal and a trace of sodium sulfite are added to the aqueous phase and it is heated on the steam bath to remove traces of ether.

After the charcoal is removed by filtration, the solution is heated to boiling and 15 percent NH_4OH solution is added until the solution is neutral to Congo Red paper. Flakes of the amino acid begin to separate. After crystallization is complete, the crystals are filtered off and washed with a very small amount of ice-cold water. The yield of omide phenylalanine is 60-70 percent. A solution of one sample was chromatographed; only one ninhydrin spot appeared, indicating only one α-amino acid. It is hoped that the yield can be improved. Reduction of other azlactones is now being carried out.

<u>Tristearin-T</u>. The tritiated stearic acid, reported in the last Quarterly Report, UCRL-1365, was converted to tristearin for future feeding experiments. A solution of stearic-T^3 acid (2.0 gm., 0.0069 mole) and thionyl chloride (1.0 gm.) in 10 ml chloroform was refluxed for two hours. The solvent was distilled and the crude acid chloride was dissolved in 5 ml of chloroform and added, with stirring, to a solution of glycerol (0.172 gm., 0.0028 mole) in 2 ml pyridine and 5 ml chloroform. After standing at room temperature for three days, the solution was poured into water and the triglyceride extracted with ether. The ether was washed with acid and with base, dried and evaporated. There was obtained 0.92 gm. of tristearin (49 percent). Saponification equivalent; calculated 890; found 873.

<u>Irradiation of Labeled Di-glycine</u>. A solution of 2.030 mg. of glycycle-1-C^{14} glycine-1-C^{14} (sp. act. = 1.5 μc/mg.) in 1.991 g. of water was irradiated in a Co^{60} source (4.5 c.) for 2-1/2 days. Counting of the solution before and

after irradiation indicated that 6 percent of the activity had been lost. The irradiated solution was chromatographed first in phenol, then in butanol-propionic acid, and put on film.

No activity was observed at the orgin and no glycine activity was observed. Seven other radioactive compounds were, however, noted. The same pattern was produced from irradiation of other samples of radio di-glycine, except that one of the spots was missing. Acetylglycine was prepared and found to run to about the same place as the seventh spot on the chromatogram. Inactive acetyl-glycine can be detected by means of bromcresol green indicator.

The irradiation of glycycl-l-tryptophan reported earlier was repeated and the irradiation of glycycl-l-tryosine was carried out. The irradiated solutions were analyzed for amino acid and peptide nitrogen by the method reported previously. The glycycl-l-tryptophane solution showed a decrease in concentration of amino acid and peptide nitrogen of 7 percent, after irradiation, and the glycycl-l-tyrosine solution showed a decrease of 5 percent. Ultraviolet analysis of the solution of glycycl-l-tryptophan before and after irradiation indicated that 10 percent of the indole nucleus had been destroyed.

Irradiation of Simple Organic Compounds. This work has been planned to show whether or not more complex organic compounds can be built up from the most simple forms. We are currently investigating the possibility that labeled oxalic acid may be formed when a mixture of radioactive CO_2 and dead formic acid are irradiated.

1.01 mc. of CO_2 and one ml of formic acid were sealed in a tube and placed in a 2.8 C. Co^{60} source for 27 days. Assay for radioactive products other than CO_2 is indicated that only about 1.5×10^{-2} μc. had become fixed. Hydrolysis of aliquots in both acid and base did not appreciably change this value. There is a radioactive residue which remains after the formic acid has been removed by evaporation. The original solution has been chromatographed and its radioautograph is now being taken. One major compound has been formed and it could be oxalic acid from the position on the paper.

Other samples composed of various aqueous mixtures have been prepared and irradiated. They are also being analyzed by paper chromatography and radioautography.

Low-level Carbon 14 Assay. It is often necessary in biological research to work with low-specific activities of C^{14}. Sometimes the radioactive tracer, for economic reasons, must be used in relatively small amounts and in other cases even when relatively high activities are used in the initiation of a biological problem, the specific activities of some of the products may be quite low. Thus, this work can be greatly enhanced when low levels of activity can be accurately assayed. This laboratory is currently exploring several possibilities in the determination of low-level C^{14} activity. The main problems in this field are the reduction of background in the counting instrument and using large samples efficiently. Ionization chambers and proportional counters seem most practical for use in such low-level counting.

The background of the ionization chambers depends partly on the material of the walls. It has been found that mild steel chambers have lower

backgrounds than brass chambers. Currently a number of new steel ionization chambers are being made. CO_2 evolved from $CaCO_3$ has a lower amount of radioactive contaminants (principally radon) than the CO_2 evolved from $BaCO_3$. The ionization chamber counting equipment is being calibrated for the use of $CaCO_3$. The ionization chambers will then be used for either $BaCO_3$ or $CaCO_3$.

Higher pressures for the ionization chambers are contemplated. If a higher sample pressure is used, more material can be placed in a constant volume. This will reduce part of the chamber background relative to the sample and will permit measurements in a more accurate range of the vibrating reed electrometer.

Nucleometers, which are methane-flow proportional counters, are used in this laboratory for the determination of the activity in low-level solid samples. $BaCO_3$ has been the standard medium for carbon 14. However, it was felt that $CaCO_3$ might make a better counting material than $BaCO_3$. $CaCO_3$ has twice the carbon weight per milligram. Since self-absorption of solid samples depends mostly on sample thickness (mg/cm^2), $CaCO_3$ should be the better of the two compounds. A self-absorption curve was determined for $CaCO_3$. The $CaCO_3$ used was precipitated by the drop-wise addition of two-times excess 0.3 M $CaCl_2$ to the NaOH absorption solution. The solution was buffered with a 20 percent excess of NH_4Cl. The precipitation was carried out at 80° C. The precipitate was washed with water and alcohol. It was dried over-night at 110° C. The plates for counting were made with the same technique as is used in making $BaCO_3$ plates at this laboratory. The $CaCO_3$ plates did not have the physical stability of $BaCO_3$ plates and deteriorated rapidly with ordinary handling. Often $CaCO_3$ is pulled off the plates by the static charge developed on the lucite plate holders. The $CaCO_3$ self-absorption curve was found to lie on the $BaCO_3$ absorption curve. It is questionable whether the advantage of low self-absorption per unit weight of carbon is worth the greater difficulty in handling the calcium carbonate.

In the near future it is hoped that a proportional counter using a C^*O_2 sample plus a diluent as the counter tube gas mixture will be set up and counting measurements tried.

Biological Chemistry

M. Calvin, E. Bennett, L. Daus, S. Ikeda, M. Kirk, D. Kritchevsky, B. Kruekel, M. Lazarus, D. Pack

Metabolic Rate Studies in Normal and Hypophysectomized Rats. Experiments are being carried out to determine the difference in the rate of metabolism to $C^{14}O_2$ of certain C^{14}-labeled compounds by normal and hypophysectomized rats and the effect of injections of cortisone on these rates.

The rats being studied are Long-Evans strain females; hypophysectomy was performed when animals weighed approximately 150 gms. Intraperitoneal

injections of 2 mg. quantities of the labeled compounds were used. Animals are placed in metabolism cages, the CO_2 collected in 1 N NaOH, precipitated, weighed and counted as $BaCO_3$.

Both normal and hypophysectomized rats injected with sodium acetate-2-C^{14} eliminated between 70 percent and 80 percent of the injected activity as $C^{14}O_2$ in the first seven hours after injection. The rate of recovery of $C^{14}O_2$ was approximately the same for both types of animals; however, the over-all metabolism, based on total weight of $BaCO_3$ recovered, was found to be about 45 percent lower in the hypophysectomized rats. Conversely, the specific activity (dis./min./mg.) of the $BaCO_3$ recovered was higher in about the same proportion; the greatest difference occurred about one hour after injection. Injection of 1.25 mg. cortisone acetate six hours before the injection of sodium acetate-2-C^{14} caused a marked increase in the utilization of the acetate during the first half hour, but no apparent difference after that.

When normal and hypophysectomized rats were injected with uniformly labeled glucose total recovery of $C^{14}O_2$ in seven hours amounted to 55-65 percent of the injected activity and showed proportionally the same differences as were found when acetate was used. However, the normal rats reached the peak of elimination of activity about 45 minutes after injection, whereas this peak was not reached by the hypophysectomized animals for about one hour or longer. When these same animals were injected with 1.25 mg. cortisone acetate six hours before receiving the sugar injection, utilization of sugar seemed to be slowed considerably in both types of animals.

The accompanying chart gives representative data for two animals each receiving 2 mg. sugar containing 3.52 μc of C^{14} at two different times, i.e., once without cortisone and once with cortisone six hours before the sugar.

Table I

Sugar Metabolism Rates for
Normal and Hypophysectomized Rats

		Av. mg. $BaCO_3$ per min.	Total % Act. in CO_2 in 2 hrs.	Highest sp. act. $BaCO_3$ and time after injection
Normal	without cortisone	53	48	873 dis./min. at 50 min.
	with crotisone	49	22	483 dis./min. at 30 min.
Hypophy-sectomized	without cortisone	29	41	1510 dis./min. at 1 hr. 15 min.
	with cortisone	26	18	764 dis./min. at 30 min.

Experiments using labeled sucrose showed no very marked differences other than the type of difference already pointed out. Use of cortisone made no readily discernible change from the normal metabolism rate; however, utilization of sucrose was very slow in any event--about 3 percent recovery of activity in 3 hours--and even a considerable difference might not be readily apparent.

Acetate Metabolism in Chickens. In order to obtain more information concerning the incorporation of acetate into yolks, the glycerol obtained from the various yolks was subjected to oxidative degradation. Treatment with lead tetraacetate oxidized the glycerol to one mole carbon dioxide (from C 2) and to 2 moles of formaldehyde (from C 1 and 3). Oxidation with periodate yielded 2 moles of formaldehyde (from C 1 and 3) and one of formic acid (C 2). The formaldehyde formed was oxidized with permanganate to give CO_2. Inasmuch as these methods cannot distinguish between the first and third carbon atoms of glycerol, the total activity was divided in two and half was assigned to each of the end carbon atoms. The activities are listed below.

Table II

Degradation of Glycerol

Yolk	C_1 and C_3 dis./min./mg. $BaCO_3$	Percent	C_2 dis./min./mg. $BaCO_3$	Percent
1*	4	50	0	0
2	23	44	6	12
6	34	43	11	14
7	68	43	22	14
9	30	44	8	12

(*) Due to the low activity, the results in this degradation are not very accurate.

Work is in progress on the degradation of glycerol from yolks 5, 8 and 10.

Steroid Studies. Cholesterol Metabolism in Rats. The breath curves for two rats each fed 0.94 μc of random labeled cholesterol-C^{14} have been drawn and analyzed. The feces material and the carcass of each animal have been split into fatty acid and non-saponifiable fractions by fractionation over IRA-400 resin. (See Table III). In the case of Rat A, some solid material was isolated in the working up of the carcass fractions. This material was from the non-saponifiable fraction and was shown to be cholesterol (m.p. 142-4°). The specific activity of this material was 2.16×10^{-4} μc/mg.

In Table IV the activity of the several other body fractions from these two rats is shown.

Table III

Distribution of Radioactivity in Rats Fed Cholesterol-C^{14}

	RAT A			RAT B		
	Wt. mg.	Sp. act. μc/mg.	Total act. μc	Wt. mg.	Sp. act. μc/mg.	Total act. μc
Feces						
Non-sap.	478.9	6.76×10^{-4}	3.24×10^{-2}	441.3	6.25×10^{-4}	2.76×10^{-1}
Fatty acid	375.3	1.20×10^{-4}	4.50×10^{-2}	243.0	2.60×10^{-4}	6.32×10^{-2}
Carcass						
Non-sap.	264.2	8.74×10^{-2}	2.31×10^{-2}	1426.0	4.46×10^{-5}	6.36×10^{-2}
Fatty acid	1307.1	1.13×10^{-5}	8.26×10^{-2}	9853.5	3.11×10^{-5}	3.06×10^{-1}
Breath in 96 hrs.			0.012			0.006

Table IV

Distribution of Activity in Rats Fed Cholesterol-C^{14}

	RAT A		RAT B	
	μc (total)	% fed	μc (total)	% fed
Breath	0.012	1.28	0.006	0.64
Feces-NS*	0.324	34.46	0.276	29.36
Feces-FA*	0.045	4.79	0.063	6.70
Carc-NS	0.023	2.45	0.064	6.81
Carc-FA	0.083	8.83	0.306	32.55
Urine (total)	0.077	8.19	0.138	14.68
Liver-FFA*	0.024	2.55	0.037	3.94
Liver-FA	0.020	2.13	0.029	3.09
Liver-NS	0.394	41.91	0.017	1.81
Liver-PL*	0.014	1.49	0.071	7.55
Adrenal-FA	0.017	1.81	0.017	1.81
Adrenal-NS	0.030	3.19	0.023	2.45
Liver-Glycogen	0.006	0.64	0.008	0.85
	1.069	113.72	1.055	112.24

(*) NS = Non-saponifiable, FA = Fatty acid, FFA = Free fatty acid, PL = Phospholipids.

The excess of 12-13 percent is well within experimental error, considering that all counts were direct plates with no care taken for exceptional accuracy. The values do show, however, that most of the activity was accounted for and that cholesterol is rapidly broken down in the body, going to fatty acids, glycogen and probably to other steriods. As would be expected, however, a considerable portion of the material was excreted in the feces.

Metabolism of Doubly Labeled Cholesterol. The purpose of this investigation was to see what limitations must be placed on observations of cholesterol metabolism made with tritium labeled cholesterol, due to the fact that the label is in the hydrogen component of the molecule.

A female rabbit, weighing 4,230 gm., was fed 0.5-0.6 gm. of cholesterol in 5 ml. of Wesson oil daily for 101 days. The initial serum cholesterol was 27 mg. percent and was 1,256 mg. percent after 101 days. A mixture of labeled cholesterol in Wesson oil (9 ml) was prepared using 206 mg. of Cholesterol-H^3 (Spec. act. 0.924 μc/mg.) and 80 mg. of cholesterol-C^{14} (sp. act. 6.60×10^{-2} μc/mg.), and administered via stomach tube. The ratio of specific activity H^3/C^{14} was 33.0:1. Blood samples were taken every day for six days, then on the 8th and 10th. The animal was killed and the viscera removed and frozen for future study. The data are tabulated below:

Table V

Source of Cholesterol	C^{14} sp. act. ($\times 10^{+4}$) μc/mg	H^3 sp. act. ($\times 10^{+2}$) μc/mg	Ratio
1st day serum, total	5.32	1.70	31:1
1st day serum, free	2.89	1.02	28:1
1st day serum, esterified	5.85	1.85	32:1
2nd day serum, total	6.23	1.86	33:1
3rd day serum, total	5.39	1.70	32:1
4th day serum, total	4.40	1.50	29:1
5th day serum, total	3.61	1.31	28:1
6th day serum, total	3.30	1.08	31:1
8th day serum, total	2.94	0.92	32:1
10th day serum, total	2.58	0.81	32:1
10th day serum, free	2.62	0.80	33:1
Liver, total cholesterol	1.68	0.63	27:1

These results show that tritium labeled cholesterol is a suitable tracer compound for the investigation of many aspects of cholesterol absorption and tissue turnover.

Isolation of Sterols from Algae. A petroleum ether solution representing the soluble portion from 100 cc. of cells of Scenedesmus was distilled and the residue saponified with 10 percent potassium hydroxide. The saponification mixture was extracted with ether and the ether dried and distilled. The residue was dissolved in equal parts of petroleum ether and methanol and, upon addition of enough water to make the methanol 90 percent, the petroleum ether layer separated. From this solution, it was possible to isolate 5 mg. of pure chondrillasterol, needles from petroleum ether, m.p. 168° C. This corresponds with the melting point given in the literature by Bergmann.

Cholesteryl Laurate. A solution of 28 gm. of cholesterol and 17.5 gm. of lauroyl chloride in 25 cc. of pyridine was brought to a boil and allowed to cool. The resulting solid was dissolved in ether and washed free of pyridine and acid. The cholesteryl laurate was crystallized from ethanol, acetone or chloroform-methanol, m.p. 78-78.5°. This value was not in agreement with the m.p. reported in the literature (91°; 92°; 100°; 110°).
Analysis: Calculated for $C_{39}H_{68}O_2$: C, 82.33; H, 12.05

Found: C, 82.21; H, 11.99.

The preparation was repeated using the method described by other authors (Page and Rudy, Biochem. Z. 220, 304 (1930)). Cholesterol (2 gm.) and lauric acid (4 gm.) were heated together at 200° for 3 hours in a stream of carbon dioxide. The material obtained melted at 75-6°. Crystallization from various solvents did not alter the melting point. This material did not depress the melting point of the ester prepared previously. Sublimation at low pressure did not alter the melting point of the initial preparation. Experiments are under way to prove the isomorphism of these esters.

Paper Chromatography of Steroids. In the paper chromatography of steroids, the detection of these compounds is a problem that parallels that of separation. In order to investigate the paper chromatography, we first attempted to devise suitable color tests for various steroids which we have managed to obtain. For running these tests, the individual steroids (none in amounts exceeding 10γ) were applied to circles 3 cm^2 in area, which had been drawn on Whatman No. 1 paper and "Quilon" treated paper. The tests are summarized below. In all, six methods of detection were tried.

1) Ultraviolet light: Inasmuch as "Quilon" impregnated paper is opaque to UV light, the results with Whatman alone are summarized.

2) Iodine: The papers were suspended for 1-2 minutes in iodine vapor. Brown spots.

3) Picrate: A solution consisting of equal parts of saturated alc. picric acid and 1 N solium hydroxide was used. The entire strip was rendered yellow and, in the case of the Whatman paper, detection was impossible.

4) Antimony pentachloride: A 20 percent chloroform solution of this material was used.

5) Phosphomolybdic acid: A 10 percent alcoholic solution of this reagent was used. After spraying, the papers were dried at 110° for one minute. Blue spots of varying intensity were noted. This is, to our knowledge, the first time that this reagent has been used as a test reagent for steroids.

6) Silicotungstic Acid: A 25 percent alcoholic solution was used. The spots were dried for 1 minute periods at 110°. Often the color came out after the second or third minute. More than three minutes caused the papers to char. In some cases, colors changed after a minute.

Eight non-steroids are included at the end of Table VI for comparison purposes

Table VI

Compounds	UV W	IODINE W	IODINE Q	PICRATE Q	PHOSPHOMOLYBDIC W	PHOSPHOMOLYBDIC Q	SILICOTUNGSTIC ACID W 2 min.	SILICOTUNGSTIC ACID Q 2 min.	ANTIMONY PENTACHLORIDE W	ANTIMONY PENTACHLORIDE Q
1. Dehydroepiandrosterone	+2	+4	0	0	+4	+4	brown	brown	purple	purple
2. Dehydroepiandrosterone acetate	0	+2	0	0	+3	+3	brown	brown	purple	purple
3. Testosterone	+4	+2	+1	+1	+2	+2	pink	pink	---	---
4. Testosterone propionate	+4	+2	+4	+3	+2	+2	tan	tan	v.p.yellow	v.p.yellow
5. Progesterone	+4	+2	+4	+3	+1	+2	yellow	yellow	v.p.yellow	v.p. yellow
6. Estradiol	0	0	+1	0	+4	+3	brown	orange	brown	brown
7. Estradiol dipropionate	0	0	+1	+2	+4	+3	brown	tan	v.p.yellow	brown
8. Androsterone	0	+1	+1	+2	+4	+3	brown	tan	gray	gray
9. Desoxycholic acid	0	0	+1	0	+3	+3	tan	v.p.pink	p.yellow	p.yellow
10. Dehydrocholic acid	0	0	0	0	0	0	---	---	---	---
11. Coprostanone	0	0	0	0	0	0	---	---	---	---
12. Cholestenone	+3	+2	+4	+2	+1	+1	p.yellow	v.p.yellow	v.p.tan	tan
13. Cortisone	+3	+1	0	0	+1	+1	---	---	---	---
14. Cholestanol	0	0	0	0	+3	+3	pink	p.yellow	v.p.tan	v.p.tan
15. Estrone	0	+1	+2	0	+3	+3	orange	orange	brown	brown
16. Equilenin	+2	+2	+2	+2	+3	+3	pink	pink	black	black
17. Stigmasterol	0	0	0	0	+1	+3	violet	violet	brick	brick
18. Desoxycorticosterone acetate	+4	+1	+2	+4	+1	+1	blue	blue	---	---
19. Acetoxy bisnorcholenic acid	0	0	0	0	+2	+3	tan	brick	pink	tan
20. Pregnandiol	0	0	0	0	+2	+3	pink	tan	tan	p.tan
21. Cholic acid	0	0	0	0	+2	+2	p.tan	yellow	p.yellow	v.p.yellow
22. Desoxycorticosterone	+2	0	+1	0	+1	+1	blue	---	---	---
23. Pregnenolone	0	0	+1	0	+4	+3	tan	tan	brown	brown
24. Pregnane-3α, 11β, 20α-triol	0	0	+1	0	+2	+3	tan	yellow	v.p.yellow	v.p.tan
25. Pregnane-3α, 11β, 20α-triacetate	0	0	0	0	+1	+1	white	---	---	---
26. Pregnane-3α, 17α, 20β-21 tetrol-11-one triacetate	0	0	0	0	+2	+1	---	---	---	---
27. Pregnane-3α, 17α-diol-11, 20-dione 3 acetate	0	0	0	0	+2	+1	---	---	---	---
28. 17-Hydroxycorticosterone	+4	0	+1	+1	+2	+1	---	gray	---	---
29. 11-Dehydrocorticosterone	+4	0	+1	+1	+1	+1	---	---	---	---
30. Corticosterone	+4	0	+1	0	+1	+1	white	---	---	---
31. Cholesterol	0	+1	+4	0	+3	+3	pink	pink	brown	brown
32. Dextrose		0	0		+1	0	---	---	---	---

Table VI (con't)

Compounds	UV	IODINE		PICRATE	PHOSPHOMOLYBDIC		SILICOTUNGSTIC ACID		ANTIMONY PENTACHLORIDE	
	W	W	Q	Q	W	Q	W 2 min.	Q 2 min.	W	Q
33. Sucrose		0	0		0	+1	---	---	---	---
34. DL-Alanine		+1	0		0	0	v.p.yellow	---	---	---
35. M-hydroxybenzoic acid		0	0		+1	0	---	---	---	---
36. P-amino benzoic acid		+2	+3		+1	+0	v.p.yellow	---	yellow	yellow
37. Urea		+2	+2		+1	0	---	---	---	v.p.tan
38. Stearic acid		+1	+2		+1	0	---	---	---	---
39. Oleic acid		+4	+4		+2	0	p.pink	---	tan	tan

Photosynthesis Chemistry

M. Calvin, A. Benson, J. Bassham, M. Goodman, V. Lynch
W. Stepka, L. Poel, P. Hayes, L. Daus and E. Schallenberg

The principal work of this laboratory has been an investigation of the processes by which carbon dioxide is converted by green plants to simple organic molecules. Previous reports have described the techniques of administering $C^{14}O_2$ to the plant and studying the resulting products. The last report included the identification of the phosphate esters of two sugars, ribulose and sedoheptulose, which appeared to be of importance in the early phases of carbon reduction during photosynthesis. The study of these compounds has been continued, by kinetic experiments, and by degradation of the molecules. In addition, work has been carried out with other organisms and tissues, both in light and dark, to investigate further and classify the bioreactions of respiration and of phytosynthesis, including light-enhanced reduction of carbon (photosynthesis).

Chromatographic Positions of Phosphate Esters. The present status of the separation and identification of phosphate esters is summarized in Fig. 1 The absolute R_f's are not indicated on this figure as these are variable and of little actual value in identification. Rather, the map is intended to indicate the relative positions of these compounds. It is, in fact, a sketch of an ideal chromatogram which would be obtained if all the phosphate esters that we have identified wholly or partially were found present in one solution and were resolved chromatographically as well as they have been resolved in practice in the best chromatograms.

Kinetic Studies. The kinetic studies described in previous reports have been continued and somewhat more accurate results obtained. These results are shown in Fig. 2. The fact that sedoheptulose phosphates and ribulose phosphates are soon labeled at their saturation value indicates that these compounds occur in small concentrations in the plant and are formed by relatively rapid reactions from carbon dioxide. Both of these conditions are indicative that these compounds could be intermediates in the path of carbon dioxide reduction during photosynthesis. Unfortunately, the kinetic data do not permit any decision as to the sequence of these compounds.

Degradation Studies. Degradations of sedoheptulose and ribulose, both uniformly labeled and non-uniformly labeled have been carried out. The methods and results follow.

Sedoheptulose. An aqueous solution containing a tracer quantity of C^{14}-labeled sedoheptulose obtained by elution from a chromatogram, enough formaldehyde to give 17 mg. formaldimedon and enough formic acid to give 20 mg. barium formate were place in a small flask. To this was added 0.02 millequivalents of periodic acid in solution, and the resulting 0.5 ml of solution allowed to stand overnight. The solution was neutralized, the formaldehyde distilled into a second flask and precipitated as formaldimedon, recrystallized and counted. The original reaction flask was acidified and the formic acid distilled out, recrystallized and counted as barium formate. The residue in the flask was taken up in water and an aliquot counted. In this case, the barium formate is a measure of carbons 3, 4, 5 and 6, the residue a measure of carbons 1 and 2, and the formaldimedon a measure of carbon 7.

A. Gives pyruvic acid on acid hydrolysis.

B. Gives glycolic acid on acid hydrolysis.

C. On enzymatic hydrolysis gives unidentified sugar in area where one might expect tetrose.

D. Enzymatic hydrolysis gives ribulose.

E. Enzymatic hydrolysis gives mixture of fructose and sedoheptulose.

F. Enzymatic hydrolysis gives mixture of glucose and sedoheptulose.

G. Acid hydrolysis for 7 minutes in 1 N H^+ completely hydrolyzes to give glucose. Not attacked by Polydase. Elution from paper and rechromatography gives some glucose, some of original compound and another compound I, contains glucose and phosphorus. G is not glucose-1-phosphate according to co-chromatography.

H. Enzymatic hydrolysis gives ribulose.

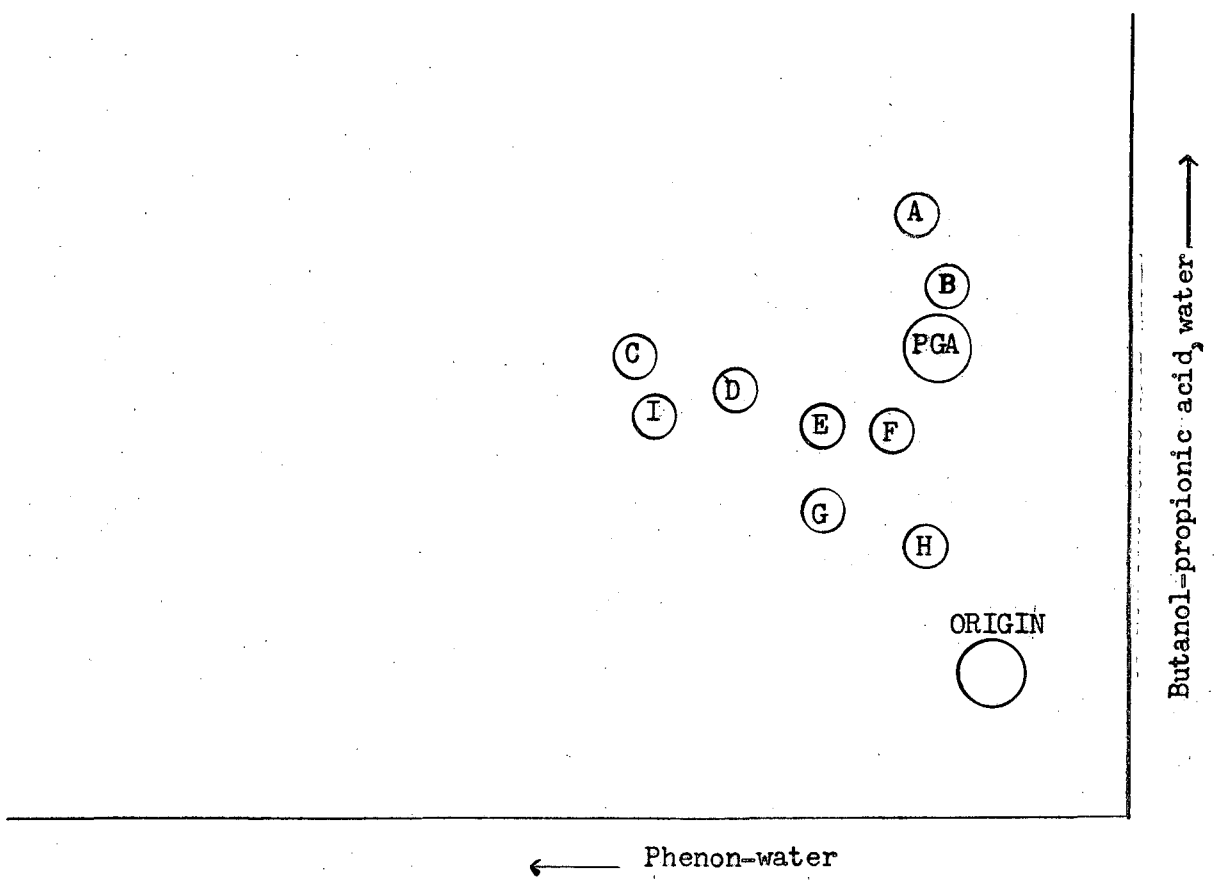


Figure 1

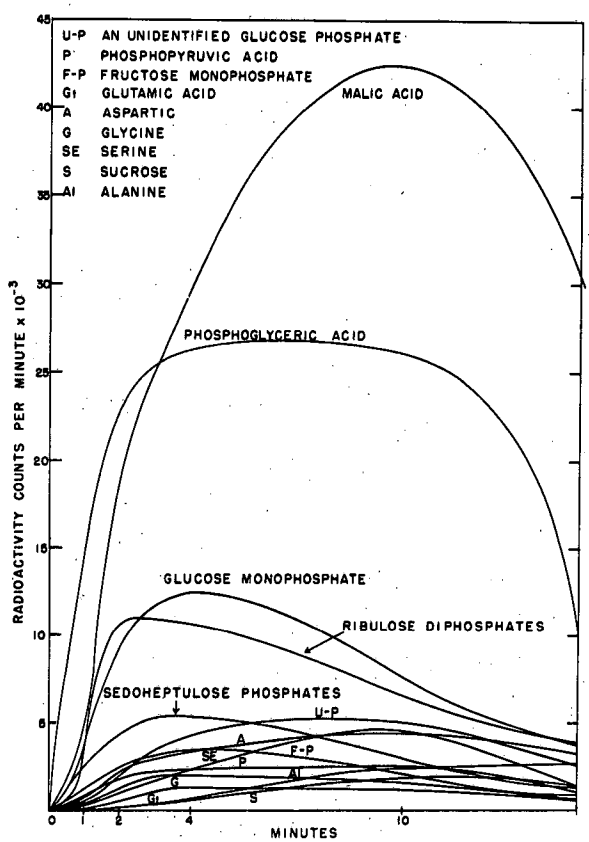

U-P AN UNIDENTIFIED GLUCOSE PHOSPHATE
P PHOSPHOPYRUVIC ACID
F-P FRUCTOSE MONOPHOSPHATE
Gt GLUTAMIC ACID
A ASPARTIC
G GLYCINE
SE SERINE
S SUCROSE
Al ALANINE
MALIC ACID
PHOSPHOGLYCERIC ACID
GLUCOSE MONOPHOSPHATE
RIBULOSE DIPHOSPHATES
SEDOHEPTULOSE PHOSPHATES
U-P
A
SE
P
F-P
Al
G
Gt
S
RADIOACTIVITY COUNTS PER MINUTE x 10^-3
45
40
35
30
25
20
15
10
5
0
1
2
4
10
MINUTES

Fig. 2

A second portion of sedoheptulose was converted to sedoheptulosan and a degradation similar to the one described above carried out, except for omission of formaldehyde. In this case the barium formate is a measure of carbon 4 only so that by combining the two degradations one gets the following results.

Table VII

	5 min. PS Soy Bean		15 sec. PS Soy Bean
$C_1 + C_2$	25%	(28)*	8
$C_3 + C_5 + C_6$	41	(43)*	26
C_4	14	(14)*	62
C_7	14	(14)*	4

(*) Expected values based on uniform labeling.

These values are based on the experimental results reported last time for 5 M PS Soy bean and upon the results given by Table VIII for 15 sec. PS Soy bean.

Table VIII

Sedoheptulose (15 sec. PS Soy Bean)	
Ba formate	88%
Formal dimedon	4%
Residue	8%
Sedoheptulosan (15 sec. PS Soy Bean)	
Ba Formate	62%
Residue	4%
Missing	34%

The missing 34 percent make the latter result less reliable. Additional short-term experiments are underway to obtain more sedoheptulose for further degradation studies.

Ribulose. To a solution of C^{14}-labeled ribulose in a small flask was added formic acid and formaldehyde as in the above degradation and then 20 mg. glycolic acid, 25 mg. iodic acid and 0.02 millequivalents periodic acid were added, the flask stoppered and left at room temperature for three hours. At

the end of this time the flask was frozen and 100 mg. $NaHCO_3$ added, the flask connected through a stopcock and U-tube to a second flask containing $Ba(OH)_2$ in solution. Both flasks were frozen and the system evacuated. The reaction flask was warmed and the CO_2 allowed to distill into the $Ba(OH)_2$ solution. A small amount of water was distilled to insure complete transfer of the CO_2. The $Ba(OH)_2$ flask was removed, the $BaCO_3$ precipitated and counted and a third flask attached in its place. The entire remaining volatile contents of the reaction flask were distilled into this third flask and the distillate neutralized with $Ba(OH)_2$ after which the formaldehyde was distilled into a fourth flask. Barium formate and formaldimedon were prepared and counted as before.

The glycolic acid which remained in the reaction flask was then dissolved in about 1 ml glacial acetic acid and 2.5 g. lead tetraacetate added. The flask was stoppered and heated at 80° C. for 2 hours. The CO_2 and a small amount of acetic were distilled from the flask, then the remaining volatile contents were distilled into another flask, in which the distillate was neutralized. Dimedon solution was added and formaldimedon prepared and counted. The several results are tabulated in Table IX.

Table IX

Degradation of Ribulose (from Scenedesmus)

Periodate Oxidation		2-1/2 M PS		60 S PS I	60 S PS II	60 S PS III	30 S PS I	30 S PS II	30 S PS III
$C_3 + C_4$	HCOOH	37.0	(40)	44.0	45.0	43.0	54.0	50.0	53.0
C_1	HCHO	18.0	(20)	9.6	11.7	12.9	6.0	4.4	7.1
	CO_2	2.2	(0)	1.5	1.5	--	0.3	--	--
Lead tetraacetate									
C_5	HCHO	18.0	(20)	16.0	10.0	16.0	11.0	13.0	10.0
$C_2 = 100 - (C_1+C_3+C_4+C_5)$		17.0	(20)	30.0	33.0	28.0	29.0	33.0	30.0

Thus it appears that in both sedoheptulose and ribulose the center of the molecule is first labeled and later the ends.

Blue-gree Algae. Blue-green algae have always attracted a great deal of interest because of their very simple structure. They contain no organized nucleus and no chloroplasts. However, because of the difficulty of obtaining pure cultures and their slow growth under laboratory conditions, little work has been done on this group of algae.

Through the courtesy of Dr. Marybelle Allen of the Hopkins Marine Station, we obtained a pure culture of Synechococcus cedorum. This blue-green alga

has proven to be a very rapid growing organism under our laboratory conditions so that sufficient quantities of material are readily available for experimentation.

The organism is grown in the following medium:

5 cc.	1 M KNO_3
5 cc.	0.2 M Na_2HPO_4
1 cc.	1 M $MgSO_4$
0.1 cc.	1 M $Ca(NO_3)_2$
1 cc.	0.5% $FeSO_4 \cdot 7H_2O$ and 0.2% tartaric acid
1 cc.	Arnon's A_5 solution (Trace elements)
1000 cc.	cation-free distilled water

pH - 8.5

with constant shaking and air bubbling through the solution. The illumination is 10,000 lux. The greatest difficulty in this continuous culturing is keeping the solution at a suitable pH. When nitrate is the source of nitrogen, the medium soon becomes alkaline and growth stops when the pH reaches 11.2. However, the cells remain viable and growth continues when new medium at a suitable pH (8-8.5) is added. When ammonia serves as a nitrogen source, the medium becomes acid and death of the cells soon follows. The culture solution becomes acid when NH_4NO_3 is present. Therefore we use a nitrate medium, harvesting every day and using a 10 percent inoculum. The pH of the medium at harvesting is 10.6. Approximately 2 cc. of packed cells are obtained per 900 cc. of culture medium.

Preliminary manometric experiments have been performed to test the photosynthetic ability of the blue-greens. One-day old cells harvested at pH 10.6 give a rapid and constant evolution of oxygen when exposed to the light. However, two-day old cells harvested from a solution at pH 11.2 but tested at pH 9.0 have a slower rate of oxygen production which slowly increases. These manometric data confirm the observations made on growing cultures. The rate of O_2 production by young cells is approximately 10 µl. of O_2 per hour per µl. of cells at 50,000 lux. This compares favorably with the rate of O_2 evolution by Chlorella whose maximum yield has been reported to be 20 µl. of O_2 per hour per µl. of cells.

The metabolic pattern of these blue-green algae (Synechococcus cedorum) has been investigated using C^{14} as a tracer. Carbon dioxide fixation in the dark was measured for resting cells suspended in distilled water. After 40 minutes, 70 percent of the activity fixed remained soluble in 80 percent ethanol. The radioactive compounds in this soluble fraction were aspartic, glutamic, succinic and malic acids. These are the same compounds formed during dark CO_2 fixation by other photosynthetic and non-photosynthetic organisms.

The path of carbon during photosynthesis by blue-green algae is also being investigated. Results from a five-minute photosynthetic experiment at 7,500 lux showed that similar compounds were formed by the blue-green algae as by other photosynthetic tissues studied. After five minutes only 30 percent of the fixed activity was soluble in alcohol and water. Of this soluble activity 61 percent was present in phosphorylated compounds. Enzymatic hydrolysis and chromatographic position indicates that these phosphates are the same as formed by other photosynthetic tissues, namely phosphates of glyceric acid, glucose and fructose. The other soluble compounds are amino acids and di- and tri-carboxylic acids. No free sugars were found after five minutes photosynthesis at this low light intensity. Photosynthetic experiments for shorter time intervals are now in progress. Therefore, it can be concluded that the metabolism of the Synechococcus cedorum is very similar to other organisms already investigated.

The pigments of blue-green algae have been considered as diffused throughout the cytoplasm and not located in discrete chloroplasts as in other algae and higher plants. However, electron photographs of Synechococcus cedorum taken in Dr. R. William's laboratory showed distinct particles scattered throughout the protoplasm of the organism. Their size was approximately 2600 A°. These particles were isolated by the following technique. The cells were ground with alumina and the slurry centrifuged at low speed to remove the alumina, cell debris and unbroken cells. The supernatant blue-green solution was then centrifuged in an ultracentrifuge at 36,000 g. for 30 minutes. A clear blue flourescent solution was obtained and a green precipitate. Electron photographs showed that the green precipitate was composed of the particles which had been seen in the whole cell. Spectrophotometric curves of the whole cells and the two centrifuged fractions showed that the precipitate contained the chlorophyll and carotenoids and the supernatant fraction contained the phycocyanin. Therefore, the chlorophyll of the blue-green algae is contained in particles and not uniformly distributed throughout the cell, while the phycocyanin appears to be in solution distributed uniformly throughout the cell.

Dark CO_2 Fixation by Euglena. During the comparative study of CO_2 fixation by various organisms, the green flagellate, Euglena gracilis, was investigated. The dark fixation of CO_2 by this organism was strikingly different from that of any other organism tested. Not only were the characteristic dark CO_2 fixation products, di and tri-carboxylic acids and related amino acids found, but also phosphorylated compounds typical of photosynthesis. Chlorella and Scenedesmus form phosphoglyceric acid and sugar phosphates from CO_2 in the dark after preillumination in the absence of CO_2. No activity is fixed if the preilluminated cells are kept in the dark for a few minutes before the CO_2 is added. To test whether the fixation of CO_2 into phosphates by Euglena might also be preillumination effect, the washed cells were resuspended in distilled water and air bubbled through the suspension while in complete darkness for 16 hours. After this time, radioactive CO_2 was added while still in the dark and the cells killed after 40 minutes. All operations were carried out in complete darkness. Chromatographs of this experiment again showed that phosphorylated compounds, as well as the usual organic acids and amino acids, had become radioactive. Enzymatic hydrolyses and chromatographic positions of the individual phosphates showed that the same phosphates were present as are formed during photosynthesis. Phosphoglyceric acid accounted for 5 percent of the total soluble activity and the sugar phosphates for 25 percent of the soluble activity. The organic acids and amino acids represented 43 percent of the

fixed soluble activity. Serine and glycine, formed at most only in small amounts in dark fixation experiments, accounted for 12 percent of the activity. Experiments are now in progress to determine whether these compounds and phosphoglyceric acid in particular, are labeled in all carbon atoms or whether they are only carboxyl labeled.

The Heterotrophic Fixation of Carbon Dioxide by Barley Roots. The fact of fixation in plant roots has been established for barley by Ruben and Kamen (1941) and Overstreet et. al. (1941), and for parsley by Gollub and Vennesland (1947) and Vennessland et. al. (1947). In the present investigation studies of the influence of various factors on the magnitude of fixation are reported and an account is given of the products of fixation as revealed by paper chromatography and radioautography.

Preliminary Experiment. Plant Material. Barley seedlings (variety "Atlas") grown in a greenhouse, were kindly provided by the Division of Plant Nutrition, Berkeley. They were produced on screens over unaerated tap water and were utilized when one week old. At an early stage in the investigation, seedlings were also grown in the laboratory in half-strength Hoagland solution with iron omitted, in a constant temperature bath maintained at 24° C., the purpose being to control more closely the conditions of growth and thus to produce very uniform material. It was found, however, that superior roots for metabolic studies were those produced in the greenhouse, being more numerous, uniform and almost devoid of root hairs. When used, the roots were approximately 7 cm. in length. They were then excised and placed en masse in fresh full-strength Hoagland solution minus iron, which was aerated continuously at 24° C. for 24 hours.

Experimental Technique. A quantity of excised roots was taken at random from the equilibration medium, lightly blotted and an arbitrarily-chosen weight of 0.7 g. of material removed. The roots in the sample were then cut randomly into short pieces and placed in a closed vessel containing 10 ml of Hoagland solution minus iron (pH = 4.6). The contents of the vessel were equilibrated for 1 hour on a Warburg shaker at 24° C. and 130 oscillations per minute with an amplitude of 2.5 cm. At the end of this period, the vessel was detached from the shaker, opened and 250λ of $NaHC^{14}O_3$ solution, containing 0.0311 millimoles $C^{14}O_2$ (0.2 mc.) per ml, added from a syringe pipette. The vessel was quickly closed and returned to the shaker for a further period of 1 hour.

Having opened the flask under a well-ventilated hood, the root material was rapidly rinsed once with distilled water, drained, and boiling 80 percent ethanol poured on. The roots and added ethanol were then transferred with rinsings of 80 percent ethanol to a mortar, where the material was ground thoroughly. Quantitative transfer to a stoppered graduated cylinder followed, the volume of the extract being recorded when cool. 100λ aliquots of centrifuged extract were plated on aluminum disc for radioactive assay, using a thin-window Geiger tube.

In this initial experiment, total activity of C^{14} fixed in ethanol-soluble non-volatile compounds was 235,800 counts per minute, representing 1.6 percent of the activity supplied. No attempt was made in this experiment to determine insoluble activity, and later experiments showed that this was considerable.

Fixation under Aerobic and Anaerabic Conditions. A preliminary comparison was made of fixation under the conditions of the initial experiment with that occurring when the gas volume of the vessel was occupied by cylinder nitrogen (containing approximately 0.5 percent oxygen). The results are presented in Table X.

Table X

Fixation of CO_2 by Barley Roots in Air and in Cylinder Nitrogen

Expt. No.	Gas Phase	Radioactivity of 80% ethanol extract	Approx. % of activity supplied
4	Air plus $C^{14}O_2$	129,600 counts/min.	0.9
5	Nitrogen plus $C^{14}O_2$	41,500 counts/min.	0.3

The material used in Experiments 4 and 5 was derived from the same culture. Although replicates are required, it would seen that a virtually anaerobic medium markedly inhibits CO_2 uptake.

Fixation Intensity in Relation to the Medium of Pre-equilibration. At this stage in the investigation, the Hoagland solution in the reaction vessel was replaced by phosphate buffer having a pH of 5.6 in order to preclude unknown variations in hydrogen ion concentration during the period of fixation. This raised the question of the validity of retaining half-strength Hoagland solution as the medium of pre-equilibration of the roots after excision. The effect of substituting tap water for this stage was accordingly examined. In the light of experience, other modifications in the general method were also made in this and subsequent experiments. Exposures were carried out in vessels possessing a side arm connected by a standard taper joint, and a second standard taper enabling the vessels to be attached to Warburg manometers, so that the CO_2 pressure could be followed, and any discrepancies in this respect between the various units of an experiment detected. The $NaHC^{14}O_3$ solution was pipetted into the side arm and, by swivelling the latter, could be discharged into the supporting medium after the system was closed. This was necessary as trials showed that variable amounts of the CO_2 may escape from the vessel before closure when the bicarbonate is added directly.

The first experiment consisted of four flasks, two containing excised roots pre-equilibrated in Hoagland solution minus iron, and the remaining two, material pre-equilibrated in tap water. The results are shown in Table XI.

Table XI

Effect of Pre-equilibration in Hoagland Solution Minus Iron versus Pre-equilibration in Tap Water on CO_2 Fixation in Excised Barley Roots

		Duration of Exposure (mins.)	Fixation in Soluble Products (counts per minute)	Approx. % of activity supplied
Hoagland	1)	60	139,300	0.9
	2)	60	163,600	1.1
Tap Water	1)	60	909,000	6.0
	2)	60	748,000	5.0

These results show that not only may the Hoagland solution stage be omitted from the procedure, but also pre-equilibration in this medium actually inhibits the fixation capacity of the material to a marked extent. As a possible explanation of this effect, it may be suggested that the supplying of mineral nutrients to starved roots will exert a stimulating influence on the rate of respiration, in providing energy for absorption salt respiration, leading to depletion of already-meagre reserves of substrate within the cells. This would involve a decrease in available energy for subsequent fixation of CO_2.

Fixation in Relation to Time of Exposure. In their studies of fixation of C^{11} by yeast, Ruben and Kamen (1941) found a linear relationship between radioactivity incorporated and time, over a period of 1 hour, whereas for dark fixation by Chlorella, the rate of fixation declined steadily. Although ground barley root material was investigated by these workers, they do not refer to experiments on the time factor in this species.

Experiments were carried out in which the exposure times varied between 5 minutes and 45 minutes, the data obtained being presented in graphical form in Fig. 3. The form of the curve corresponds to that obtained by Ruben and Kamen for Chlorella. The dissimilarity between the present results and those of Ruben and Kamen for yeast is probably only apparent in that had the fixation in the latter organism been extended over a longer period, a decline in the rate of fixation might well have been observed. These data lend some support to the view that dark fixation in heterotrophic and autotrophic systems is essentially similar.

The Effect of Added Pyruvate. Since pyruvic acid is a common starting point in schemes involving the fixation of carbon dioxide in non-green cells, e.g. by the b-carboxylase system or by the "malic enxyme," experiments were performed in which fixation occured in the presence of sodium pyruvate, it being expected that this would lead to an increase in CO_2 uptake.

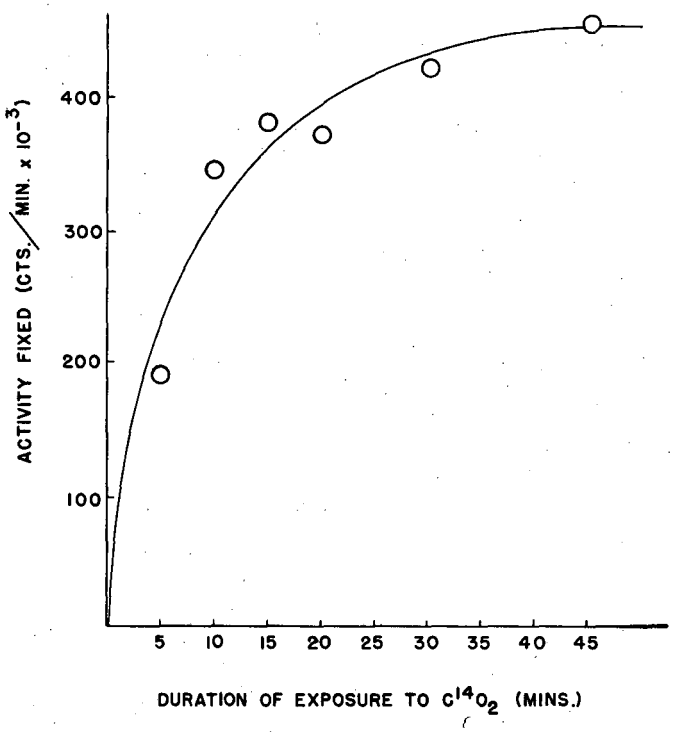

ACTIVITY FIXED (CTS./MIN. x 10^-3)
400
300
200
100
5
10
15
20
25
30
35
40
45
DURATION OF EXPOSURE TO C14O2 (MINS.)

Fig. 3

Pre-equilibration of the material was carried out in Hoagland minus iron, and the roots were transferred to phosphate buffer at pH 5.6 where they were exposed to $C^{14}O_2$ administered as sodium bicarbonate, for 1 hour.

Table XII

The Effect of Added Pyruvate on the Intensity of Fixation of CO_2 by Excised Barley Roots

Expt. No.	Exposure Medium	Fixation in Soluble Products (counts per min.)	Approx. % of activity supplied
12	a) Phosphate buffer	282,000	1.9
	b) Phosphate buffer plus 0.0028 M. sodium pyruvate	425,000	2.8
13	a) Phosphate buffer	193,200	1.3
	b) Phosphate buffer plus 0.0028 M. sodium pyruvate	144,300	1.0
14	a) Phosphate buffer	168,200	1.1
	b) Phosphate buffer plus 0.0028 M. sodium pyruvate	174,700	1.2
15	a) Phosphate buffer	199,600	1.3
	b) Phosphate buffer plus 0.0028 M. sodium pyruvate	218,300	1.5
	c) Phosphate buffer plus 0.0055 M. sodium pyruvate	198,000	1.3

The inconsistent nature of the data for this experiment further demonstrates the wide variability in fixation power possessed by roots from a single culture. One may conclude either that the addition of pyruvate is without effect on the magnitude of fixation or that the effect, if present, is too small to show above the inherent variation of the material. Again, the computing of values for total fixation (soluble and insoluble) may regularize the data and thus provide an unequivocal answer on the influence of pyruvate. Determinations of insoluble fixation are being made.

The Products of Fixation. The 80 percent ethanol extracts of root material prepared as described in the previous section were filtered and the residues retained for estimating insoluble fixation. The filtrates were reduced to a volume of 1 to 2 ml by vacuum distillation on a steam bath, two-dimensional chromatograms prepared from an aliquot of the concentrated filtrate and radioautographs produced. Some of the chromatograms were sprayed with ninhydrin or bromocresol green to reveal amino acids and carboxylic acids respectively. Others were retained for counting of spots and for elution.

Results. The various radioactive spots on the chromatograms were counted using a large end-window counter. The data for the initial experiment (#1) are shown in Table XIII.

Table XIII

Radioactivity Fixed in 80 Percent Ethanol-soluble Compounds During 1 Hour by Excised Barley Roots

	Malic	Citric or Isocitric	Aspartic	Glutamic	Serine	Asparagine	Glutamine
Activity (counts per min.)	2205	124	892	766	223	455	1579
Activity relative to malic acid (x100)	100	5.6	40	35	10	21	72

These seven compounds have been found consistently in all the experiments with the addition in certain cases of small amounts of succinic acid and several as yet undetermined substances. Malic acid is always dominant. Although alanine is present on the chromatograms, it is in no instance radioactive, in contrast to photosynthetic fixation. This part of the investigation is proceeding.

II QUARTERLY PROGRESS REPORT Project 48B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics

Leo Brewer, LeRoy Bromley, Guy Elliott, Russell Edwards, Norman LeRoy, James Robbers, Albert Rothman, and John Margrave (AEC Fellow)

Gaseous Oxides. The vapor pressures of Na_2O and Li_2O have been determined. The results indicate that these oxides vaporize by decomposition to the elements.

Gaseous Hydroxides. Measurements of the stabilities of hydroxides of Mo are continuing.

Refractories. Tests of TaC heating elements are being made for use in a very high temperature furnace. ZrC is being prepared for thermodynamic measurements.

Oxide Phase Diagrams. A paper discussing the solid suboxides of Si and Al has been completed.

Publications. The following papers have been published:

A Study of Refractory Borides by L. Brewer, D. L. Sawyer, D. H. Templeton and C. H. Dauben, J. Amer. Ceramic Soc. 34, 173-179 (1951).

Stability of Gaseous Diatomic Oxides by L. Brewer and D. F. Mastick, J. Phys. Chem. 19, 834 (1951).

Oxide-Sulfides of Ce, Th, U, and Zr by E. D. Eastman, L. Brewer, L. A. Bromley, P. W. Gilles, and N. L. Lofgren, J. Amer. Chem. Soc. 75, 3896 (1951).

Purification of Na by Distillation by L. Brewer and J. Margrave, August issue of Journal of Reactor Techniques.

Thermal Conductivity of Gases at High Temperatures. A literature survey has been made, and the initial design and ordering of equipment has begun. Based on this survey, the most suitable apparatus for measurement of conductivity at high temperature appears to be the concentric cylinder type of Keys and Sandell. A modified cylindrical cell about 10 cm long and 2 cm diameter will be constructed of polished silver, to reduce radiation through the annulus to a minimum. This cell should be suitable for temperatures up to about 800° C and several atmospheres pressure. A White double potentiometer will be ordered as soon as possible, and will be used in conjunction with platinum and platinum rhodium thermocouples to measure the temperature level and temperature drop of the gas annulus. Radiation and metal conduction corrections will be applied by operating the cell at about 10^{-3} mm Hg and measuring the heat flow and temperature drop.

Molten tin contained in a graphite pot and agitated will be used as a thermostated bath for the conductivity cell. We will attempt to maintain the bath at specified temperatures within a few hundredths of a degree. The control circuit, however, has not yet been selected.

Heat Transfer in Forced Convection Film Boiling. Experimental data have been completed on forced convection film boiling from horizontal tubes. Four liquids were studied, ethyl alcohol, benzene, hexane and carbon tetrachloride. The results have been calculated and the developed theory has been found adequate. The results will be issued as a UCRL report.

UCRL-1463, which describes the new centrifuged sodium pump and contacting tray for maintaining an inert atmosphere has been issued.

B. Basic Chemistry, including Metal Chelates

Z. Z. Hugus, R. E. Connick, William Jolly, Albin Zielen, Frank Owings, Howard Mel, Lorin Hepler, and John Kury

Thermodynamics of Rhenium. The electrolytic preparation of Re^{-I} has been attempted in basic solution, using a Re bar as cathode. Current densities have been varied over several powers of ten, and the KOH concentration varied from 1 M to 10 M. At lower current reduction of an oxide film on the rhenium was accompanied by a rise in the potential to a value which permitted the discharge of hydrogen. Under these conditions no electrode process taking place prior to hydrogen discharge could be detected. At high current densities after violent hydrogen evolution, no detectable reducing power or rhenium content could be found in the solution in the cathode compartment. It thus appears that one cannot produce Re^{-I} by electrolytic reduction of the metal under what would appear to be the most suitable conditions, low (H^+) and low temperature.

The Hydrolytic Polymerization of Zirconium. Work is proceeding on the polymerization of Zr(IV) in 1 M $HClO_4$ plus 1 M $LiClO_4$ solution. As outlined in the June Quarterly Progress Report, the experimental method employs the aqueous equilibrium between Zr and its first TTA (thenoyltrifluoroacetone) complex.

Utilizing the results of a completed series of experiments in 2 M $HClO_4$, it has been possible to make a tentative analysis of the data. The results so obtained are in disagreement with the previous work on this problem by Connick and Reas (AECD-2491), who interpreted their data in terms of an infinite series of polymers commencing with a dimer. The present work indicates that the dimer, if it exists, is never an important species. Experiments are continuing to determine the formulas of the higher polymers.

Germanium Chemistry. The vapor pressure of GeO(s) has been measured by the Knudsen effusion method, using a glass cell. The results may be expressed as thermodynamic equations for

$$GeO(s) = GeO(g)$$

$$\Delta H^o = 55,500 - 3T$$

$$\Delta F^o = 55,500 + 6.9\ T \log_{10}T - 62\ T$$

$$\Delta S^o = 59 - 6.9 \log_{10}T$$

Using Herzberg's value of 159 kcal/mole as an upper limit for the dissociation energy of GeO(g) (because he used a liner Birge-Sponer extrapolation) and Searcy's value of 85.2 kcal/mole for the heat of sublimation of Ge(s), we calculate that the heat of formation of GeO(s) at 25° C is more positive than -69 kcal/mole. Since the heat of formation of GeO_2(s) is -128.4 kcal/mole, a germanous oxide is just barely stable, or actually unstable, with respect to disproportionation.

The solubility of hydrous germanous oxide (germanous hydroxide) in hydrochloric acid solutions and in solutions containing various concentrations of H^+ ion and Cl^- ion is being measured. This study is nearing completion.

Static equilibrium studies are being made of the reactions between gaseous HCl and GeO_2 and between gaseous HCl and GeO. The extents of reaction at various temperatures are determined from pressure changes.

Electron Exchange Rate Between Fe^{2+} and Fe^{3+}. In deriving the equation for the fraction exchanged in terms of the change in specific Fe^{59} activity and change in ferrous and ferric concentrations, it is necessary to assume that negligible electron exchange occurs during the time of exposure to the resin. Since it is now known that the exchange is very fast, in order to make the above assumption valid, it has been necessary to work with much lower iron concentrations than were previously used. Spectrophotometric analysis using the ferrous bipyridyl complex has proven very difficult at these low concentrations of 10^{-6} M, although the method is quite accurate at 10^{-4} M. A small amount of an oxidizing impurity in the stock $HClO_4$ or conductivity water used in making dilutions is presumed to be responsible for the inaccuracy, although errors due to surface adsorption of iron by the glass containers is possible.

Means of eliminating or correcting for these errors are being investigated.

Thermodynamics of Indium. An attempt is being made to measure the equilibria:

$$In^{+3} + 2\ In(m) = 3\ In^{+} \quad \text{and}$$

$$2\ In^{+3} + In(m) = 3\ In^{+2}$$ and thence calculate the E^o values for the couples:

$$In = In^{+} + \epsilon^{-} \quad \text{and}$$

$$In = In^{+2} + 2\epsilon^{-}$$. This is done by equilibrating solutions with various concentrations in In^{+3}, with finely divided Indium metal and measuring the reducing power of the solution. Reducing power has been detected indicating the presence of Indium in the lower oxidation states although not enough data has been accumulated to evaluate the equilibrium constants.

The hydrolysis constant of In^{+3} is being recalculated, taking account of halide complexing, from the data of Moellor. $In(ClO_4)_{3(c)}$ is also being prepared so that the hydrolysis constant can be measured without interference from complexing.

Thermodynamics of Thiosulfates. In an effort to determine concordant values for ΔF_f^o and ΔH_f^o of $S_2O_3^{=}$ ion, several possible equilibria are being considered including:

1) $CaS_2O_3\ (aq.) = CaSO_3 \cdot ?H_2O(s) + S\ (r)$
Studied by Bichowski: JACS 45, 2225 (1923).

2) $S_2O_3^{=}(aq.) + Ag(s) = Ag_2S(s) = Ag_2S(s) + SO_3^{=}(aq.)$

3) $S_2O_3 = (aq.) + Hg(l) = HgS + SO_3 = (aq.)$

Preliminary investigations indicate that Reaction 3 appears to be accompanied by side reactions and hence is unsuitable. Reaction 2 looks more promising and further work is being done on Reactions 1 and 2.

Indications are that a thermoregulated and stirred air bath may not give adequate temperature regulation for high precision at the elevated temperatures necessary for rapid establishment of equilibrium in the above reactions; consequently an oil bath with proportional temperature controller is being readied for future work.

Attempts have been made by numerous methods to prepare crystals of slightly soluble $CaSO_3 \cdot ?H_2O$ (the amount of hydration is in doubt) with only a slow diffusion method showing any promise.

Solubility of the Rare Earth Fluorides. An experiment designed to measure the equilibrium constant for the following reaction is in progress:

$$CeF_3 \cdot 1/2\ H_2O + 3H^+ \longrightarrow Ce^{+3} + 3HF + 1/2\ H_2O$$

This experiment should also shed light on the nature and extent of ions of the type $CeF_x^{(3-x)}$. This is being done by means of cell measurements with Fe^{++} and Fe^{+++}.

In preparing some $CeF_3 \cdot 1/2\ H_2O$ it was found that the solid gives an anhydrous CeF_3 x-ray pattern even though it is still in contact with the solution from which it was precipitated. The possibility of molecules of H_2O fitting into "holes" in the crystal lattice without changing the cell constants (as is the case with $AlF_3 \cdot 1/2\ H_2O$) is being investigated.

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