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UCRL-1196
Chemistry-Transuranic Elements

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CHEMISTRY DIVISION QUARTERLY REPORT

December, 1950, January and February, 1951

April 4, 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 ElementsMagnetic Susceptibility of CmF₃

W. W. T. Crane and B. B. Cunningham

The molar susceptibility of Cm⁺³ in curium trifluoride has been found to be $24,000 \times 10^{-6} \pm 500 \times 10^{-6}$ cgs units at 295°K. The molar susceptibility of Cm⁺³ in curium trifluoride diluted with 90 percent lanthanum trifluoride has been found to be $26,500 \times 10^{-6} \pm 700 \times 10^{-6}$ cgs units at 295°K.

If one calculates the molecular Field Constant due to the Heisenberg interaction forces, an upper limit of 40 degrees can be set. The reason only a maximum value can be calculated is due to an uncertainty in the temperature of the sample of pure CmF₃. It is possible for the sample to be many degrees above the surrounding temperature due to its intense radioactivity.

Microcalorimetry

S. R. Gunn and B. B. Cunningham

Further work has been done with the adiabatic microcalorimeter built here by Westrum and Eyring in 1949. Efforts to seal the vacuum jacket were unsuccessful, and all tests were made at atmospheric pressure. Under these conditions the thermal leakage modulus of the instrument is approximately 0.060 degrees per degree-minute; this high value results in large heat transfers with any deviation from adiabatic conditions.

The calorimeter consists basically of a 7-ml inner chamber contained in a submarine of 5/16 in. larger radius. Between the walls of the two there is located a 24-couple iron-constantan thermopile having one set of junctions in juxtaposition to the inner chamber and the other set adjacent to the submarine wall. The current through the thermopile is measured with a high-sensitivity galvanometer, resulting in a sensitivity of about 2×10^{-4} °C per mm. on the galvanometer scale. The submarine is immersed in an adiabat bath of about 2 liters of 0.001 M Na₂SO₄ which is heated by the passage of alternating current, the submarine serving as one electrode and the adiabat container as the other. Operation of the adiabat is manual; it has been found possible to maintain the temperature differential at less than 10^{-4} °C during steady periods and the deviation during energy inputs at a maximum of 2×10^{-3} °C and usually considerably less.

A considerable lag in the dissipation of heat from the electric heater to the calorimeter chamber during calibration inputs has been found, together

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with an uncertainty as to the amount of heat lost from the heater to other parts of the instrument. Several test runs have been made with high-purity magnesium metal whose heat of solution in 1.0 M HCl at 25°C is given as -111.322 ± 0.041 kcal per mole by Shomate and Huffman. The results have all been low and of poor reproducibility. It cannot be said as yet that the instrument is in satisfactory operating condition.

A new vacuum line has been built for the semi-adiabatic calorimeter previously described. A vacuum of approximately 2×10^{-5} mm Hg was obtained in the jacket and the thermal leakage modulus found to be 0.010 per minute, compared with a value of 0.030 at atmospheric pressure.

Anion Exchange of Complex Chloro-Ions of Zirconium, Hafnium
Niobium and Tantalum

E. H. Huffman, G. M. Iddings and R. C. Lilly

Zirconium, hafnium, niobium and tantalum have been found to form complex ions in strong hydrochloric acid which can be exchanged on anion exchange resins. The plots of K_D vs. HCl concentration show a different type of curve for niobium and tantalum than for zirconium and hafnium, as presented in Fig. 1.

The relative adsorptions for each of these pairs of elements is the reverse of those which have been found for fluo-ions in mixtures of hydrochloric and hydrofluoric acids. It seems probable that the equilibria in the low acid concentrations for niobium and tantalum involve the simple displacement of oxy-ions from the resin by chloride, with no chloride complexing. This hypothesis is supported by the negative slope of 0.8-0.9 when $\log K_D$ is plotted against $\log (HCl)$ below 2.5 molar and could be represented by the simplified reaction



In the high acid concentrations the log-log slopes are as high as +12, and the exchange equilibria probably include hydrolysis of complex chloro-ions, represented by



There is also the possibility that radio-colloids account for the behavior in the low acid range. The dependence on several variables is needed to clear up these points.

A good separation of zirconium and hafnium can be made by elution from a Dowex-2 resin column, as shown for 9M hydrochloric acid in Fig. 2. A similar column separation for niobium and tantalum has not been found. Although the niobium can be eluted in a normal fashion with high-concentration hydrochloric acid, the tantalum behaves erratically. Both of these elements are immediately washed from the column in the low acid range.

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The different characteristics of zirconium and niobium allow a very convenient separation of Zr^{95} from its daughter Nb^{95} . The mixture in one ml of 6-7 M hydrochloric acid is passed through a column 8 cm long and washed with 3 ml more of acid. The elutriant and wash contain the zirconium, and the niobium is then washed from the column with about 4 ml of 1.5 - 4.0 M hydrochloric acid.

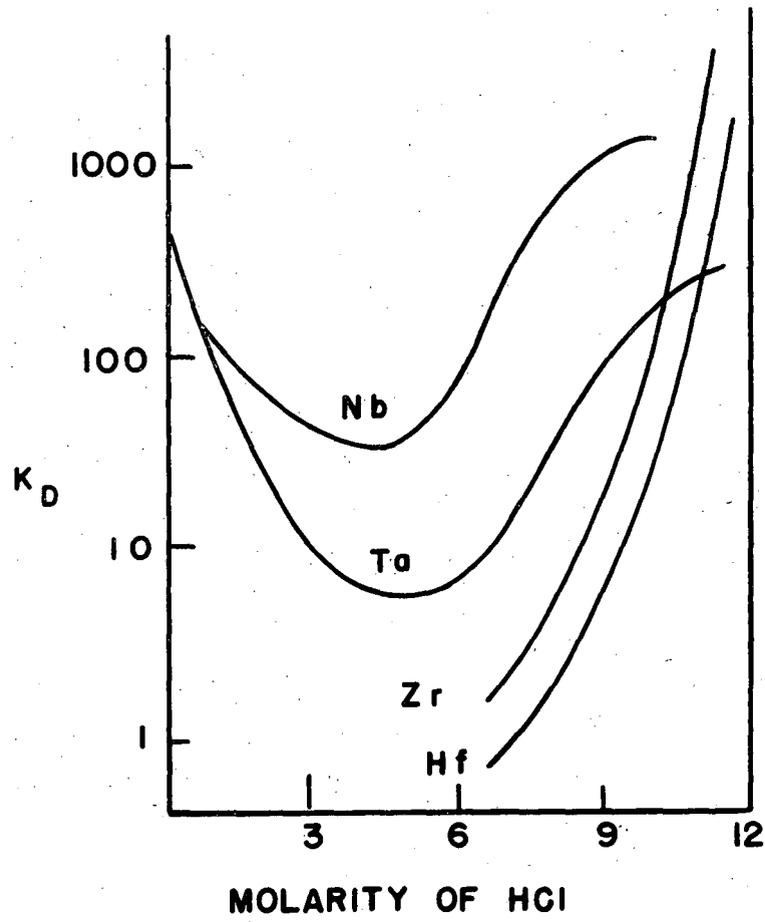


FIG. 1

MU 1663

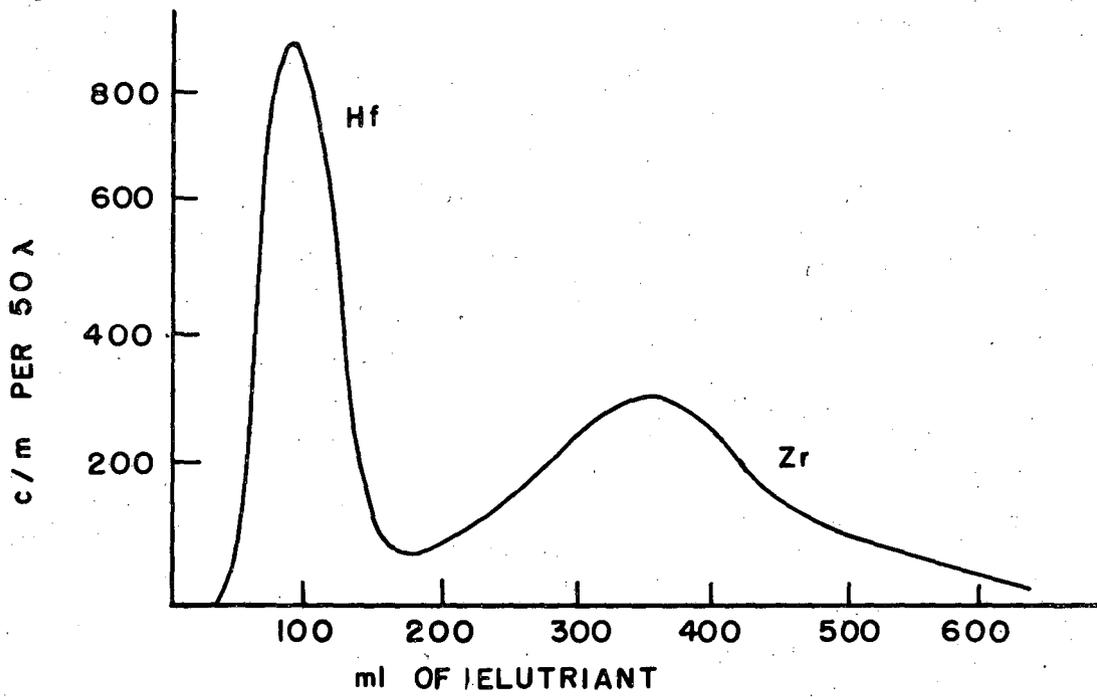


FIG. 2

MU 1664

Spectrographic Analyses of Radioactive Materials

John G. Conway

A new pair of gloved boxes has been placed in service. An improved arc spark chamber has been built for these boxes which affords greater ease of operation. The filter systems are made up of CWS filter units. Previous installation on this laboratory used glass wool, but recent work indicates that CWS filters are more efficient. One CWS unit filters the box air and a double unit, (2 in series) specially enclosed for vacuum, filters the air which passes through the arc-spark chamber. Two monitor filters have also been incorporated in the chamber filter train, one directly after the chamber and before the CWS the second monitor after the CWS and before the vacuum pump.

Fig. 1 shows a general view of the box positioned at the end of the optical bench of the spectrograph. Above is the box filter connected to the duct and a double blower system on the roof. One blower acts as a standby and should the first blower fail either electrically or mechanically the standby will automatically start up. On the shelf below the boxes may be seen the holder for the two CWS filters. In the small white gloved box is the second monitor filter. On the floor is the vac pump which is the air mover for the chamber. The right hand box is for sample preparation. Fig. 2 shows the interior of the left hand box. The first monitor filter is shown on the right hand side. A close up of the spark chamber is shown in Fig. 3. The lid has three windows, two pyrex, one quartz and it is counter-weighted for ease of handling. The electrodes are held against a stainless "V" block by a spring. The "V" blocks are mounted on Lavite insulators which are pivoted on 1/2 in. steel shafts. Horizontal alignment can be made by loosening the pivot screw and positioning the electrodes. Vertical adjustment of the shafts is through a metal bellows and regulated by bevel gears. The lid is fastened to the base by a quarter turn breech thread and gasketed by means of "O" Ring.

The chamber is mounted on rails so that it may be positioned on the optical axis.

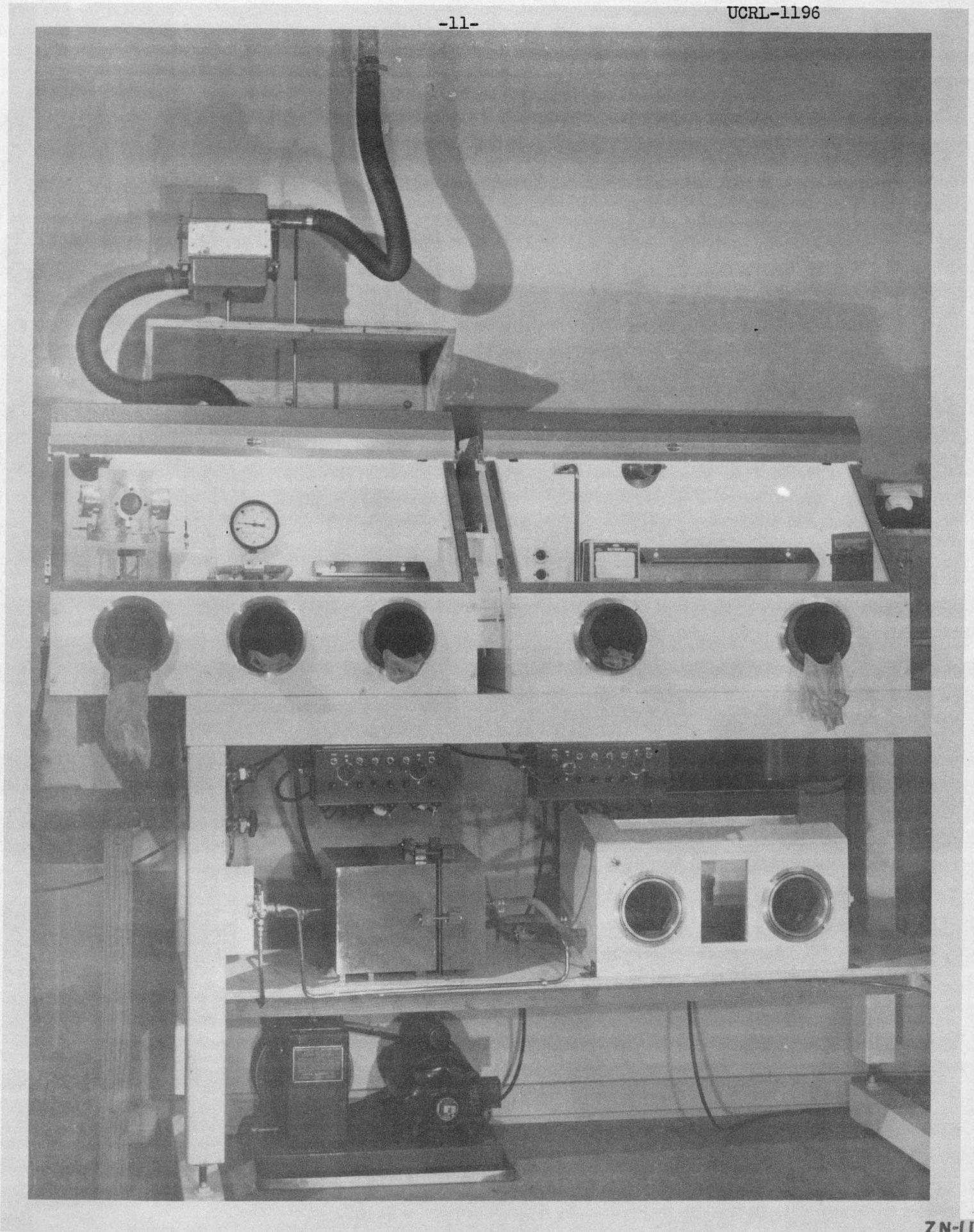


Fig. 1

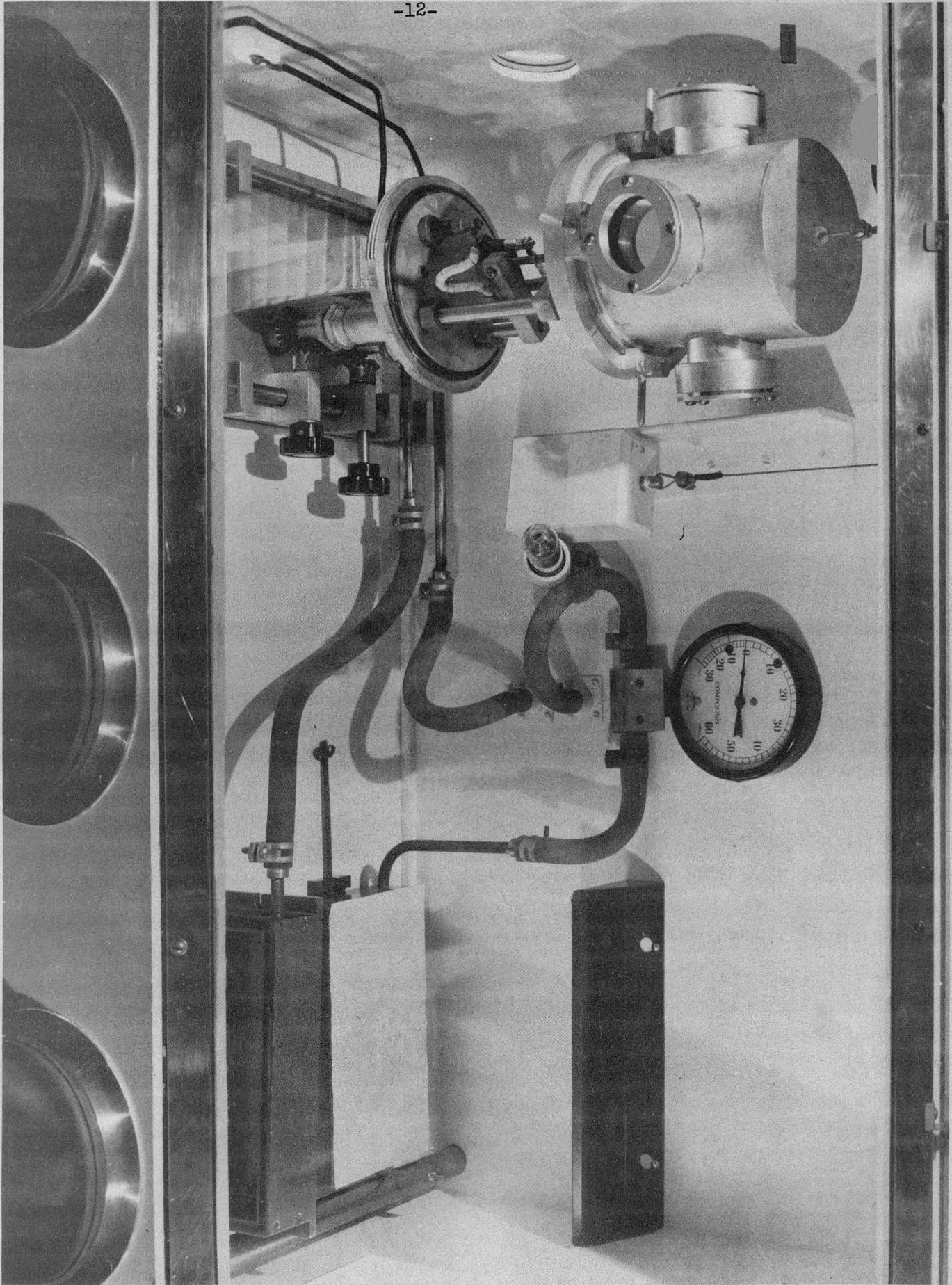


Fig. 2

01 N10

CHEM 2197

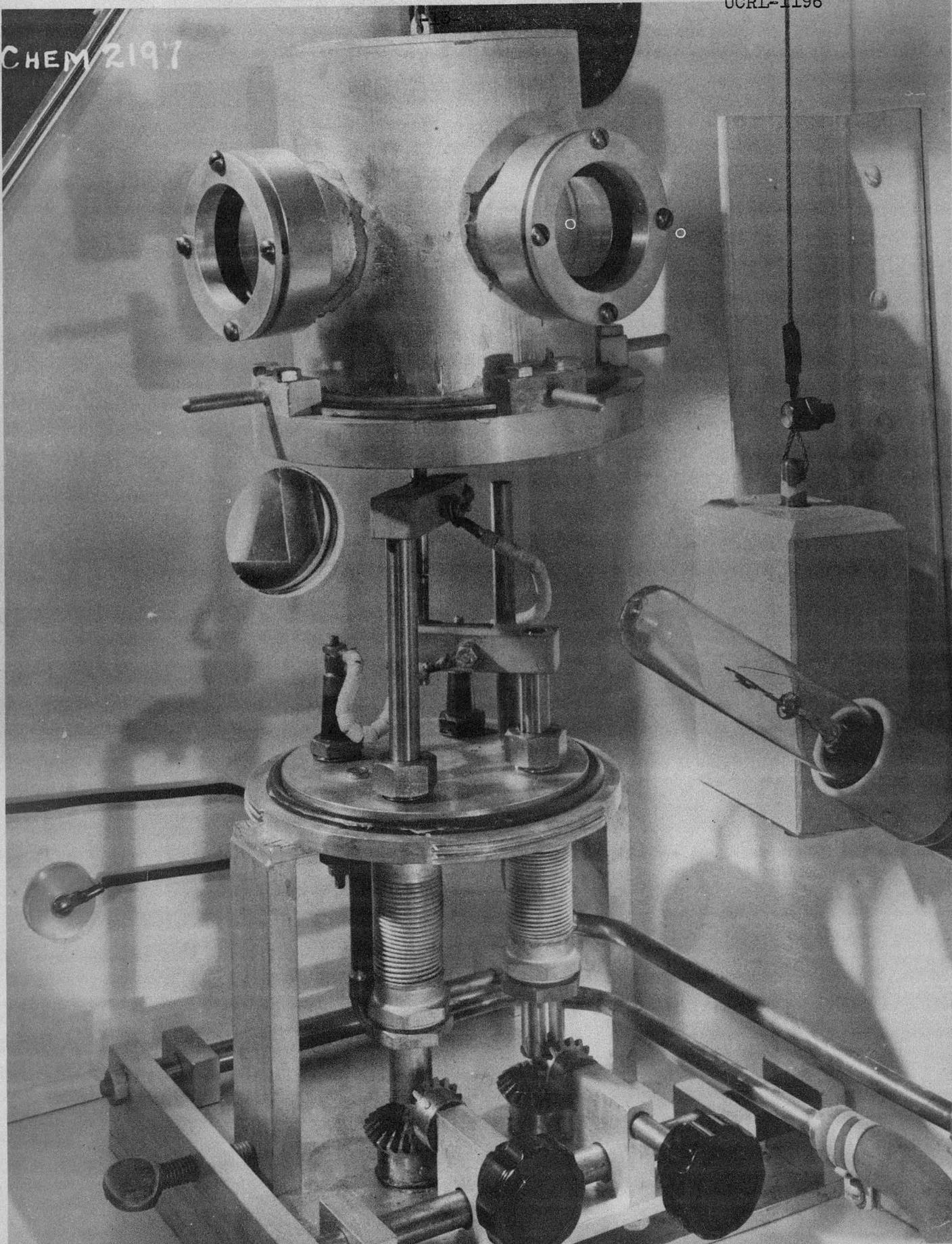


Fig. 3

Radiation Chemistry of Alcohols

A. S. Newton and W. R. McDonell

The study of the radiolysis products of alcohols mentioned in previous quarterly reports is continuing and a survey has been made of all alcohols through the butanols plus normal octyl and decyl alcohols. The results are still under study to get as complete a picture as possible of the effects of chain length and branching on the radiolysis products, so only generalizations concerning the products will be described at the present time. The complete data will be presented in a separate report when some doubtful points have been checked and an adequate interpretation made.

As mentioned in previous quarterly reports the principal reduced product in normal alcohols is hydrogen though as increased branching is introduced in the alcohols, hydrocarbons become more important until in tertiary butanol, the methane yield is actually higher than the hydrogen yield. The source of all of this hydrogen has not yet been adequately explained, though about 20 to 40 percent of the oxidized products have apparently not been identified. The total G (molecules/100 e.v.) for reduced products is from 3 to 4 indicating that there are no chain reactions occurring. No polymers have been seen except higher hydrocarbons and glycols which can be formed by ordinary free radical reactions.

The yield of acid and CO_2 is small in all cases though some acid is always found. The only positive identification of peroxides and ethers has been in methanol where dimethyl ether and dimethyl peroxide have been found in low yield and in ethanol where diethyl ether and methyl ethyl ether are present in low yields. No diethyl peroxide could be identified from ethanol but the method of detection is very inefficient. All glycols seen have been adjacent glycols; non adjacent glycols, if present, are at least a factor of ten or more lower in abundance than the adjacent glycols. Unsaturated hydrocarbons are seen in all cases, and are about equal (within a factor of 2) to the saturated hydrocarbons in all cases except isopropyl alcohol where the propylene yield is much higher than the propane yield. Acetylene is formed from ethanol in very small yield and there is some evidence for an extremely small yield of methyl acetylene in the same bombardment. These are the only acetylenic hydrocarbons found.

A study of these points as well as the relative formation of ketones and aldehydes in branched chain alcohols indicates that the most vulnerable point in the molecule is the carbon to which the OH is attached, and that groups attached to this carbon are split off during the radiolysis. This same vulnerability of groups attached to the COH group is shown in the mass spectra of these alcohols leading to the possibility that, to a first approximation, the primary steps in the two processes are similar and if the secondary steps are understood (free radical recombinations and reactions), the major radiolysis products might be predictable from mass spectral data.

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B. Nuclear Properties and TransformationsSpallation of Zinc and Nickel

B. C. Haldar, W. J. Worthington, Jr. and P. C. Stevenson

Zinc Spallation. One bombardment of zinc with 340 Mev protons was carried out. Gallium, zinc, copper, nickel and iron were separated from the target chemically. From these the following isotopes have been tentatively identified by following the decay curves of the various fractions:

Ni⁵⁷, Ni⁶⁵
Zn⁶²
Ga⁶⁶ and Ga⁶⁷
Fe⁵⁹
Cu⁶¹, Cu⁶⁴ and Cu⁶⁷

The approximate cross sections for these reactions are in the process of being determined.

Particle emissions of iron and nickel fractions were measured on a crude beta ray spectrometer. The presence of β^+ particles emitted from the iron fraction approximately 8 hours after bombardment indicates the presence of Fe⁵². The presence of β^- particles at the same time indicates the presence of Fe⁵⁹ or a higher isotope. The presence of β^+ particles emitted from the nickel fraction approximately 5 hours after bombardment confirms the presence of Ni⁵⁷.

Nickel Spallation. Three bombardments of nickel with 340 Mev protons have been carried out. All three bombardments have been with "thin" targets. Manganese, copper, cobalt, chromium, titanium, iron and nickel were separated chemically from these targets.

The following isotopes have been tentatively identified by following the decay curves of the various fractions:

Bombardment and σ in mb (uncorrected for absorption)

Isotope	I	II	III
Mn ⁵¹	11.52	12.47	---
Mn ⁵²	12.87	14.41	---
Mn ⁵⁶	0.49	0.89	---
Cu ⁶¹	0.12	0.118	---
Cu ⁶⁴	0.025	---	---
Co ⁵⁵	6.38	---	6.62
Cr ⁴⁹	---	8.91	5.53
Ti ⁴⁵	---	---	2.42
Fe ⁵²	1.2	1.67	---
Ni ⁵⁷	31.78	35.12	---

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In calculating the above cross sections, observed counting rates were corrected for

- (1) coincidence
- (2) background
- (3) backscattering
- (4) time, to give disintegration rate at end of bombardment.

Correction factors for absorption are being determined.

Gamma Radiation in Rb⁸² Decay

C. I. Browne and D. G. Karraker

The conversion lines from Rb⁸² have been examined in the β -ray spectrometer in order to determine what correlation can be found with the lines observed by Siegbahn et al.¹ in the decay of Br⁸².

We have observed gamma radiation of the following energies, in fair agreement with Siegbahn's values: 579, 616, 700, 834, 1042, 1319, 765 Kev. Our data indicate that five additional gamma rays are present though the spectrum is made so complex by the presence of Rb⁸¹ that the number cannot be stated firmly.

The relative intensities of the radiation corresponding in energy to that reported by Siegbahn are quite different from those observed in the Br⁸² decay. It was the original purpose of this investigation to elucidate the decay scheme of Kr⁸²; the complexity of the spectrum and lack of time has forced the abandonment of further research on this problem.

¹ Siegbahn et al, Phys. Rev. 76, 1263, 1949.

Alpha Activity in the Rare Earth Elements

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

The alpha activity of 2.9 Mev energy in a neutron-deficient europium isotope reported in UCRL-1054, p.17, has been further studied. Its half-life has been determined as 23.5 ± 1 days. Its principal mode of decay is electron capture. By assuming a counting efficiency for the electron capture decay of 0.3 when counted in a windowless methane proportional counter (nucleometer) the branching ratio of alpha to electron capture decay for this isotope is about 1×10^{-5} , from which the partial half-life for alpha decay is calculated to be about 6×10^5 years. The 23.5 day europium isotope has been shown to have mass number 147 from yield considerations in bombardments of Oak Ridge separated samarium isotopes by 10 Mev protons.¹ The best value by ion chamber

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measurements for the alpha particle energy of the Eu^{147} activity is 2.92 ± 0.04 Mev.

An estimate of the partial alpha half-life of the 3.21 Mev Gd^{148} alpha activity can be made from the absolute yield of the activity from alpha particle bombardment of Sm^{147} , if a cross section for the $(\alpha, 3n)$ reaction is assumed. The assumption of a one barn cross section at 36 Mev would give a half-life value of about 140 years. This estimate may be in error by as much as a factor of two.

The isotopically separated samarium isotopes used as target materials were made available by the Oak Ridge Isotope Research and Production Division.

¹ R. Hoff et al. UCRL Quarterly Report (this issue).

Light Europium Activities

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

A europium activity with an approximate 53-day half-life has been observed in deuteron bombardments on natural samarium oxide by Marinsky and Glendenin.¹ They report 0.38 Mev conversion electrons and γ -rays with energies of 0.42 and 1.0 Mev. On the basis of energy considerations using the Bohr-Wheeler equation, they have assigned this activity to Eu^{147} . Wilkinson and Hicks have also reported a 54 ± 1 day europium activity from proton bombardments on natural samarium oxide.² They find radiation characteristics similar to those found by Marinsky and Glendenin, but did not attempt to predict the mass assignment.

Using enriched Sm^{148} (Oak Ridge, 76 percent) an ~ 53 day activity was found to be the predominant activity formed in a bombardment with 10 Mev protons. This activity has been followed through 1-1/2 half-lives and actually shows a 47 day half-life. Absorption curves using beryllium and aluminum show conversion electrons of about 0.5 Mev, which are assumed to agree with the above data, the difference being due to the uncertainty in interpreting the absorption curves. It was impractical to attempt a lead absorption for γ -ray energies because of the small amount of activity present. The reaction $\text{Sm}^{148} (p, n)\text{Eu}^{148}$ is assumed with 10 Mev protons. Therefore, the 53 day europium activity is assigned to Eu^{148} .

The 15 hr. Eu^{149} and the 9.2 hr. Eu^{150} were also formed in small amounts in the bombardment as a result of the other stable samarium isotopes present in the enriched Sm^{148} .

Using enriched Sm^{147} (Oak Ridge, 81 percent) in the same bombardment with 10 Mev protons, the predominant activity formed had a 23.5 day half-life. Alpha branching was found in this isotope giving 2.92 Mev alpha particles.³ The best value for the half-life was found by following the alpha decay in the pulse analyzer. Aluminum and beryllium absorption curves show the presence

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of 0.2 Mev conversion electrons. A p,n reaction is assumed with 10 Mev protons on Sm^{147} and the activity is assigned to Eu^{147} . This activity has also been seen in an alpha bombardment of enriched Sm^{144} .

- ¹Marinsky, J. A. and L. E. Glendenin, MDDC-1775-J.
- ²Wilkinson, Geoffrey and Harry G. Hicks, UCRL-872.
- ³Rasmussen, J. O., S. G. Thompson, A. Ghiorso, and G. T. Seaborg, UCRL Quarterly Report, (Dec., Jan., Feb., 1950-51).

Alpha Decay Energies of Polonium Isotopes

D. G. Karraker, A. Ghiorso and D. H. Templeton

Previously published values^{1,2} for the alpha particle energies of Po^{206} , Po^{207} , Po^{208} , and Po^{209} were measured in the 48 channel differential pulse analyzer before improvement of techniques and the apparatus made possible the precision of the measurements reported here. Some of the increased resolution is due to care taken to produce thin, uniform samples, but improved design of the ionization chamber and the preamplifier are mainly responsible for the improvement. At the present time, the precision of a measurement is about 10 Kev, with the accuracy estimated as about 20 Kev.

Energies are obtained from the pulse analyzer by a standardization of the relation of the output pulse height to the energy of the alpha particle causing the pulse. This calibration was determined using as primary standards alpha emitters whose alpha energies have been previously determined by alpha ray spectroscopy.

The polonium samples used for measurement were produced by proton and deuteron bombardment of bismuth. Bismuth oxide targets were dissolved in 6M HCl, and the polonium extracted into an organic phase composed of 20 percent tributyl phosphate and 80 percent dibutyl ether. Plates for pulse analysis were prepared by distilling the polonium from one platinum plate to another platinum plate about one-half inch distant in air. A collimator restricted the activity on the second plate to an area 15 mm in diameter.

The isotopic composition of the polonium used in the measurements was determined by the manner of preparation, which was varied to produce samples containing a reasonable amount of the activity of interest. The alpha energies of Po^{209} and Po^{208} were determined from a sample prepared by bombardment of bismuth with 18 Mev deuterons; Po^{207} and Po^{206} were determined from a sample prepared by 32 Mev proton bombardment of bismuth. Po^{207} was discovered to have the same alpha-particle energy, within experimental error, as Po^{208} . The alpha particles of Po^{207} were distinguished from those of Po^{208} by observing their decay in the pulse analyzer. The results of these measurements are listed in Table I.

¹Templeton, Howland and Perlman, Phys. Rev. 72, 758 (1947).
²E. L. Kelly and E. Segrè, Phys. Rev. 75, 999 (1949).

TABLE I

Isotope	Half-life	Alpha Particle Energy (Mev)
Po ²⁰⁹	~200 yr	4.86 ± 0.02
Po ²⁰⁸	2.93 yr	5.10 ± 0.02
Po ²⁰⁷	5.7 hr	5.10 ± 0.02
Po ²⁰⁶	9 days	5.21 ± 0.02

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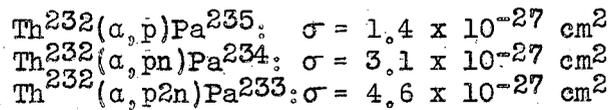
Helium Ion Reactions on Thorium

L. M. Slater

Thorium was bombarded with full energy helium ions (about 38 Mev) from the 60-inch cyclotron. Cross sections for the (α, p) , (α, pn) , and $(\alpha, p2n)$ reactions were measured. The results of two bombardments are recorded below.

Bombardment 1.

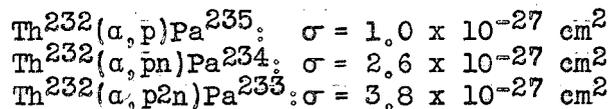
25 mil thorium metal was bombarded for 62 min. with approximately 38 Mev alpha particles. The beam was degraded from about 38 Mev to 0 Mev by the 25 mil. thorium metal, so the cross section reported for this bombardment covers the energy range from about 38 Mev to about 21 Mev which is about the threshold determined by the coulomb barrier.



About a hundred counts a minute of Pa^{232} was found due to deuteron contamination in the beam, but the amount of contamination that would cause this much activity would not affect to any significant extent the cross section for the $\text{Th}^{232}(\alpha, p2n)\text{Pa}^{233}$ reaction.

Bombardment 2.

5 mil thorium metal was bombarded for 24 min with approximately 38 Mev alpha particles. The cross sections reported cover the range of energy of about 38 Mev to about 27 Mev.



No Pa^{232} was found in this bombardment.

It should be noted the cross section for the $\text{Th}^{232}(\alpha, pn)\text{Pa}^{234}$ reaction is only that for the formation of the ground state form of $\text{Pa}^{234}(\text{UZ})$ and not for the isomeric form (UX_2).

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Specific Activity of U²³⁵

E. H. Fleming, Jr., A. Ghirso, B. B. Cunningham

A counting chamber, which has a counting yield of 8.313 percent for electro-deposited samples of uranium of a certain diameter, was described in the previous quarterly report.

Five separate deposits of uranium highly enriched in U²³⁵ were counted in this instrument, and the specific activity of U²³⁵ was calculated. A careful pulse analysis of the uranium showed that U²³⁵ comprised 65.3 percent of the mixture by activity.

The following data were obtained:

<u>Plate</u>	<u>Counts/min/mgm</u>
1	601.2
2	612.3
3	607.7
4	616.3
5	601.6

The average specific activity in this instrument for these deposits then is 607.8 counts/min/mgm for the mixture. This yields a specific activity for U²³⁵ of (4774 ± 72) disintegrations/min/mgm. The corresponding half-life is then $(7.07 \pm 0.11) \times 10^8$ yr.

Further measurements are being taken with deposits about 2-1/2 times the diameter of those used above. These values, together with a more precise value of the half-life, are to be reported in the next Quarterly Report.

Fission Products of Uranium Bombarded with 340 Mev Protons

P. C. Stevenson and R. L. Folger

Investigation of the fission of uranium with 340 Mev protons has been continued. Protective devices for safe handling of highly radioactive targets by remote control have been completed by the Health Chemistry group and by their use a number of product nuclides formed in low yield have been isolated and identified. During the course of this investigation a number of previously unreported isotopes have been observed and in a few cases identified. These activities are listed in Tables I and II.

A number of additional formation cross sections have been measured and will be reported at a later date.

TABLE I

New Isotopes Observed:

Yb ¹⁶⁶	61.2 hr	K, γ , e?
Tm [?]	2-3 days	β^- ?
Er [?]	17 hr	β^+ ?
Er [?]	65 hr	β^+ ?
Pm ^{150?}	43 da	β^- 0.6, 2.4 Mev γ 0.9 Mev
Pm [?]	13 hr	?
Fe [?]	8.4 hr	β^- 1.5 Mev

TABLE II

Nuclides formed by Fission of Uranium with 340 Mev Protons

Br ^{80m}	4.4 hr I.T.
Br ⁸²	35 hr β^-
Br ⁸³	2.4 hr β^-
Br ⁸⁴	33 Mev β^-
Cd ¹¹⁵	53 hr β^-
Cd ^{115m}	43 da β^-
Ce ¹⁴¹	30 da β^-
Ce ¹⁴⁴	275 da β^-
Pm ¹⁴⁸	5.3 da β^-
Eu ¹⁵⁶	15.4 da β^-
Tb ¹⁶⁰	74 da β^-
Tb ¹⁵³	5.1 da K, γ , e
Tb ¹⁶¹	6.8 da β^-
Dy ¹⁶⁶	80 hr β^-
Ho ¹⁶⁶	27 hr. β^-
Er ¹⁶⁹	9.4 da β^-
Tm ¹⁶⁷	9.6 da K, e ⁻
Yb ¹⁶⁹	32.5 da K, e ⁻
Zr ⁹⁵	65 da β^-

The Self-Scattering and Self-Absorption of Beta Particles

W. E. Nervik

During the period December through February a technique was developed for measuring the self-scattering and self-absorption correction factors for active sample thicknesses between 1-20 mg/cm².

The procedure in use at the present time is as follows:

1. An aliquot (usually about 20,000 counts) of the carrier free active material is placed on a very thin (approximately 5 µg/cm²), Zapon film, evaporated to dryness, and counted. It is assumed that there is zero backscattering from the zapon film and zero self-absorption and self-scattering from the active material.
2. A stainless steel filter, which has previously been washed with 1 N HNO₃ and distilled H₂O, dried to constant weight (± 0.01 mg), and "filled" with about 1.5 mg of inert salt (NaCl) to minimize penetration by the active salt in subsequent operation, is mounted directly beneath the Zapon film and a second count is taken. The difference between the first and second counts will give the backscattering factor for the steel plate.
3. A solution is made up of a precisely weighed amount of an inert salt and an aliquot (about 150,000 counts) of the active material. This solution is placed in the atomizer unit of a special "dust" making apparatus when it is atomized to extremely small droplets. These droplets are drawn into a heating chamber, when the water is completely evaporated and the resulting "dust," consisting of a homogeneous mixture of inert salt and active material, collected on the stainless steel filter. When approximately 1 mg/cm² of salt has been collected the filter is removed, counted, and weighed, and the salt's specific activity computed. From the original weight of salt used, the aliquot taken, and the count of step (2), the specific activity of the "dust" with zero self-scattering and zero self-absorption is known. The ratio of these specific activities is then plotted against thickness of the inert salt. This process of depositing the "dust" and computing its specific activity is repeated at 1 mg/cm² intervals until the sample thickness is approximately 20 mg/cm².

So far, curves of this type have been obtained with mixtures of Pm¹⁴⁷ (0.223 Mev β^-) and NaCl, KCl, Ba(NO₃)₂, and Cs(NO₃). The curves have yet to be completely analyzed, but they are all of the same general shape as shown in Fig. 1. The curves pass through a peak of about 102 percent at 2 mg/cm² and decrease to about 45 percent at 20 mg/cm². Preliminary analysis of the NaCl data indicates that the curves may be fitted by an equation of the form

$$C = \left(\frac{C_0}{\lambda}\right) (1 - e^{-\lambda t}) (1 + B e^{-\lambda t}) (1 + b(1 - e^{-\lambda t}))$$

where C = counts observed

C₀ = counts with zero self-scattering and zero self-absorption

$\lambda = 0.693/T_{1/2}$, where $T_{1/2}$ is the half-thickness in Al for beta particles of this energy.

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B = backscattering factor for the plate on which the sample is mounted.

b and K are analogous to a backscattering factor and absorption coefficient respectively for the salt with which the active material is mixed.

It is expected that the remaining curves for Pm^{147} ($\text{Gd}(\text{NO}_3)_2$, RbCl , NaNO_3 , KNO_3 ,) will be completed shortly. When they are finished, similar curves will be run using the same salts mixed with active materials having beta energies between 0.17 and 2.4 Mev.

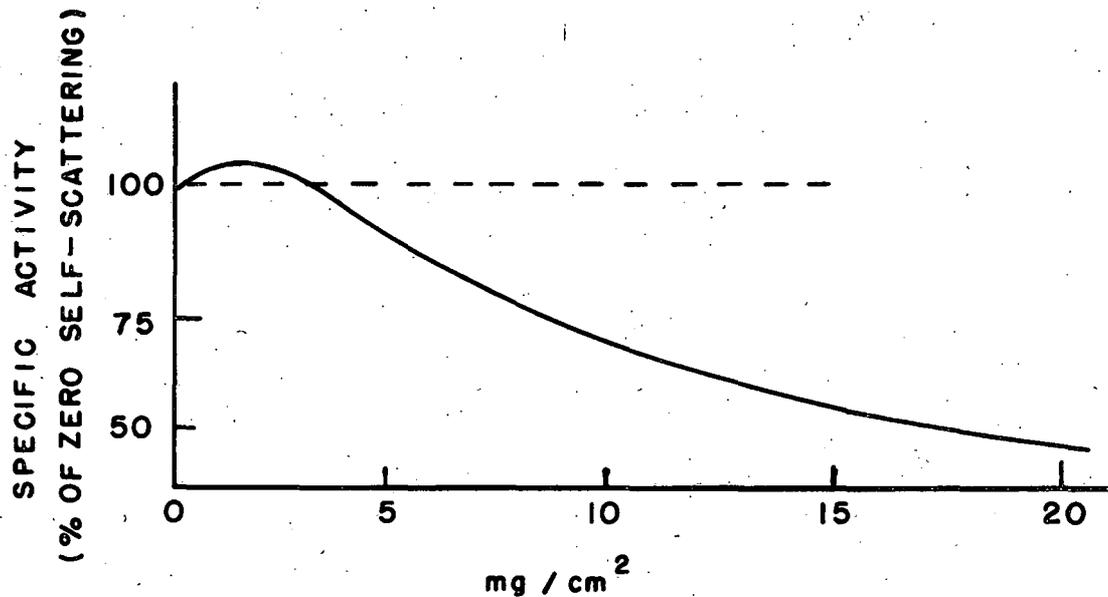


FIG. 1

MU 1665

Calibration of β Spectrometer with Co^{60} Gamma Radiation

C. I. Browne

In order to have a high energy calibration point to check the linearity of our β -ray spectrometer, we have measured the energies of the photo-electrons obtained from Co^{60} gamma radiation on a 1/2 mil uranium foil radiator. The gamma-ray energies derived from the observed photo-electrons are 1.175 and 1.340 Mev, in close agreement with the accepted values of 1.171 and 1.332 Mev.

Mass Spectrograph

F. L. Reynolds

In the last quarterly report (UCRL-1054) mention was made of a collection of the isotopes of curium on a metal foil. This foil was subsequently cut between the isotope deposits and submitted for pulse analysis. Insufficient counts were obtained at masses 243 and 244 to make the experiment clean-cut for the determination of the half-life of these isotopes, also there was considerable tailing of the curium 242 peak to cause experimental troubles. It was also considered that errors could have been made in cutting the foil at the exact division point between the collected isotopes.

At this writing another collection is to be tried, using a different collection technique from the previous run. A series of stacked 10 mil platinum foils, supported in a carriage so that the edges of the foil are presented to the beam is to be used for the surface collecting of the isotopes. This surface was ground and polished. This method will allow plotting of the spread of the tailing of the Cm^{242} peak, by counting the deposit on each segment of foil in 10 mil segments. This should give a plot of the extent of Cm^{242} tail which can be corrected for in estimating the $\text{Cm}^{243, 244}$ peaks.

Considerable trouble is still experienced in obtaining good positive ion emission of curium from surface ionization sources. In trying to get abundance ratio determinations on curium samples, the photographic platephotometer method had to be used because of insufficient lasting of the emission. The following results on a curium sample were obtained by this method. This curium was made by neutron bombardment of Am in the Chalk River Pile. The analysis was made on January 9, 1951.

Cm 242	243	244
93.6	4.6	1.7

If curium 245 was present, it existed in quantities less than 0.05 percent in this sample.

Some experiments, not as yet completed, were attempted on a double filament type of surface ionization source. For many samples placed on a single filament it is thought that the sample is lost by evaporation before sufficient thermal energy is obtained from the filament to produce thermal or surface

ionization. A source was constructed, the higher temperature emitting filament a spiral made of 4 mil wolfram, and a ribbon filament of wolfram mounted behind the spiral filament holding the sample. From experiments using U_3O_8 as the sample U^+ and UO^+ ion currents were obtained from this source when the sample holding filament was at an insufficient temperature to produce any detectable ion beam. These results, while semi-quantitative, are worth further study on this method of producing positive ions from surface ionization sources.

Abundance ratio determination of a plutonium sample Chalk River No. 3 was made. The results follow, and were made on February 21, 1951.

Pu 239	240	241	242
44.99	43.92	8.53	2.63

An attempt at collection of these isotopes, which involved operating the spectrograph for about 16 hours produced insufficient amounts of the separated plutonium isotopes for intended fission studies. From the amount of measured beta activity of the Pu^{241} collection there was collected the following amounts of these isotopes:

<u>Pu</u>	<u>Amount</u>
239	7.58×10^{-12} grams
240	7.5×10^{-12} grams
241	1.47×10^{-12} grams
242	4.5×10^{-13} grams

The limiting factor is the beam intensity and efficiency obtainable from plutonium samples on a surface ionization source. In this run the calculated efficiency was one ion over for every 10^6 atoms of plutonium placed on the filament.

Alpha Particle Spectrometer

F. Asaro, F. L. Reynolds and I. Perlman

For the past few weeks we have been using the large 75 centimeter radius mass spectrograph, as an alpha particle spectrograph. Using alpha track sensitive plates at the receiver we have been able to detect at least one fine structure alpha energy associated with the isotope curium 242. We have been able to get the half-width of these track deposits down to approximately 8 Kev in width, which means that the resolution of two alpha energies differing by 10 to 12 Kev can be detected by this instrument. The $H\rho$ values of this spectrograph are such as to focus all known energies of alpha particles.

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C. Experimental and Service Chemical Work Relatedto the Research Program

O. Chamberlain

Preparation of Stilbene Crystals

R. Leininger

A number of large stilbene crystals have been prepared during this period. The method of preparation and the experiences and results are analyzed in a summary report numbered UCRL-1104.

D. Bio-Organic Chemistry Group

Edited by B. M. Tolbert

Synthetic and Experimental Chemistry

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

During the past quarter the synthesis of a number of compounds has been studied. Only those on which major progress has been made will be discussed here.

Norvaline-3-C¹⁴. From n-propyl-1-C¹⁴ iodide on a 16 mmole scale norvaline-3-C¹⁴ hydrochloride with a specific activity of 18 $\mu\text{c}/\text{mg}$ has been prepared. The method of preparation used was that described in the last Quarterly Report. The crude norvaline from the hot run was found to be contaminated with dead glycine arising from the excess ethyl acetamido-malonate used in the preparation, with radioactive alanine, α -aminobutyric acid and with two or three other unknown radioactive compounds. These last materials presumably came from side reactions in the condensation. All but one of these materials were separated from the norvaline by fractional elution from a Dowex 50 ion exchange column. The remaining impurity represented no more than 1 percent of the total activity and was not conveniently removable.

Valine-4,4'-C¹⁴. Work has continued during the past quarter on the preparation of this important amino acid and of the intermediate used in its synthesis, isopropyl-methyl-C¹⁴ iodide.

In order to check the purity of the isopropyl iodide being used in this synthesis and, at the same time, to prepare additional material for exploratory low activity runs, 0.5 mc of isopropyl iodide was prepared from sodium acetate-2-C¹⁴ by the pyrolysis of the barium salt, reduction of acetone and conversion of the isopropyl alcohol to the iodide with phosphorus and iodine in an overall yield of 75 percent. A solution of the product was redistilled and analyzed.

Anal. Calcd. for C₃H₇I: C, 21.18; H, 4.12; I, 74.71.
Found: C, 21.14; H, 4.18; I, 74.78.

Mass spectrographic analysis revealed no detectable traces of any other halides in this sample.

Samples of the purified halide and of the unpurified halide were then condensed with ethyl acetamidomalonate. Both samples gave a variety of amino acid and amino acid-like byproducts which were very difficult to separate from the valine. In addition, only about 20 percent of the activity of the halide was found in the valine itself which represents a very low yield for this condensation step.

It is known that the condensation of branched chain halides with ethyl

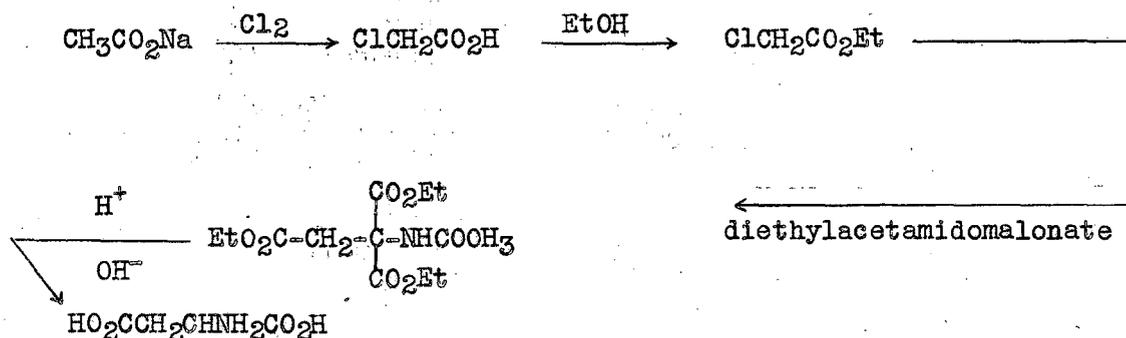
-29-

acetamidomalonate is a difficult reaction. It is presumed that the halide undergoes a number of side reactions and it is not certain that there is an answer to these low yield and poor product qualities. At present, we are assaying a couple of modified condensation conditions in order to get better results. If these conditions do not work satisfactorily we may have to abandon this synthetic method entirely with the branched chain halides.

Preparation of Intermediates. On a 20 mmole scale n-butyl-1-C¹⁴ bromide was prepared in 54 percent yield from sodium butyrate-1-C¹⁴ with a specific activity of 4×10^4 dis/min/mg. Sodium isobutyrate-1-C¹⁴ was converted to isobutyl-1-C¹⁴ iodide (8×10^5 dis/min/mg) in 35 percent yield. Sodium caproate-2-C¹⁴ was prepared in an 89 percent yield from 10.4 mc of amyl-1-C¹⁴ bromide. Methanol-C¹⁴ (14.5 mc) was converted to methyl-C¹⁴ iodide in nearly quantitative yield.

Low activity preparations of ethylene-1-C¹⁴ have been carried out from ethyl iodide and trimethylamine. The ethyl iodide and a water mixture of the trimethylamine with enough alcohol to make a single phase solution were heated in a steam bath in a sealed tube for 16 hours. Following that, the reaction mixture was treated with silver oxide, filtered and then decomposed by boiling to dryness. Yields on both steps are essentially quantitative.

Synthesis of Labeled dl-aspartic Acids. The small scale synthesis of aspartic acid labeled in either the β - or γ -position with C¹⁴ has been studied via the following method:



The acetic acid is the radioactive starting material and may be labeled in either the one- or two- position.

The preparation of the sodium acetate and the conversion of this material to chloroacetic acid has been previously worked out in this laboratory. The preparation of the ethyl chloroacetate in a state of sufficient purity to use for this reaction offers more difficulty.

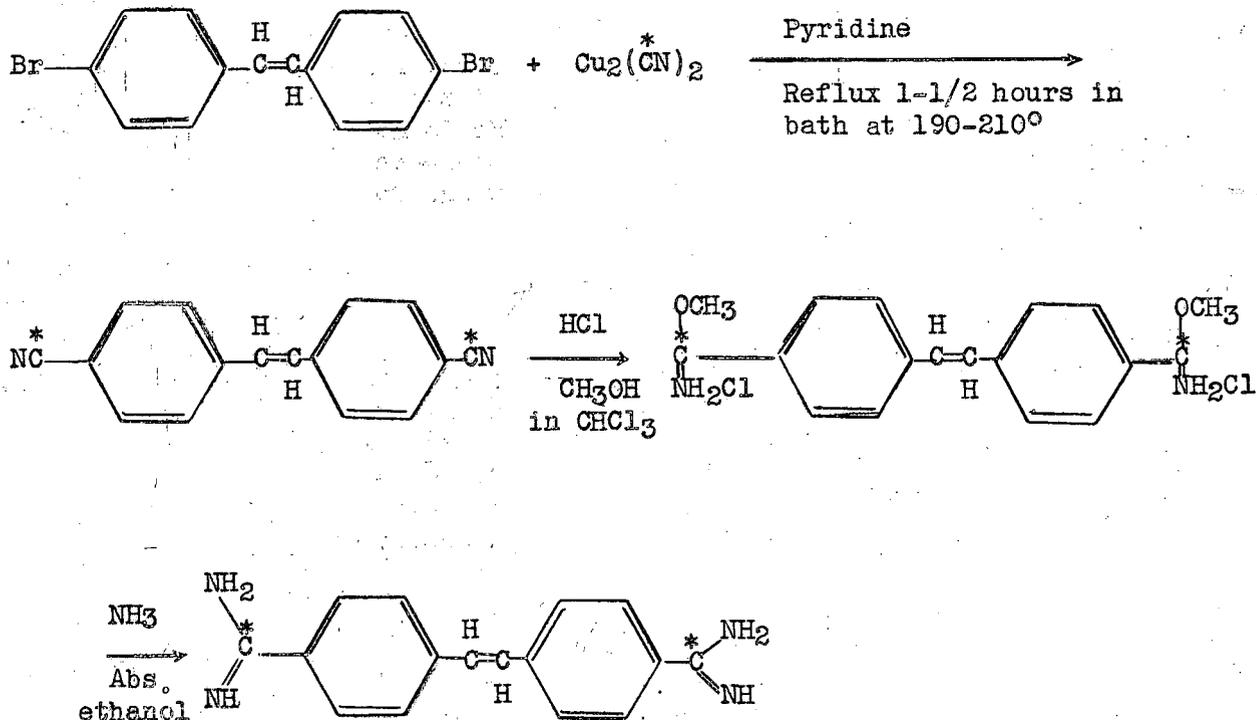
The possibility of chlorinating ethyl acetate directly was first investigated, but it was found there were so many side reactions under the conditions tried that purification of the products was not possible nor could it be used directly. Esterification of the potassium chloroacetate with diethyl sulfate was next tried. Intermittent yields as high as 99 percent could be obtained by this method. There are a number of technical difficulties

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involved which lead to inconsistent results. At an unpredictable time the diethyl sulfate mixture decomposes very rapidly. When the reaction is run at reduced pressure the bumping problem has led to difficulties in equipment design and the presence of both ether and alcohol from the decomposition of the diethyl sulfate has contaminated the product.

At present, we are studying the direct esterification of chloroacetic acid with absolute ethanol in the presence of zinc chloride. Preliminary yields of over 90 percent have been obtained and the product is quite pure. The condensation of ethyl chloroacetate with ethyl acetamidomalonate is quite satisfactory, and if the halide is pure, glycine and aspartic acid are the only product. These can be separated very satisfactorily by fractional elution using 0.5 N hydrochloric acid on a Dowex 50 ion exchange resin column. The extent to which the columns can be loaded without interfering with the separation is now being investigated.

Stilbamidine-amidine- C_{14}^3 . In order to continue a study which was being carried out with the Division of Medical Physics on the metabolism of stilbamidine-amidine- C_{14}^3 in normal and tumor-bearing mice and in human beings, a repeat of the preparation of this drug which was originally carried out by Dr. J. C. Reid of this group (UCRL-465) was attempted. In the original preparation the following reactions were used:



An overall yield of 28 percent based on the cyanide used was obtained.

In an attempt to repeat this preparation difficulty was encountered at several points. Thus, analysis of the dicyanostilbene for bromine indicated that under the conditions of the reaction only about 25 percent of the hydrogen had been replaced. Although the imino ether could be obtained from the dicyanostilbene in good yield on a weight basis, analysis of the several products obtained indicated that the material was not pure and that the actual yield was much lower. Direct conversion of the imino ether to stilbamidine yielded a large amount of material which could not be extracted by dilute hydrochloric acid.

Therefore, a new set of conditions for the preparation of the dicyanostilbene was determined and the direct conversion of this material to stilbamidine with thiocyanate was investigated. It was found that upon heating dibromostilbene and cuprous cyanide in the presence of pyridine and copper sulfate in a sealed tube at 250° for 2-3 hours dicyanostilbene in nearly quantitative yields could be obtained. The method for the direct conversion of the dicyanostilbene to stilbamidine is essentially that described by Partridge and Short (J. Chem. Soc., 1947, 390).

Dicyanostilbene was converted into stilbamidine easily and satisfactorily by heating with ammonium thiocyanate at 185° for 12-14 hours in a sealed tube. The tube was cooled in liquid nitrogen before opening, and the excess ammonium thiocyanate removed by extraction with dilute ammonium hydroxide. The product was then extracted with dilute hydrochloric acid and crystallized by the addition of concentrated hydrochloric acid. It was subsequently reprecipitated with ammonium hydroxide and converted to the diisethionate as previously described. A 32 percent overall yield from cuprous cyanide-C¹⁴ was obtained (specific activity, 19.5 µc/mg. or 10 mc/mmmole). The product isolated is chromatographically pure as determined by a radioautograph after chromatography on paper using butanol-propionic acid-water as the solvent system. A higher yield may be expected in future syntheses since, in actuality, this synthesis included several unnecessary, unreported steps. Partridge and Short report a 70 percent yield of stilbamidine hydrochloride from dicyanostilbene. We have obtained 56 percent yield in this step and a 75 percent yield in the conversion of the hydrochloride to the diisethionate.

Action of Ionizing Radiation on Peptides. This investigation was undertaken in order to study the action of ionizing radiation on peptides of known structure. It is planned to study the action of radiation on peptides in the solid state as well as in aqueous solution since it is conceivable that the effects of "direct hits" on the solid material might be quite different from effects observed in solution.

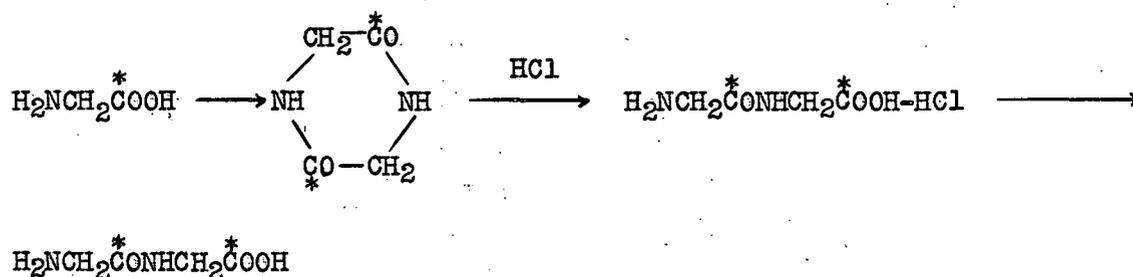
Preliminary work involving the use of unlabeled peptides has been carried out. In each case the peptide was irradiated in an aqueous solution which was then analyzed by the method of paper chromatography. The paper was sprayed with ninhydrin in order to locate the position of the product on the paper. Since ninhydrin gives a colored spot only with amino acids or peptides, any product which resulted from the breaking of any bond other than the peptide bond could not be located in this manner. Irradiation of

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a solution of glycyl-L-tryptophan (4 mg in 10 ml) by a deuteron beam for five minutes proved to be an overdose, and the original peptide was destroyed completely. Irradiation of a solution of triglycine, both with x-rays and with a smaller dose of deuterons, was carried out, but the only compound which could be detected was the original peptide.

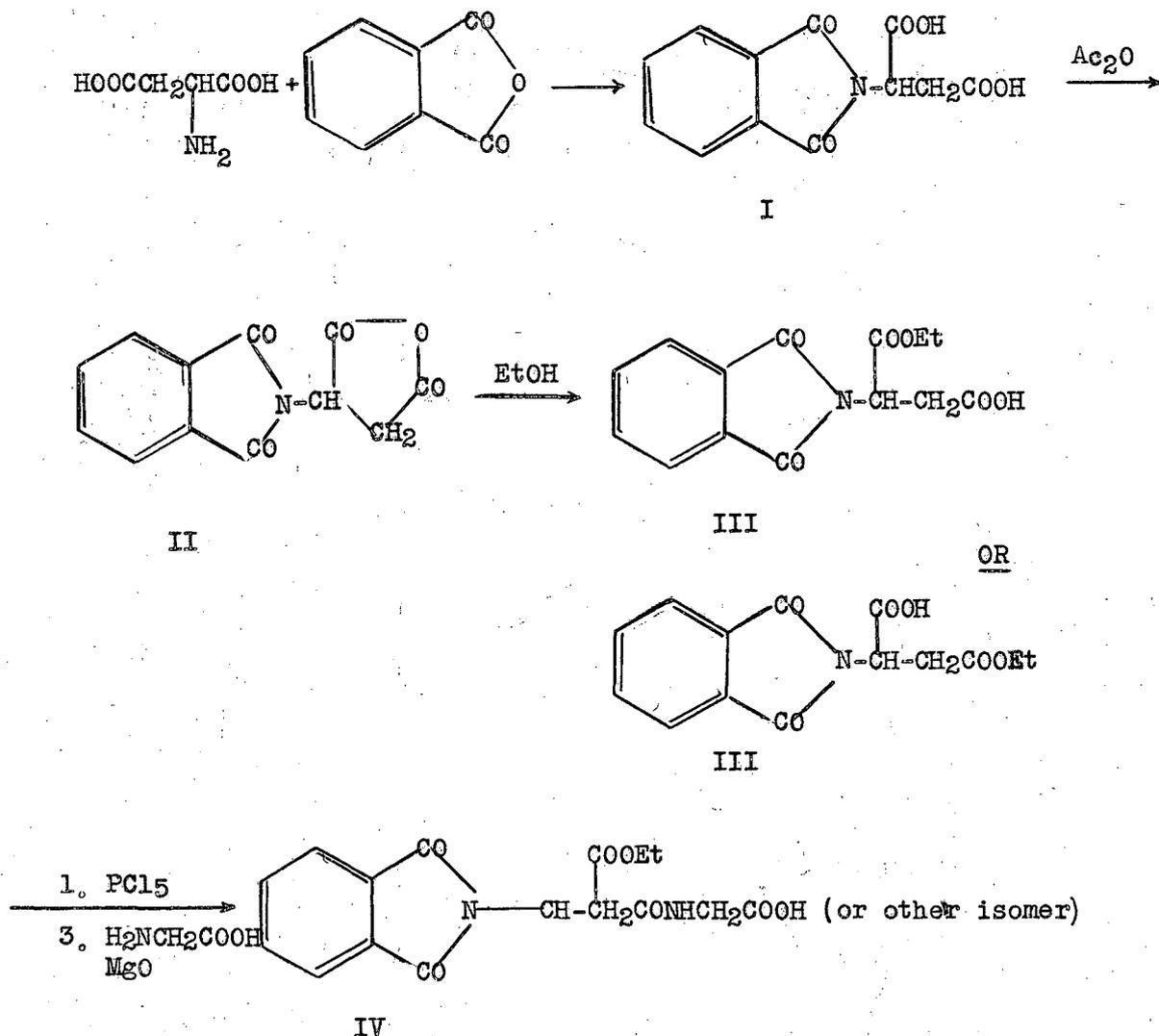
In the present investigation of the action of ionizing radiation on the biologically important peptides the solvent plays an important role. It is generally believed that when water is the solvent the primary effect of ionizing radiation is to produce hydroxyl radicals and hydrogen atoms. Therefore, any change produced in the solute will be a secondary effect brought about by these fragments. Stein and Weiss have studied the action of x-rays on dilute solutions of amino acids, and studies have also been made on enzyme systems, but there have been no reports of similar investigations carried out on smaller compounds which contain the peptide bond and thus offer some possibility for identification of the products of irradiation.

a. Synthetic Procedures. The convenient detection of all of the products of such an irradiation can most easily be accomplished by labeling the original peptide, and toward this end diglycine has been prepared from glycine labeled with C^{14} in the carboxyl group, according to the following scheme:



Synthetic procedures which will lead to peptides of aspartic acid are also being studied. The following sequence has been worked out:

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Work is in progress to obtain the free peptide, aspartylglycine, from IV and to determine which isomer of III was obtained. The synthesis will then be carried out with labeled amino acids.

b. Diketopiperazine. A mixture of 428 mg of glycine and 1.8 cc of ethylene glycol was refluxed for 30 minutes. The glycine went into solution after the first two minutes, and the solution became progressively darker. The solution was cooled and 20 cc of acetone was added. The solid was removed by filtration and washed with acetone to give 200 mg (90 percent) of black powder. The product was treated with charcoal in 60 percent methanol and the solvent evaporated to leave 139 mg of diketopiperazine (62 percent).

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c. Diglycine-hydrochloride. A mixture of 139 mg of diketopiperazine and 0.7 cc of concentrated hydrochloric acid was boiled for 45 seconds, and then cooled in ice immediately. The diglycine hydrochloride was removed by filtration and dried to give 158 mg of diglycine hydrochloride (77 percent).

d. Diglycine. Ethylene oxide was bubbled through a solution of 158 mg of diglycine hydrochloride in 10 cc of 70 percent ethanol for one hour. Most of the solvent remained in vacuo, and the product precipitated by the addition of absolute ethanol. There was obtained in the first crop 72 mg of diglycine (58 percent).

e. Phthalyl-dl-aspartic Acid. A mixture of 3.00 g. of dl-aspartic acid and 3.66 g. of phthalic anhydride was heated at 180-185° for 15 minutes. The melt was then cooled and recrystallized from 10 percent ethanol. There was obtained 3.58 g. of phthalyl-dl-aspartic acid (65 percent) in the first crop, m.p. 223-224.5° (dec.).

f. Phthalyl-dl-aspartic Anhydride. The anhydride was prepared by refluxing phthalyl-dl-aspartic acid in acetic anhydride for 30 minutes. The acetic anhydride was removed in vacuo to leave a solid which was suitable for the use in the next step.

g. Mono Ethyl Ester of Phthalyl-dl-aspartic Acid. The mono ester was prepared by refluxing the anhydride with ethanol for 1-1/2 hours. The solvent was removed in vacuo to leave an oil. Crystallization from a petroleum ether-ethanol mixture gave about half of the material as a solid, m.p. 143-145°. The material in the mother liquor remained an oil after evaporation of the solvent, and presumably consists mainly of the other isomer of III. The crystalline product was purified by recrystallization from benzene-cyclohexane, and melted at 149-149.5°.

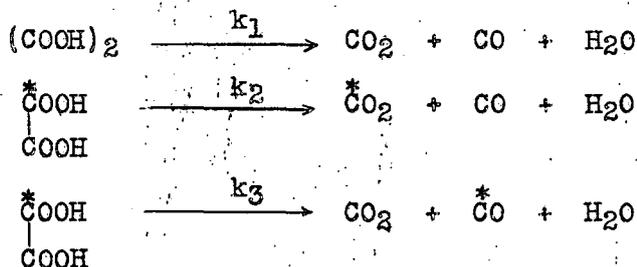
Anal. Calcd. for $C_{14}H_{13}O_6N$: C, 57.73; H, 4.50; N, 4.81.
Found: C, 57.85; H, 4.47; N, 4.78.

h. Mono Ethyl Ester of Phthalyl-dl-aspartylglycine. To a suspension of 409 mg of phthalyl-dl-aspartic acid, mono ethyl ester, in 3.0 cc of dry benzene was added 292 mg of phosphorus pentachloride. Evolution of hydrogen chloride began almost immediately, and the material went into solution. The solution was heated at 60° for one hour and fifty minutes. The benzene was then removed in vacuo to leave an oil which was dissolved in 2.5 cc of dioxane. The dioxane solution was added all at once to a cooled suspension of 106 mg of glycine and 85 mg of magnesium oxide in 5 cc of water. The mixture was swirled at room temperature for one-half hour and then acidified with 6 N hydrochloric acid. The oil which separated was extracted with four portions of chloroform. The solvent was evaporated to leave 440 mg of oil.

One hundred milligrams of the oil was dissolved in chloroform and separated into its components by partition chromatography on a silicic acid column (20 g.). The first fractions, eluted with chloroform, represented about 60 percent of recovered starting material. The remainder of the material was eluted with 5 percent butanol in chloroform and consisted of the desired product of the coupling. It was purified by recrystallization from benzene-ethanol and melted at 151.5-152.5°.

Anal. Calcd. for $C_{16}H_{16}O_7N_2$: C, 55.17; H, 4.63.
 Found: C, 55.31; H, 4.72.

Isotope Effect in the Decomposition of Oxalic Acid. The decomposition of oxalic acid into CO_2 , CO and H_2O by 100 percent sulfuric acid has been carried out at 103.0 and at 80.1°C. The CO_2 was collected as barium carbonate, and the CO was oxidized to CO_2 and also collected as barium carbonate. Carbon dioxide was regenerated from the samples, and the specific activities were determined by ionization chamber measurements. The C^{13} content of the above samples was measured by means of a mass spectrometer. Considering the three equations:



where (*) indicates the presence of a labeled atom, the ratio of k_2/k_3 for complete reaction can be readily determined from the measured isotopic contents. These values are given in Table I. Each of the values in the table is the average of several independent measurements of isotopic content.

TABLE I

Sample	T	k_2/k_3	
		C^{13}	C^{14}
1	103.0 ± 0.2	1.0235	1.057 ⁵
2	103.0 ± 0.2	1.032	1.060 ⁵
3	103.0 ± 0.2	1.027 ⁵	1.052
4	103.0 ± 0.2	1.027	1.051
Average	103.0 ± 0.2	1.027 ± 0.0022	1.055 ± 0.0037
5	80.1 ± 0.2	1.035	1.072
6	80.1 ± 0.2	1.030	1.069 ⁵
7	80.1 ± 0.2	1.032	1.060 ⁵
8	80.1 ± 0.2	1.032 ⁵	1.060 ⁵
Average	80.1 ± 0.2	1.032 ± 0.0014	1.065 ± 0.0051

Isotope Effect in the Decarboxylation of Substituted Malonic Acids. The kinetics of the hydrolysis of diethyl α -naphthalene malonate have been extended and refined by carrying out the reaction in a thermostated bath at 24.95 and 36.95°C. The second order rate constants

$$\frac{-d \text{ ester}}{dt} = k_1 (\text{ester}) (\text{OH}^-); \quad \frac{-d (1/2 \text{ ester-ion})}{dt} = k_2 (1/2 \text{ ester-ion}) (\text{OH}^-)$$

at 24.95°C are, $k_1 = 32/\text{moles hr.}$, and $k_2 = 3.2/\text{moles hr.}$; at 36.95°C, $k_2 = 11.5/\text{moles hr.}$ k_1 at 37°C was too fast to be measured by the procedure used in this experiment.

The basic carbonate split previously mentioned has been found to occur only during the hydrolysis of monoethyl α -naphthalene malonate. The rate of production of carbonate is directly proportional to the rate of hydrolysis of monoethyl α -naphthalene malonate. No carbonate is produced during the hydrolysis of the diester to the mono-ester and no additional carbonate is produced after the monoester has been completely hydrolyzed to the di-ion. It should be mentioned that the rate constants (k_2 above) include both the normal hydrolysis and the carbonate split.

Naphthalene malonic acid has been prepared in 60 percent yield from di-ethyl α -naphthalene malonate by a transesterification procedure, by allowing a solution of the ester in glacial acetic acid and concentrated hydrochloric acid to stand for two weeks at room temperature. The free acid is isolated by blowing off the acetic acid and the hydrochloric acid, treating the residue with benzene and filtering off the resulting precipitate of α -naphthalene malonic acid. The benzene solution contains unreacted ester and mono ethyl α -naphthalene malonate which may be recycled, thus increasing the yield.

Diethyl α -naphthalene methyl malonate has been prepared by alkylation of the sodium derivative of diethyl α -naphthalene malonate with methyl iodide in benzene solution. The hydrolysis of this ester is now being studied.

Biological Chemistry

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

Chicken Feeding Experiments. In a previous Quarterly Report the feeding of a laying hen sodium acetate-1-C¹⁴ has been described. Data have been presented on the incorporation of activity in the yolks and whites of the ten eggs obtained. This report continues the presentation of the results obtained.

The crude cholesterol fractions which were obtained in varying amounts from all yolks were recrystallized and the activity was determined in every case. (Direct plating from acetone was used.) See Table II. All R_F values were determined on Quilon-treated paper using methanol as the developing solvent. In all cases the spot was located by a spray test using a 25 percent solution of SbCl₅ in chloroform. In all cases except 1 and 2, the location of the spot was verified by a radioautograph. The radioautographs showed that no other active material was present. Several experiments utilizing this material are now in the planning stage.

For determination of the activity of the protein fractions, aliquots were converted to carbon dioxide by combustion and the resulting carbon dioxide collected and plated as barium carbonate. In the case of egg No. 1 the barium carbonate obtained had to be counted in an ionization chamber. The results are given in Table III. The phospholipids were available in the form of a petroleum ether solution. Aliquots of this solution were placed in combustion boats, the solvent evaporated and the residue burned. The activity was determined in the form of barium carbonate. The data are given in Table III.

For separation of the glycerol, two methods were tried. In one case, the aqueous material following saponification was distilled to dryness and the resulting salt extracted with pyridine. The pyridine solution was dried and then an excess of benzoyl chloride was added to it. The glyceryl tribenzoate was separated by acidification, extraction with ether and the ether was washed with sodium carbonate solution until no more benzoic acid was obtained. The ether was dried and distilled and the tribenzoate recrystallized from alcohol. This method was tried with yolks No. 1, 2, 3, 4 and 6. In the other case, the salt remaining after the aqueous material had been distilled was extracted with hot acetone and the acetone evaporated to give glycerol. This was done in the case of yolks No. 5, 7, 8, 9 and 10. The latter five were combusted and the remainder treated with benzoyl chloride in pyridine. To date, crystalline esters have been obtained from No. 5 and No. 8 and No. 7, 9 and 10 are still being worked up.

The combustion data gave results shown in Table IV. The tribenzoate obtained from yolk No. 1 was too little to be purified, so it was plated directly on a weighed plate. It was found to have a specific activity of about 2.3×10^{-3} $\mu\text{c}/\text{mg}$. The weights of other tribenzoates obtained are: No. 2, 335 mg; No. 3, 1 mg; No. 4, 3 mg; No. 5, 43 mg, No. 6, 127 mg; No. 8, 96 mg. These will be plated and combusted, to check relative accuracy.

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TABLE II

Yolk No.	Mg Pure Cholesterol	Sp. Act. $\mu\text{c}/\text{mg.} \times 10^2$	R _f
1	46	0.351	0.57
2	12	0.387	0.55
3	47	0.786	0.54
4	75	2.99	0.54
5	98	4.59	0.55
6	39	6.77	0.54
7	99	10.12	0.56
8	46	6.00	0.58
9	21	5.56	0.54
10	8	4.54	0.53

TABLE III

Yolk No.	Protein Specific Activity, $\mu\text{c}/\text{mg}$ $\times 10^3$	Protein Total Activity, $\mu\text{c}/\text{mg.}$	Phospholipid Specific Activity, $\mu\text{c}/\text{mg.} \times 10^3$
1	0.0285	0.086	0.174
2	0.438	1.29	1.17
3	1.13	3.39	3.44
4	1.67	4.63	6.16
5	2.28	6.66	12.72
6	3.23	10.5	11.08
7	2.80	8.74	10.16
8	2.52	4.48	10.70
9	2.07	1.86	7.15
10	1.85	0.87	7.22

TABLE IV

Yolk No.	Specific Activity $\mu\text{c}/\text{mg} \times 10^3$	Total Activity $\mu\text{c} \times 10$
5	2.38	4.16
7	2.25	3.87
8	2.36	4.6
9	1.82	1.71
10	1.22	3.49

Separation of the acidic from the neutral portion of the yolks was achieved by passing the material through an ion exchange column (resin IRA-400). A column of about 35 g. of resin was washed twice with a 200 cc portion of 6 N sodium hydroxide and the column was then washed with water until the washings were neutral to phenolphthalein. The water was forced out and an ether solution of the crude material was passed through the column twice, under slight pressure; the column was then washed with 200 ml of ether. The acids were eluted from the column with a mixture of 70 parts ether, 30 parts 95 percent ethanol and 8 parts 12 N hydrochloric acid. This mixture was passed through the column until a few drops of eluate caught on a glass plate and dried contained no activity. It was found that in all cases there was some non-acidic material present. This would indicate incomplete saponification and make the activity values for the glycerol which have been given open to question. The neutral material was non-acidic; this was shown by titration with a very weak base. An immediate color was obtained with phenolphthalein. Infra-red spectra of several of these neutral fractions were similar to spectra of cholesterol esters and of fats. Investigation of the neutral material is underway. The combustion of the acids obtained is also in progress. Plans for separation of the acidic material into saturated and unsaturated fractions have been abandoned for the present time.

Paper Chromatography of Steroids. Paper chromatography of steroids on Quilon impregnated paper is still under investigation. Several color tests have been tried with varying degrees of success. In some cases, the tests work very well on a test-spot, but are not visible when applied to a regular chromatogram. The five compounds which have been tested most thoroughly and which will be referred to by their numbers hereafter, are: (1) Dehydroepiandrosterone; (2) Dehydroepiandrosterone acetate; (3) Testosterone; (4) Testosterone acetate; and (5) Progesterone. The most satisfactory method of identification consists of suspending the strip in a vapor of iodine and then allowing the background to fade in air. The area where the steroid is holds the color much longer. Table V below gives R_f values as found in a number of solvents, the number in parentheses is the number of strips run in that solvent. Testing of other solvents and other steroids is in progress.

Metabolism in vitro of Labeled Sodium Propionate. In concluding the study of the metabolism of labeled sodium propionate incubated with liver slices, work has continued on identification of the one remaining major unknown radioactive compound appearing on chromatograms of metabolic products.

Previous degradations of this activity indicated a structure:

$\text{CH}_3\text{-CH}_2\text{-}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{-}\text{?}-\text{COOH}$ containing no adjacent hydroxyl groups. A silicic acid chromatogram of the activity with carrier α -ketobutyric acid showed a large difference in position of activity and acidity, eliminating this compound as a possibility. Tests were undertaken to show the hydroxy- or keto-nature of the oxygenated group. Formation of the acetyl derivative of hydroxyacids was found to be satisfactory and suitable to chromatography on the silicic acid column (benzoyl derivatives moved too quickly to chromatogram), working in both carrier and trace quantities. The unknown activity formed on acetyl derivative, therefore establishing the presence of a hydroxy group.

TABLE V

Compound	MeOH	EtOH-water 7:3	THF (*)	THF-water 9:1	MeOH-water 9:1
1	0.75 (8)	0.96 (6)	0.96 (2)	1 (2)	0.87 (6)
2	0.76 (8)	0.97 (6)	0.96 (2)	1 (2)	0.85 (7)
3	0.78 (9)	0.98 (6)	0.98 (2)	1 (2)	0.83 (7)
4	0.81 (8)	1 (10)	0.97 (2)	1 (2)	0.92 (6)
5	0.79 (9)	0.98 (6)	0.97 (2)	1 (2)	0.95 (6)
		MeOH-CHCl ₃ 9:1		CHCl ₃ -MeOH 9:1	
1		0.86 (4)		0.99 (2)	
2		0.87 (6)		0.99 (2)	
3		0.87 (6)		0.99 (2)	
4		0.97 (4)		0.99 (2)	
5		0.97 (6)		0.99 (2)	

(*) Tetrahydrofuran

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To recheck α -hydroxybutyric acid, previously discarded as a possibility, some of this acid was synthesized and chromatographed as such and as the acetylated acid with the unknown activity. Neither the acid itself nor the acetyl derivative corresponded to the activity or its derivative. This confirmed that the activity is not α -hydroxybutyric acid.

A small portion of ethyl- β -ketovalerate was obtained and hydrogenated with Raney nickel as catalyst. This hydrolyzed and chromatographed on the silicic acid column. The synthetic mixture proved to contain a mixture of acids. One of these, presumably β -hydroxyvaleric acid, corresponded to the unknown activity on the column. The eluted peak was degraded with chromic acid and gave propionic acid with the same specific activity (activity/acidity) as the acid the the previous chromatogram.

A synthesis of the β -hydroxyvaleric acid is now in progress in order to obtain the acid in a pure state so that final identification of the unknown activity will be possible.

Metabolism of Stilbamidine-amidine- C_2^{14} . The work reported here has been carried out in cooperation with Dr. J. C. Weaver and the Division of Medical Physics under Dr. J. H. Lawrence. In previous studies on the metabolism of labeled stilbamidine in mice it was found that there is no appreciable metabolic degradation to $C^{14}O_2$ of this drug in mice and in humans and that there is found an abnormal high liver concentration of radioactivity in tumor-bearing animals four days after intravenous injection of stilbamidine-amidine- C_2^{14} . In order to localize this liver concentration of radioactivity additional fractionations of the livers of normal and S-1 sarcoma-bearing "A" strain mice have been carried out after the injection of labeled stilbamidine. The fractionation procedure is that of Schneider and Hogeboom, (J. Biol. Chem., 183, 123 (1950)) in 0.25 M sucrose.

The data from several typical experiments are presented in Table VI. The activity of the various fractions is determined by direct plating the sucrose solutions unless otherwise noted, and the values are necessarily approximate due to self-absorption. However, such errors are rather constant and cross checks available in the fractionation technique and by combustion to carbon dioxide and plating of the resulting barium carbonate confirm this.

Succinic oxidase determinations confirm that the mitochondria are largely in F-4-P, with a small amount (10-15 percent) remaining in F-2-A, presumably in unbroken cells. Thus, the succinic oxidase activity data and the radioactivity data have a similar distribution. At the present time we are standardizing the procedures necessary for pentose nucleic acid (PNA), desoxypentose nucleic acid (DNA) and nitrogen determinations in order to check the fractionation procedure further, and the distribution of these elements as this procedure is carried out by us. Tentatively the PNA distribution is: F-2-A, \sim 15 percent; F-4-P, \sim 30 percent; and F-5, \sim 55 percent. The DNA distribution is: F-2-A, \sim 80 percent; F-4-P, \sim 10 percent and F-5, \sim 10 percent. The nitrogen determinations have not been done as yet.

From the data obtained to date, several preliminary conclusions appear to be made: (1) The stilbamidine is largely, probably essentially completely, localized either in the mitochondria or in or as a particle of similar

TABLE VI

Distribution of Activity in Normal and Sarcoma-Bearing "A"
Strain Mice Livers after Injection of Stilbamidine-Amidine- C_{14}^2

Fraction No. and Description	M-16*		M-17		M-65		M-66	
	total dis/min in fract. $\times 10^{-4}$	% of total liver						
F-1 Homogenate in 0.25 M sucrose	200	100	360	100	79	100	62	100
F-2 Homogenate centri- fuged at 1500 G. Nuclear ppt.	42	21	60	16	11	14	11	17
F-2-A F-2 rehomogenized and recentrifuged at 1500 G. Nuclear ppt (plated from water)	33	17	31	9	8.5	11	7.6	12
F-2-B F-2 rehomogenized and recentrifuged at 1500 G. Super- natant.	13	7	39	11	3.7	5	4.7	8
F-3 Homogenate centri- fuged at 1500 G. Supernatant	14	70	250	69	46	58	47	76
F-3-S F-3 centrifuged at 10,000 RPM for 15 minutes. Super- natant	20	10	15	4	5.3	7	5.7	9
F-2-B-S F-2-B centrifuged at 10,000 RPM for 15 minutes. Super- natant	--	--	--	--	0.65	1	0.92	1

TABLE VI
(Continued)

F-4 F-3 and F-2-B centrifuged at 10,000 RPM for 15 minutes. Combined precipitate. (Mito- chondrial fraction)	92	46	190	41	40	51	43	69
F-4-S F-4 rehomogenized and recentrifuged at 20,000 RPM for 15 minutes. Super- natant.	1.5	1	4.3	1	0.58	1	0.62	1
F-4-P F-4 rehomogenized and recentrifuged at 20,000 RPM for 15 minutes. Preci- pitate (plated from water).	150	75	310	86	46	58	51	82

* M-16 and M-17 were S-1 sarcoma-bearing "A" strain male mice, weight 25 and 20 g., respectively. 19.3×10^6 dis/min of stilbamidine diisethionate injected 7 days after the tumors had been transplanted; sacrificed 4 days later.

M-65 and M-66 were normal "A" strain male mice, weight 25 g. each. 23.1×10^6 dis/min of stilbamidine diisethionate injected. Sacrificed 4 days after injection.

physical properties. This is true of both normal and sarcoma-bearing mice; (2) The stilbamidine is not bound to the desoxyribonucleic acid; (3) The stilbamidine is not bound, if at all, to all the pentose nucleic acid equally.

Further experiments are in progress to study the binding site and method of binding of the stilbamidine in the mice livers. Also, it remains to be shown that the radioactive material being studied in the livers is stilbamidine rather than a slightly modified compound.

Metabolism of Labeled Purines. Due to the time that has been spent on the synthesis and metabolic studies of stilbamidine-amidine- C_2^{14} , few experiments have been carried out with labeled purines during the period covered by this report. The general metabolism and distribution of adenine-4,6- C^{14} and guanine-4- C^{14} in "A" strain mice with and without S-1 sarcomas have been studied. In the case of adenine-4,6- C^{14} no marked differences between the metabolism in the normal and tumorous mice were noted after 24 hours. A large amount (~10 percent) of the activity was found in the hot TCA extract of the tumors. The results of experiments with guanine and adenine were similar in C-57 and "A" strain male mice. Further work is in progress on the metabolism of these compounds.

Effect of Ionizing Radiation on $C^{14}O_2$. In cooperation with Dr. W. M. Garrison and the Crocker Laboratory under the direction of Dr. J. G. Hamilton a series of experiments has been carried out in which $C^{14}O_2$ has been subjected to cyclotron irradiation. Formation of formaldehyde and formic acid has been observed under these conditions and the possibility of formation of carbon-carbon bonds is being explored. Details of this work will be reported in the quarterly report from Crocker Laboratory.

Photosynthesis Chemistry

M. Calvin, A. Benson, J. Bassham, M. Goodman, S. Kawaguchi, V. Lynch and W. Stepka

The mechanism of carbon dioxide absorption by plants during photosynthesis has been under investigation in this laboratory during the past four years. During this time it has been shown that the products of at least two carboxylation reactions are reduced through a series of intermediates to give the primary carbohydrate, fat, and protein constituents of plants. The energy required for the phosphorylation and reduction steps involved derive from the photochemical phase of photosynthesis and the plant has been shown capable of storing this energy for subsequent dark reduction of carbon dioxide to the normal products of photosynthesis. A number of the intermediate compounds in this process have been previously identified and the present work of the laboratory is directed towards expansion of our knowledge of the reactions and intermediates involved.

Since a large number of compounds exist in plants and become radioactive after extended photosynthesis with $C^{14}O_2$ our efforts are focused upon those compounds observed to acquire C^{14} in short periods of photosynthesis. Hence the kinetics of the formation of various intermediates are being investigated during steady state photosynthesis. The kinetic relationships of unidentified compounds give some clues to their identity.

The examination of the relationships of intermediates of CO_2 reduction during non-steady state photosynthesis has been continued. By calculated distortion of the conditions of photosynthesis it has been possible to gain information regarding normal relationships. Work on the effect of changes in external conditions of photosynthesis such as pH, temperature and poison has been extended and is reported briefly below.

Kinetics of Formation of the Intermediates of Steady State Photosynthesis.

Kinetic studies of the intermediates of CO_2 reduction have been made a number of times in this laboratory. As refinements in separation and analysis techniques are made, it becomes expedient to re-examine the kinetics. Each time new advances are made. The improved methods for separation of phosphate esters described in the previous Quarterly Report from this laboratory have been applied to several series of experiments with Scenedesmus.

a. Experimental Methods. A suspension of 2 g. of Scenedesmus (two days old) in 200 ml of deionized water was illuminated (8000 f.c.) 30 minutes in the presence of 4 percent CO_2 in air. The excess CO_2 was removed by sweeping with air for 5 minutes. Two minutes after adding 2 mg KH_2PO_4 0.7 millimole of $NaHC^{14}O_3$ solution was injected into the suspension and the illumination vessel agitated by shaking. Samples were withdrawn at 5 second intervals and killed as they flowed into boiling ethanol. The amount of algae taken in each sample was determined colorimetrically. The total fixation curve, Fig. 1, represents the specific activity of the algae as a function of time. Each aliquot of cells was centrifuged and re-extracted with 20 percent ethanol. The soluble activity is also plotted in Fig. 1. Extracts of appropriate amounts of cells (20-30 mg) were chromatographed two-dimensionally in phenol-water and in butanol-propionic acid-water and radiograms were prepared. The radioactivities, determined by direct counting of the major products, are plotted in Fig. 2. It is known that separation of some hexose monophosphates is not complete under the conditions of our

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chromatography and the analysis is extended by examining the sugars formed by enzymatic hydrolysis of each phosphate area. The results of this work will be described in a future report.

b. Discussion. When the radioactivity incorporated in a given intermediate is plotted as percent of total fixed radioactivity, those compounds whose activity results from primary carboxylation reactions will give curves with negative initial slopes. Such is observed for phosphoglyceric acid and malic acid in Fig. 2.

Each of the other compounds has a positive initial slope. The inversion in the curve for the diphosphorylated compound appears to be anomalous but may well represent reality.

The negative initial slope for malic acid has not been observed before except at very low light intensities. It is now clear that the C_3 carboxylation to give malic acid or a closely related compound in rapid equilibrium with it is an essential step in steady state photosynthesis. The ratio of the rates of the two reactions appears to be about two if one ventures to extrapolate the phosphoglycerate curve to 66 percent and the malic acid curve to 33 percent. Examination of the cyclic path for regeneration of C_2 - CO_2 acceptor first proposed in this laboratory shows that each $C_3 + C_1$ carboxylation results in a source of C_2 acceptors for two $C_2 + C_1$ carboxylations. It appears then that refinement of experiments such as that plotted in Fig. 2 may provide indirect evidence for the proposed cycle.

A second time series has been prepared for photosynthesis by Scenedesmus at 20°C. The results shown in Fig. 3 show the anticipated negligible rate of synthesis of insoluble products. After chromatography the curves shown in Figs. 4 and 5 were obtained. Of major interest is the rapid rate of phosphoglycerate synthesis, even at low temperature. This rate is found to be as great as that observed in Fig. 2 for phosphoglycerate synthesis at 25°. The temperature coefficient of the $C_2 + C_1$ carboxylation reaction is thus very low compared to those for other simultaneous reactions.

The Relationships of Fructose and Glucose Phosphates involved in Sucrose Synthesis. Considerable effort has been expended by biochemists to determine which is the first sugar of photosynthesis. Numerous researches have led to the general conclusion that sucrose is probably the first free sugar. However this conclusion has been based upon indirect evidence. Using $C^{14}O_2$ we have been able to show that sucrose is formed without prior accumulation of free hexoses.

The answer to the question of which hexose phosphate involved in sucrose synthesis is the earliest to accumulate C^{14} may be indicated by the amounts of C^{14} in each of the hexoses of sucrose. The phosphate esters have now been hydrolyzed and the amounts of fructose and glucose activity determined as a function of time. In the time series experiment with Scenedesmus it is clear that the specific activity of fructose monophosphates is much higher than that of glucose monophosphates in the shorter times of photosynthesis (5-15 Sec.). Relative specific activities are determined from measurements of the amount of C^{14} in a compound at a given time compared to that when it is saturated with C^{14} in steady state photosynthesis.

Evidence has recently been published (Aronoff and Vernon, Arch. Biochem., 28 424 (1950)) which suggests that the mechanism of sucrose synthesis in soy bean and cane leaves may differ markedly from that in Chlorella, Scenedesmus and barley leaves. The evidence presented in this publication suggested prior synthesis of the glucose fraction of sucrose. Although a sequence of reactions similar to the reversal of glycolysis could conceivably result in prior labeling of the glucose moiety, there is as yet no reason to suspect such a unique system of reactions.

We have repeated our earlier experiments with soy bean leaves (var. Hawkeye) and find labeling of fructose phosphates and the fructose moiety of sucrose prior to that of glucose compounds. As in algae, barley and geranium, no appreciable amounts of free trioses or hexoses are obtained without enzymatic hydrolysis of phosphate esters. The ratio of fructose to glucose radioactivity in sucrose was found to be 1.8 in 30 sec. photosynthesis Chlorella, 3.0 in 15 sec. photosynthesis barley, 3.3 in 15 sec. photosynthesis soy bean leaf and 1.05 in 60 sec. soy bean leaf. The ratio of fructose phosphates to glucose phosphates was 1.0 in 5 sec. photosynthesis Scenedesmus, 0.3 in 60 sec. photosynthesis Scenedesmus, 14 in 2 sec. photosynthesis barley, 0.3 in 30 sec. photosynthesis barley, 5 in 5 sec. photosynthesis soy bean, 0.8 in 15 sec. photosynthesis soy bean and 0.8 in 60 sec. photosynthesis soy bean. The prior appearance of C^{14} in fructose phosphates is in accord with its early predominance in the fructose of sucrose.

The general pattern of compounds and the similarity of the kinetics of their synthesis strongly suggests that hexoses are formed by the same pathways in most plants. Although their formation from phosphoglyceric acid by the precise reversal of glycolysis is open to question, it appears that three-carbon compounds are condensed to form ketoses rather than glucose.

Preliminary Report on Effect of pH on Size of Réservoirs of Intermediates in Steady State of Photosynthesis in Scenedesmus. The results obtained by Ouellet in this laboratory indicate more rapid sucrose synthesis by Scenedesmus at low than at high pH. This can be interpreted as an enhancement of synthetic rate or inhibition of side reactions but the net rate of CO_2 uptake is not enhanced at low pH. Hence it is reasonable to expect that the early appearance of sucrose results from a diminution in the concentrations of the intermediates involved. These are readily measured using algae previously saturated with radiophosphate.

Algae were illuminated in nutrient, pH 6, overnight in the presence of radiophosphate. Identical aliquots were adjusted to pH 1.8 and 10.6 with HCl and NaOH and allowed to stand 30 minutes in dim light with 4 percent CO_2 in air bubbling through suspension. The algae were centrifuged as quickly as possible and killed in hot ethanol.

The amount of $P^{32}GA$ in equal volumes of cells was 2.5 times as great at pH 10.6 as at pH 2.8 while the corresponding ratio of activity in hexose monophosphates (largely glucose monophosphates) was ~ 1.7 .

Non-steady State Photosynthesis. Products of Dark $C^{14}O_2$ Fixation Immediately Following Steady State $C^{12}O_2$ Saturated Photosynthesis. The nature of the effect of preillumination in the absence of CO_2 and oxygen has been studied earlier and we have extended this work to include a determination of the "reducing power"

stored during steady state photosynthesis. The major effect of changes in external conditions during photosynthesis is reflected in changes in the concentrations of the various intermediates involved in both the energy transfer and in the carbon dioxide reduction process.

A suspension of *Scenedesmus* (1.0 g/100 ml) was given 0.5 percent CO₂ in air for 30 minutes at 8000-10,000 f.c. At the instant the illumination was ceased, an excess of NaHC¹⁴O₃ solution was injected with the suspension which was shaken vigorously for two minutes. The algae were killed in boiling ethanol. The radiograms were similar to those for 5-10 seconds of steady state photosynthesis. The majority of the C¹⁴ was found in the phosphoglyceric acid and phosphopyruvic acid. About 25 percent of the C¹⁴ was in malic and aspartic acids. Lesser amounts of labeled serine, alanine, sucrose and citric acid were formed. The absence of glycine and glycolic acid was striking.

The phosphate esters were hydrolyzed enzymatically to give largely glyceric acid and small amounts of hexoses. The activities of these hexoses were in the ratio observed after the hexose monophosphates are saturated with C¹⁴. That is, glucose activity was predominant. It is clear that the amount of energy available for hexose formation during CO₂ saturation is small but finite. Less than 1 percent of the C¹⁴ was observed in hexoses.

Effect of Lack of CO₂ on Products of Photosynthesis. A suspension of *Scenedesmus* in steady state photosynthesis in air was given an amount of NaHC¹⁴O₃ calculated to be sufficient for about three minutes of photosynthesis. The algae were maintained in light of 8000 f.c. for a total of 5 minutes and killed in ethanol. The major result observed in radiograms was a great diminution in the amount of phosphoglycerate and the appearance of a series of 11-12 discrete - radioactive polysaccharides containing only glucose. The synthesis of three such polyglucose compounds had been observed before during anaerobic illumination of algae but the separation of such a large series was not observed. The interpretation of this phenomenon is not yet clear.

The Phosphorous-carbon Ratios in the Intermediates of Sucrose Synthesis. The identified intermediates of sucrose synthesis have been studied in a number of chemical ways. One of the most promising seemed to be direct measurement of the number of carbon atoms per phosphorous atom in the molecules. Preliminary determinations have exposed several difficulties not previously anticipated.

Scenedesmus cells were equilibrated with P³² during 20 hours of photosynthesis at moderate light intensity. The C¹²O₂ was removed and NaHC¹⁴O₃ shaken with the algae at 8000 f.c. for 5 minutes. Since this time is sufficient to give uniformly labeled intermediates, it was expected that they would be saturated with C¹⁴ as well as P³². Radiograms were prepared and the separated phosphates eluted for accurate P³² and C¹⁴ measurement. The C¹⁴/P³² ratios observed were: phosphoglycerate, 1.60; glucose monophosphate, 1.12; fructose monophosphate, 1.3; diphosphates (largely one compound), 1.05; phosphopyruvate, 0.31; phosphoglycolate, 0.38.

If the expected results had been obtained the ratios of the hexose monophosphates would have been twice that for phosphoglycerate and phosphoglycolic acid would have been two-thirds that of phosphoglycerate. These results

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indicate that phosphoglycerate is relatively rapidly saturated with C^{14} and that slow equilibration with polysaccharide reservoirs probably retards the C^{14} labeling of hexose phosphates.

Assimilation of Methanol- C^{14} and Formaldehyde- C^{14} by Scenedesmus. Formaldehyde- C^{14} . Historical interest and current availability of labeled formalin solutions prompted a preliminary study of formaldehyde assimilation by plants. The toxicity of this substance precludes macrodetermination of assimilated formaldehyde but the products formed from the labeled compound can be readily detected and identified.

A suspension of Scenedesmus stirred with a nitrogen stream was illuminated after photosynthesis with $C^{14}O_2$ in nitrogen. Sixty microcuries of formalin solution (210 μ c/mmol.) (10 percent methanol impurity) was added and the nitrogen flow was continued for 10 minutes. The algae were filtered from the suspension and killed in boiling ethanol. In the alcohol extract 32 μ c (54 percent) of the activity was found but the radiograms of this material indicated that over 90 percent of the activity was formaldehyde in one of two forms. One, with a low R_f in our solvents is presumed to be linear polymer; the other with a much higher R_f is probably trioxymethylene. The original formalin solution contained a large fraction of its activity in trioxymethylene but the formation of the linear polymer is apparently a result of action of the algae themselves.

The remaining products are those normally observed in $C^{14}O_2$ photosynthesis experiments. Sucrose was identified by hydrolysis to glucose and fructose. Amino acids were identified with ninhydrin.

The conclusions to be drawn from this experiment are that algae may catalyze a change in the polymeric form of formaldehyde. An unequivocal proof of conversion of formaldehyde to sucrose is not possible since a ten percent impurity of methanol- C^{14} was stated to be in the original formalin preparation.

Methanol- C^{14} . A suspension of photosynthesizing Scenedesmus was illuminated for 20 minutes in nitrogen. Carbon dioxide-free air was then bubbled through the suspension and 44 μ c of methanol- C^{14} (1 mg.) (12×10^6 cts/min) was added. The total fixed activity in 10 minutes was 0.73×10^6 cts/min and the total insoluble products was 0.45×10^6 cts/min. The products were the phosphorylated esters, amino acids and polysaccharides normally resulting from $C^{14}O_2$ assimilation.

The rapid assimilation of methanol may be due to its oxidation to $C^{14}O_2$ and normal photosynthesis, although the rapid flushing with air and the high rate of respiration lessens this possibility. If methanol is actually incorporated into the metabolic intermediates of photosynthesis to the extent indicated by this experiment, further work is certainly worth while to investigate the pathways through which it enters the metabolic cycle. This could be a real clue to unknown or short lived photosynthesis intermediates.

Assimilation of Malic Acid- C^{14} by Scenedesmus. A preliminary feeding experiment with C^{14} -labeled malic acid was performed. About 0.2 g. wet packed cells (Scenedesmus) was suspended in 20 ml of a sodium malate solution. This solution

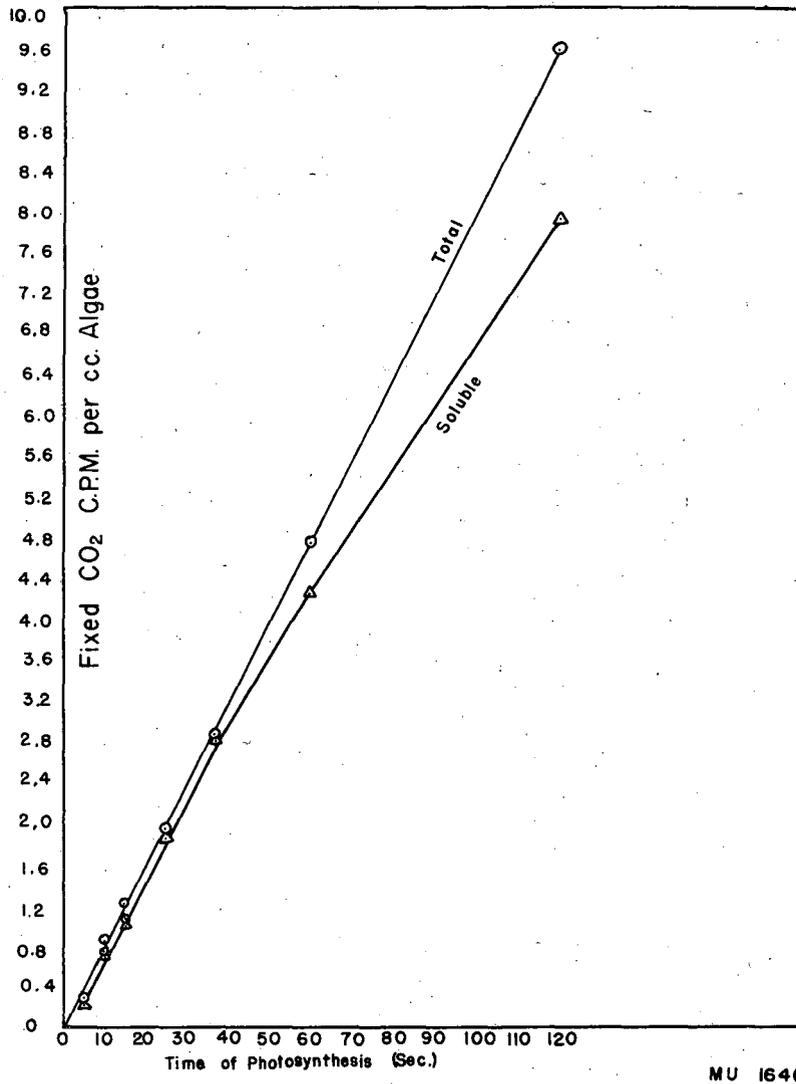
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was made up by dissolving 22 mg (80 μ c) $\text{COOH-CHOH-CH}_2\text{-C}^{14}\text{OH}$ in 20 ml H_2O and adjusting the pH to 3.5 with 2 N NaOH solution.

The cells were illuminated immediately on suspension and allowed to photosynthesize in air with 4 percent CO_2 for 10 minutes after which they were rapidly filtered and killed with boiling alcohol. They were extracted successively with alcohol, 50 percent alcohol, and hot water, the extracts were combined, concentrated, counted and analyzed by paper chromatography. In this cell extract 28 μ c of C^{14} or 35 percent of the administered dose was found.

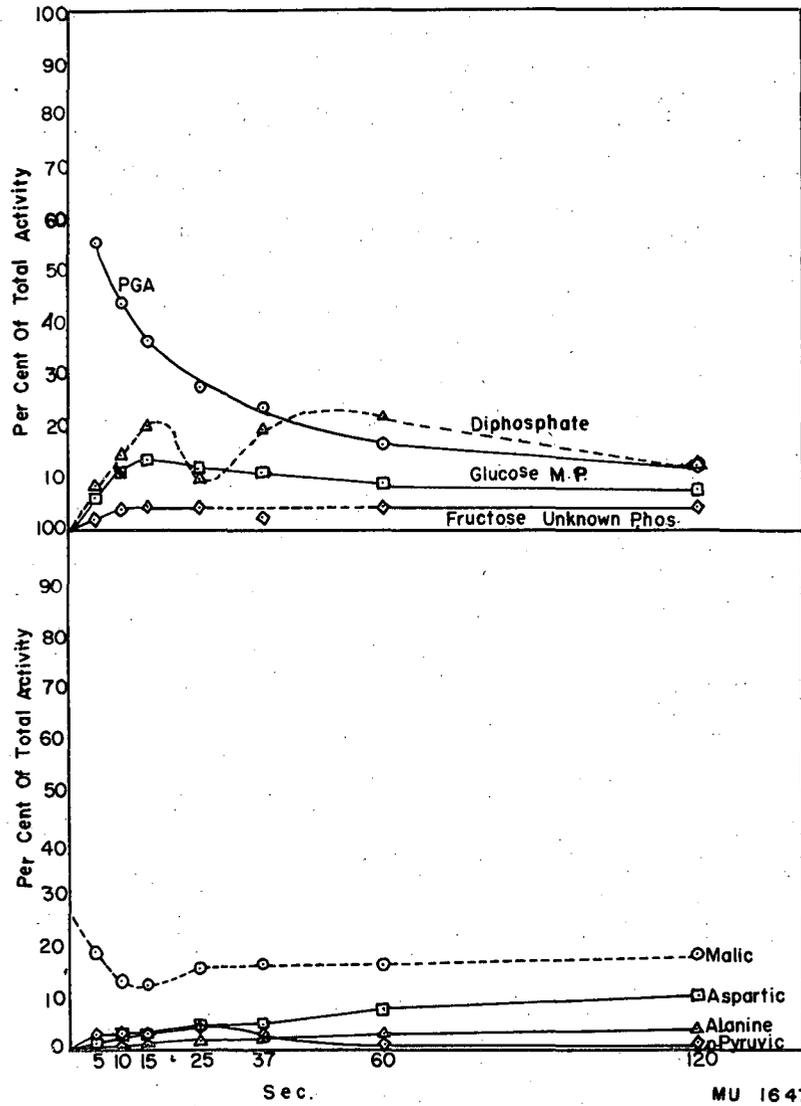
However, the radiograms indicated that virtually all the radioactivity remained as unchanged malic acid. After two months exposure of the x-ray film to the paper chromatogram, faint spots of the normal photosynthetic products could be seen but whether these substances arise from direct conversion of malic acid without prior oxidation or from conversion of malic acid to carbon dioxide and subsequent reduction by photosynthesis cannot be easily determined. After the filtration of the cells, the volume of cells, filter pad, and interstitial liquid was only about one-tenth of the volume of the cell suspension, so this could not have been the source of this malic acid.

The uptake of malic acid by the cells indicated by this experiment is interesting, if true, but additional experiments are necessary to verify the observation.



MU 1646

Fig. 1



MU 1647

Fig. 2

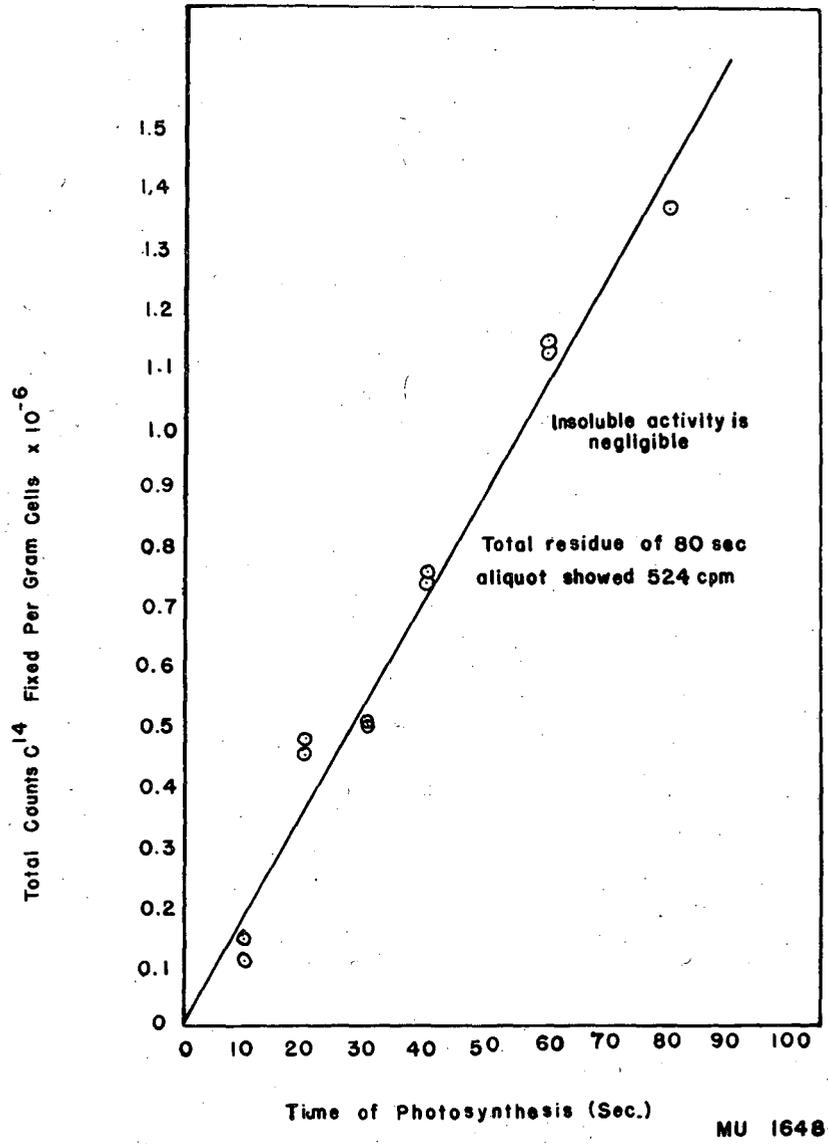


Fig. 3

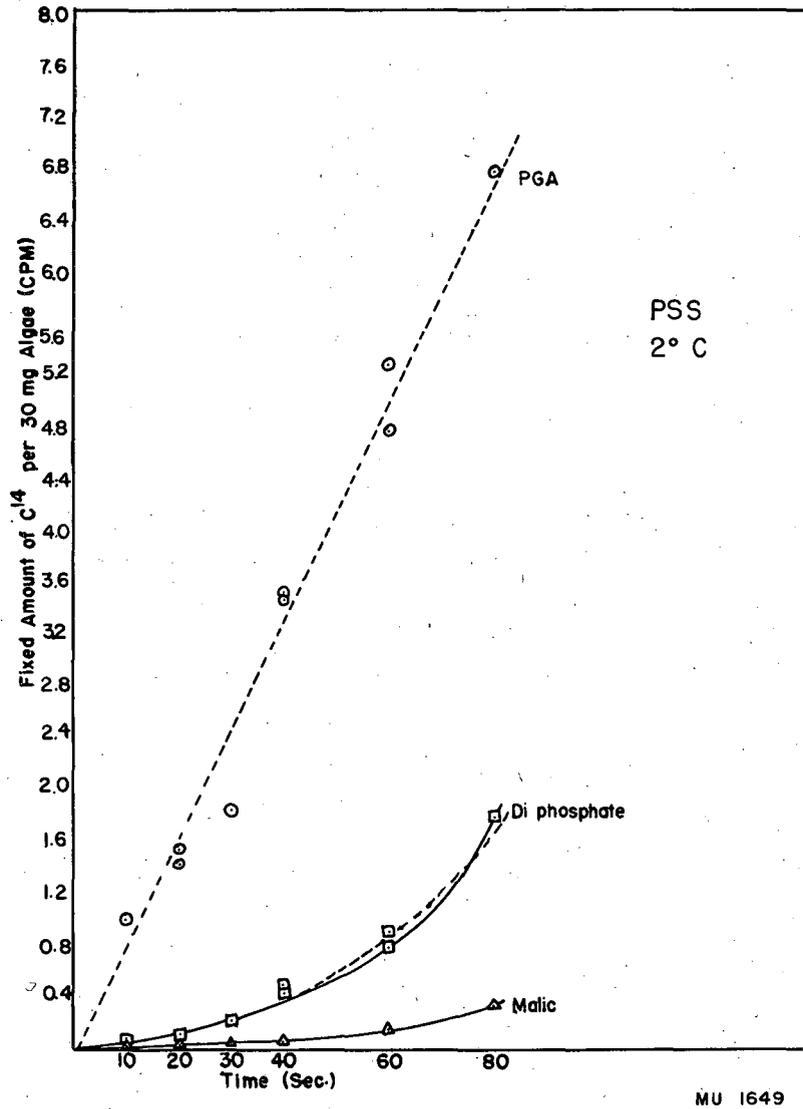


Fig. 4

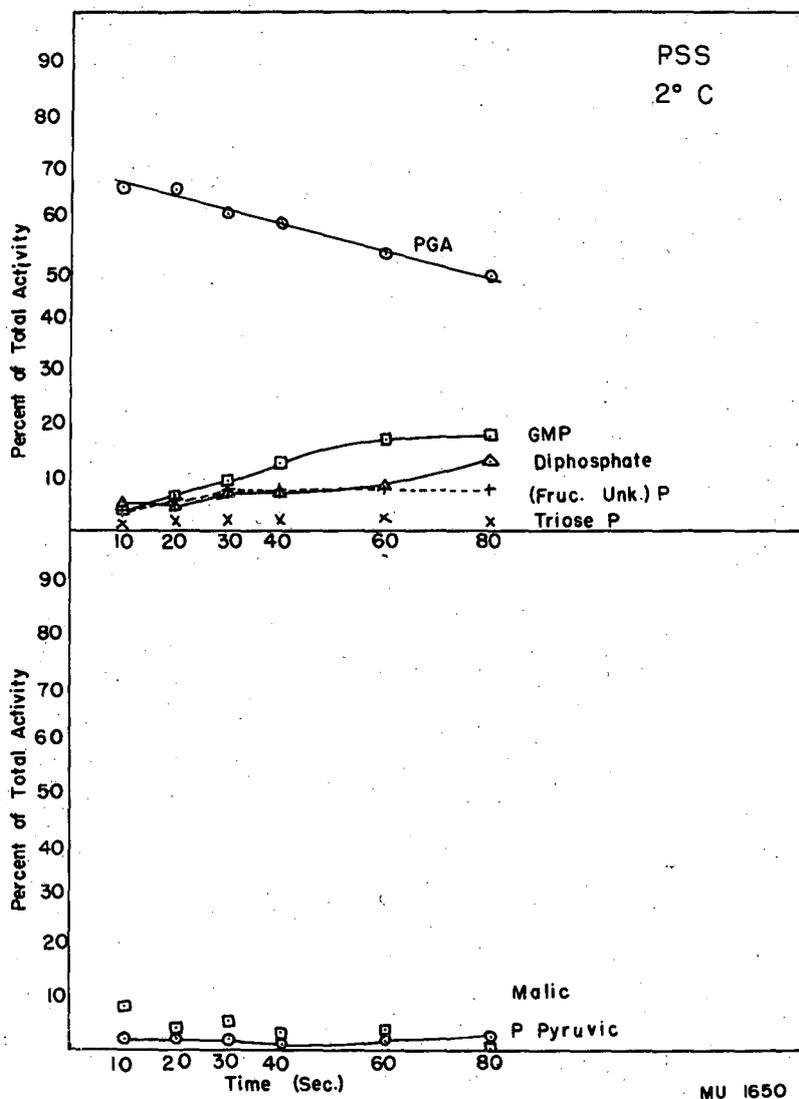


Fig. 5

II QUARTERLY PROGRESS REPORT. Project 48B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics

L. A. Bromley, G. Elliott, Russell Edwards, Norman Leroy, James Robbers
and Leo Brewer

Oxide Phase Diagrams. A study of the Na-O and K-O system is underway to determine the phases of these systems and their relationships.

Gaseous Hydroxides of Mo and W. Work is in progress on the determination of the composition of the gaseous hydroxides of Mo and W and their thermodynamic properties.

High Temperature X-ray Studies. The high temperature x-ray camera has been used to study the controversial questions of the existence of stable AlO or SiO . Results to date indicate that these compounds do not exist as stable solid phases below $1000^{\circ}C$.

Liquid Metal Systems. Evidence has been found for a solid solution range for the phase UBi_2 in the ternary diagram U-Na-Bi.

Heat Transfer in Forced Convection Film Boiling. The experimental work has been completed for determining the heattransfer coefficients for the film boiling of ethyl alcohol from a $3/8$ inch diameter graphite heating tube. The measurements covered a liquid velocity range from 2 ft/sec to 14.6 ft/sec and a temperature range for the heating surface from $700^{\circ}F$ to $1275^{\circ}F$. The results showed overall heat transfer coefficients from 64.7 to 148.1 $BTU/HR-FT^2-^{\circ}F$. The values of the coefficients due to convection alone were from 61.0 to 138.6 $BTU/HR-FT^2-^{\circ}F$, respectively. These last values are larger than those predicted by our theoretical development by an approximate constant factor of 1.55.

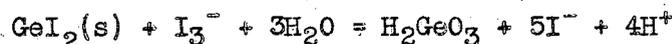
Velocity measurements were made, by means of a pilot tube, of the uniform liquid flow impinging on the heating tube. As a result of these measurements, we are modifying the equipment before continuing the determination of heat transfer coefficients from larger diameter heating tubes.

Work has been undertaken for the construction of a liquid proof wall to surround the present apparatus. This wall is in conjunction with the installation of an exhaust system which will decrease the operational hazards, due to the toxicity and the flammable nature of the liquids involved, by the removal of their vapors from the immediate area.

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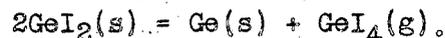
B. Basic Chemistry, including Metal Chelates

R. E. Connick, Z. Z. Hugus, William Jolly, Frank Owings, and Albin Zielen

Germanium Chemistry. The heat of the reaction

has been measured calorimetrically and the results are being published in UCRL-1152.

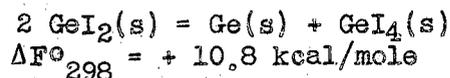
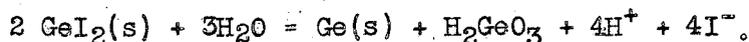
A high temperature flow method has successfully been used to study the reaction:



The data yield:

$$\begin{aligned}\Delta H^\circ &= 37,800 - 11T \text{ cal/mole} \\ \Delta F^\circ &= 37,800 + 11T \ln T - 123T \text{ cal/mole} \\ \Delta S^\circ &= 112 - 11 \ln T \text{ e.u.}\end{aligned}$$

Combining these with the data presented in the December Quarterly Report, we find for

The heat of solution of $\text{GeI}_4(\text{s})$ in water has been found to be 9.64 kcal. Estimating an entropy of 33 for the same reaction, we obtain $\Delta F^\circ_{298} = - 8.6$ kcal forInasmuch as this reaction has never been observed, it is suspected that $\text{GeI}_2(\text{s})$ is actually not a stable phase in the presence of water, and that it undergoes hydrolysis to germanous hydroxide (which is stable with respect to disproportionation). GeI_2 is prepared by precipitating it from concentrated aqueous hydriodic acid, but in such a solution the activity of the HI is more than high enough to reverse the sign of the free energy of disproportionation.Thermodynamics of Rhenium. The cells $\text{Hg}/\text{HgO}/\text{KOH}(\text{m}), \text{KReO}_4(\text{m})/\text{ReO}_2\text{Pt}$ and $\text{Hg}/\text{HgO}/\text{KOH}(\text{m}), \text{KReO}_4(\text{m})/\text{ReO}_2/\text{Au}$ have been under investigation; it was hoped that consistent results would be obtained after removal of oxygen from the solutions by passing hydrogen through them. A reproducible potential was obtained for a cell solution .01 molal in KOH , and KReO_4 , however, for more dilute solutions the potential is erratic and the measured potential does not agree with that calculated from the Nernst equation for the assumed cell reaction. Substitution of gold for the platinum electrode has not resolved this difficulty, and other remedies are currently under investigation.

Due to an insufficient supply of rhenium compounds we have been forced to reclaim approximately 20 gm. of rhenium from 20 liters of waste solutions containing large quantities of various anions as well as ferric iron. Electrolysis did not prove to be practicable on such a scale and reduction with 10 mesh zinc was attempted with satisfactory results.

Electron Exchange Rate Between Fe^{2+} and Fe^{3+} . Very poor reproducibility of blanks for the spectrophotometric iron analysis was found when using a sintered glass filter funnel to separate the iron solution from the resin. When extensive treatment of the filter with various cleaning agents and solvents failed to remedy this situation, a new filtering method was employed. The solution is now pipetted directly from the mixture using a glass wool filter tip; the reproducibility of the blanks is much improved. However, even using the new filtering method, the fraction of complete exchange was found to be $\alpha = 1.18 \pm 7$ percent for a reaction time of one minute. Since the probable error in the calculated value of α is very sensitive to errors in the iron analyses, it was felt that a more reliably accurate method should be found than the present determination of (Fe^{3+}) spectrophotometrically at 2400Å, where error due to absorption by dust or organic impurities is extremely difficult to control.

Determination of the red ferrous bipyridyl complex at 5220Å is at present being investigated. The analysis for total iron by reduction of ferric with hydroxylamine is quite satisfactory, however the measurement of the ferrous complex in the presence of ferric is complicated by a slow reduction of ferric by some unknown reducing agent. This reduction is photosensitive, and at the present stage of the investigation it appears that by keeping the solutions protected from light, an accurate analysis may be made.