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September, October and November 1951

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Carbide and Carbon Chemicals Company (Y-12 Area)	24
Chicago Patent Group	25
Dow Chemical Company (Rocky Flats)	26
duPont Company	27 - 29
General Electric Company, Richland	30 - 33
Hanford Operations Office	34
Idaho Operations Office	35 - 36
Iowa State College	37
Knolls Atomic Power Laboratory	38 - 41
Los Alamos	42 - 44
Mound Laboratory	45 - 47
Naval Research Laboratory	48
New York Operations Office	49 - 50
North American Aviation, Inc.	51 - 52
Patent Branch, Washington	53
Savannah River Operations Office	54
U. S. Naval Radiological Defense Laboratory	55
UCLA Medical Research Laboratory (Warren)	56
University of California Radiation Laboratory	57 - 60
University of Rochester	61 - 62
Vitro Corporation of America	63 - 64
Westinghouse Electric Corporation	65 - 66
Wright Air Development Center	67 - 68
Technical Information Service, Oak Ridge	69 - 83

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TABLE OF CONTENTS

*Previous Chemistry Quarterly Report, UCRL-1507

II QUARTERLY PROGRESS REPORT Project 48B

I QUARTERLY PROGRESS REPORT. Project 48

G. T. Seaborg and I. Perlman

A. Chemistry of the Rare Earth and Actinide Elements.

Chemistry of the Lanthanide Elements

B. B. Cunningham, D. C. Feay, and M. A. Rollier

Because of the fundamental relation between the electronic configurations in the lanthanide and actinide series of elements, this laboratory has a continuing interest in the chemistry of the latter series, especially concerning the thermodynamic stability of their nontripositive compounds and ions.

Chemistry of Praseodymium. Previous work[1] showed that the potential of the couple:

$$Pr^{+3}_{(aq)} + H^{+}_{(aq)} = Pr^{+4}_{(aq)} + 1/2\ H_{2(g)}$$

is 3.0 ± 0.2 v at room temperature. The free energy of the reaction

$$PrF_{4(s)} = PrF_{3(s)} + 1/2\ F_{2(g)}$$

is estimated to be -4 ± 4.5 kcal at room temperature, from the potential given above. A negative value of ΔF is consistent with the observation by Klemm and Heukel[2] that PrF_4 is not formed at room temperature from $PrCl_{3(s)} + F_{2(g)}$, although CeF_4 is readily obtained by the analogous reaction. We find also that PrF_4 is not formed from PrF_3 and F_2 at 320°C (described below).

Chemistry of Terbium. Although praseodymium dioxide apparently is more stable with respect to partial decomposition to a lower oxide than is TbO_2 (since the oxides obtained under similar conditions of ignition have the approximate compositions $PrO_{1.83}$ and $TbO_{1.75}$) it was anticipated that TbF_4 might be more stable than PrF_4, because of more prominent anion repulsion in the dioxides.

The preparation of TbF_4 by treatment of terbium compounds with elemental fluorine was therefore attempted as described below.

The apparatus used is shown in Fig. 1. It was constructed entirely of nickel except for brass fittings, teflon washers in the reaction

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[1] L. Eyring, B. B. Cunningham, and H. Lohr, University of California Radiation Laboratory Report UCRL-327 Revised, April, 1949.

[2] Wilhelm Klemm and Paul Heukel, Z. anorg. u. allgem. Chem. 220, 180-182 (1934).

chamber and various traps. "Kereotest" valves were used to control the argon and nitrogen flow. The calcium bromide chamber was used to remove excess fluorine.

About five mg of pure Tb_4O_7[*] was dissolved in 20 microliters of HCl and 20 microliters of H_2O. The solution was transferred to a lusteroid cone and diluted to approximately 200 microliters with H_2O. Excess concentrated HF solution was added. The resulting precipitate was washed several times with H_2O and dried for several hours at 80°C. The compound was identified as TbF_3 by x-ray diffraction analysis.

Approximately 500 micrograms of the TbF_3, prepared as described, was placed in a BaF_2 semimicro crucible, which was then placed in a hole in the nickel boat. The boat was placed in the reaction chamber, and the system was completely assembled. The system was flushed with dry argon for 8 to 10 minutes. Then the argon flow was stopped, and a slow fluorine[**] flow was started. The temperature of the reaction chamber was initially about 23°C but was raised to about 320°C in 55 minutes. The reaction chamber was maintained at this temperature for 40 minutes and then cooled down to 110°C slowly. At this cooler temperature, the fluorine flow was stopped, the furnace was turned off, and the argon flow was started. After about ten minutes the system was opened, and the white reaction product was removed from the crucible and sealed in vacuum in a Pyrex capillary.

The terbium reaction product was identified by x-ray diffraction analysis as being isostructural with ZrF_4. Approximate dimensions of the unit cell are:

$$\left.\begin{array}{l} a = 12.1 \text{ Å} \\ b = 10.3 \text{ Å} \\ c = 7.9 \text{ Å} \\ \beta = 126^{\circ} \end{array}\right\} \quad \pm 2 \text{ percent}$$

When the experiment described above was repeated using Tb_4O_7 in place of TbF_3, the resulting product gave an uninterpretable x-ray diffraction picture. Similar treatment of PrF_3 produced only the trifluoride.

Terbium tetrafluoride prepared in the manner described above was a white powder which showed no obvious reaction with air in several hours at room temperature.

The solubility of TbF_4 in several solvents was tested with the results shown in Table I. Comparison tests were made with CeF_4, prepared in a similar manner.

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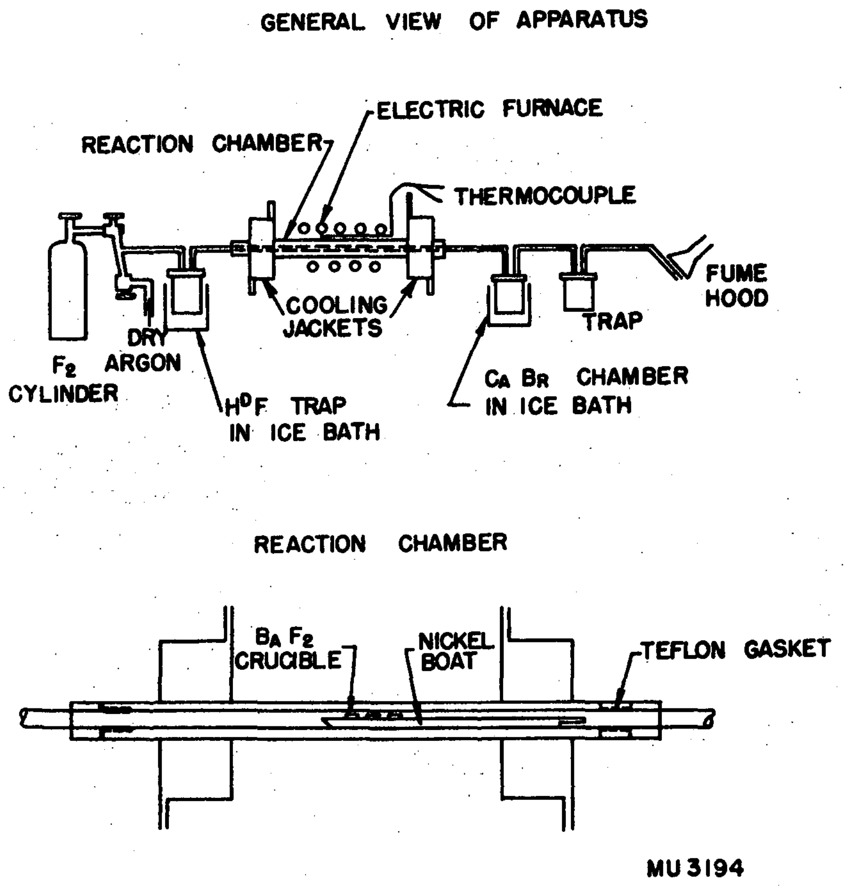
[*] The Tb_4O_7 used was purchased from Johnson, Matthey, and Co., Limited.

[**] The fluorine used was purchased in a half pound cylinder from the Pennsylvania salt manufacturing company.



GENERAL VIEW OF APPARATUS

REACTION CHAMBER

MU 3194

Fig. 1

Table I

Solubility of TbF_4 and CaF_4 in Various Solvents

Solvent	TbF_4	CeF_4
H_2O	No visible rx. with warm or cold water.	
1 M HCl(aq)	Dissolved slowly. No evidence of bubbling. Appearance of solid changed from white to semi transparent. Material then dissolved.	No definite reaction
1 M HNO_3(aq)	Dissolved very slowly. No evidence of bubbling. Same color change as in HCl.	
KI(aq)	A few of the solid particles became yellow as if coated with iodine.	The solution and particles became distinctly yellow.
KI in 1 M HCl(aq)	Dissolved slowly forming a yellow solution (I_3^-).	
$Al(NO_3)_3$(aq)	Dissolved slowly. Some bubbling observed. Same color change as observed with HCl.	No visible reaction.
$Al(NO_3)_3$ in 1 M HNO_3 (aq)	Dissolved moderately rapidly. Some bubbling observed. Same color change as with HCl.	Dissolved moderately rapidly. Some bubbling observed.

ACKNOWLEDGMENT

The x-ray diffraction pictures were taken and analyzed by the x-ray group under the direction of D. H. Templeton.

Microcalorimetry

S. R. Gunn and B. B. Cunningham

Significant improvements have been made in the semi-adiabatic microcalorimeter. The thermometer and heater coils have been wound on a copper cylinder approximately 0.5 inches in diameter and 0.6 inch in length mounted on the cap inside the chamber. The thermometer winding is about 25 ohms, and the heater about 40 ohms, both being of No. 42 enameled copper wire. The coils are separated and insulated by two or three coats of baked Glyptal varnish, and the whole assembly is coated with a thin layer of Ceresin wax. While this insulation has proved satisfactory, it is planned to cover the windings with a copper jacket and gold plate the entire assembly for permanence and greater resistance to corrosion. The previous sustem used as single winding on the outside of the chamber for both thermometric and heating purposes. The present system eliminates the necessity of disconnecting the easily damaged leads when disassembling the calorimeter, permits disconnection of the external heater-control circuit when electrical energy inputs are not being made, and provides a much more efficient thermal contact with the contents of the calorimeter, thus reducing the lag to a nearly negligible value.

The resistance thermometer bridge circuit has been mounted in a 1/8 inch iron chassis to provide magnetic and electrostatic shielding. Special care was taken to insure thorough insulation of the circuits, and all important measuring resistors are immersed in a constant temperature oil bath. The net effect of these improvements has been to practically eliminate transient currents and to increase the stability of the thermometer to the point where drift rates are constant within the sensitivity of the galvanometer over indefinite periods of time.

The operation of the instrument was checked by measuring the heat of dilution of constant boiling sulfuric acid, as described previously.[1] An apparatus has been constructed for filling the small sample bulbs which consists essentially of a clamp for holding the latter and a remotely controlled pipet mounted on a micro-manipulator. The entire assembly is mounted inside a nitrogen drybox. Five test runs were made, four using sulfuric acid and one blank. The mean deviation of the temperature rise of five to eight inputs made during each run, each of about 1 calorie and 16 minute duration, was 0.08 percent. Four sulfuric acid dilution runs with samples of from 1.8 to 7.0 mg gave values of -0.1617, -0.1606, -0.1616, and -0.1613 cal/mg. The reproducibility of these runs greatly improved over that previously attained. The deviation of the results from the expected value of -0.1655 cal/mg is undoubtedly to be attributed to the fact that the constant boiling acid had absorbed some water in the two months since it had been prepared.

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[1] Chemistry Division Quarterly Report, June, July, August, 1951, UCRL-1507.

Crystal Structure of the Rare Earth Trichlorides

D. H. Templeton, G. F. Carter, H. W. Ruben, and C. H. Dauben

In connection with the work of C. Koch and associates, diffraction patterns of all the rare earth trichlorides have been made. Although previous workers[1] failed to report specific structures, their diffraction patterns were in complete agreement with those found in this work. It has been reported[2] that the trichlorides of lanthanum, cerium, praseodymium and neodymium have the UCl_3 structure and it has been shown that samarium, europium, and gadolinium trichlorides have the same structure. The lattice dimensions are:

	a	c
$SmCl_3$*	7.377 ± 0.006 Å	4.159 ± 0.004 Å
$EuCl_3$	7.354 ± 0.016 Å	4.141 ± 0.008 Å
$GdCl_3$	7.335 ± 0.018 Å	4.108 ± 0.009 Å

It was found that $TbCl_3$ had the beta form, in agreement with Bommer and Hohmann, and that $DyCl_3$, $HoDl_3$, $ErCl_3$, $TmCl_3$, $YbCl_3$, and $LuCl_3$ have the gamma form-YCl_3 structure. The beta form of $DyCl_3$ has not yet been observed although Bommer and Hohmann have reported it to be dimorphic. It is hoped the dimorphic character of $TbCl_3$ may be shown by diffraction photographs taken at high temperatures. The samples of the gamma form reported above are not all pure, but the main lines show these compounds to be isostructural.

Single crystals of YCl_3 have been prepared and rotation and Weissenberg photographs indicate a monoclinic structure. Dimensions of the A centered cell are:

$a = 6.47$ Å

$b = 11.9$

$c = 6.98$

$\beta = 112^\circ$

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[1] H. Bommer and E. Hohmann, Z. anorg. allgem. chem. 248, 373 (1941)

[2] W. H. Zachariasen, Acta Cryst. 1, 265 (1948)

* Produced by H. Lohr

The Crystal Structures of Sodium Superoxide

G. F. Carter and D. H. Templeton

The crystal structures of NaO_2 at low temperatures have been determined. Previously, the structure of the phase which is stable at room temperature had been shown[1] to be face-centered cubic, NaCl structure with the O_2 groups in disordered orientations.

Two low temperature forms have been found:

(1) NaO_2 II, stable between -50° and -75°, having the primitive cubic, pyrite structure,

$$a = 5.47 \pm 0.01 \text{ Å}$$

(2) NaO_2 III, an orthorhombic structure, stable below -75° , with

$$a = 4.26 \pm 0.01 \text{ Å}$$

$$b = 5.54 \pm 0.01$$

$$c = 3.44 \pm 0.01$$

Its structure was determined from rotation and Weissenberg photographs of highly twinned crystals obtained by cooling cubic single crystals. The structure is D_{2h}^{12} - Pnnm with

Na in 2 (a) 0,0,0; 1/2, 1/2, 1/2;

O in 4 (g) $\pm$ (u,v,0; 1/2 - u, 1/2 + v, 1/2;) with u = 0.12
v = 0.42

This is the $FeAs_2$ type structure; marcasite (FeS_2) possesses the same structure.

---

[1] D. H. Templeton and C. H. Dauben, J. Am. Chem. Soc. 73, 2251 (1950).

B. Nuclear Properties and Transformations

Spontaneous Fission of Pu^{236}

Kenneth Street, Almon E. Larsh, Gary H. Higgins, and Glenn T. Seaborg

U^{235} of very high purity was bombarded with deuterons to produce Np^{235-6} which were allowed to decay for several half-lives. Very pure Pu^{236} was then chemically separated from all other activities and the spontaneous fission rate was determined in an ionization chamber type fission counter. The total alpha activity was measured and the fission half-life calculated from the ratio of alpha counts to fission counts times the alpha half-life. The value obtained was $1.8 \pm 0.8 \times 10^9$ years.

Spontaneous Fission of Cm^{240}

Gary H. Higgins, Kenneth Street, Almon E. Larsh, and Glenn T. Seaborg

Cm^{240}, along with Cm^{241} and Cm^{242}, was produced by the Pu^{239} $(\alpha,xn)Cm^{\leq 243}$ reaction and was chemically purified. A sample was prepared and fission pulses counted in an ionization type fission chamber. The fission pulse rate was recorded and the pulses were found to decay with the half-life of Cm^{240} (27 d). The half-life for fission, calculated from the alpha half-life was found to be $2.5 \pm 0.5 \times 10^6$ years. This value is corrected both for the contribution to the alpha count and the fission rate for the small amount of Cm^{242} and Am^{241} present in the sample.

Investigation of the Alpha Particles of Bi^{203} and Cm^{242}

Dean C. Dunlavey and Glenn T. Seaborg

Bi^{203}. An alpha emitting isotope with alpha particle energy of 4.8 ± 0.1 Mev has been found in the bismuth fraction produced by the bombardment of natural lead with 60 Mev protons. The carrier free bismuth was diluted, in neutral solution, to a concentration which was previously determined (100 microliter aliquot/2cm^2 emulsion surface) to be the limit tolerated by the C-2 alpha emulsion without excessive fogging due to the electron capture activity. After allowing 14 hours after bombardment for the decay of Bi^{201} and shorter lived activities, the first of six aliquots was impregnated in the emulsion. Thirteen hours of decay were allowed between each impregnation. Drying of the emulsions was completed within 30 minutes of taking the aliquot and development was immediately after each 13 hour period. Thorough microscope investigation of each emulsion indicated that the alpha activity diminished with a half-life of $\sim$12 hours. Consideration of this half-life with the energy predictions of alpha systematics indicate the isotope to be Bi^{203}.

Cm^{242}. Nuclear emulsions were also used to determine the number of conversion electrons that accompanied the alpha particle emission of Cm^{242}. It was found that of 4100 alpha particles observed, 942 or 23 percent were accompanied by a conversion electron. Hence, at leeast 23 percent of the alpha particle decay of Cm^{242} leads to an excited level of Pu^{238}. This compares favorably with the presence of a low energy alpha particle group, determined by alpha ray spectroscopy to be of abundance of $\sim$27 percent.[1] The conversion coefficient of the

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[1] I. Perlman and F. Asaro, to be published.

gamma ray is indicated to be ~100 percent.

A Study of the Isotopes of Promethium

Vera K. Fischer

Isotopically enriched samples of neodymium have been bombarded with protons from the 60-inch cyclotron and from the linear accelerator. Promethium was bombarded with helium ions from the 60-inch cyclotron. The half-lives and radiation characteristics of the promethium isotopes thus produced have been measured. The isotopes were identified chemically and the mass allocations were made on the basis of yield considerations. Nuclear shell theory applied to explain ambiguities and to estimate decay characteristics of unobserved promethium isotopes.

The method of production and the properties of these isotopes is summarized in the following table where the notation is identical to that used in the Table of Isotopes[1] with the exception that an asterisk (*) indicates results previously reported in the literature.

---

[1] G. S. Seaborg and I. Perlman, Rev. Mod. Phys., 20, 585 (1948).

Table I

Summary of Results

Radioactive Characteristics of Promethium Isotopes

A	Class	Type of Radiation	Half-life	Energy of Radiation		Produced by
				Particles	Electromagnetic	
142		β^+(calc)	5 to 240 min (calc)			
143	a	K,L x-ray γ,e^-	long 285$\pm$1 d*	0.6 Mev (abs) 0.74 Mev (calc)	0.9 Mev (abs) 0.79 (calc) L and K x-rays	Pr(α,2n) Nd^{143}(p,n) (?)
144		K,L x-ray(calc) β^- (calc)	long (calc)			
145	a*	K,L x-ray[31] e^-(?)	~30 y*	low energy*	K and L x-rays*	Sm(n,γ)Sm^{145*} oec
146	a	β^- γ(calc) K,L x-rays (calc)	~285 d	0.7 Mev (abs)		Nd^{146}(p,n)
147	^{a}MS*	β^-	long 3.7 y*	0.223 Mev (spec)* 0.2 Mev		Nd^{148}(p,2n)
	b(calc)	β^-,γ K x-rays (?)	42 $\pm$ 1 d	2.7 Mev(abs) (7.4%) 0.7 Mev(abs) (92.6%)	~1 Mev (abs) ~0.8 Mev (abs)* K x-rays (?)	Nd^{148}(p,2n)
148	^{a}MS*	β^-,γ K x-rays(?)	5.3 $\pm$ 0.1 d	2.3 Mev (abs) 2.5 Mev (abs)*	~1 Mev (abs) ~0.8 Mev (abs)* K x-rays(?)	Nd^{148}(p,n)
149	^{a}MS*	β^-,γ K x-rays (?)	54.4 $\pm$ 1.1 h	1.0 Mev (abs + spec)*	0.3 to 0.35Mev(spec) K x-rays (?)	Nd^{150}(p,2n)
150	b	β^-,γ K x-rays (?)	2.7 h	2.01$\pm$0.03 Mev(84%)(spec) 3.00$\pm$0.01 Mev(16%)(spec)	~1.4 Mev (abs) ~0.3 Mev (abs) K x-rays (?)	Nd^{150}(p,n)
151	a*	β^-,γ*	27.5 h*	1.1 Mev (spec)*	0.615 kev(abs)(weak)*	Nd^{150}(nγ)* Nd^{151} β^-

The Emission of Light Nuclei During High Energy Proton Bombardment

Harold Jaffe

An attempt is being made to identify the various light nuclei given off in high energy proton bombardments (340 Mev). The target, in this case aluminum, is mounted on a cart that moves into the cyclotron tank. Photographic emulsions, suitably shielded, are placed on the cart at a known distance from the target. During bombardment a small fraction of the positively charged particles leaving the target will spiral downward under the influence of the cylotron magnetic field and enter the emulsion. From the angle of entry and the known distance between the target and the track one can calculate $H\rho$ for the particle. By assuming a definite nucleus and normalizing the axes of the range energy curve for protons one can obtain the energy of the assumed nucleus. Knowing the strength of the magnetic field one can then calculate $H\rho$ for the assumed particle. If the $H\rho$ obtained from geometrical considerations has the same value as that calculated for the assumed nucleus, we have identified the particle. We can, therefore, determine the identity of the nucleus leaving the target by trial and error calculations.

Preliminary experiments to identify Li^8 nuclei given off in the high energy bombardment of aluminum with protons are underway. This isotope of lithium lends itself well to such a preliminary experiment in that the characteristic hammer tracks are so readily identified.

Tantalum Spallation and Fission

W. E. Nervik

Up to the present time, eight bombardments of tantalum with 340 Mev protons have been run. In all bombardments a thin traget consisting of four 2 mil tantalum foils and a stack of three 1/2 mil aluminum monitor foils in front and in back was used. Cross sections (in mb) for the formation of isotopes isolated in these bombardments are given in Table I.

(Figures in parentheses are doubtful, either because of a low absolute counting rate, or a low chemical yield). Decay curves for TaP9, and for several elements of TaP7 and TaP8, have not been followed long enough to allow the various isotopes to be identified.

Table I

Bombardment No.	TaP2	TaP3	TaP4	TaP5	TaP7	TaP8	TaP9
Isotope							
Br^{80}	(0.049)	0.11	0.065	0.11			
Br^{82}	(0.042)	0.078	0.055	0.11			
Br^{83}	(0.015)	0.041	0.041	0.068			
Fe^{59}	0.063		0.051				
Ce^{134}		0.018	0.020				
Ce^{135}		0.16	0.28				
Ce^{141}		$\leq$0.0036	0.0025				
W^{178}		(65.2)	39.4				
W^{177}		(35.8)	23.7				
W^{176}		(79.8)	50.8				
Pd^{109}				0.00475	(0.0039)	0.0061	
Ni^{65}					0.038	0.043	
Ni^{66}					0.017		
Cu^{64}					0.057	0.069	
Cu^{67}					0.048	0.055	
Ag^{111}					(0.0145)		

Two Ta(340 Mev protons) bombardments in which carbon was separated gave cross sections for formation of C^{11} of 0.025 and 0.018 mg, where the cross sections were taken relative to Pd^{109}. Suspecting that these values were too high, a bombardment was made at 50 Mev to check for impurities in the tantalum. In this bombardment Ni^{65} was used as an internal monitor since it has a higher cross section at 340 Mev and a shorter half-life than Pd^{109}. If the tantalum were pure, and C^{11} and Ni^{65} were obtained by the same type of reactions on tantalum; i.e., a fission process which had the same isotope fragment distribution at 50 Mev as at 340 Mev, the ratio of cross sections of C^{11} and Ni^{65} at 50 Mev and at 340 Mev should be identical. In the 50 Mev bombardment, essentially zero counts of Ni^{65} were seen, while the observed counts of C^{11} were approximately the same as were obtained at 340 Mev. Assuming that the impurity in the tantalum is carbon, and using a cross section of 83 mb for the reaction C^{12}(50 Mev p,d)C^{11}, a carbon impurity of approximately 0.05 percent would account for the C^{11} counts observed. Since this is not at all unreasonable, an attempt has been made to determine the cross section of C^{11} from Au^{197} at 340 Mev. Results are not available on these bombardments as yet, but it should be possible to get pure gold which will give a reliable cross section for the formation of C^{11} from target elements in this region of the periodic table.

X-ray Spectrum of Pu^{239}

C. I. Browne and I. Perlman

The radioactive nuclide Pu^{239} has been reported[1] to emit x-rays and soft gamma rays in the course of its decay by alpha emission to U^{235}. A sample

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[1] D. West and J. K. Dawson, Proc. Phys. Soc., 64A, 586 (1951).

(2 gt) of Pu^{239} was studied with the bent crystal x-ray spectrometer in order to determine the details of the L x-ray spectrum as well as to search for gamma radiation which might be masked to ordinary methods of search by the x-rays themselves.

The low specific activity of Pu^{239} - particularly with respect to electromagnetic radiation - coupled with the inherent low geometry of the spectrometer prohibited the observation of many lines. X-rays observed are given in Table I.

Table I

Line Assignment	Energy Observed (kev)	Accepted Values (kev)
U L_{β_1}	17.24 ± 0.04	17.22
U L_{β_3}	17.44 ± 0.08	17.45
U L_{α_1}	13.69 ± 0.06	13.61

The intensities of the x-rays were too low to permit calculation of quantitative relative intensities with any reasonable accuracy. Qualitatively, the L_{β_1} was in highest abundance, with the L_{α_1} and L_{β_3} in perhaps comparable intensity.

With the exception of the L_{α_1}, the energies observed agree very well indeed with the accepted values for uranium x-rays. In the case of the L_{α_1}, geometrical considerations and the use of a topaz diffraction crystal prohibit observation on both sides of the midpoint with energies less than 16 kev. This fact, together with the low intensity of the line, would be expected to give rise to larger errors in energy determination.

The alpha particle spectrum observed by Asaro and Perlman[1] suggests the presence of a 14 kev gamma ray in Pu^{239} decay in an abundance of 0.20 per alpha particle. No such radiation was observed in this experiment, and an upper intensity limit of 0.01 per alpha particle may be set if the values of West and Dawson[2] are accepted for x-ray intensities. This very probably means that the gamma ray is very highly converted, which might well be anticipated.

---

[1] F. Asaro and I. Perlman, to be published.

[2] D. West and J. K. Dawson, Proc. Phys. Soc., <u>64A</u>, 586 (1951).

The Spallation of Zinc

William J. Worthington, Jr., and Glenn T. Seaborg

The investigation of the products of the spallation of zinc with 240 Mev protons is in progress. Table I indicates the isotopes thus far studied and the cross sections (in mb) for their formation.

Table I

Ga^{66}	0.65	Fe^{59}	0.65
Ga^{67}	1.4		
Zn^{62}	6.9	Mn^{51}	2
Zn^{65}	87	Mn^{52}	9
Cu^{61}	32	Mn^{54}	18
Cu^{64}	23	Mn^{56}	1.8
Cu^{67}	4.5	Cr^{48}	0.19
Ni^{56}	0.8	Cr^{49}	1.9
Ni^{57}	1.6	Cr^{51}	14.5
Ni^{65}	0.39	V^{47}	0.79
Ni^{66}	0.18	V^{48}	4.6
Co^{55}	1.4	Ti^{45}	0.91
Co^{56}	1.6	Ga^{45}	0.21
Co^{58}	44	Ca^{47}	0.0053
Co^{61}	1.5	K^{42}	0.23
Fe^{52}	0.20	K^{43}	0.19
		S^{35}	0.06
		P^{32}	0.01

It will be noted that Ni^{56} has not been previously reported. This isotope proved to decay by ~24 percent positron emission and ~76 percent electron capture with a half-life of 6.0 ± 0.5 days. The mass was assigned on the basis of the Co^{56} which was milked from a sample which had been previously free of cobalt. The Co^{56} was identified by its characteristic 72 day half-life and aluminum absorption curve.

Mass Spectrograph

F. L. Reynolds

One method of making mass assignments of radioactive isotopes with the mass spectrograph is to collect the active and inactive mass markers on a photographic plate, place another plate emulsion to emulsion with the original plate and look for line image development in the second plate due to the radioactivity of the active masses.

In many cyclotron induced activities there is insufficient total activity for this type of transfer technique since most emulsions require about 5×10^5 beta events per square millimeter of emulsion surface to just produce a visable image.

The use of alpha track sensitive plates has been employed with good success as transfer plates for locating alpha active mass spectra deposits, but no effort has been made to date to study the possibility of using electron track sensitive plates for locating beta active line deposits.

The processing treatment of Ilford G-5 plates for the detection of small amounts of beta activity by track counting methods proved involved compared to similar techniques with alpha track plates. The electron sensitive plate has to first be cleaned of all existing tracks, which consists in treating the plate at 35° C and at nearly 100 percent humidity for a period of 3 to 4 days. Water must not collect on the plates as drops because the emulsion will be washed free from the glass at 35° and excess water increases the surface fog upon development. The best eradication technique seems to be that of having just sufficient water present to swell the gelatin and maintain this amount of water in a sealed light tight container at the proper temperature.

To return the plate to electron track sensitivity requires proper drying methods. If the plate is not properly dried it will not record beta tracks. Also, if the plate is not properly dried the random surface grain development is heavy which decreases the sensitivity to track detection.

Using artificially made spectra lines of Sr^{90}, the processed G-5 plates were exposed to various amounts of beta events. About 6000 beta tracks in an area of 2 sq mm (a line 0.2 mm by 1 mm) will just produce a visible image to the eye, and by track counting methods at 1500 magnification as few as 500 tracks will be required to make a mass assignment provided other factors such as processing and background track build-up can be controlled.

The storage and background track build-up problem has not been completely studied as yet. Plates stored in 1 inch walled iron containers surrounded by 2 to 3 inches of lead qualitatively show a minimum of background tracks after 48 hours. Data on longer periods of storage have yet to be ascertained.

At this writing the process seems workable as a means of detecting small amounts of activity from mass spectrograph collections and warrants further study and experiment.

Determination of Trace Impurities in Gases

Amos S. Newton and Laurin F. Tolman

The accurate determination of trace impurities in gases is a frequently encountered problem in any gas analysis laboratory. With the mass spectrometer the accurate determination of such impurities can be carried to parts per million provided the impurities can be concentrated or separated from the bulk gas as is the case with condensible impurities in noncondensible gases or noncondensible impurities in condensible gases. In the first case of condensible impurities in air (noncondensible), Happ, Stewart, and Brockmeyer[1] have bescribed a method of determining solvent vapors in air in the parts per million concentration range and compared their results with chemical methods. Shepherd, et al.,[2] described a method in which parts per billion condensible impurities in air were, in principle, determinable. Both of these methods utilized volume determinations to link the impurities to the air for the absolute determination of percentage composition.

In the range of a few parts per hundred thousand it has been found quite useful to provide the connecting link between the impurities and the main component by use of an added internal standard rather than attempting to use volume calibrations This is particularly effective in the determination of noncondensible impurities in condensible gases where the main component must be held back in a liquid nitrogen trap and the correction for temperature of the trap volume, always a dubious decision, must be made.

To check the method, known mixtures of various types have been made and analyzed on the mass spectrometer. These mixtures were made by seating a fritted glass disc on the side of a 500 ml flask. The disc was then covered with mercury and the flask calibrated for volume by expansion into a previously calibrated system. The bulb was evacuated and the impurities were introduced through the disc from calibrated gas pipettes (Consolidated Engineering Corporation, Pasadena, California), for this purpose. After the gases were introduced the bulk gas which had been previously purified to remove all condensible or noncondensible gases respectively was introduced through the stopcock and the total pressure measured. Thus the composition could be calculated and the impurities added are estimated to be accurate to 1 percent of the value calculated.

For the analysis a few millimeters of a suitable internal standard gas, neon in the case of noncondensibles and sulphur dioxide in the case of condensibles was introduced into the mixing bulb and then the gas mixture introduced, the mixture allowed to equilibrate and the analysis run on the spectrometer. Two spectrometer runs are required. The first giving the ratio of standard to the basic component, and the second giving the ratio of the impurities to the standard after a suitable separation process, in this case freezing with liquid nitrogen. This freezing is done on the inlet system of the mass spectrometer. In the case of noncondensible impurities the gas is simple slowly passed through a liquid air trap directly into the sample bottles until the fifty microns or so pressure was indicated on the Piriani gauge. In the case of condensible impurities in noncondensible gases the mixing bulb was provided with a freeze-out finger and after running the total gas, the condensible impurities were frozen out and the noncondensible gas pumped off. The bulb was then allowed to warm and equilibrate before sampling the condensible gases.

The gases used were of commercial or better purity.

CO_2-liquid carbon Inc. tank CO_2 was redistilled 3 times in vacuum.

Propane - Phillips research grade propane was redistilled three times

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[1] G. P. Happ, D. W. Stewart, and H.F. Brockmeyer, Anal. Chem., 22, 1224 (1950).

[2] Martin Shepherd, S. M. Rock, Royce Howard, and John Stornes; Anal. Chem. 23, 1431 (1951).

in vacuum.

SO_2 - Matheson tank SO_2 was redistilled three times in vacuum.

He - Tank helium was used.

H_2 - Tank hydrogen was used.

N_2 - Matheson tank nitrogen was used.

Air - Air was passed over potassium hydroxide pellets and through a liquid nitrogen trap.

Methane - Phillip's research grade methane was used.

Argon - Linde tank argon was used.

CO - Matheson tank carbon monoxide was used. This was independently analyzed for oxygen and nitrogen and these components added to the oxygen and nitrogen respectively. The calibrating gas used was pure synthetic carbon monoxide.

Mixture No. 1	Added (%)	Found (%)	Δ
CO_2	99.66	99.67(by diff)	+0.01
Air	0.127	0.129	+0.002
CO	0.208	0.197	-0.011

Mixture No. 2

Propane	99.74	99.74(by diff)	0.00
He	0.112	0.114	+0.002
H_2	0.112	0.117	+0.005
O_2	0.034	0.032	-0.002

Neon internal standard; 1.37 percent He in Ne considered part of neon spectrum.

Mixture No. 3

CO_2	99.45	99.46	+0.06
O_2	0.111	0.109	-0.002
N_2	0.222	0.222	-0.000
CO	0.215	0.205	-0.010

Neon internal standard.

Mixture No. 4

SO_2			
O_2	0.036	0.034	-0.002
N_2	0.037	0.045	+0.007
CO	0.116	0.102	-0.014
H_2	0.205	0.198	-0.007
CH_4	0.114	0.086	-0.028

Mixture No. 4 (continued)

	Added (%)	Found (%)	Δ
A	0.035	0.034	-0.001

Neon internal standard.

Mixture No. 5

	Added (%)	Found (%)	Δ
CO_2 free air	99.62		
Ethanol	0.087	0.072	-0.015
Acetone	0.032	0.028	-0.004
Ethyl ether	0.048	0.043	-0.005
n-Pentane	0.084	0.074	-0.010
Ethyl bromide	0.130	0.125	-0.005

SO_2 internal standard.

It is seen that the accuracy of the method is a few parts per hundred thousand in the original mixture in most cases. Carbon monoxide seems to be consistently low and methane is very low and this is probably inherent in considering methane a noncondensible gas. In Mixture No. 2, the propane used was found to contain a trace of methane and similar analyses on the original propane and the propane, He, O_2, H_2 mixture gave 0.0153 percent methane in the original propane and 0.0053 percent methane in the mixture. This wide divergence of results is understandable considering the high solubility of methane in the propane.

The mass spectrometer used was a Consolidated Engineering Corporation Model 21-102 spectrometer which has been converted to the 21-103 model except for the inlet system.

C. Bio-Organic Chemistry Group

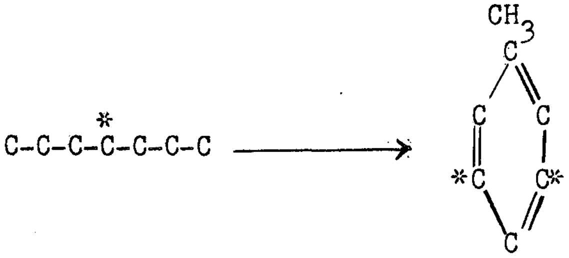
Edited by B. M. Tolbert

Synthetic and Experimental Chemistry

M. Calvin, B. Tolbert, P. Adams, R. Bartsch, P. Chiado, P. Christensen, J. Fairley, D. Kritchevsky, R. Noller, R. Ostwald, and D. Pack

1. Cyclodehydrogenation of Heptene.

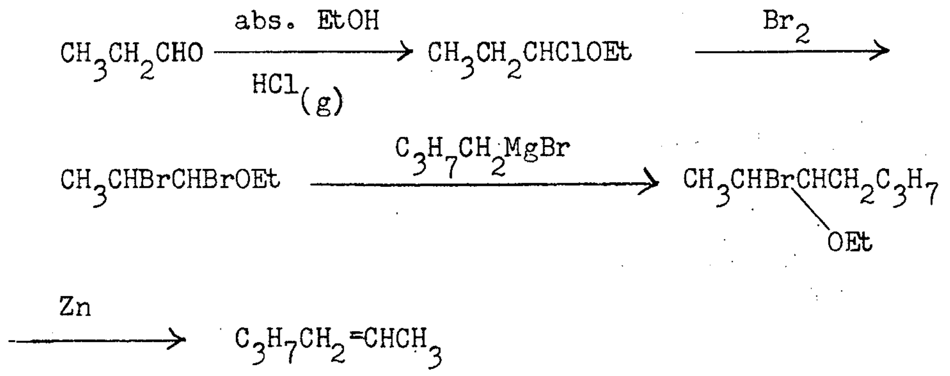
A number of procedures for the preparation in good yields of ring-labeled compounds from $C^{14}O_2$ have been studied in this laboratory. These include (1) the condensation of pyruvic acid, (2) Diels-Alder synthesis, (3) the Tiffeneau ring expansion reaction, and (4) cyclodehydrogenation of heptene over chromous oxide. This last method is currently being investigated to determine the best procedure for the synthesis of the intermediate heptene and the optimum conditions for the cyclization. In the cyclization of heptene (or heptane which is dehydrogenated under the conditions of the experiment to heptene), the position of the double bond in the molecule is not critical, since by proton shift an equilibrium mixture is rapidly reached under the conditions of the cyclization. Only the heptene-1 fraction of this equilibrium mixture will cyclize in an appreciable quantity. The position of label, however, is important. Thus, heptene-4-C^{14} will give toluene labeled in the meta position. Heptene-3-C^{14} will give ortho-para labeled toluene. One labeled heptene will give a mixture of ring and side chain labeled toluene and so is of lesser interest in biological studies.



This labeled toluene will be used in mechanism studies and in the preparation of ring labeled amino acids.

A number of methods for preparation of the labeled heptene have been considered, but the only procedures that appeared of interest on the basis of the library work were (1) the Boord synthesis, (2) the reaction of acid chlorides with organicadmium reagents, and (3) acylation of malonic esters (benzyl esters).

Boord Synthesis



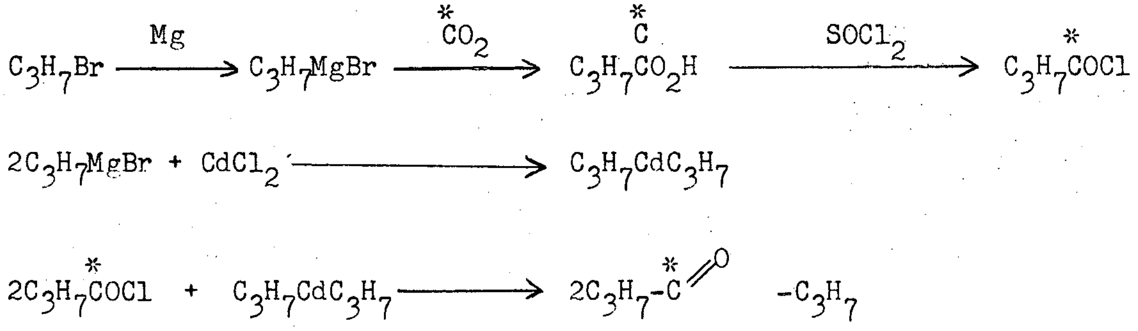
$$CH_3CH_2CHO \xrightarrow[HCl_{(g)}]{abs.\ EtOH} CH_3CH_2CHClOEt \xrightarrow{Br_2}$$

$$CH_3CHBrCHBrOEt \xrightarrow{C_3H_7CH_2MgBr} CH_3CHBr\underset{OEt}{C}HCH_2C_3H_7$$

$$\xrightarrow{Zn} C_3H_7CH_2{=}CHCH_3$$

Yields given in the literature are very good and this method leads directly to the alkene in a short series of steps which in themselves involve rather simple manipulations. The intermediates for this synthesis are currently being prepared and are described elsewhere in this progress report. The preparation of the labeled butyl bromide has been worked out in yields of 75 percent to 78 percent yield based on $C^{14}O_2$ used to begin the synthesis.

Acid Chloride-Organocadmium Reaction



$$C_3H_7Br \xrightarrow{Mg} C_3H_7MgBr \xrightarrow{\overset{*}{C}O_2} C_3H_7\overset{*}{C}O_2H \xrightarrow{SOCl_2} C_3H_7\overset{*}{C}OCl$$

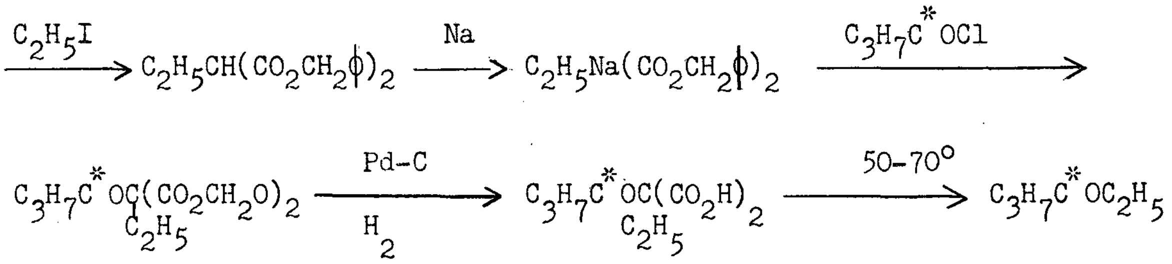
$$2C_3H_7MgBr + CdCl_2 \longrightarrow C_3H_7CdC_3H_7$$

$$2C_3H_7\overset{*}{C}OCl + C_3H_7CdC_3H_7 \longrightarrow 2C_3H_7-\overset{*}{C}{=}O\quad -C_3H_7$$

The butyryl chloride was prepared in 87 percent field from $SOCl_2$ and butyric acid. This is a very clean-cut preparation and is considered to be better than using PCl_3, $POCl_3$, or PCl_5. In a first trial heptanone was prepared by the above method in 30 percent yield. This was considerably lower than originally expected, but was probably due to the fact that stirring was inefficient. During much of the reaction there was a bulky precipitate, and it was impossible to break it up properly during the addition of the acid chloride to the cadmium reagent. Therefore, when this method is tried again, different equipment will be used as well as a greater amount of solvent.

Dibenzyl Malonate Synthesis. The other method which is now being investigated is the acylation of dibenzyl malonate. Only the preliminary steps have been done to date.

$$CH_2(CO_2H)_2 + 2\phi CH_2OH \xrightarrow{H_2SO_4} CH_2(CO_2CH_2\phi)_2 \xrightarrow{Na} NaCH(CO_2CH_2\phi)_2$$



$$\xrightarrow{C_2H_5I} C_2H_5CH(CO_2CH_2\phi)_2 \xrightarrow{Na} C_2H_5Na(CO_2CH_2\phi)_2 \xrightarrow{C_3H_7C^*OCl}$$

$$C_3H_7C^*O\underset{C_2H_5}{C}(CO_2CH_2O)_2 \xrightarrow[H_2]{Pd-C} C_3H_7C^*O\underset{C_2H_5}{C}(CO_2H)_2 \xrightarrow{50-70^\circ} C_3H_7C^*OC_2H_5$$

The dibenzylmalonate obtained was a sirupy, yellow glass. It was impossible to distill at a bath temperature of 220°C and pressure of 10mm and was very difficult to transfer. It was washed with water to remove sulfuric acid and 10 percent sodium carbonate to remove any unreacted acid or monoester. In both cases emulsions which were very hard to break were obtained. The α-ethyldibenzylmalonate was likewise a sticky, hard-to-handle mass. To reduce the difficulties encountered in handling benzyl esters, it was decided to form the sodio-benzyl ester, $C_2H_5CNa(CO_2CH_2O)_2$, from the corresponding ethyl ester by ester interchange[1]. This will involve the following steps:

$$CH_2(CO_2H)_2 \xrightarrow[H_2SO_4]{EtOH} CH_2(CO_2Et)_2 \xrightarrow[EtOH]{Na} NaCH(CO_2Et)_2 \xrightarrow{EtBr}$$

$$EtCH(CO_2Et)_2 \xrightarrow{Na} NaCEt(CO_2Et)_2 \xrightarrow[OH]{OCH_2OH} Na\ \ EtC(CO_2CH_2O)_2$$

It is hoped that the condensation steps with the ethyl ester will prove to be successful. The heptanone will probably be reduced with $LiAlH_4$ followed by the dehydration of the alcohol to give the heptene-4-C^{14}.

2. Valine-4,4'-C_2^{14} Hydrochloride.

A high specific activity preparation of valine-4-4'$C^{14}{}_2$ hydrochloride has been completed. Methyl labeled isopropyl iodide was condensed with ethyl acetamidomalonate in t-butanol and hydrolyzed to give valine. The product was purified by fractional elution from a Dowex-50 ion exchange column.

$$(C^*H_3)_2CH_2I \xrightarrow{\text{Ethylacetamidomatonate}} (C^*H_3)_2CHC(NHCOCH_3)\ \ (CO_2Et)_2$$

$$\xrightarrow[H_2O]{H^+} (C^*H_3)_2\ CHCHNH_2CO_2H$$

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[1] R. E. Bowman, J. Chem. Soc., 325 (1950).

Freshly cut potassium (0.52 g., 13.2 millimoles) was added to 15 ml t-butanol in a three-neck flask fitted with a stirrer, a dropping funnel and a reflux condenser protected by a drying tube. Nitrogen was swept slowly through the system as the mixture was stirred and warmed on a steam bath until evolution of gas had ceased. The condenser was replaced by a wide-mouth funnel and 5.65 g. ethyl acetamidomalonate (26 millimoles) was quickly added and rinsed into the reaction mixture with 5 ml t-butanol. The condenser was replaced and the mixture allowed to reflux under a slow nitrogen stream for 45 minutes.

A solution of 2.24 g. isopropyl-methyl-C_2^{14} iodide (13.2 millimoles, 11.1 mc.) in 5 ml t-butanol was transferred to the dropping funnel and added slowly, with stirring, to the reaction mixture. A small amount of the solvent was used to rinse the funnel. Potassium iodide began to precipitate from the reaction almost immediately.

The reaction mixture was allowed to reflux 24 hours, at which time it was cooled and filtered. The insoluble residue contained 12μc or 0.1 percent of the initial activity. The filtrate was evaporated to dryness, dissolved in 50 ml concentrated hydrochloric acid and heated under reflux for 48 hours. The acid solution was then distilled to dryness and dissolved in 250 ml water. This crude hydrolysis product (8.2 mc., 73 percent of the initial activity) was found to contain valine, non-radioactive glycine, a small amount of radiactive alanine, a trace of a radioactive amino acid believed to be aminobutyric acid, and less than 3 percent of a radioactive compound which did not give a positive ninhydrin test for amino acids.

Preliminary experiments indicated that while fractional elution of this amino acid mixture from Dowex-2 anion exchange resin was not suitable for separation of these compounds, acid elution from Dowex-50 cation exchange resin gave a satisfactory purification. A glass column 2.5 cm diameter and 160 cm high was filled with 700 ml Dowex-50 cation exchange resin (100-200 mesh; 3 milliequivalents/ml). After treatment with alternate portions of 2 N hydrochloric acid and 2 N sodium hydroxide, the resin was washed with 3 liters 6 N hydrochloric acid and then with 10 liters of water. The last liter of water was found to contain no inorganic salts.

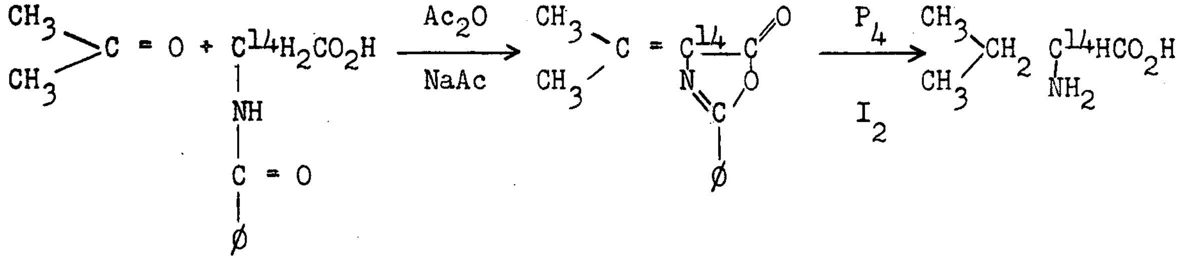
The column was charged with the crude amino acid mixture and washed with 1500 ml water. This water wash contained 2.1 μc. The amino acids were eluted from the column with 1 N hydrochloric acid at a rate of 20 ml/hour (0.068 cm/min.). The first 2275 ml of eluate (1.08X column capacity) contained 2.1 μc. The fraction from 2275 ml to 2785 ml (1.08-1.33 times column capacity) contained all of the glycine and all of the alanine (28.4 μc; 0.3 percent). The fraction from 2785 to 2870 ml contained no amino acids and no radioactivity. Between 2870 and 3190 ml (1.33-1.52 times column capacity) a mixture of valine and "amino-butyric acid" was eluted and found to contain 1.725 mc (15 percent). Pure valine was eluted between 3190 and 4745 ml (1.52-2.26 times column capacity).

The hydrochloric acid solution of pure valine was distilled to dryness, dissolved in water, evaporated to dryness on a steam bath and dried in vacuo. The dry valine-4,4'-C^{14}_2 hydrochloride weighed 1.07 g. and had a specific activity of 6.0 μc/mg (theory: 5.5 μc/mg.). The discrepancy in specific activity is within the limits of accuracy of the assay of the isopropyl iodide. The yield of pure valine was 6.41 mc. (58 percent). Two-dimensional paper chromatographic analysis showed the valine to be completely free from radioactive and amino acid contamination.

An additional 1.73 mc. of valine (15 percent) contaminated by a trace (less than 1 percent) of amino butyric acid was isolated from the earlier elution fraction.

3. Valine-2-C^{14}.

A new preparation of valine, used to prepare valine-2-C^{14} and adaptable also to preparations of valine-1-C^{14}, has been developed. The azlactone of acetone is prepared using labeled hippuric acid. The oxazalone is then reduced and hydrolyzed to valine in good yields.



2-phenyl-4-isopropylidine oxazalone-4-C^{14} was prepared by a modification of the method of Ramage and Simonsen[2]. Glycine-2-C^{14} (120 μc, 0.76 g., 10 millimoles) was converted to hippuric acid by a method described in a previous report. The product was dissolved in 35 ml of dry, redistilled acetone in a flask fitted with a reflux condenser. After addition of 0.6 g. freshly fused powered sodium acetate and 5 ml acetic anhydride, the mixture was allowed to reflux 15 hours.

The hot solution was then poured into a beaker containing 100 g. cracked ice and the volume adjusted to 250 ml with water. The precipitate was collected by vacuum filtration and dried in vacuo. The yield of oxazalone was 1.16 g. (71 μc) or 59 percent from glycine. It was demonstrated to be free from radioactive contaminants by two-dimensional paper chromatography.

The filtrate contained 36 μc (30 percent) of which 28 μc (23 percent) was glycine and could be recovered as the pure compounds.

The oxazalone was combined in a small flask with 2.0 g. red phosphorus and 12.5 ml acetic anhydride. Very slowly 12.5 ml HI (specific gravity 1.7) was dropped into the solution and the mixture heated on the steam bath under reflux for 20 hours.

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[2] G. R. Ramage and J. L. Simonsen, J. Chem. Soc., 534, (1935).

The reaction mixture was filtered by gravity through Whatman #40 filter paper and the filtrate vacuum-distilled to dryness. The distillation residue was dissolved in 100 ml 70 percent ethanol and poured slowly onto a 60 ml Dowex-50 ion exchange column (50-100 mesh, acid form). The resin was washed free of anions by washing first with 100 ml 70 percent ethanol (since benzoic acid crystallizes out onto the resin in water solution), followed by 250 ml water.

The amino acid was eluted from the resin with 250 ml 2N ammonium hydroxide followed by 250 ml water. The eluate was evaporated to dryness on a steam bath, using an air stream, and dried *in vacuo*. The resulting valine-2-C^{14}, shown to be free from radioactive or amino acid contaminants by two-dimensional paper chromatographic analysis, contained 50 μc of carbon-14. This is an 83 percent yield from the oxazalone or 65 percent from unrecovered glycine.

4. Purification of Glycine-2-C^{14}.

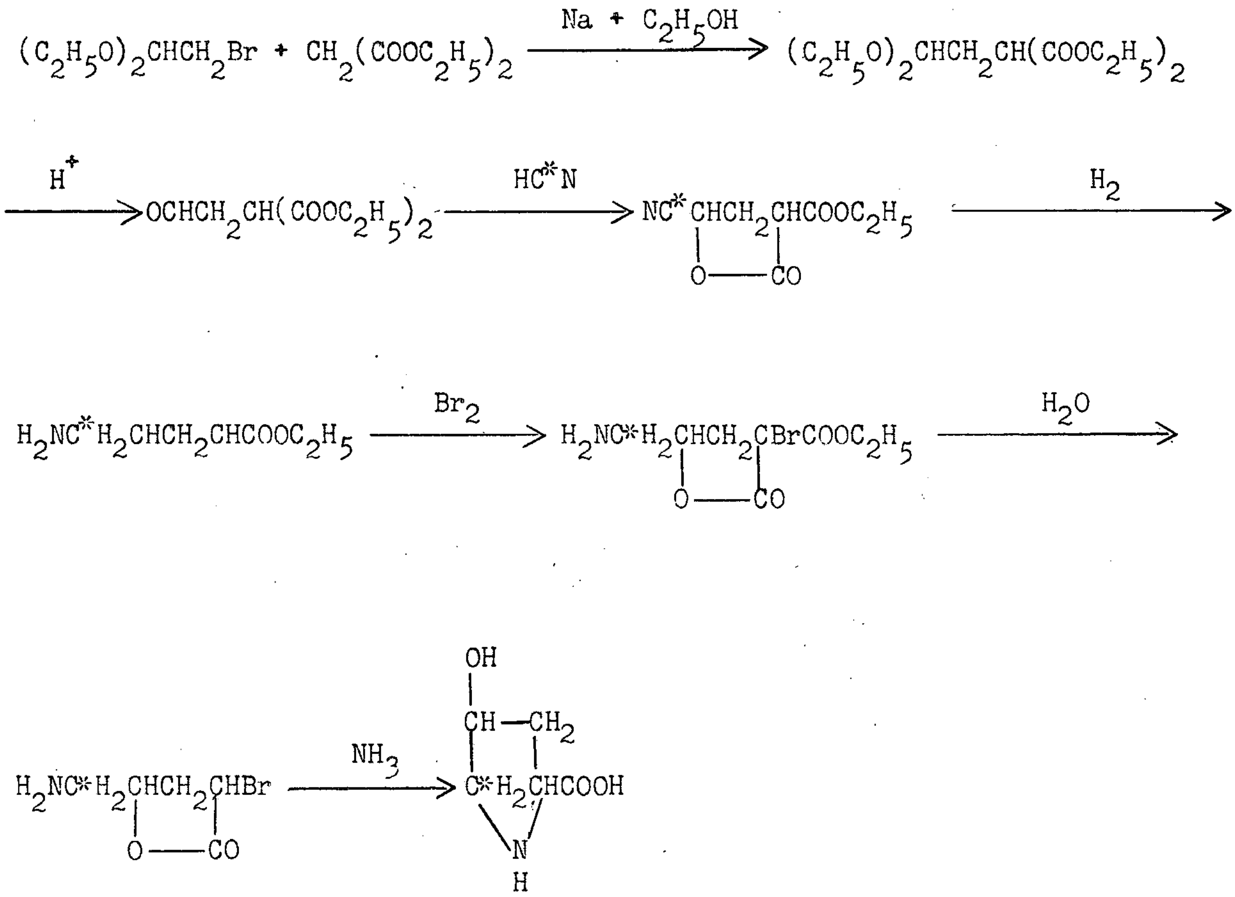
It was found that a sample of crude glycine-2-C^{14} prepared by chlorination and amination of acetic-2-C^{14} acid contained no less than six major impurities, totaling about 15 percent of the total activity of the sample. Fractional elution of this mixture from a 700 ml Dowex-50 cation exchange column (100-200 mesh; acid form) with 1 N hydrochloric acid gave 3 percent of the activity in the water wash, and 12 percent in the first 2500 ml of eluate (1.19 times column capacity).

The eluate from 2500 ml to 4000 ml (1.19-1.9 times column capacity) was collected and distilled to dryness. The distillation residue, although paper chromatographic analysis showed it to be radioactively pure glycine hydrochloride, was darkly colored and glassy in form. The glycine was dissolved in water, the solution adjusted to pH 5.0 evaporated to dryness and the residue subjected to sublimation at a pressure of 10 microns and a temperature of 150-200° C.

Combined sublimation products, representing 70 percent of the activity in the glycine elution fraction, were found to contain diketopiperazine representing 30-40 percent of the glycine activity. Hydrolysis with concentrated hydrochloric acid, however, gave a pure crystalline glycine hydrochloride quantitatively from the sublimation product. This pure glycine-2-C^{14} hydrochloride represented 60 percent of the activity in the original crude glycine sample.

5. 4-Hydroxyproline-5-C^{14}.

The synthesis of hydroxyproline labeled with C^{14} in the 5 position is being undertaken by the following series of reactions.



Hydroxyproline

The formation of the nitrile by this method has not been done before, but the remaining reactions in this sequence are described by Traube, Johow, and Tephol[3]. By slight modification of this series of reactions, this preparation could be extended to the synthesis of proline and ornithine. Thus far, the preparation of the labeled cyanide on a 10 mml scale has been worked out in yields of 70-75 percent, based on barium carbonate used to begin the synthesis

6. Synthesis of Alcohols and Halides. Butyl and isobutyl alcohol.

In the last Quarterly Report, the reduction of acetic acid and carbon dioxide with lithium aluminum hydride in tetrahydrofuroxy tetrahydropyran and the decomposition of the resulting complex with tetrahydrofurfuryl alcohol was described. This procedure has been modified and extended to the reduction of butyric and isobutyric acid. Using the same flow system as was used with acetic acid, the reduction and bromination of n-butyric acid gave only 60-80 percent yields in somewhat erratic manner. Therefore, the system was changed to carry out the reaction in two steps, i.e., the butanol was isolated by nitrogen sweeping and then the alcohol was brominated by refluxing with 48 percent hydrobromic

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[3] W. Traube, R. Johow, and W. Tephol, Ber., 56, 1861 (1923).

acid. This gave yields of 80-90 percent, based on the starting n-butyric acid. After these preliminary experiments, the procedure was tested, starting with sodium butyrate which was converted into the free acid with phosphoric acid saturated with phosphorus pentoxide and then reduced by the above procedure. Yields of 70-78 percent were obtained, and although this is slightly lower than those obtained starting with the acid, tests showed that the generation of the acid itself from the sodium salt was essentially quantitative. By this procedure a total of 50 millicuries of sodium butyrate-1-C^{14} were reduced to the alcohol and brominated in two 11 mmole scale reactions to give n-butyl-1-C^{14} bromide in 74 and 78 percent yields. This procedure is now being investigated with sodium isobutyrate-1-C^{14}.

Methanol. Carbon dioxide has been reduced and iodinated to methyl iodide in the flow system previously described for acetic acid. Barium carbonate was converted to carbon dioxide with sulfuric acid and the carbon dioxide was absorbed in the hydride solution on the vacuum manifold. After decomposition of excess hydride, the methanol was swept into refluxing 58 percent hydriodic acid and the methyl iodide collected in a liquid nitrogen trap. After washing and drying, the product was weighed and analyzed. The yield was 70 percent based on barium carbonate and had a refractive index identical with that of pure methyl iodide ($N_D^{17.7}$ = 1.5308). Ten millicuries of $C^{14}O_2$ has been converted into methyl iodide in 70 percent yield by this method.

7. Azlactone Synthesis of Alpha-labeled Amino Acids.

Several additional runs have been made on the preparation of amino acids by the reduction of azlactone formed by the condensation of aromatic aldehydes and hippuric acid in the presence of acetic anhydride and sodium acetate. In the following table the yields on the hydrolysis reduction step for the preparation of a number of amino acids are listed.

Table I

Product	Yield
Phenylalanine	69-84 percent
2,3-dihydroxyphenylalanine	89 percent
o-hydroxyphenylalanine	62-81 percent
3,4-dihydroxyphenylalanine	0
Tyrosine	32-44 percent first crop 46-55 percent 2nd crops
Dimethylaminophenylalanine	23 perdent first crop 81 percent 1st and 2nd crops

It has not been possible to obtain tyrosine in good yields by this procedure, and no product has been isolated in the attempted preparation of 3,4-dihydroxyphenylalanine.

8. Acetamidomalonate Synthesis of Amino Acids. Leucine-3-C^{14}.

Two low-activity preparations of leucine-3-C^{14} have been carried out. The aim of these preparations was to investigate the influence on the yield and purity of the product of the two steps in the synthesis: the condensation and hydrolysis of the resulting intermediate. The method of synthesis as previously described consisted of the condensation of acetamidomalonate with isobutyl iodide-1-C^{14} in tertiary butanol with potassium tertiary butoxide and consequent hydrolysis of the condensation product to the amino acids. Two condensation procedures were tested, as follows: in the first, a mixture of 10 millimole potassium and 20 millimoles of acetamidomalonate dissolved in butanol were frozen in a long bomb tube, connected to the vacuum line, and 10 millimoles of the halide distilled into the container. The condensation was then carried out in the closed tube on a steam bath *in vacuo*.

In the second procedure the halide was added dropwise over a period of 45 minutes to the reaction mixture in a three-neck flask which was fitted with a dropping funnel, a reflux condenser, and a magnetic stirrer. The condensation was carried out at atmospheric pressure and at steam bath temperature. Our results show that the first procedure gives a considerably higher yield of the amino acids. Thus, the first procedure gave yields of 70 percent of the condensation product, whereas the second gave yields of approximately 20 percent. In another run 80 percent of the starting activity was found in the amino acid when done by the first procedure and 35 percent of the starting activity was found in the amino acid when done by the second procedure.

In the investigation of the hydrolysis of the condensation product, the value of 48 percent hydrobromic acid versus concentrated hydrochloric acid was investigated. The yield of the product was slightly higher in the concentrated hydrochloric acid hydrolysis and the product was much clearer looking. The high specific activity run will be carried out as soon as the halide has been prepared.

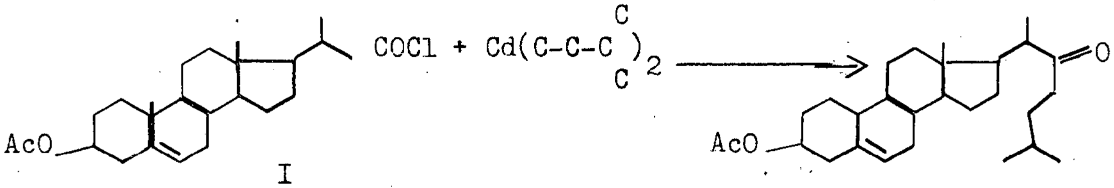
Norleucine-3-C^{14}. The work on the preparation of norleucine-3-C^{14} has continued. The method of synthesis is analogous to that described for leucine. Warm runs have yielded about 60 percent of the starting activity in the amino acid. At present, two runs of 15 and 20 millicuries, respectively, are in progress.

Side Reactions. During the preparation of amino acids by the acetamidomalonate synthesis, a number of unexpected compounds have appeared in paper chromatograms of various purification fractions. These have been observed in the course of the preparation of alanine-3-C^{14}, valine-4-4'-C_2^{14}, norvaline-3-C^{14}, leucine-3-C^{14}, and norleucine-3-C^{14}. Both radioactive and non-radioactive ninhydrine positive impurities have been observed, as well as radioactive ninhydrine negative compounds. The identity of three of these materials has been definitely established by a combination of chemical tests and paper chromatography, and by fractional elution from Dowex-50 ion exchange columns. They are: alanine and α-aminobutyric acid observed in the preparation of valine and norvaline; and valine, alanine and α-aminobutyric acid observed in the preparation of leucine and norleucine.

The origin of these compounds is not clear. It is thought that they do not arise from impurities in the halides, but this point is being determined by synthetic samples of special purity and cross-checked by means of mass spectroscopic analysis. It is hoped to decide by these procedures whether they truly originate from the condensation or whether they are derived from the halides. A detailed report is in the process of preparation.

9. Studies Towards the Preparation of Cholesterol-23-C^{14}.

The proposed synthesis would involve the reaction between 3β-acetoxybisnorcholenic acid chloride (I) with labeled isoamyl cadmium to yield 22-ketocholesterol, which could then be reduced via the mercaptal to the desired steroid.



I

Using equimolar amounts of the halide and the acid chloride (in cold runs) only amorphous masses were obtained from which some acid could be reclaimed, but which had a wide melting point range and must contain very little ketone if any. One run using the proportions initially used by Cole and Julian[4], namely an eight mole excess of the halide gave a 40 percent yield of the desired ketone. Since that time it has been reported[5] that this method had been attempted before and after considerable investigation had been dropped. Reasons given were very great difficulty in separating the ketone from the reaction mixture, difficulties in purification and difficulties in forming mercaptal. Since those would also apply in this case, we have abandoned this approach.

10. Cholesteryl laurate.

This ester was prepared according to the method of Cataline, *et.al.*[6] Cholesterol (5.8 gm), lauric acid (3.0 gm) and 0.13 gm of p-toluenesulfonic acid in 75 ml of benzene were refluxed for 3 hours under constant water take-off. The product, crystallized from chloroform-methanol, melted at 74-5°. Mixed melting point with initial preparation, 74-5°. Mixed melting point with material prepared after the method of Page and Rudy (see last Quarterly Report), 75-6°.

Summary

Method	Melting Point	$(\alpha)_D$
Kritchevsky + Anderson	78-78.5°	-30.3° ($CHCl_3$)
Page and Rudy	75-76°	-30.2° ($CHCl_3$)
Cataline, et.al.	74-75°	-28.8° ($CHCl_3$)

A sample of the original material (K+A) maintained at 75° for 100 hours had a

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[4] Wayne Cole and Percy L. Julian, JACS 67, 1369 (1945).

[5] B. Riegel, private communication.

[6] E. L. Cataline, *et. al.*, J. Am. Pharm. Assoc., 33, 107 (1944).

melting point of 75-85°. Another sample maintained at 85° for 100 hours melted, after solidification, at 60-78°. It would appear that this preparation represents a hitherto unreported isomorphic modification of cholesteryl laurate.

11. Radiation Chemistry. Gamma Ray Source.

For studies in radiation experiments, both of a chemical and biological nature, a cobalt-60 gamma ray source is being constructed by the Health Chemistry Group. The unit is modeled after that in existence at the X-10 laboratory at Oak Ridge. Initially, it will contain a 100 curie cobalt source. This should permit experiments such as those described below to continue more rapidly.

Amino Acids. Preliminary experiments on the effect of ionizing radiation upon simple peptides in aqueous solution have been described in previous Quarterly Reports. To provide a foundation for the continuation of this work, it was necessary to study the chemical reactions brought about by the irradiation of solutions of the individual amino acids.

The irradiation of glycine-1-C^{14} solutions has been examined in the presence of dissolved oxygen, nitrogen, and hydrogen. Each atmosphere was obtained by bubbling the gas through the glycine solution for five minutes, followed by rapid sealing of the containing tube. The three samples were then subjected to a radiation dosage of 1.4×10^6 rep. from a Co^{60} source. The standard chromatographic and radioautographic techniques of the laboratory was applied to these solutions and to a fourth non-irradiated control and gave evidence that at least three compounds other than glycine were present in the irradiated samples. No significant differences were found among the samples irradiated in the presence of the various gases. This may possibly be explained by incomplete removal of oxygen from the hydrogen and nitrogen solutions, although even then a quantitative difference in the results might have been expected. For all samples the bulk of the glycine was recovered unchanged, indicating the need for higher radiation dosages. In fact, only 3 percent of the total activity recovered was found to be associated with the compounds formed during the irradiation procedure. Experiments designed to identify these new compounds are in progress.

Purines. Exploratory experiments were also initiated to determine the effect of ionizing radiation upon solutions of the purines, adenine-4,6-C^{14} and guanine-4-C^{14}. Each of these compounds was dissolved in water with the aid of a drop of hydrochloric acid and subjected to 2.5×10^6 rep. from the Co^{60} source. After chromatography of the resulting solutions and of non-irradiated controls in isopropanol-hydrochloric acid-water, small but significant amounts of fast-moving compounds were found to be present in only irradiated samples. Further aliquots of the solutions were chromatographed by the ascending method, using 5 percent Na_2HPO_4 layered with isoamyl alcohol as the solvent. Samples of xanthine and hyperxanthine were run in lanes adjacent to those of guanine and adenine respectively. For neither irradiated aminopurine sample, adenine nor guanine, could radio-activity be found in the corresponding oxypurine areas, eliminating hydrolytic deamination as a major reaction. However, a radioactive area of high R_f (0.8 for adenine, 0.7 for guanine) was again found present only in the irradiated samples. The preparation of conventional phenol-water and butanol-propionic acid-water chromatograms and radioautographs indicated that the radioactive areas found above consisted of three new compounds. In these solvents the R_f's of the unknown compounds were lower than those of the parent purines, suggesting that the new substances are hydrophilic in nature.

Synthesis by Radiation. In the last Quarterly Report, an experiment on the irradiation of a solution of $C^{14}O_2$ and inactive formic acid was described. Paper chromatographs and radioautographs of this material showed two rather faint spots after an exposure of one month. These materials have not been identified. Carrier oxalic acid was added to a portion of the sample and the material then recrystallized four times from dioxane. The last two fractions of recrystallized acid were then converted to barium carbonate and counted in the ionization chambers, but these were found to contain very little, if any, activity.

Four samples containing the following mixtures of materials have been irradiated with about 10^6 roentgens. 1) Active Na formate, NaCN, H_2O; 2) active Na formate, NaCN, NH_4OH, H_2O; 3) active CO_2, NaCN, H_2O; 4) active CO_2, NaCN, NH_4OH, H_2O. Half of each of these irradiated samples and the blanks were hydrolized and made salt free. The other half was simply made salt free. Paper chromatographs and radioautographs were made from each of these.

Determinations of the radioactivity in these several samples showed that there was considerable activity fixed in the non-irradiated tubes, amounting to about one-third of that fixed in the radiated samples. Part, but not all, of this may be due to self-irradiation from the carbon -14. The radioautographs were complicated and difficult to interpret from these experiments. While no more work has been done on this particular series of samples, they have established bases for additional experiments, and with this information in mind, we have prepared three tubes, each containing 10 mg of sodium formate (200 microcuries carbon-14), 10 mg sodium cyanide, and 0.25 milliliters, and 7.5 N NH_4OH. One of these tubes will serve as a blank, the second will be heated in a steam bath, and the third will be irradiated in a cobalt-60 source. We have also prepared three tubes, each containing 1 milliliter CO_2 gas (700 microcuries C^{14}) and 0.5 milliliters of formic acid. These are being treated in a manner similar to the other three.

Biological Chemistry

M. Calvin, M. Anderson, J. Fairley, J. Gilbert, M. Kirk, D. Kritchevsky

B. Krueckel and B. Tolbert

1. Cholesterol Metabolism in Rats.

Two rats, both Curtis-Dunning females, were each given 13.1 mg of cholesterol-C^{14} (7.64×10^{-2} μc/mg, 1μc total) in 0.5 cc. Wesson oil via stomach tube. The tube was rinsed with another 0.5 cc. of Wesson oil. The rats were then placed in individual metabolism cages and allowed to feed and water ad. lib. The urine and feces were collected daily, the feces were extracted daily, the urine pooled and the pool extracted with ether. After four days the rats were sacrificed, the adrenals and liver extirpated and the carcass dissolved in conc. KOH. In the case of the rat C, it was discovered that the animal was pregnant. The foeti were removed (there were 8) and dissolved in KOH. The data which has been accumulated to date is given in Table I.

Table I

Total Feces

	Rat C			Rat D		
Day	mg	Act. (µc)	S.A.(µc/mg)	mg	Act.(µc)	S.A.(µc/mg)
1	503.8	0.264	5.2×10^{-4}	509.8	0.204	4.0×10^{-4}
2	84.5	0.058	6.8 "	61.0	0.022	3.7 "
3	127.7	0.014	1.1 "	124.0	0.014	1.1 "
4	125.0	0.003	0.25 "	146.0	0.003	1.7 "

Saponified Feces

	Rat C	Rat D
Non-Sap.	0.328 µc, 5.4×10^{-4} µc/mg	0.227 µc, 3.9×10^{-4} µc/mg
Fatty Acids	0.011 µc, 5×10^{-5} µc/mg	0.016 µc, 4.7×10^{-5} µc/mg

Other Fractions

	mg	µc total	µc/mg	mg	µc total	µc/mg
Urine						
	129.0	0.005	4.3×10^{-5}	225.1	0.329	1.5×10^{-3}
Liver						
NS	103.5	0.005	4.5×10^{-5}	82.1	0.003	3.65×10^{-5}
FA	86.4	0.004	5.1×10^{-5}	118.0	0.0003	2.3×10^{-6}
PS	2.0	0.0002	1.0×10^{-4}	21.5	0.0005	2.5×10^{-5}
Adrenal						
NS	40.3	0.053	1.32×10^{-3}	29.4	0.047	1.6×10^{-3}
FA	27.2	0.065	2.38×10^{-3}	9.8	0.014	1.4×10^{-3}
Foeti						
NS	240.0	0.095	4.0×10^{-4}			
FA	400.0	0.028	7.0×10^{-5}			

Legend: NS = non-saponifiable material
FA = fatty acids
PL = phospholipids

Results on liver glycogen and carcass fractions are not yet available.

In another experiment, Rats E and F, female, Curtis-Dunning strain, were each fed 20 mg. of cholesterol-H^3(1 µc/mg, 20 µc fed) in 0.5 cc. Wesson oil via stomach tube. The tube was rinsed with an additional 0.5 cc. of Wesson oil. The animals were placed in metabolism cages, allowed to feed and water ad. lib. and the urine and feces were collected daily. The feces were combined, the urine from each day's collection was lyophylized and the water saved for analysis. The resulting solids were pooled for each rat, dissolved in water, acidified and extracted with ether. After four days the rats were sacrificed and worked up as indicated previously. No tritium counting data is yet available.

2. Cholesterol plus Inositol in Rabbits.

This experiment was designed as a "pilot" experiment to test the efficacy of inositol in treatment of atherosclerosis. This substance has been reported to reduce blood cholesterol levels, but not much data is available on the lipoproteins. Four rabbits were taken. KP-1 and KP-2 were fed i-Inositol plus cholesterol (1 percent of each in food) for 4 weeks. They were then sacrificed. KP-3 and KP-4 were kept on cholesterol for 4 weeks and i-inositol (only) for 6 weeks, then sacrificed.

Ultracentrifugal analysis of the macro serum molecule fraction are as follows: KP-1 and KP-2 showed much faster development of lipoprotein defect in 4 weeks. KP-1 was positive toward the atherosclerosis cholesterol factor within 2 days. Autopsy showed 3+ atheroma in both animals. KP-3 showed the usual slow disappearance of the defect after removal of the cholesterol. KP-4 showed disappearance within 7 days. In order to make this experiment significant, we would need a) more rabbits and b) pill inositol and cholesterol in the food.

3. Cholesterol-H^3 Metabolism in Humans.

Cholesterol-H^3 (1.2761 gm, 0.61 mc/gm) was dissolved in 300 ml of chocolate milk and fed, via stomach tube, to Mr. B., victim of a stroke. The urine was collected for 12 days, acidified to pH 1 and heated under reflux for 48 hours. The cooled hydrolysate was extracted continuously with ether for 49 hours, the ether was dried over anhydrous sodium sulfate and the solvent stripped from the extract. The extracted material was separated into ketonic and non-ketonic fractions using Girard's T reagent. The oily ketonic fraction was subjected to crude fractionation on an alumina column. There was not enough material in any fraction for characterization, but activities were determined as given in Table I.

Table I

Fraction	Solvent	Activity
1	pet.ether-ø 9:1	0.108 mc/gm
2	benzene	0.0097 mc/gm
3	ø-ether 8:2	0.0183 mc/gm
4	methanol	0.0117 mc/gm

Fraction 3 corresponds to etiocholanolone and fraction 2 to androsterone according to the chromatography scheme of Konrad Dobriner et.al.[1] This phase of the experiment is being repeated with a patient who should, theoretically, excrete more ketosteroids. It does, however, indicate conversion of labeled cholesterol to ketosteroid. This must be confirmed by actual characterization of the compounds.

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[1] Konrad Dobriner, Seymour Liberman, and C. P. Rhoades, J. Biol. Chem. 172, 241 (1948).

Table II

Serum Data

Day	Cholesterol S.A.(mc/gm)	Free cholest. mg percent	Total mg percent
1	0.0315	47	167
2	0.0331	43	151
3	0.0292	43	153
4	0.0296	38	147
5	0.0267	43	149
7	0.0258	42	147
14	0.0186	39	137
21	0.0149	30	135

4. Chicken Feeding Experiments.

Degradation of the glycerol has been completed. Date hitherto unreported is shown in Table III.

Table III

Degradation of Glycerol

	cts./min.mg CO_2			percent of activity		
Yolk No.	C1	C2	C3	C1	C2	C3
4	30	8	30	44	12	44
5	31	10	31	43	14	43
8	23	6	23	44	12	44
10	4.6	1.5	4.6	43	14	43

5. Nucleic Acid Studies.

Studies were begun on the incorporation of C^{14} into the nucleic acids of the green alga, Scenedesmus, during photosynthesis in the presence of $C^{14}O_2$. It was found that the tissue fractionation procedure of Schmidt and Thannhauser applied to the alga yielded ribonucleic acid fractions contaminated with considerable quantities of radioactive impurities, presumably of protein origin. The method of Ogur and Rosen, which employs cold N perchloric acid to extract the ribonucleic acid (RNA) from other acid-insoluble, lipid free cellular materials, gave purer RNA fractions; however, the yield of RNA from the alga appeared to be lower than those obtained by the alkaline extraction procedure of Schmidt and Thannhauser.

Aliquots of the various RNA fractions obtained by use of these extraction procedures were hydrolyzed by 12 N perchloric acid to give the free nitrogenous bases, while other aliquots were treated with N hydrochloric acid to free the purine-bound ribose. Two dimensional chromatographs of the latter solutions were prepared by the standard method; while for chromatography of the bases, isopropanol-hydrochloric acid-water and butanol-propionic acid-water solvents were employed. The bases were located by their fluorescence under U. V. radiation. The ribose was detected by spraying with Tollens reagent.

Final results for the ribose fractions are not yet available; those for the bases indicate that in 5 and 10 minute photosynthetic periods, very little C^{14} from the $C^{14}O_2$ is fixed in any of the four nitrogenous constituents of RNA.

Similar experiments with canna leaves which had been exposed to 30 mc of $C^{14}O_2$ in a long-term photosynthesis period, yielded very little RNA by use of the Schmidt-Thannhauser procedure. However, addition of carrier ribose to an aliquot of the canna ribose fraction resulted in a chromatogram for which the Tollens reagent spot was coincident with a radioactive area. Two other Tollens reagent spots were found associated with high radioactivity; presumably these are glucose and fructose. The base fraction of the canna RNA was too contaminated with active impurities to yield much information. A preparation of the RNA fraction by use of the method of Ogus and Rosen is planned, as is the determination of the distribution of C^{14} in the ribose.

6. Metabolic Rate Studies in Normal and Hypophysectomized Rats.

Experiments to determine the difference in metabolism rates between normal and hypophysectomized rats are being continued. As reported in a previous Quarterly Report, both normal and hypophysectomized rats injected with sodium acetate-2-C^{14} eliminate between 70 percent and 80 percent of the injected activity as $C^{14}O_2$ during the first seven hours after injection and the rate of elimination of activity is approximately the same for both types of animals. Animals chosen were of the same age, but the weights of the normal rats were about double that of the operated animals. In order to be sure that the difference in CO_2 recovered-as $BaCO_3$ - from the two types of animals was really a difference in metabolism rate and not simply a function of the weights of the animals used, two young normal rats, weighing about 125 gm each (corresponding to the weight of the hypophysectomized rats) were injected with sodium acetate-2-C^{14} and the expired carbon dioxide collected as barium carbonate, filtered, weighed and counted. The rate of elimination of $C^{14}O_2$ and total percent activity recovered averaged about the same as for previous animals, but comparative amounts of barium carbonate recovered in 3 hours were as follows:

Hypophysectomized rats	Av. 5.0 gms
Normal rats of same age as operated rats	Av. 7.5 gms
Normal rats of same weight as operated rats	Av. 6.5 gms

This would seem to indicate that, while weight makes some difference in overall metabolism, it is by no means the only determining factor.

Injection of hypophysectomized rats with 1 mg ACTH 3 hours before injection of acetate appeared to speed the initial rate of metabolism as shown in Table I.

Table I

	Total percent of injected dose recovered			Max. rate of Elimination
	in 30 min.	in 1 hr.	in 1-1/2 hrs.	
With ACTH	21	43	57	0.88% /min. at 20 min.
Without ACTH	14	32	45	0.71% /min. at 25 min.

The fact that the rate of $C^{14}O_2$ expiration was slower in the ACTH treated animals after the first 1-1/2 hours was probably due simply to the earlier, more rapid depletion of C^{14} sources.

Similar injections of ACTH caused no such variations in metabolism rates of normal rats. ACTH also had no discernible effect on rate of metabolism of glucose in either normal or hypophysectomized rats.

Table II shows average metabolism rates for normal and hypophysectomized rats, each injected with 2 mg glycine-2-C^{14}. Rats used were fasted 24 hours before injection of glycine and those which were given ACTH received 2 mg/100 gm of body weight in 2 equal doses - six hours and two hours before injection of glycine.

Table II

		Percent activity recovered in 3 hours	Max. rate of elimination
Normal	With ACTH	10	0.74%/min. at 50 min.
	Without ACTH	10.6	0.080%/min. at 50 min.
Hypophysectomized	With ACTH	20.2	0.165%/min. at 55 min.
	Without ACTH	19.7	0.170%/min. at 45 min.

Results: Hypophysectomized rats utilize glycine at twice the rate it is used by normal rats, but under the conditions of these experiments, ACTH has no appreciable effect on rate of utilization by either. Further experiments, varying the amount of ACTH and the relationship between time of injection of ACTH and $C^{14}H_3COONa$ are under way at the present time.

Two normal rats were each injected with 0.1 mg heparin mixed with the regular solution containing 2 mg sodium acetate-2-C^{14}. The result was an increase in initial rate of expiration of $C^{14}O_2$ from an average of 37 percent of the total activity recovered in one hour without heparin to 47 percent recovered in one hour with heparin. After about one hour the average rate of $C^{14}O_2$ expiration for the heparinized animals fell below that for the normal animals, but whether this was due to the exhaustion of the heparin or simply of the sources of C^{14} remains to be shown.

7. Paper Chromatography of Squalene.

Recently, there has been some speculation whether the hydrocarbon squalene, $C_{30}H_{50}$, is involved in the biosynthesis of cholesterol. It was then thought desirable to have a method for paper chromatography of squalene and separation from cholesterol, if possible. All samples of squalene and cholesterol described below were obtained from Dr. W. G. Dauben. The squalene was run on "Quilon" impregnated filter paper and could be detected with iodine vapor, showing up as a brown spot. Methanol was the solvent in all experiments.

A) Regenerated natural squalene: This material gave two spots when run. In all 12 strips were run and the R_f's averaged to: spot I, 0.71; spot II, 0.88.

B) Synthetic squalene: Average of 12 papers, R_f 0.75.

C) Natural, redistilled, Average of 11 papers, R_f 0.71.

D) Squalene-C^{14}, average of 12 papers, R_f 0.72. The R_f values are based on the iodine spot, the radioactivity not being enough to show up on short exposure, the films will be developed at a later date.

E) Squalene-C^{14} and Cholesterol-C^{14}: The cholesterol showed up quickly as a definite radioactive spot, R_f 0.65 (aver. 6 papers). The squalene gave an iodine spot, R_f 0.75 and a faint radioactive spot, R_f 0.86. The cholesterol spot gave a pink color with silicotungstic acid which coincided with the spot. Although the cholesterol spots overlapped the tails of the first squalene spot in some cases, the two compounds could easily be defined. The chlesterol R_f value was considerably higher than that normally obtained in methanol (0.55). This might be due to solvent action of the squalene and might be an error, although it is not likely that an error would show up with such good precision on six strips.

These experiments are being repeated and some of the cholesterol-C^{14} is being run by itself to help acquaint us with the characteristics of this particular sample. This method seems to be a promising one for the separation of cholesterol from squalene and for the identification of squalene. The other runs (cold squalene) will also be repeated with samples whose history is better known than that of the material used in these runs.

8. Paper Chromatography of Steroids.

Several new chromic complexes were received from the duPont Company. These were the furoic, salicylic, gluconic and p-aminobenzoic acid complexes. Papers were treated with each of these complexes, but in methanol a green color moved with the front, which would indicate that some of the complex was washing down. The preparation of papers was repeated, using double the amount of neutralizer and still all except the gluconic acid complex ran.

Six strips of the gluconic treated paper were used for the chromatography of progesterone, the average of the six values gave an R_f of 0.86. (The R_f of this material on stearic acid quilon paper is 0.80). The R_f values for cholestanol and stigmasterol were determined on stearic quilon. In both cases a silicotungstic acid spray was used to detect the steroids. Each gave a faint pink spot: Cholestanol (average of 12 papers) R_f 0.62; Stigmasterol (average of 12 papers) R_f 0.52. The R_f of cholestenone was again determined, inasmuch as the color test used in the initial determinations left some doubts. Again the R_f was found to be 0.80.

9. Lathosterol Content of Serum Cholesterol.

This work is being carried out in collaboration with Prof. L. F. Fieser of Harvard University and all lathosterol analyses are being done in his laboratory. Recently, Prof. Fieser announced the discovery of lathosterol (Δ^7-cholestenol) as a companion steroid of cholesterol, one which had not previously been detected.

Cholesterol was isolated from the following sources and sent to Harvard for analyses. The results are given in Table I.

Table I

Source	mg cholesterol	percent lathosterol
Normal female serum	52	0.60
Female serum high in S_f 10-20. Xanthoma patient	27	0.10
Cholesterol from atherosclerotic human aorta	800	0.56

Other samples are being prepared for this program. Dr. Fieser has also sent us his exact analytical method which will be set up by the Medical Physics Group.

Photosynthesis Chemistry

M. Calvin, J. Bassham, V. Lynch, M. Goodman, P. Hayes, L. Norris, G. Buchanan,
P. Massini, A. Wilson, A. Zweifler, D. Bradley.

Previous reports from this laboratory have described the investigation of carbon reduction by plants. Techniques of analyzing C^{14}-labeled compounds formed by plants during exposure to $C^{14}O_2$ were reported. Most important of the early products of $C^{14}O_2$ reduction are the phosphate esters of simple organic compounds. These include phosphate esters of two, three, five, six and seven carbon and perhaps four carbon compounds. Problems under consideration at the last report which are receiving continued attention include the following:

1) Better separation of all the phosphate esters by use of improved paper chromatographic technique,

2) Isolation of macro-amounts of some phosphate esters by ion-exchange columns,

3) Detection and identification of new compounds, particularly four carbon compounds,

4) Refinement of kinetic studies of the order of occurrence of these

substances during reduction of radiocarbon by plants,

5) Improvement of degradation procedures for the more prominently occurring compounds.

In addition to the detailed analysis of these compounds, the effects of other variables on the pattern of photosynthesis are being studied. These studies include the dark CO_2 fixation with different organisms, and the effects of inhibitors. Some preliminary experiments on the photochemical mechanism of photosynthesis have been undertaken.

1. Ion-exchange Separations of Phosphates.

Thirty grams of algae were extracted with 80 percent ethyl alcohol. The cells were then extracted with hot H_2O (90° C.) which contained 20 percent ethanol. These extracts were treated with petroleum ether. The water and alcohol solutions were then combined and concentrated to 100 ml. The solution was placed on an ion-exchange column 350 mm long x 2 cm diameter, Dow A-1, 100 mesh.

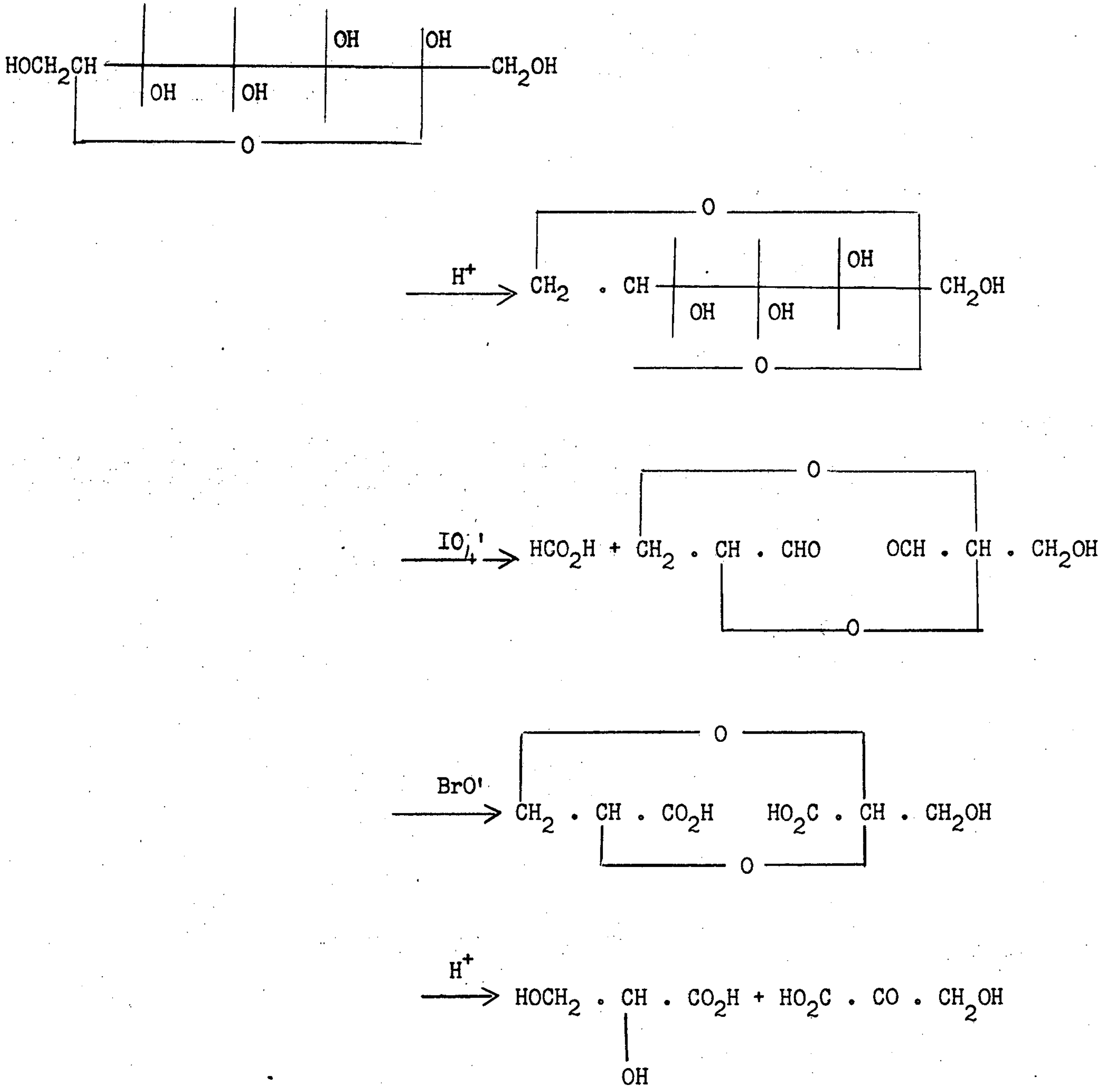
The column was washed with water and eluted with 0.2 $\underline{N}$ $Cl^{(-)}$ 0.05 $\underline{N}$ $H^{(+)}$. Six hundred ml of eluting agent were run through the column with a recovery of only 50 percent of the amount of phosphorus placed on the column. At this point the eluting agent was changed to 1 $\underline{N}$ HCl. The remaining 50 percent of the phosphorus came off the column. The material gave no ortho-phosphate test without strong oxidation. This indicates that the substance is either an organic phosphate or a poly phosphate (inorganic) such as tetra-phosphate, etc. A carbon analysis indicated that the maximum carbon-phosphorus ratio was 1 to 1. The only phosphate that corresponds to this is inositol hexaphosphate. Tests are now being run to identify this compound.

2. Spectrophotometric Analysis.

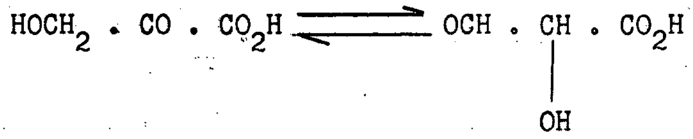
In view of the difficulties in identifying unknown photosynthetic products on the microgram scale by ordinary analytical methods, it appeared desirable to utilize structure-spectra correlations in the identification. Our first experiments have centered on adaptation of the laboratory's Cary recording spectrophotometer. Approximate calculations indicated that it would suffice merely to reduce the cuvette diameter to 1 mm and preliminary experiments indicate that absorption spectra in the visible and near ultraviolet can be obtained with such a capillary cuvette. Techniques are now being developed for transferring the unknowns from the chromatograms to the capillary cell with minimal manipulation.

3. Sedoheptulose Degradation.

The following degradation of sedoheptulose is being attempted:



Estimation of the formic acid has already been carried out and the estimation of glyceric acid should present no difficulties. The other oxidation product, hydroxypyruvic acid, will probably be more difficult to detect and measure. Even oxidation of this compound to glycollic acid may prove difficult because of the tautomerism

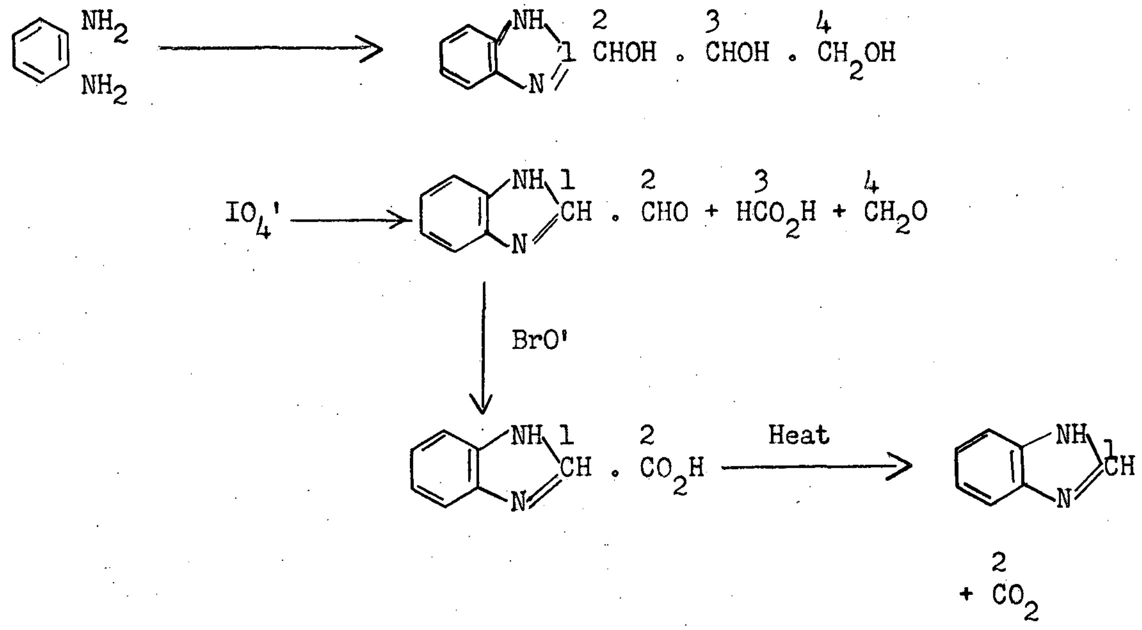


$$HOCH_2 \cdot CO \cdot CO_2H \rightleftharpoons OCH \cdot \underset{\displaystyle OH}{\underset{|}{CH}} \cdot CO_2H$$

4. Aldonic Acid.

A method for the detection and estimation of aldonic acids is being worked out. Reaction of aldonic acids with o-phenylenediamine yields the corresponding benzimidazoles. The latter have the following useful physical properties: High crystallinity, ability to form cyrstalline salts with acids and insoluble copper salts, solubility characteristics suitable for paper chromatography. They can be estimated in micro quantities by means of ultraviolet absorption spectrophotometers.

Periodate oxidation can be used for the determination of radioactivity, e.g., in the hypothetical erythronic acid:



The benzimidazoles from gluconic and glyceric acids have been synthesized, and suitable paper chromatographic solvents are being investigated. Experiments are also in progress to find out the best conditions for the reactions between aldonic acids and o-phenylenediamine in tracer quantities.

5. Phosphoglyceraldehyde.

An attempt has been made to synthesize phosphoglycolaldehyde with a view to determining its behavior in paper chromatography. Sodium glycerophosphate containing 50 percent of the α-compound was oxidized with periodate and from the reaction mixture after treatment with p-nitrophenylhydrazine was isolated a compound having the properties of the barium salt of phosphoglycolaldehyde p-nitrophenylhydrazone.

This compound is rapidly hydrolyzed by dilute acid at 100° to give a water-insoluble nitrophenylhydrazone. Further work on this subject is in progress.

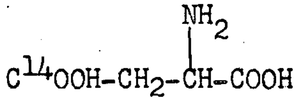
6. Kinetic Studies.

The kinetic studies described in previous reports have been continued with the view to refining the experiments and reducing some of the inherent experimental errors. A search has been made for a suitable labeled compound which could be added to the algae and be carried through all the manipulations finally ending up on the paper chromatogram. Thus, the only accurate measurement required would be the counting of the relative activity of the compound we are interested in to the aliquoting compound. The reliability of counting on paper has also been investigated and it has been found that the apparent activity of the malic spot is independent of carrier (up to 100 μg) to within 5 percent.

7. Feeding Experiments.

Six mg $C^{14}OOH\text{-}CH_2\text{-}CHOH\text{-}COOH$ (specific activity = 3.8 μcuries/mg) was dissolved in 1 ml H_2O and added to a suspension of 0.5 cc. wet packed cells (Scenedesmus) in 15 ml solution buffered with KH_2PO_4 and NaOH at pH 3.4. The cells had been illuminated 30 minutes prior to the addition of malic acid by 1500 ft. candles from each side of a thin illumination vessel, a stream of air being bubbled through the suspension meanwhile. After administration of the malic acid, the illumination and air streams were continued for 20 minutes, after which the algal suspension was filtered rapidly and the residue of cells quickly killed and extracted with boiling ethanol. After further extractions of the cells with alcohol-water mixtures, the extracts were combined and analyzed by paper chromatography. About 20 percent of the administered radioactivity was found in the extracts. Of the radioactivity found on the paper chromatogram, 95 percent was found as unchanged malic acid and 5 percent as aspartic acid. Insignificant amounts (less than 0.5 percent) were found in other compounds.

Similar experiments were performed with labeled aspartic acid,



$$C^{14}OOH\text{-}CH_2\text{-}\underset{}{\overset{NH_2}{\overset{|}{C}}}H\text{-}COOH$$

In this case, the radioactivity found in the extracts of the cells was comparable and of the radioactivity found on the paper chromatograms, 4 percent was converted to malic acid, about 1 percent to glutamic acid, and the rest remained as aspartic acid, save for traces of normal photosynthetic products.

These results indicate that both malic acid and aspartic acid enter the cell (though not necessarily the site of photosynthesis) under the conditions used and are partially metabolized, but are not converted to the usual photosynthetic products except perhaps through carbon dioxide.

8. The Effect of Culture Age on CO_2 Fixation.

Experiments on the effect of culture age on CO_2 fixation are continuing.

The fixation of one and three day old cultures of Chlorella are shown in Table I.

Table I

	Total fix.	Soluble	% Solubles
1 day cultures	8.44×10^6	6.1×10^6	72
3 day cultures	3.07×10^6	2.3×10^6	75

As shown above, the actively growing cells of the one day cultures fix about two and one-half times as much as the older resting cells. Chromatographic analyses of the products of fixation in the two conditions are being carried out.

9. Dark CO_2 Fixation by *Euglena gracilis*.

Experiments on the fixation of carbon dioxide by the green flagellate, *Euglena gracilis*, in the dark is continuing.

The rate of fixation has been determined over the time interval of 5 to 40 minutes. The amount of activity fixed is shown in Fig. 1. The total amount of activity fixed and the activity fixed into 80 percent alcohol soluble compounds are plotted. The total amount of activity increases steadily with time. The alcohol soluble compounds increase rapidly for the first 10 minutes with a very slow increase at later time intervals. Therefore, the greater increase with time is in the insolubles, 38 percent of the activity insoluble at 5 min. to 68 percent insoluble in 40 min. The alcohol-soluble compounds are now being separated by the use of paper chromatography so that the rate of change of radio carbon of individual compounds may be determined.

Carbon dioxide fixation by photosynthetic organisms in the light results primarily in the formation of phosphorylated sugars and hydroxy acids. During carbon dioxide fixation in the dark by either photosynthetic or non-photosynthetic organisms, the major radioactive compounds formed are carboxylic acids and amino acids.

The dark fixation of radioactive carbon dioxide by *Euglena* differs from this normal pattern as both phosphorylated compounds and di- and tricarboxylic acids and related amino acids are formed. In an attempt to determine whether the formation of these two general types of compounds are inter-related or whether they arise by two different pathways, malonate pre-treatment of the cells was tried. Since malonate is a known inhibitor of succinic dehydrogenase and is also reported to inhibit the reduction of oxalacetic acid to malic acid, the formation of the carboxylic acids and related amino acids might be inhibited without affecting the formation of the phosphorylated compounds. Pretreatment of the cells with 0.05 *M* malonate at pH 4.3 for 105 minutes before the addition of radio-carbon dioxide did not alter the amount of alcohol soluble activity fixed during 15 minutes. The effect of this malonate treatment on individual compounds is now in progress. As yet no conclusions can be drawn on the effect of malonate on the dark CO_2 fixation by *Euglena*.

When radioactive carbon dioxide is administered to photosynthetic or non-photosynthetic tissues, radioactive sugars may be found. However, the distribution of activity in the individual carbons of the sugar skeleton is strikingly different for the two types of reactions. The radioactive glucose obtained by

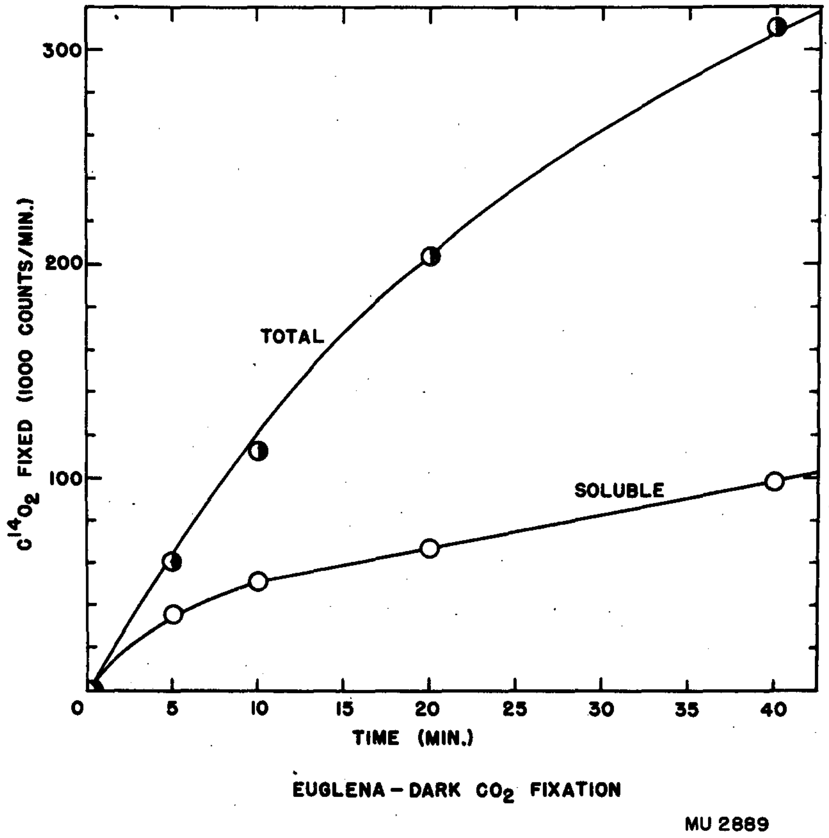

300
200
100
$C^{14}O_2$ FIXED (1000 COUNTS/MIN.)
TOTAL
SOLUBLE
0
5
10
15
20
25
30
35
40
TIME (MIN.)
EUGLENA - DARK CO_2 FIXATION
MU 2889

Fig. 1

hydrolysis of the phosphates formed by Euglena during a 40 minute carbon dioxide fixation in the dark was oxidized with periodate. Only the carbon of the primary alcohol group of glucose will be oxidized to formaldehyde by this treatment. This formaldehyde was trapped with dimedon and its activity determined. The formyldimedon contained no radioactivity. The activity of the other carbon atoms was not determined. If carbon dioxide were fixed by the same reactions as occur during photosynthesis, the number 6 carbon of glucose would be radioactive. Therefore, although radioactive sugars are formed in relatively large amounts in the dark by Euglena, the mechanism of carbon dioxide fixation differs from that occurring in the light.

10. Effect of Anti-folics on Photosynthesis.

Folic acid has been shown to affect the condensation of C_1 compounds. Therefore, the effect of anti-folics on photosynthesis was of interest. The following compounds are being investigated: aminopterin, A-methopterin, amino-an-fol, and 9 methyl pterylglutamic acid.

The effect of these compounds on photosynthesis has been tested. When photosynthesis was determined by measuring the rate of oxygen evolution in the light by algae suspended in bicarbonate buffer, there was no effect on oxygen production by either Scenedesmus or Synechococcus. The antifolics were tested over a concentration range of 0.1 mg to 100 mg per liter.

Experiments are now in progress using radioactive carbon dioxide to determine whether these antifolic compounds affect the carbon dioxide fixing mechanism of the organism.

11. Blue-green Algae.

Photosynthesis of green plants is characterized by the evolution of oxygen with the simultaneous reduction of carbon dioxide. In 1937 Hill found that the oxygen liberating mechanism of photosynthesis could be dissociated from the carbon dioxide reducing mechanism. Using leaf chloroplasts and ferric potassium oxalate as the oxidizing agent, oxygen was liberated when the system was illuminated. In the following years many other oxidizing agents have been found that will substitute for ferric ion in the "Hill reaction." Quinone is one of these compounds.

Using suspensions of intact cells of Synechococcus cedorum, the rates of photosynthesis and the "Hill reaction" were compared. To measure the rate of photosynthesis, 0.2 cc. of cells was suspended in 0.1 M $NaHCO_3$ in air. The rate of oxygen evolution from quinone ("Hill reaction") was determined using 0.2 cc. of cells suspended in 1/10 M phosphate buffer at pH 6.5 containing 0.08 percent quinone. The system was flushed with nitrogen and KOH added to remove any carbon dioxide present. The same intensity of illumination was used for both reactions. Fig. 2 shows the rate of oxygen evolution for the two reactions. Curve A shows the rate of oxygen evolution by photo-synthesizing cells and Curve B the amount of oxygen evolved from quinone by an equivalent volume of cells. Photosynthesis proceeds at approximately four times the rate of the "Hill reaction." Oxygen evolution by the "Hill reaction" stopped after 40 minutes. The amount of oxygen liberated at this time corresponds to the theoretical amount expected if all of the quinone were converted to hydroquinone. Therefore, in the light intact cells of Synechococcus cedorum can use quinone as a substitute hydrogen acceptor with the evolution of oxygen. The ability of broken cells and "grana" obtained for blue-green algae to carry out "Hill reactions" is now under investigation.

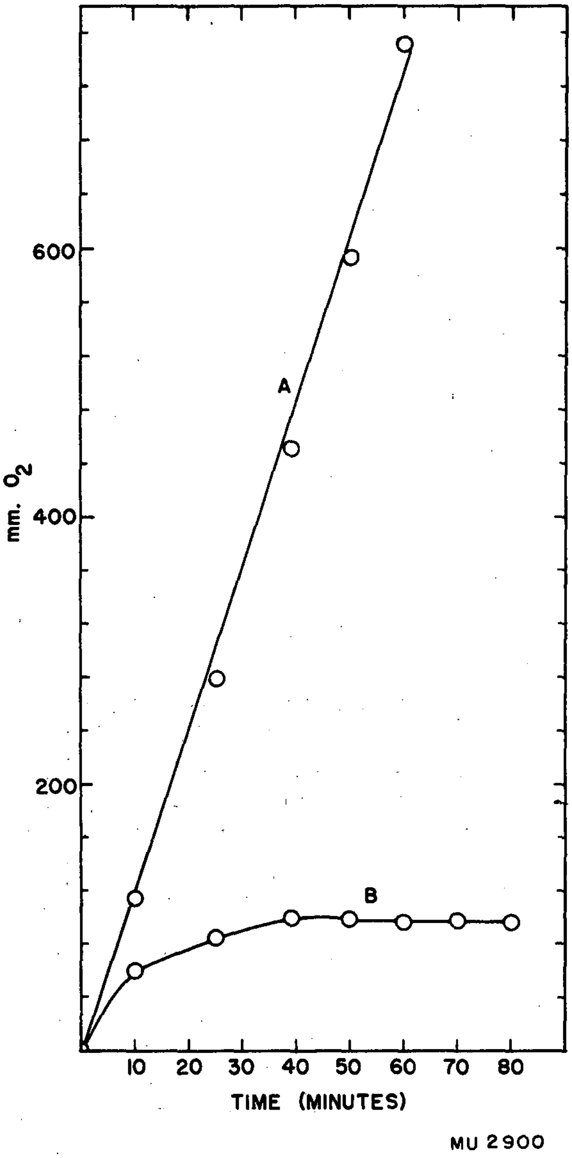


Fig. 2

12. Dependence of Photosynthesis on the Wave Length of the Light Used.

Certain algae contain more than one colored substance capable of converting light-energy in chemical energy (e.g. *Chroococcus* and *Synechococcus* containing chlorophyll and phycocyanin). Emerson and Lewis[1] showed that light absorbed by phycocyanin in *Chroococcus* is used in the process of photosynthesis with an efficiency almost as high as if it had been absorbed by chlorophyll, by measuring the gas exchange rate of the algae illuminated with light of different wave lengths. The question of whether light absorbed by different substances acts on the same photosynthetic mechanism or not has not yet been studied. It is the purpose of this investigation to attack this problem by means of the radiochromatographic method developed in this laboratory. As monochromatic light source, the monochromater described recently by Heisler, et. al.[2] was tried. With an opening of about 0.8, the efficiency of the monochromater is very low, due mainly to the lens errors and the reflections.

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[1] R. Emerson and C. M. Lewis, J. Gen Physiol. 25, 579 (1942).

[2] M. Heisler, Chr. Schmelzer, and K Schuhmann, Z. F. Naturf. 6a, 513 (1951).

II QUARTERLY PROGRESS REPORT Project 48 B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics.

Leo Brewer, LeRoy Bromley, Guy Elliott, Albert Rothman, Richard Porter, John Margrave, Haakon Haraldsen, Karl Wieland.

Gaseous Hydroxides. The study of gaseous hydroxides of Mo and of other elements is continuing.

Vapor Pressure of Iron Telluride. The vapor pressure of iron telluride has been determined and work has started on the determination of the vapor pressures of refractory systems.

Gaseous Molecules at High Temperatures. Studies of gaseous molecules which exist at high temperarures are being carried out using spectroscopic methods of determining the heats of formation. Some of the molecules being studied are CF, CF_2, CCl, CS, SiCl and it is hoped to extend this work to CH and CH_2. Also atomic carbon in the D state is being used to determine the heat of sublimation of graphite. The general procedure for all these studies is to measure the absorption or emission due to a molecule as a function of temperature. The molecule is produced in a King furnace using a graphite heating element. It is hoped to extend the temperature range of study by using TaC and other carbide heating elements.

Publications. Requests have been received for a list of publications describing the work of this group. The following list of papers has appeared in the journals:

The Crystal Structure of Zirconium Oxysulfide: ZrOS. Acta Cryst. 1, 287. (Dept. Reprint No. 1588).

The Vapor Pressure and Heat of Sublimation of Graphite. J. Chem. Physics, 16, No. 8, 797-807 (1948). (Information Division Reprint No. 1948-51 and Dept. Reprint No. 1485).

The Vapor Pressure and Melting Point of Graphite. J. Chem. Phys., 16, 1165-1166.

The Halides of Neptunium. National Nuclear Energy Series, Plutonium Project Record, Vol. 14B, Paper 15.7, pgs. 1111-1118 (1949). (Information Division Reprint No. 1949-174.)

The Thermodynamic Properties and Equilibria at High Temperatures of the Compounds of Plutonium, N.N.E.S., P.P.R., Vol. 14B, Paper 6.40, pgs. 861-886 (1949). (Information Division Reprint No. 1949-109.)

Utilization of Equilibrium Vapor Pressure Data. J. Chem. Education, 26, 548-552 (1949).

The Thermodynamic and Physical Properties of the Elements. National Nuclear Energy Series, Plutonium Project Record, Division IV, Vol. 19B, Paper 3, pgs. 14-39 (1950). (Previously listed as MDDC-438C). Information Division Reprint No. 1949-157.

Thermodynamic and Physical Properties of Nitrides, Carbides, Sulfides, Silicides, and Phosphides. N.N.E.S., P.P.R., Vol. 19B, Paper 4, pgs. 40-59 (1950). Information Division Reprint No. 1949-158). (Previously listed as MDDC-438D.)

The Thermodynamic Properties of Common Gases. N.N.E.S., P.P.R., Vol. 19B, Paper 5, pgs. 60-75 (1950). Information Division Reprint No. 1949-159. (Previously listed as MDDC-438E.)

The Thermodynamic Properties of the Halides. N.N.E.S., P.P.R. Vol. 19B, Paper 6, pgs. 76-192 (1950). (Previously listed as MDDC-438F). Information Division Reprint No. 1949-160.

The Fusion and Vaporization Data of the Halides. N.N.E.S., P.P.R., Vol. 19B, Paper 7, pgs. 193-275 (1950). (Previously listed as MDDC-438G). Information Division Reprint No. 1949-161.

The Thermodynamic Properties of Molybdenum and Tungsten Halides and the Use of the Metals as Refractories. N.N.E.S., P.P.R., Vol. 19B, Paper 8, pgs. 193-275 (1950). Previously listed as MDDC-438H. Information Division Reprint No. 1949-162.

Preparation and Properties of Refractory Cerium Sulfides. J. Amer. Chem. Soc., 72, 2248-2250 (1950).

The Thermodynamics of Gaseous Cuprous Chloride, Monomer and Trimer. J. Amer. Chem. Soc., 72, 3038-3045 (1950).

Preparation and Properties of the Sulfides of Thorium and Uranium. J. Amer. Chem. Soc., 72, 4019-4023 (1950).

Heterogeneous Equilibria and Phase Diagrams, Annual Reviews of Physical Chemistry, 1, 41-58 (1950).

High Melting Silicides. J. Amer. Ceramic Soc. 33, 291-294 (1950).

Preparation and Tests of Refractory Sulfide Crucibles. J. Amer. Ceramic Soc., 34, 128-134 (1951).

Theoretical Calculation of the Stability of Solid and Gaseous Species in the Alkali and Alkaline Earth Oxide Systems. J. Am. Chem. Soc., 73, 2045-2050 (1951).

The Heat of Formation of CN and the Dissociation Energies of N_2 and C_2N_2. J. Am. Chem. Soc., 73, 1462 (1951).

Heat Transfer in Forced Convection Film Boiling. The final UCRL report is in preparation.

Variation of Equilibrium Constants on Vapor Pressure with Temperature. UCRL-1406 which summarizes a rapid method for estimation of variation of equilibrium constants or vapor pressure with temperature has been issued.

Thermal Conductivity of Gases at High Temperatures. Work is being continued on the high temperature apparatus. The furnace is almost completely designed, and will be constructed shortly. The design of the cell proper is being continued. Tin for the high temperature bath, and a suitable propeller agitator have been received. The White Double Potentiometer is expected in January.

B. Basic Chemistry, including Metal Chelates.

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

Thermodynamics of Rhenium.

Work on the synthesis of a solid rhenide is underway with the object of determining the nature of the (-I) species.

Heat measurements on rhenium oxides are continuing and the tentative heats of formation are in agreement with the chemical behavior of the oxides at moderate temperatures.

The electrolytic work on the attempted preparation of the (-I) state will be described at length in a report, when the present work on rhenide is completed.

The Hydrolytic Polymerization of Zirconium.

The investigation of the polymerization of Zr(IV) in 1 M $HClO_4$ plus 1 M $LiClO_4$ solutions by means of the aqueous equilibrium between Zr and its first TTA (thenoyltrifluoro-acetone) complex is continuing. Work completed to date covers a range of Zr concentrations from 0.13 to 10^{-2} M. Additional experiments to be completed will extend the concentration range down to 10^{-4} or 10^{-5} M.

A few additional determinations in 2 M $HClO_4$ to establish doubtful points in that series of experiments have been made. The new points add weight to the interpretation reported previously (September Quarterly Progress Report) that the dimer, if it exists at all, is never an important species. This is in agreement with the earlier conclusions of Kraus based on centrifugation experiments.

It is expected that the present series of experiments in 1 M $HClO_4$ plus 1 M $LiClO_4$ will furnish information on the number of hydroxide groups per Zr in the polymer.

Germanium Chemistry.

The study of the solubility of germanous hydroxide in solutions containing various concentrations of H^+ ion and Cl^- ion has been completed. An average of 2×10^{-4} molar was obtained for the solubility of germanous hydroxide in water. The solubility in hydrochloric acid remains fairly low below 3 M HCl and suddenly begins to increase at about 4 M HCl. Even in 4

M HCl, however, the solubility is only ca. 0.01 molar. A series of solubility measurements were carried out at constant ionic strength ($\mu = 4.00$) in which the chloride and hydrogen ion concentrations were varied independently of one another. Only one generalization can be gleaned from the data: when the chloride ion concentration is held constant at 4 molar while the hydrogen ion concentration is increased (or vice versa), the solubility of germanous hydroxide goes up. This behavior is consistent with the formation of a complex ion of the type $GeCl_X^{\pm(2-x)}$, where $X > 0$.

A study of the equilibrium $GeO_2 + 4HCl(g) = GeCl_4(g) + 2H_2O(g)$ has shown that the reaction is complete up to the highest temperature investigated, 1363°K. Studies of the system GeO - HCl(g) proved inconclusive. It was impossible to distinguish between the various possible reactions, e.g.

$$GeO + 3HCl = GeHCl_3 + H_2O$$

$$4GeO + 12\ HCl = GeH_4 + 3GeCl_4 + 4H_2O$$

$$2GeO + 6\ HCl = GeH_2Cl_2 + GeCl_4 + 2H_2O \quad \text{etc.}$$

The heat of formation of $GeO_2(s)$ has been determined calorimetrically by oxidizing finely powdered Ge in an alkaline solution of hypochlorite. The value $\Delta H_f = -130 \pm 3$ kcal./mole was obtained.

The absorption spectrum of GeI_4 in various organic solvents was studied. The spectra of these solutions were very similar; each exhibited absorption maxima at approximately 2800Å and 3600Å.

An attempt was made to determine the Raman spectrum of a saturated solution of GeI_4 in carbon tetrachloride. No success was obtained because the solution absorbs the exciting lines all the way up to about 5100Å and the tetraiodide is markedly decomposed by ultraviolet light. Solid GeI_4 is too deeply colored for a Raman spectrum.

Studies of Non-Aqueous Solvents.

The absorption of iodide in aqueous solution at about 2270Å has been interpreted as being due to the process

$$I^- + h\nu = I + e^-.$$

Hence the heat of formation of the electron in water can be calculated. It was decided to check the validity of this hypothesis by measuring the absorption spectrum of iodide in liquid ammonia. From the known value for the heat of formation of the electron in liquid ammonia (cf. UCRL-1402), we calculate that the absorption in liquid ammonia should occur at about 2600Å. Preliminary studies have definitely shown that the absorption peak in liquid ammonia is at 2540Å, representing good agreement with the theory.

Electron Exchange Rate Between Fe^{2+} and Fe^{3+}.

A source of oxidizing impurity causing errors in the iron analysis was graced to the conductivity water used in making up solutions. This difficulty has been eliminated by making the final distillation in the preparation of conductivity water, from a large glass still, without added permanganate.

The spectrophotometric method for the analysis of Fe^{++} using bipyridyl now gives accurate results at low iron concentrations ($\sim 10^{-5}$ M to 10^{-6} M) although the preparation and order of mixing of the reagents is very critical.

At this low iron concentration, oxidation of ferrous or reduction of ferric in the resin treatment could cause large errors in the calculated fractional exchange. Blank runs using pure ferrous and pure ferric solutions have shown no oxidation or reduction occurs within the limits of experimental error (± 1 percent)

Thermodynamics of Indium.

Measurements of the pH of $In(ClO_4)_3$ solutions as a function of In^{+++} concentration and also as a function of ionic strength are in progress. This will make it possible to calculate the hydrolysis constant of In^{+++} and thence, using Moellor's data, to calculate the constants for the complexing of In^{+++} by Cl^-, Br^-, and I^-.

Work is proceeding on the determination of the equilibria between In(m), In^{+1}, In^{+2}, and In^{+3}. It has not yet been shown whether (a) an equilibrium or (b) a steady state gives rise to the +1 and +2 species. Further experiments including some runs involving the disproportionation of +1 compounds will be made to decide if the process is actually an equilibrium.

Thermodynamics of Thiosulfate.

From preliminary measurements, on the following reaction:

1. $S_2O_3^=$ (aq.) + Ag(s) = Ag_2S(s) + $SO_3^=$ (aq.), it appears that equilibrium is approached from either side and at 120°C lies to the right. The work suggests that equilibrium is established more rapidly than in the reaction:

2. CaS_2O_3(aq.) = $CaSO_3$(s) + S(r), and that Reaction 1 may be less troubled by side reactions, so further study of Reaction 2 has been temporarily discontinued.

"Aeroshell 120" SAE 60 (Aircraft Lub. Oil) has been found satisfactory for oil bath temperatures up to at least 150°C. This oil becomes quite viscous, however, much below 100°C.

Solubility of the Rare Earth Fluorides.

Cell measurements to determine the equilibrium constant for the reaction

$$CeF_3 \cdot 1/2\ H_2O(s) + 3H^+ = Ce^{+3} + 3HF + 1/2\ H_2O \quad K.$$

are in progress. The first set of measurements approaching the above equilibrium from the left lead to an approximate value of K, but due to the slow rate of solution of $CeF_3 \cdot 1/2\ H_2O$ the reverse reaction is now being investigated. NaF is added to a solution of $Ce(ClO_4)_3$, $Fe(ClO_4)_2$, $Fe(ClO_4)_3$ and $HClO_4$ in a half cell containing a gold electrode. The change in potential of this electrode with reference to another half cell containing $Fe(ClO_4)_2$,

$Fe(ClO_4)_3$ and $HClO_4$ can be utilized to calculate an equilibrium constant for the above reaction.

Hydrated CeF_4 is also being investigated.

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