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CHEMISTRY DIVISION QUARTERLY REPORT

March, April and May, 1951

June 26, 1951

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I QUARTERLY PROGRESS REPORT. Project 48

G. T. Seaborg and I. Perlman

A. Chemistry of the Rare Earth and Actinide Elements

Vapor Pressure of Americium Fluoride

Merle Jones and B. B. Cunningham

Preliminary measurements on the vapor pressure of a sample of AmF_3 have been carried out by the effusion method in the temperature range 800-1000°C. The apparatus used is similar to that built by Westrum and Wallman as described by Cunningham (Nucleonics 5, 62, 1949). The sample was contained in a cylindrical tantalum effusion vessel 5 mm o.d. and about 1 cm high. The top of the container was covered with 1/4 mil tantalum foil perforated to form an effusion hole of ca. 2×10^{-4} cm^2 area. The effusion vessel was surrounded by a 1 mm thick beryllia shell, which in turn rested inside a slightly larger beryllia crucible. The effusion vessel and surrounding crucible were drilled to admit a Pt-90 percent Pt, 10 percent Rh thermocouple into a 2 mm deep well in the bottom of the tantalum vessel. The effusion vessel was heated by a wolfram coil filament wrapped around the outer beryllia crucible. The spacing of the coils was such as to maintain a higher temperature at the top of the effusion vessel than at the bottom.

The thermocouple was calibrated to within $\pm 3^\circ$ against the melting points of $K_2Cr_2O_7$, LiCl, $CaCl_2$, Na_2CO_3, Na_2SO_4, and K_2CrO_4.

Four runs were made on a 300 μg sample of AmF_3 prepared by wet precipitation, and drying at 300°.

The first run agreed with the latter three to within ± 20 percent, and the latter agreed among themselves to within ± 5 percent.

Within the indicated limit of experimental error the vapor pressure was found to obey the relation

$$\text{Log } P_{mm\ Hg} = 10.95 - \frac{20090}{T}.$$

Spectrographic analysis of the fluoride, carried out on the material after the measurements showed it to contain several percent of iron, and the crystal structure was found to be different from the normal hexagonal AmF_3 structure.

The vapor pressures observed were slightly lower than those for PuF_3 as reported by Phipps, Siefert and Simpson (CN 3223, September 26, 1945) whereas somewhat higher values are to be expected.

The heat of vaporization corresponding to the vapor pressure relation given above is rather closely similar to that for plutonium trifluoride.

At the present time it seems most likely that impurites in the sample reduced the vapor pressure somewhat below that of pure AmF_3 and also produced a stabilization of an abnormal crystalline form.

B. Nuclear Properties and Transformations

Artifically Produced Alpha Activities in Elements Below Lead

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

In the course of investigation of the problem of alpha emission in the neutron deficient isotopes of medium heavy elements during the past quarter several bombardments have been made. Alpha particle energies of several alpha emitters have been redetermined with greater precision. Limits on the mass numbers of three dysprosium alpha emitters have been roughly determined from the observation that all three are produced in good yield by 100 Mev proton bombardment of terbium oxide and essentially not at all by 70 Mev protons. Table I gives the best values at present for various properties of all the well-established artificial alpha emitters found among medium heavy elements.

The Production of Bromine Nuclides with Carbon Ion Bombardments

Jack M. Hollander

Studies are being made on the light mass isotopes of bromine, including a previously unreported activity, produced by bombardment of copper with accelerated carbon ions.

	72	73	74	75	76	77	78	79
Br				1.7 hr?	16 hr?	57 hr	6.4 min	
Se	9.5 day	7.0 hr		127 days				

On the nuclides shown, only the 6.4 minute Br^{78} and the 127 day Se^{75} had been uniquely assigned; a "probable" rating had been given to 57 hour Br^{77} and to Se^{72} and Se^{73}. A 16-hour activity had been reported[1] in Br, but the mass number had not been assigned, although it was suspected to be Br^{76}. Pool et al.[2] report a 1.7 hour Br^{75} as the result of a (p,γ) reaction on enriched Se^{74}. Also, in this laboratory, a 30 minute isotope of bromine has been produced by the (C,xn) reaction on copper and must have a mass less than 77.

By means of excitation experiments with (α,n), $(\alpha,2n)$ and $(\alpha,3n)$ reactions on As^{75}, the 16 hour activity has been definitely assigned to Br^{76}, and the 57 hour activity verified as Br^{77}. These experiments could not be continued to the $(\alpha,4n)$ and $(\alpha,5n)$ reactions because of the limitation in energy available from the 60-inch cyclotron.

TABLE I

Artificial Alpha Activities in Elements Below Lead

Element	Atomic Number	Mass Number	Alpha Particle Energy (Mev)	Half-life	Other Modes	Branching Ratio α/Total	Partial Alpha Decay Half-life
Europium	63	147	$2.8_8 \pm 0.1$	22 D±3	E.C.	$\sim 10^{-5}$	$\sim 6 \times 10^3$ Y (within factor of 2)
Gadolinium	64	148	$3.1_6 \pm 0.1$	>35 Y	--	--	$\sim 1.4 \times 10^2$ Y (within factor of 2)
Terbium	65	149	$3.9_5 \pm 0.04$	4.1 H±0.2	E.C., prob. no β^+	--	--
	65	150 or 151	$3.4_4 \pm 0.1$	19 H± 1	--	--	--
Dysprosium	66	$149 \leq A \leq 153$	$4.2_1 \pm 0.06$	7 M±2	--	--	--
	66	$149 \leq A \leq 153$	$4.0_6 \pm 0.04$	19 M±4	--	--	--
	66	$149 \leq A \leq 153$	$3.6_1 \pm 0.08$	2.3 H±0.2	--	--	--
Gold	79	$183 \leq A \leq 187$	$5.0_7 \pm 0.1$	4.3 M±0.2	E.C., β^+	α/K x-rays. $\sim 10^{-4}$	~30 D(within factor of 4)
Mercury	80	$A < 185$	$5.6_0 \pm 0.1$	0.7 M±0.2	--	--	--

Carbon ion bombardments of copper (Cu^{65}) have produced the 1.7 hour (we find 1.5 hour) and 30 minute bromine activities. Bombardment of zinc with carbon ions produced these two, and also the 16 hour Br^{76}. However, the energy distribution of the carbon beam is wide, and reaction yields are at present difficult to interpret, so no definite mass assignments can yet be made on the basis of these data.

Attempts to work out these assignments on the basis of proton bombardments of selenium (enriched in Se^{76}) gave inconclusive results, because of interference from heavier isotopes of selenium which were present even in the enriched samples.

If one assumes that Pool's assignment of the 1.7 hour bromine to Br^{75} is correct, then the 30 minute activity might tentatively be placed at Br^{74}. Br^{73} seems to be ruled out by the fact that no 7.0 hour daughter (Se^{73}) is observed to grow into samples containing only the 30 minute and 1.7 hour activities.

Work is being continued in this region.

---

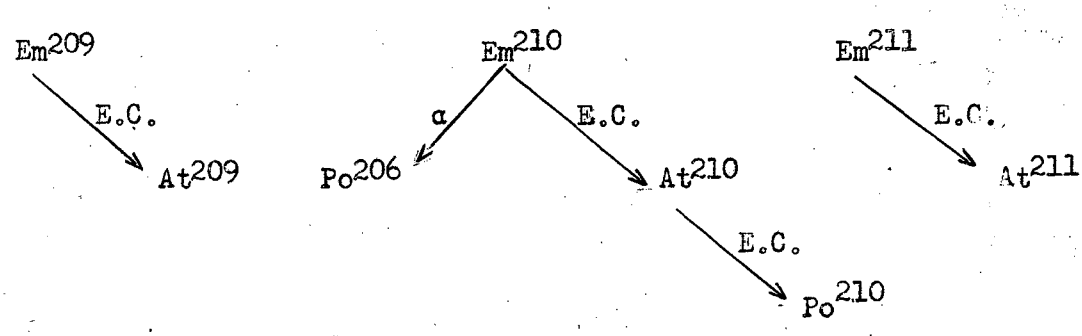
[1] R. H. Geockermann, private communication to G. T. Seaborg and I. Perlman (1948).
[2] L. L. Woodward, D. A. McCowan, and M. L. Pool, Phys. Rev. 74, 870 (1948).

The Production Of Neutron Deficient Emanation Nuclides

D. F. Martin, A. Ghiorso, E. K. Hyde, F. F. Momyer

Samples of Em^{209} have been obtained from the alpha decay of Ra^{213}, which was prepared by bombarding thorium with 340 Mev protons. These samples were sealed in glass ampules and the decay was followed by observing the K x-ray and gamma radiation on the gamma ray spectrometer. At present the half-life of Em^{209} appears to be 30 ± 5 minutes. The isotope decays mainly by electron capture to At^{209}, which has been identified as to half-life and alpha energy. The half-life of the Ra^{213} parent is about 2 minutes.

In emanation fractions isolated directly from thorium after 340 Mev proton bombardments, At^{209}, At^{211}, Po^{206}, and Po^{210} were identified by half-life and alpha energy on the alpha pulse analyzer. This is evidence for the presence Em^{209}, Em^{210}, and Em^{211}. Thus:



Successive milkings of the daughters from the emanation indicated that Em^{210} and Em^{211} have comparable half-lives, probably of about two hours. Po^{206} and Po^{210} were found in the emanation decay products in comparable abundance so that Em^{210} E.C./α appears to be of the order of 1.

Investigations are continuing on the properties of emanation, francium, and radium isotopes in this region, with a view of characterizing such isotopes as Ra^{212}, Ra^{213}, Fr^{211}, Fr^{213}, Em^{208}, Em^{209}, Em^{210}, and Em^{211} as to thir half-lives and alpha energies.

Radiation Chemistry of Alcohols

A. S. Newton and W. R. McDonell

The survey of the radiation chemistry of alcohols is essentially complete and a report on the work to date will be issued in the near future. The products from the radiolysis of the lower alcohols, methyl, ethyl and isopropyl, show a good balance of oxidized vs. reduced products. However, the balance of oxidation vs. reduction becomes poorer in the higher normal alcohols. Since the reduced products consist of hydrogen and hydrocarbons which are relatively easy to analyze, the reduced products can be used as a measure of the total reaction occurring in the system and represent a true measure of the reduction occurring. The oxidized products unaccounted for must represent unsaturated oxygenated compounds not detected in our scheme of analysis. Recently a semi-micro Podbielniak column has been obtained, and it is hoped that some of these products can be concentrated enough to allow chemical or mass spectrometric identification of these components.

Spontaneous Fission of Cm^{244}

A. E. Larsh, A. Ghiorso and S. G. Thompson

The heaviest isotope of curium, Cm^{244}, has been shown to undergo spontaneous fission with the extremely high ratio of 1.3 ± 0.2 spontaneous fissions per 10^6 alpha disintegrations. This value is about 20 times higher per alpha disintegration than that for Cm^{242} known from early work[1] to be 0.062 spontaneous fissions per 10^6 alpha disintegrations. The measurements were made on almost pure Cm^{244} produced in the Hanford pile by neutron bombardment of Pu^{242} through the reactions: $Pu^{242}(n,\gamma)Pu^{243} \xrightarrow{\beta-} Am^{243}(n,\gamma)Am^{244} \xrightarrow{\beta-} Cm^{244}$. A small amount of Cm^{242} by weight was present in the sample, having been formed by other neutron reactions, but its contribution to the spontaneous fission rate was only about 20 percent. Assuming a half-life of some 40 years, as discussed in another section of this report, the half-life for spontaneous fission of Cm^{244} is approximately 3×10^7 years.

---

[1] Ghiorso and Robinson, University of California Radiation Laboratory, Progress Report, Chemistry Section, BC-84 (October 14, 1947).

The Decay Schemes of Pu^{239} and Cm^{242}

Charles Prohaska

A coincidence counter for measuring alpha-gamma and alpha-electron coincidences has been constructed and data have been obtained on Pu^{239} and Cm^{242}.

Pu^{239}. The alpha-gamma coincidence counter shows the presence of a 65-70 Kev γ in coincidence with the alpha particles. The abundance is $4(10^{-4})$ coincidence per alpha. There is no evidence of any coincidences between alpha particles and conversion electrons from the 65 Kev gamma.

The alpha-electron coincidence counter shows the presence of many soft electrons (energies up to 20 Kev) in coincidence with alpha particles. The 20 Kev electrons may be conversion electrons from a highly converted 40 Kev gamma (not observed), but there are far too many soft electrons present to be accounted for in this manner. Apparently, a large number of these soft electrons are Auger electrons, which cannot be resolved from L-conversion electrons by absorbtion methods.

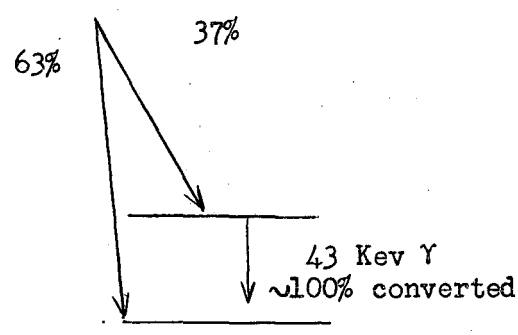
Cm^{242}. No alpha-gamma coincidences are observed. If any gamma rays are in coincidence with alpha particles, they are in too low abundance to be observed with this apparatus.

A considerable number of alpha-electron coincidences are observed in Cm^{242}. These may be resolved into two components: 37.5 Kev electrons and 25 Kev electrons. These correspond to M and L conversion electrons, respectively, of a 43 Kev gamma.

The abundance of these coincidences is as follows:

37.5 Kev electron	0.09 coin/α
25 Kev electron	0.28 coin/α

This gives an abundance for the 43 Kev transition of 0.37/α, and a ratio of L conversion/M conversion = 2.9. These data are consistent with the following decay scheme:



The Recoil of Na^{24} Produced in Aluminum Foil by the Bombardment with High Energy Nuclear Particles

S. C. Fung

During the investigation of the excitation function of (α,α2pn) reaction on bismuth with high energy alpha particles in the 184-inch cyclotron, it was discovered that the amount of Na^{24} found in the first of a stack of aluminum monitor foils was less than that in the following foils. It was of interest to us to determine the cause of this effect. A standard procedure of the experiments is as follows. Four to six layers of aluminum foils of equal thickness sandwiched between two or more sheets of ten mil graphite were mounted in a clamp type target holder. The edges of the rectangular shaped foils were flush with one another. After the bombardment (7-20 minutes) the foils were cut into equal and corresponding areas. After approximately 24 hours, all the foils were weighed and mounted separately and counted every four to eight hours for three days on the second shelf of the same Geiger-Müller counter. The aluminum foils contained essentially the same amount of Na^{24} activity (almost always within one percent), except the first one which always had much lower amount of Na^{24} activity than the others. The graphite layer right behind the last aluminum foil caught quantitatively an amount of Na^{24} corresponding to that which the first aluminum foil lost, whereas the graphite in front of the aluminum foils caught little or no activity.

Therefore it can be safely concluded that this phenomenon is due to the recoil of the Na^{24} produced in aluminum foils by the incident particles, and the range of the Na^{24} recoil particles in aluminum is less than the thickness of the foil.

The results of the experiments are found in Table I and show the dependence of the percentage of recoil of Na^{24} upon the energy of the incident particles. Starting from the low energy side, the percentage of recoil increases as the energy of incident particle increases which is consistent with the compound nucleus formation as postulated by Bohr. The percentage of recoil then passes through a maximum and decreases as the energy of the incident particle increases.

If the assumptions are made that Na^{24} results from nuclei excited only to a fraction of the energy of the incident particle and that both the degraded incident particle and Na^{24} proceed in the forward direction, this result can be explained. Experiments for determining this energy transfer by measuring the angular distribution of the recoil Na^{24} are in progress.

Modification and Use of the Alpha Particle Spectrometer

Frank Asaro

It has been found that the geometry of the alpha particle spectrometer can be increased by a factor of 3, i.e., from 1.33×10^{-6} to 4.0×10^{-6}, by decreasing the one-inch collimating slit opening to one quarter inch. This

TABLE I

Exp. No.	Nuclear Particle	Energy of Particle	Thickness of Al Foils	% of loss of Na^{24} from first Al foil	Na^{24} in Graphite Right Behind the Al Foils	Na^{24} in Graphite in Front of Al Foils
1	Proton	60 Mev	0.00025 in.	27.8%	28.0%	<2.0%
2	"	"	"	26.4%	28.3%	<2.2%
3	"	70 Mev	"	29.2%	29.0%	<1.5%
4	"	90 Mev	0.0005 in.	12.9%	13.2%	
5	"	"	0.00025 in.	26.8%	26.2%	<2.0%
6	"	140 Mev	"	20.9%	19.9%	<2.1%
7	"	220 Mev	"	17.6%	17.6%	
8	"	280 Mev	"	17.5%	16.4%	<3.3%
9	"	340 Mev	0.0005 in.	7.6%	7.6%	≪2.0%
10	"	"	0.00025 in.	15.0%	15.7%	≪4.0%
11	"	"	"	16.3%	17.1%	≪4.0%
12	Deuteron	194 Mev	0.00025 in.	26.4%	26.8%	
13	"	"	0.0005 in.	12.4%		
14	"	"	"	12.7%		
15	"	"	0.001 in.	6.4%		<0.5%
16	"	140 Mev	0.0005 in.	15.5%		
	alpha	60 Mev	0.00025 in.	23.5%	23.6%	<0.7%
	"	"	"	24.2%	21.4%	<0.7%
	"	90 Mev	"	28.1%	30.3%	<1.1%
	"	"	"	29.4%	28.5%	<1.1%
	"	140 Mev	0.0005 in.	27.5%		
	"	"	"	27.1%	26.0%	<0.7%
	"	220 Mev	0.00025 in.	35.0%	34.1%	<1.8%
	"	220 Mev	"	34.8%	35.1%	<1.8%
	"	340 Mev	0.0005 in.	16.6%		<1.5%
	"	"	"	14.9%		<1.5%
	"	"	"	16.7%		<1.5%
	"	370 Mev	"	13.0%		<0.9%
	"	"	"	14.0%		<1.0%
	"	"	"	14.9%		<1.2%
	"	full	0.00025 in.	24.2%		

geometry is defined as the ratio of tracks appearing on the photographic plate to disintegrations occurring on the source sample. There is no appreciable loss in resolution if the baffles are adjusted such that only 4.2 inches of the magnet pole face is exposed to the beam.

Several ideas for the modification of the recording techniques are under consideration and a crystal scintillation counter has been constructed and mounted. With this type of static recording device, the magnetic field is varied by changing the magnet current while the number of alpha particles is noted.

Work is continuing to determine the optimum source conditions. Construction of a new vaporizing system is partially completed which it is hoped will enable study of proper source backings, filaments and transfer temperatures.

At the present time the alpha emissions of Cm^{242}, Am^{241}, Pu^{239}, Pu^{240}, Po^{208}, Po^{209}, and Po^{210} have been investigated. No attempt has been made to improve the absolute values of the energies of the first four, rather to investigate the energy differences between the groups and their relative abundances. The data are illustrated below in Table I where the energy is in Kev and the abundance in percent

No complex structure was found for Po^{208}, Po^{209}, or Po^{210}. The ground state transition of Po^{208} is an alpha particle of 5.118 ± 0.009 Mev and for Po^{209}, 4.860 ± 0.014 Mev. These values are relative to 5.298 Mev for Po^{210}.

TABLE I

Energy	0	42.9 ± 1.01		Cm^{242}
Abundance	27 ± 2	73 ± 2		
Energy	0	41.0 ± 1.0	100.3 ± 4	Am^{241}
Abundance	14	86	0.4	
Energy	0	36 ± 2	48.5 ± 1.5	Pu^{239}
Abundance	10	21	69	
Energy	0	40.9 ± 1.0		Pu^{240}
Abundance	26	74		

Modification and Use of the Mass Spectrograph

F. L. Reynolds, S. G. Thompson, and A. Ghiorso

The mass spectrograph was used to collect separated small amounts of the curium isotopes 242, 243 and 244 by a rather unique collection method. A series of stacked 10 mil platinum foils, supported in a carriage so that the

edges of the foil are presented to the beam was used as the surface for collecting the isotopes. The method allowed pulse analysis data to be taken in unit segments on each isotopic deposit, and gave an estimate of the amount of tailing of the Cm^{242} peak. From this pulse analysis, and knowing the abundance ratio between these isotopes an estimate of the half-lives of Cm^{243} and Cm^{244} could be calculated. The estimated alpha half-lives from the above experiments are about 40 years for both Cm^{243} and Cm^{244}. Since the abundance ratio determinations are still the least accurately known the above can only be approximate.

Considerable changes have been made in the physical and electronic equipment of the spectrograph during this period: the entire pumping system has been completely redesigned and built. Additional relay racks have been installed to house existing equipment which had to be moved to mount the new pumping system, and to house in the near future a new high voltage supply in the process of design. The present high voltage supply allows only a maximum of 50 microamperes drain, and is not suitable for proposed bombardment sources planned for the spectrograph.

A new high temperature bombardment source for use with solid samples of low vapor pressure has been designed. Design studies included potential field mapping and rubber membrane studies of the trajectories. The shop work on this source is completed and the unit is being assembled.

Since the previous abundance ratio run on a Canada plutonium sample could be in error because of small amounts of Am^{241}, a recheck on this ratio was made after chemistry had removed the americium. Previous results did show a higher percentage of mass 241 compared with this run. Table I shows these results.

TABLE I

	Percentage			
	239	240	241	242
Previous Run (Beam Pu^+)	93.60	5.84	.58	< .014
Present Run (Beam PuO^+)	94.05	5.34	.52	< .01
Corrected for $(Pu^{239}O^{18})^+$	94.23	5.34	.33	--
Argonne Laboratory	93.94	5.60	.41	.019

All these abundances were run on a surface ionization type of source using a tantalum filament, the sample being plutonium oxide. No detailed knowledge is available concerning the Argonne technique on this sample, but agreement is not exact.

Sample Recovery After Spectrographic Analysis of a Radioactive Material

John G. Conway

An air filter system has been constructed to prevent the spread of radioactive materials used in an arc spectrograph. Tests of the system constructed have been very successful and the results are to be reported shortly in a UCRL report. The principal points covered are the effectiveness of the system to stop activity, the effect of flow rate and the deposition of material in the system. It was found that 75-80 percent of the material sparked will collect in the first monitor filter and is available for recovery while the over-all loss through the system is only one part in 10^{10}.

C. Bio-Organic Chemistry Group

Edited by B. M. Tolbert

Synthetic and Experimental Chemistry

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Preparation of Aspartic Acid-C^{14}. In the last Quarterly Report, development work on the synthesis of DL-aspartic acid labeled in either the β or the γ position with C^{14} was described. In the last few months a method has been worked out which would give consistent yields of 50-60 percent of the material from sodium acetate. A hot run starting with 50 mc. of the sodium acetate-1-C^{14} is now in the process of being completed.

The essential difficulty has been in the preparation of ethyl chloroacetate in a satisfactory form for the malonate condensation. Catalytic esterification of chloroacetic acid using a solution of anhydrous zinc chloride and ethanol proved to be unsatisfactory due to contaminating chlorine and HCl which could not be easily removed. Preparation of the ester by refluxing the acid and alcohol in a Soxhlet filled with Drierite was abandoned since it seemed impossible to remove all the calcium chloride from the $CaSO_4$. The method finally adopted involves the preparation of the potassium salt of the chloroacetic acid and subsequent treatment of this with diethyl sulfate; yields as high as 99 percent of ethyl chloroacetate from potassium chloroacetate are thus obtainable.

The chlorinated acetic acid is titrated with alcoholic KOH. More than the theoretical amount of base usually must be added slowly apparently due to the reaction of chlorine with lcohol and a subsequent decomposition of this alcohol in the presence of base. Loss of chloroacetic acid may occur when the solution is dried if this titration is not carried to completion. The dried potassium chloroacetate is mixed with diethyl sulfate and distilled at atmospheric pressure.

Ethyl chloroacetate is then condensed with diethylacetamidomalonate and hydrolyzed with concentrated HCl to give a mixture of glycine and aspartic acid which is separated by the use of Amberlite IR-4B resin. The aspartic acid is then precipitated from an aqueous solution at pH 2.9 with alcohol. An attempt was originally made to precipitate the free amino acid hydrochloride from alcohol solution with ethylene oxide. Under these conditions, however, various esters of the amino acid are formed.

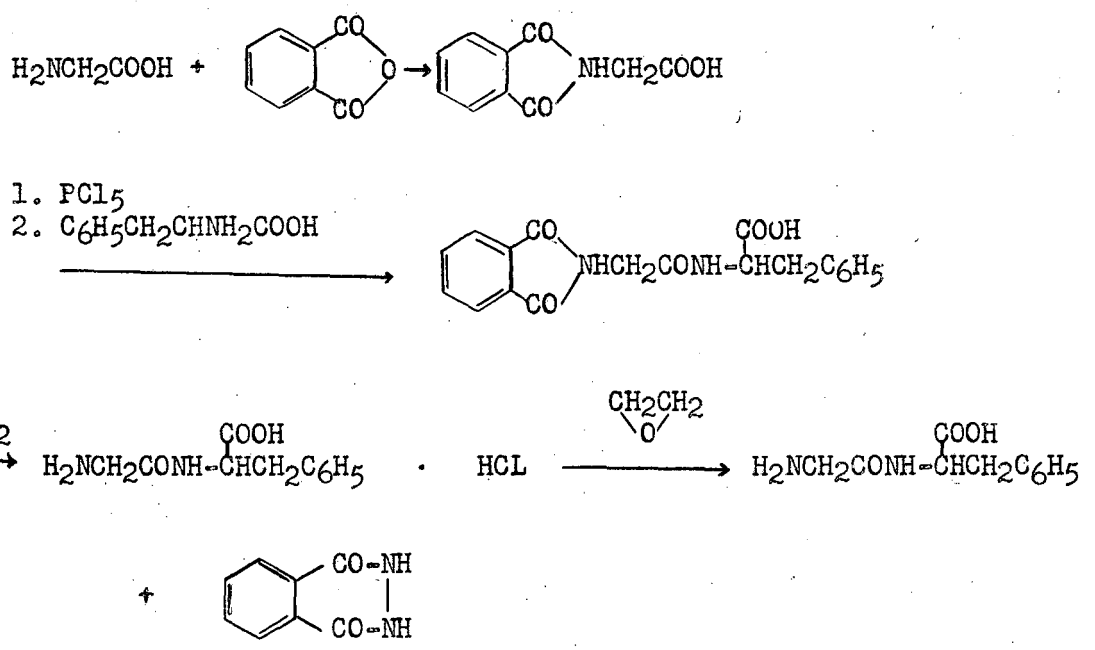
Preparation of Labeled Hippuric Acid. Because of the possibility of preparing a number of labeled amino acids through an azlactone condensation of hippuric acid with various aldehydes, a study has been made on the prepartion of hippuric acid in good yields starting with sodium acetate-2-C^{14} and going through chloroacetic acid and glycine. The conditions of the preparation of the glycine from acetate had previously been worked out by the group. It was hoped that the crude glycine could be converted to hippuric acid without any purifications, but a number of runs all gave relatively poor results, so this was given up in

favor of some type of simple purification. Ammonium chloride and nitrogen di- and tri-substituted glycine would have been contaminants present although the presence of the latter two was minimized by the condition of ammination in which a large excess of NH_4OH and $(NH_4)_2CO_3$ was used.

In one attempt at purification, the glycine was precipitated by methanol, filtered, dried and converted to hippuric acid. The yield in the latter step was 85 percent, but the overall yield from chloroacetic acid was only a little over 50 percent. It was decided to try still another method in hopes of better yields since the results in this case were so limited by solubilities, etc.

In a new attempt, the crude glycine was treated in a beaker with approximately 25-30 ml of Dowex 50 cation-exchange resin. The resin was carefully washed with water followed by washes with 1.5 N NH_4OH. The latter displaced the glycine. NH_4Cl would still be present as a contaminant. This reaction mixture, after having been evaporated to dryness, was converted to hippuric acid, which was treated with a water wash, a CCl_4 wash and then recrystallized from boiling water. The needles of hippuric acid thus obtained have a melting point of 189-190°C. The recrystallized product has been obtained in yields from 55-70 percent (based on acetic acid), and it is thought that this can be improved somewhat by improved manipulation techniques in the washings and recrystallization. The yield of 70 percent does appear good, however in view of the fact that the yield of glycine limits the overall yield considerably.

Synthesis of Peptides. Further syntheses of peptides by the phthalyl method were worked out. Glycyl-l-phenylalanine and glycyl-l-glutamic acid were prepared. The former synthesis is illustrated below.



Phthalyglycine. A mixture of 370 mg of glycine and 750 mg of phthalic annydride was heated at 185-195° for fifteen minutes. The product was recrystallized from 10 percent ethanol to give 979 mg of phthalylglycine (97 percent), m.p., 190.5-193°.

Phthalylglycyl-l-phenylalanine. A mixture of 410 mg of phthalylglycine and 416 mg of phosphorus pentachloride in 4 ml of dry benzene was heated at 60° for two and three quarters hours. The benzene was removed in vacuo, and the residue dissolved in 4 ml of dioxane. The dioxane solution was added over the course of five minutes to a cooled, stirred suspension of 320 mg of l-phenylalanine and 100 mg of magnesium dioxide in 8 ml of water. The reaction mixture was stirred an additional two hours at room temperature.

Dilute hydrochloric acid was added to dissolve the magnesium oxide, and the mixture was extracted with ethyl acetate. The organic solution was washed with water and evaporated in vacuo to leave 594 mg (87 percent) of solid residue. The product was recrystallized twice from benzene to yield 215 mg of phthalylglycyl-l-phenylalanine, m.p., 190-194°.

Glycyl-l-phenylalanine. To a suspension of 215 mg of phthalylglycyl-l-phenylalanine in 2 ml of ethanol was added 0.62 ml of 1 M alcoholic hydrazine. The mixture was heated under reflux, and within the first two minutes everything went into solution. A precipitate then began to appear, and heating was continued for one hour. Most of the ethanol was removed in vacuo, and the residue was warmed at 50° for ten minutes with 2 ml of 2 N hydrochloric acid. It was allowed to cool to room temperature over a period of fifteen minutes and then cooled to 0°. The solid phthalyhydrazide was removed by filtration and washed with cold water. There was obtained 90 mg (91 percent) of phthalyhydrazide. The aqueous filtrate was evaporated in vacuo with slight heating to leave 268 mg of solid which could not be dried any further under reduced pressure.

The solid glycyl-l-phenylalanine hydrochloride was dissolved in 70 percent ethanol, and ethylene oxide was bubbled through it for one half hour. Most of the solvent was removed in vacuo, and the residue was recrystallized from 70 percent ethanol to give 51 mg glycyl-l-phenylalanine (40 percent) in the first crop, m.p., 243° (dec.).

Phthalylglycyl-l-glutamic acid. The acid chloride of phthalylglycine was prepared as above from 512 mg of phthalylglycine and 520 mg of phosphorus pentachloride in 5 ml of benzene. The solvent was removed in vacuo, 5 ml of dioxane added, and the solution added over twenty minutes to a cooled, stirred suspension of 367 mg of l-glutamic acid and 150 mg of magnesium oxide in 10 ml of water. Stirring was continued for two hours longer.

The reaction mixture was acidified with dilute hydrochloric acid and extracted eight times with ethyl acetate. The organic solution was evaporated without further washing to leave 491 mg (59 percent) of solid material.

Glycyl-l-glutamic acid. The phthalyl group was removed as described for phthalylglycyl-l-phenylalanine using 491 mg of phthalylglycyl-l-glutamic acid and 1.47 ml of 1 M hydrazine in 5 ml of ethanol. There was obtained 465 mg of glycyl-l-glutamic acid hydrochloride, which was probably still wet.

The free dipeptide was obtained from its hydrochloride with ethylene oxide, as described above. Crystallization of the product from 70 percent ethanol gave 161 mg glycyl-l-glutamic acid, (54 percent).

Tritiated Stearic Acid. For experiments connected with the atherosclerosis research program, it was desirable to obtain labeled fat or fatty acid. It was decided to label stearic acid with tritium since the procedure is simple and the acid can be converted to triglyceride if necessary. Stearic acid (7.5 gm) potassium hydroxide (100 mg) and 11 ml of tritium water (46.94 mc/ml) were placed in a glass bomb containing 1 gram of reduced platinum and the mixture shaken at 130-135° for 200 hours. After opening of the tube, it was connected to a vacuum line and the water collected in a trap (10.9 ml recovered). The residual solid was dissolved in ether-benzene and filtered through celite to remove spent catalyst. There was obtained 7.5 gm of crude stearic acid. Recrystallation from ethanol-water yielded 7.1 gm (95 percent) of pure material. Specific activity, 10.8 μc/mg.

β-labeled amino acids. In a previous report, the preparation of a number of aliphatic-α-amino acids by acetomidomalonate condensation with the corresponding labeled halides is outlined. This section describes current progress on the synthesis of two of these compounds, norleucine-3-C^{14} and leucine-3-C^{14}.

Norleucine-3-C^{14}. The synthesis of this amino acid consists of the condensation of ethylacetoamidomalonate with n-butyl-1-C^{14} bromide in tertiary butanol and potassium butoxide as previously described for valine. After hydrolysis, the crude amino acid mixture was partially pruified by passage through a coarse Dowex 50 column and eluted with ammonium hydroxide. The resulting mixture of norleucine, glycine, radioactive alanine, radioactive valine (or norvaline) and an unknown ninhydrin negative compound was subjected to fractional crystallization from water. A 35 percent yield of norleucine free of other amino acids but containing 25 percent of its activity in the unknown compound was obtained. This represents 42 percent of the starting activity; an additional 30 percent of radioactive amino acid mixture was obtained on further workup. Attempts to either identify or remove the unknown compound from the norleucine have been unsuccessful to date. Fractional elution from ion-exchange resin is now planned.

Leucine-3-C^{14}. The condensation of ethylacetoamidomalonate with isobutyliodide, the hydrolysis of the crude condensation mixture and preliminary purification of the amino acid by passage through a course Dowex 50 column was performed as reported above. The amino acid mixture consisted of glycine, radioactive alanine, α-aminobutyric acid, radioactive valine (or norvaline) leucine and unknown radioactive ninhydrin negative compound. Fractional crystallization was not successful.

By fractional elution from a fine Dowex 50 column at a slow flow rate 30-35 percent of pure leucine was obtained. Because of the low specific activity used, it was not possible to ascertain whether the unknown compound was removed by this process. Another run is in progress.

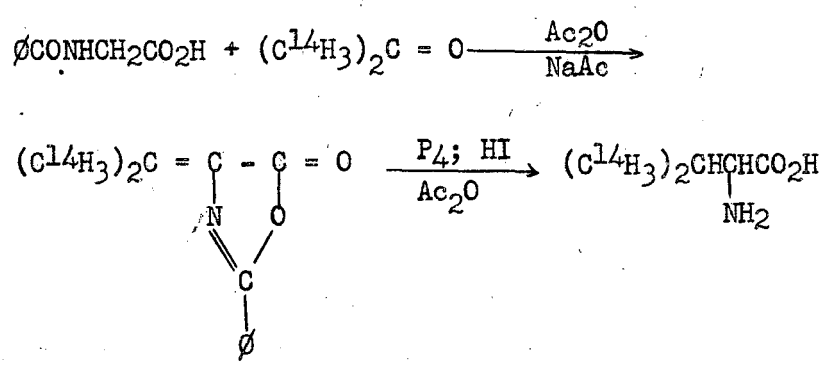
Preparation of Intermediates. During the past quarter, a number of C^{14} labeled intermediates have been prepared for use in various syntheses. These include

the following: Sodium formate-C^{14} (150 mc, yields 83-91 percent based on CO_2); methyl-C^{14} iodide (140 mc, yields 74-91 percent based on sodium formate); methyl-C^{14} iodide (11 mc >95 percent yield from methanol); isobutyl iodide-2,3-$C^{14}{}_2$ (by pyrolysis of sodium acetate); butyl-1-C^{14} iodide (by reduction of corresponding fatty acid, 50 percent yield); isoamyl-1-C^{14} bromide (by reduction of sodium isovalerate, 50 percent yield); sodium acetate-1-C^{14} (50 mc >95 percent yield).

The preparation of labeled sodium formate was modified from previously described procedures to include the addition of sufficient barium hydroxide to the reduced mixture to precipitate any sulfate that had been added to adjust a pH of the initial bicarbonate solution. The presence of this sulfate was thought to be a possible catalyst poison for the subsequent steps in which the formate is directly reduced to methanol. Although no direct evidence was present that it had harmed this reduction, its removal was felt advisable.

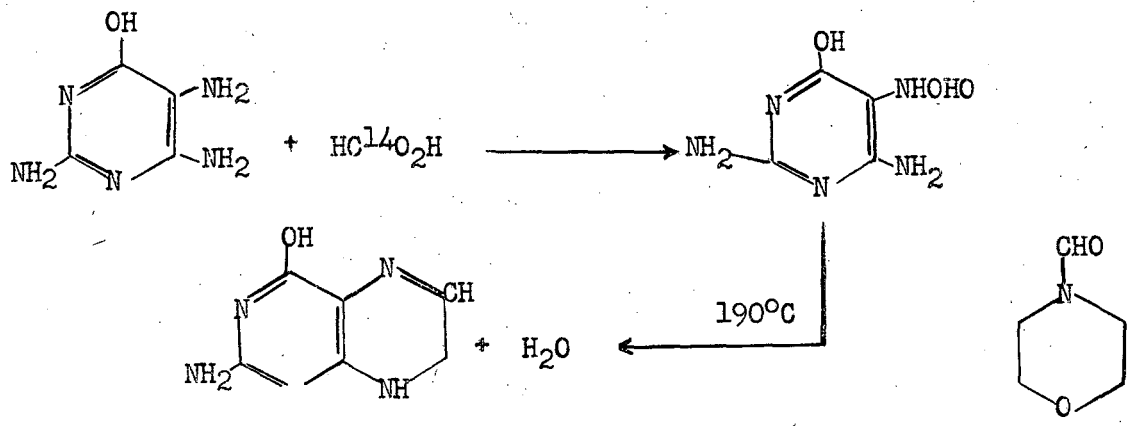
Valine-4,5-C^{14}. Two procedures are currently being studied for the synthesis of methyl-labeled valine. The condensation of isopropyl-methyl-$C^{14}{}_2$ iodide with ethyl acetamidomalonate is being investigated under varying conditions of temperature, solvent and condensing agents. Experiments at atmospheric pressure with sodium in ethanol and with potassium and t-butanol have shown a conversion of activity from the iodide to the amino acid of about 50 percent, but the purity of the product has not been established. Previous experiments in this condensation have given a variety of unsuccessful results. At first, the labeled halide used was not satisfactory so a new method of preparation of the isopropyl iodide was developed. It was then found that there were a number of side products in the condensation and these were discussed in the last quarterly report.

In addition to this standard method, preliminary experiments are under way in the synthesis of valine via the azlactone of labeled acetone. The proposed scheme is as follows:



The azlactone has been prepared previously as a synthetic intermediate but the method has not been used for the preparation of valine.

Guanine-8-C^{14}. Guanine-8-C^{14} was desired for use in purine metabolism studies. An attempt was made to prepare the labeled material according to a published procedure which involves formylation of 2,4,5-triamino-6-hydroxy-pyrimidine in aqueous solution at room temperature and subsequent cyclization to guanine by heating in 4-formylmorpholine.



One millimole each of the pyrimidine dihydrochloride, warm sodium formate (48,000 dis/mg min) and 1N HCl were dissolved in a total volume of 1 to 6 ml, stoppered and left 24 hours. The formyl pyrimidine plus unreacted pyrimidine was recovered either by precipitation with ether-ethanol or by lyophilization. Unreacted formic acid was readily recovered from the ether solution and the lyophillate. At best only 50 percent of the formate activity was fixed in the pyrimidine instead of the 80 percent reported in the procedure. Subsequent cyclization of the pyrimidine gave 50-70 percent weight yields of guanine with only 10-20 percent radioactive yield. Apparently formylmorpholine not only exchanges formate with the formyl pyrimidine but it also donates formate to the starting pyrimidine. All the guanine samples obtained by the route were colored a bright yellow, even after slow filtration through a 0.5 x 0.5 cm column of decolorizing charcoal contained in a tube surrounded by a water jacket heated by a microme wire immersion heater. To date no successful run has been made in the preparation of labeled guanine by the above procedure.

An alternate method of synthesis was tried. Half millimole amounts of the pyrimidine dihydrochloride, sodium formate plus potassium bisulfate (to acidify the formate) and 0.2 ml formylmorpholine (about 1.75 mm) were sealed in a 4 ml bomb tube and heated at 190° for 3 hours. Considerable pressure, presumably CO from formate decomposition, was released when the tube was opened after freezing in liquid nitrogen. The residue was dissolved in 30 ml boiling 2 N HCl, treated with 50 mg decolorizing charcoal, filtered and the filtrate left in the refrigerator 4 days. Pure needles were deposited in 65 percent weight yield, 10 percent radioactive yield.

Experiments are under way to investigate several possible variables, including the use of a new batch of pyrimidine.

Vinyl Acetic Acid. Carboxyl-labeled vinyl acetic acid was desired for study of the possible role of the acid in the synthesis of fatty acids by the bacterium *Clostridium kluyveri*. The acid may be prepared by carbonating allyl Grignard reagent at -70°C by the following procedure, which is more reliable than one previously described.

The Grignard reagent was prepared in 80-90 percent yield by slow addition of one mole allyl chloride to three moles magnesium turnings in a large volume of dry ether with vigorous stirring. The precipitated reagent was largely dissolved when an equal volume of dry toluene was added. Carbon dioxide was generated on a vacuum line and transferred to 0.1 mole excess of the frozen Grignard reagent, the flask closed off with a stopcock and the mixture thawed with vigorous shaking in a dry ice-isopropanol bath. After thirty minutes the carbonation complex was decomposed with 0.1 mole excess of 6 M H_2SO_4. As soon as the added water thawed, the phases were separated and the aqueous phase extracted several times with fresh ether. The ether extracts were combined and titrated to phenol red endpoint with 0.10 N alkali, the aqueous phase separated and lyophillized immediately. In low activity runs, yields of 80-90 percent were obtained. The product appeared to be homogeneous. It gave a single radioactive spot in the vinyl acetic-crotonic acid position on a paper chromatogram run with 2 percent NH_4OH-methyl cellosolve developer and a single uniform peak in a plot of acid equivalents vs. effluent volume from a continuous extraction train using ether and water as immiscible phases. The possible presence of crotonic acid in the product was not checked by the two methods available, namely: partition chromatography on a silica gel column with water-chloroform developer and bioassay with a Clostridium kluyveri preparation which catalyzes the oxidation of vinyl acetic acid only.

Irradiation of Peptides. In continuing the study of the effect of high energy irradiation on peptides, a 750 mc cobalt-60 source was set up. The source consisted of two pieces, and gave a γ-ray dosage of about 1000 r per hour. A solution of 4.9 mg of glycyl-1-C^{14}-glycine-1-C^{14} in 5.0 ml of twice distilled water was irradiated in this field for a period of seven days. Determination of t of the activity of aliquots before and after irradiation indicated that about five other components, in addition to the original dipeptide spot. The total activity in these five spots amounted to about 10 percent of the original activity. None of them gave a positive ninhydrin test. Nothing further is known about these products at the present time, but it would appear that rupture of the diglycine molecule at the peptide bond to give two molecules of glycine does not occur to any appreciable extent.

A source to provide higher levels of radiation was set up, and consisted of 4.1 c in 16 pieces. The radiation dosage in this field is not yet known. Three unlabeled dipeptides, glycyl-l-tryptophan, glycyl-l-phenylalanine, and triglycine, were irradiated as aqueous solutions at a concentration of about one milligram per ml. Irradiation was carried out for four days. No amino acids could be detected from any of them after chromatographing on paper. In order to determine whether or not any change had occurred, an analysis was made for α-amino and peptide-nitrogen. The analytical procedure used was similar to that previously described (Biochem. J., 45, 412 (1949)). The sample to be determined was treated with a suspension of cupric phosphate. The α-amino and peptide nitrogen forms a complex with copper and brings it into solution. The excess cupric phosphate is removed by filtration, and an aliquot of the filtrate treated with sodium diethyl dithiocarbamate. The resulting yellow-colored complex is extracted is isoamyl alcohol and its concentration determined spectrophotometrically. The amount of copper taken into solution is thus a measure of the α-amino and peptide nitrogen. Standard curves must be prepared for each peptide but the method appears to be good to 2-3 percent. The results of these analyses on the irradiated solutions is given in Table I.

TABLE I

Nitrogen Analysis of Irradiated Peptides

Sample	γ of Cu taken into soln. per 100 λ before irrad. (calcd.)	γ of Cu after irrad.	% change
glycyl-l-tryptophan	9.8	12.2	+24
glycyl-l-phenylalanine	13.0	11.2	-14
triglycine	16.2	16.0	- 1

Apparently, irradiation of glycyl-l-tryptophan produces products which can be detected by the above method. The nitrogen contained in the indole nucleus of tryptophan is not determined by this method, so possibly the nucleus has been altered in such a manner as to make this nitrogen detectable. As a further check, the ultraviolet absorption of the solution was measured at 280mμ, where the absorption peak of the indole nucleus occurs. It was found that the concentration of this moiety has decreased by about 30 percent. It was also noted that the solution of glycyl-l-tryptophan had turned yellow after irradiation.

Isotope Effect in the Decarboxylation of α-Naphthylmalonic Acid-l-C^{14}. The decarboxylation of α-naphthylmalonic acid-l-C^{14} has been carried out in a dioxane-1 N hydrochloric acid solution at 72.8° and 87.5°C and on the pure liquid acid at its melting point. The carbon dioxide evolved was collected as barium carbonate. Samples of the α-naphthylacetic acid-l-C^{14} produced were oxidized by a modified Van Slyke-Folch wet combusion method and collected as barium carbonate. The yields in the decarboxylation experiments and in the combustions were quantitative. Carbon dioxide was regenerated from the barium carbonate samples and the various specific activities were measured in an ionization chamber using a vibrating reed electrometer connected to a Brown recorder. The isotope effect may be expressed as a ratio of rate constants, the value of which may be calculated from the measured specific activities. If the possible modes of decarboxylation of the species present in the malonic acid is considered as shown below,

$$RCH(COOH)_2 \xrightarrow{K_1} CO_2 \quad RCH_2COOH$$

$$\begin{matrix} C^{14}OOH \\ RCH \\ COOH \end{matrix} \xrightarrow{K_2} C^{14}O_2 \quad RCH_2COOH$$

$$\begin{matrix} C^{14}OOH \\ RCH \\ COOH \end{matrix} \xrightarrow{K_3} CO_2 \quad RCH_2C^{14}OOH$$

it is seen that the ratio K_3/K_2 is a measure of the ratio of the frequency of rupture of C^{12}-C^{12} bonds to C^{12}-C^{14} bonds in the labeled molecule. This ratio can be calculated from any pair of the specific activities of the carbon dioxide, the α-naphthylmalonic acid and the α-naphthylacetic acid. These calculated values are shown in the table below. The constancy of the values of K_3/K_2 calculated from the three different pairings of the specific activities furnishes a measure of the internal consistancy of the data.

TABLE II

C^{14} Isotope Effect in the Decarboxylation of α-Naphthylmalonic acid-1-C^{14}

		K_3/K_2			
Run No.	Temp. °C	CO_2 and Diacid	Monoacid and Diacid	CO_2 and Monoacid	Average
Liquid					
1	163.0	1.074	1.070	1.072	1.072 ± 0.001
2	163.0	1.074	1.085	1.079	1.079 ± 0.005
Average	163.0				1.076 ± 0.004
Solution	85-95°				
3		1.115	1.088	1.102	1.102 ± 0.009
4	72.8	1.090	1.109	1.099	1.099 ± 0.006
5	72.8	1.083	1.110	1.096	1.096 ± 0.009
Average	72.8				1.098 ± 0.008
6	87.5	1.085	1.100	1.092	1.092 ± 0.005
7	87.5	1.106	1.089	1.098	1.098 ± 0.006
Average	87.5				1.095 ± 0.006
Average of all Soln. runs					1.097 ± 0.008

The isotope effect in the decarboxylation of liquid α-naphthyl-malonic acid-1-C^{14} at 163.0°C is thus seen to be 1.076 ± 0.004 expressed as the ratio K_3/K_2. The corresponding value in solution between 73° and ~95°C is 1.097 ± 0.008. The precision of the measurements is not great enough to show any significant temperature coefficient of the isotope effect in the solution experiments. However, there is a significant difference between the isotope effect for the liquid acid at 163°C and for the acid in solution at 73- ~95°C.

Paper Chromatography of Steroids. Chromatography of the androgens was continued. The results still show good reproducibility, but poor resolution. The compounds used were 1) dehydroepiandrosterone; 2) dehydroepiandrosterone acetate; 3) Testosterone; 4) Testosterone acetate and 5) the progestational hormone, progesterone. The results obtained in various solvents are tabulated:

TABLE III

Chromatography of Steroids

	Iso propanol	$MeOH-NH_4OH$ 95:5	$MeOH-NH_4OH$ 9:1
Cpd. 1	0.94	0.80	0.85
2	0.95	0.82	0.91
3	0.93	0.82	0.83
4	0.93	0.79	0.86
5	0.95	0.79	0.84

	MeOH-Ethanolamine 95:5	MeOH-ØH 95:5	MeOH-ØH 99:1	$MeOH-CHCl_3$ 95:5
Cpd. 1	1.00	0.86	0.80	0.82
2	1.00	0.88	0.80	0.85
3	1.00	0.84	0.82	0.83
4	1.00	0.84	0.81	0.90
5	1.00	0.67	0.81	0.86

All compounds were detected by the color produced upon exposure to iodine vapor. Ethanolamine was also tried but it did not wet the paper.

Determination of Tritium and C^{14}. (In collaboration with Dr. M. W. Biggs, Medical Physics Div.). This series of experiments were designed to develop a method by which, in a single combustion of an organic sample, both water and carbon dioxide could be collected and assayed for radioactivity. The samples were burned in a stream of oxygen, the water frozen out at the furnace mouth and the carbon dioxide trapped in sodium hydroxide. The tritium was assayed by treating the water with $LiAlH_4$ and counting the collected gas in an ionization chamber. The carbon dioxide was precipitated as barium carbonate, plated and counted in a Nucleometer.

The samples were prepared in the following manner. Alcoholic solutions of the compounds to be used (tritiated cholesterol, carbon labeled cholesterol and naphthoic-carboxyl-C^{14} acid) were prepared. An aliquot of the appropriate material was transferred to a quartz vessel and allowed to evaporate under a heat lamp. The lamp was held high enough to prevent spattering. After all sample had been introduced, the material was diluted with cholesterol and then combusted. The samples were so made up so that they covered the range from carbon labeled material containing no tritium, through varying mixtures of the two, to tritium labeled material containing no C^{14}. The labeled naphthoic acid was used to give material of a higher specific activity and to conserve the

cholesterol. The results given below are for the carbon determinations.

Series A. Cholesterol, sp. act. 6.67×10^{-3} μc/mg.

	Calc'd Sp. Act.	Found	Calcd. Act.	Found	Dev (%)
I	6.67×10^{-3}	6.25	33.35	31.23	6.4
II	6.67×10^{-3}	7.22	16.68	18.04	8.2
III	6.67×10^{-3}	6.26	50.03	46.95	6.2

The standard deviation here is 6.9 percent. The three samples were made up with 5 ml, 2.5 ml and 7.5 ml respectively of a solution containing 1 mg cholesterol/ml. For the naphthoic acid (8.25 μc/mg) the specific activities are given below, plus the volume used. The original solution contained 0.811 mg/ml.

Expt.	ml	Sp. Act. Found	Dev(%)	Expt.	ml	Sp. Act Found	Dev (%)
1	0.1	7.98	3.3	6	0.5	7.96	3.5
2	0.1	8.40	1.8	7	0.75	8.05	2.4
3	0.2	7.47	9.5	8	0.75	7.91	4.1
4	0.2	7.93	3.9	9	1.0	8.06	2.3
5	0.5	7.98	3.3	10	1.0	8.11	1.7

The standard deviation in this case was 4.2 percent. In all the tritium determinations, the standard deviation was 4.2 percent.

Low Level C^{14} Measurements. Development work on improving the reliability, increasing the sensitivity and simplifying the operation of the ion chambers used for low level C^{14} assay has continued with some success.

In very low level C^{14} analysis the α emission in chambers is both a source of error and a nuisance, since it must be subtracted out of the ion chamber current flow. This α emission has been investigated and shown to come in about equal amounts from two sources, (1) radon in the sample and (2) chamber contamination. Methods for the elimination of both have been devised.

In the current procedure for C^{14} assay 1.2 g $BaCO_3$ samples are treated with concentrated sulfuric acid and the CO_2 produced dried in dry ice cooled spiral, freed of air and expanded into an ionization chamber. An α count rate of 0.3 to 0.5 α's/min is observed in the 100 cc brass chambers. On the suspicion that radon from radium impurities in the barium could contribute part of this activity, 10 gms of $BaSO_4$ was added to one of the samples. This sample filling showed an α count of over 4 α's per minute. This radioactivity was followed over a period of time and shown to have a half life of approximately 4 days (Rn $T_{1/2}$ = 3.8 days), thus confirming our suspicion of extensive radon contamination in barium salts. Since radon has approximately the same melting and boiling point as CO_2, it would be carried along easily with CO_2. Since each radon gives three α's in going to a long half life isotope and since

about 15 α's/hour were shown to come from a "Normal" sample of 1.2 gms barium carbonate, it can be easily calculated that the radium contamination in this material would be about 4×10^{-14} gms, a not unexpected figure.

Elimination of this radon has been accomplished by several methods: (a) It has been shown that if CO_2 is fractionally distilled under vacuum, radon is selectively removed in the first fractions. The radon specific activity of a sample can be cut in about half by thus discarding the first half of the sample. (b) It has been shown that if the CO_2 is passed over activated charcol (8 mesh lumps from gas mask), almost all the radon can be absorbed while only about 1/3 of the CO_2 will be lost. (c) It was shown that almost all barium salts and commercial reagent grade calcium carbonate were extensively contaminated with radium. A systematic investigation of calcium carbonate samples prepared from many different samples of calcium chloride hydrate, reagent grade, has shown that certain of these salts are almost radium free. By precipitating carbonate sample with such carefully chosen calcium chloride radon contamination in the CO_2 sample can also be almost completely eliminated.

The α emission of a number of ion chambers has been investigated using CO_2 filling prepared from sodium bicarbonate. The results of these studies for 100 cc ion chambers are given in Table IV. Measurements were made over a 2-6 hour period.

TABLE IV

α Emission of Various Chambers

Material	α's/min
Mild steel turned from solid 2" rod	0.049, 0.051
Pure copper	0.075
Steel tubing	0.09
Aluminum, com'l pure, 99 + % ST	0.14
Aluminum alloy 2T	0.17
Brass (sample 1)	0.53
Brass (sample 2)	0.42

A number of chambers are currently being constructed of solid mild steel bar stock and will be tested. By the combination of selected calcium carbonate samples and iron chambers, the α emission problem can be virtually eliminated.

Biological Chemistry

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Metabolism of Stilbamidine-amindine-C^{14} in Mice. The localization of a large amount (20-40 percent) of the injected activity of stilbamidine-amidine-C^{14} in the livers of "A" strain mice bearing S-1 sarcomas has been reported in previous communications from these laboratories. In cooperation with Dr. J. C. Weaver of the Medical Physics Division we have been studying the localization of the radioactive material in the livers after injection in an effort to obtain knowledge of the site and method of binding of the stilbamidine, and perhaps by this method gain further knowledge of a difference between normal and tumor bearing mice. The general method of fractionation is the 0.25 M sucrose method of Schneider and Hogeboom (J. Biol. Chem. 183, 123 (1950)) and our adaptation of it has been described more completely in the previous quarterly report.

As was pointed out in the last report, the radioactivity appears to be associated largely with the mitochondrial fraction of the liver, or at least with a particle of very similar physical properties. Recent experiments have been designed to test further this apparent association of radioactivity with mitochondria. After homogenization of the liver and removal of the nuclear fraction and unbroken cells by centrifugation in a clinical centrifuge, aliquots of the supernatant were centrifuged at various speeds in the 40 rotor of a Spinco centrifuge, and analyses performed on the washed precipitate and supernatant for radioactivity, PNA (ribonucleic acid), nitrogen, and succinic oxidase activity (the latter is believed to be associated with the mitochondria and thus serves as an indicator for the separation of mitochondria). The data obtained from two series of experiments is given in Table V.

Within the limits of the experiment, it appears that the radioactivity is associated with the succinic oxidase activity (therefore the mitochondria). It appears that the radioactivity and percent N in the precipitate have a close correspondence when experessed as percent of that which will come down at 9000 RPM. The nitrogen in this fraction represents approximately 25 percent of the total liver nitrogen. The fact that the percentage PNA centrifuged down at speeds less than 9000 RPM appears to be less than the percent of radioactivity (expressed as percent of total spun down at 9000 RPM) can be explained on the basis of a small amount of the "microsome" fraction which is rich in PNA contaminating the precipitate at 9000 RPM. The data given in Table V is good evidence, we believe, that the radioactivity is bound to the mitochondria.

Qualitative experiments indicated that dilute hydrochloric acid or ammonium hydroxide would dissociate the radioactivity from the precipitate which is formed, whereas at neutral pH the radioactivity is not dialyzable. It is therefore difficult to further study the nature of binding of the radioactivity to the mitochondrial fraction. We have attempted to get the mitochondria "into solution" by several methods. One is the sonic disintegration method of disintegrating mitochondria described by Hogeboom. In this method, the mitochondria

TABLE V

Fractionation of Mice Liver Homogenates after Removal of Nuclei at Various Speeds in Spinco Centrifuge

	Speed RPM for 15 min.	% C^{14} In Supernatant	% C^{14} in Ppt.	% C^{14} in Ppt.*	% Succinic Oxidase in Ppt.*	% N in Ppt.*	% PNA in Ppt.*
Exp. I	1800	84	16	18	20	29	--
	3750	44	56	63	60	69	--
	5300	25	75	85	79	80	--
	7000	14	86	96	100	95	--
	9000	11	89	100	80	100	--
Exp. II	1800	85	15	18	25	11	12
	3500	47	53	60	82	65	40
	4400	31	59	66	87	73	50
	5200	21	79	88	92	82	63
	9000	11	89	100	100	100	100

* Expressed as percent of maximum amount found in precipitate.

in sucrose or in pH 7,8 phosphate buffer are subjected to 9 Kc sonic vibration in a sonic oscillator (Raytheon) for approximately 1 hour. Subsequently the material is recentrifuged at 10,000 RPM for 15 minutes and at 40,000 RPM for 30 minutes. The analytical data obtained is given in Table VI.

TABLE VI

Disintegration of Mitochondria by Sonic Vibrations

Fraction	% of C^{14}	% of Nitrogen	% of PNA
Run I in 0.25 M Sucrose			
Ppt. at 10,000 RPM for 15 minutes	27	11	11
Ppt. at 40,000 RPM for 30 minutes	60	46	74
Supernatant	13	33	15
Run II in pH 7.8 PO_4 buffer			
Ppt. at 10,000 RPM for 15 minutes	24	12	8
Ppt. at 40,000 RPM for 30 minutes	56	37	70
Supernatant	17	51	22

By this method a large proportion of the mitochondria are disintegrated to material of smaller size since it could no longer be centrifuged down at 10,000 RPM. However, 80-85 percent of the radioactivity is still associated with material large enough to be centrifuged down at 40,000 RPM. The supernatant contains 35-50 percent of the nitrogen present in the mitochondria, presumable largely as soluble proteins. Several papers have appeared expressing the view that the mitochondria have a membrane. Our data can be interpreted on the basis of a membrane to which the stilbamidine is bound.

The second method of disintegration attempted is adapted from that described by Fraser (Nature, 1951). The mitochondria in pH 7.8 buffer or in sucrose are subjected to a pressure of 700-800 pounds of nitrous oxide. This pressure is released rapidly, thus breaking the mitochondria open much like a balloon. This method has been shown to inactivate bacteria and possibly make them more permeable. From the data in Table VII, it does not appear that the particles are broken as completely by this method since much more of the material is centrifuged down at 10,000 RPM than is centrifuged down after sonic disintegration under similar conditions. However, nearly as much soluble nitrogen is present in the supernatant.

TABLE VII

Disintegration of Mitochondria by Release of Gas Pressure

Fraction	% of C^{14}	% of Nitrogen	% of PNA
Run I in 0.25 M Sucrose			
Ppt. at 10,000 RPM for 15 minutes	66	29	22
Ppt. at 40,000 RPM for 15 minutes	28	34	69
Supernatant	5	37	9
Run II in pH 7.8 PO_4 buffer			
Ppt. at 10,000 RPM for 15 minutes	67	31	45
Ppt. at 40,000 RPM for 15 minutes	23	26	41
Supernatant	10	42	13

By both methods of disintegration, the nucleic acid is largely (more than 80 percent) associated with material separable by centrifugation; however there is not a fixed ratio of percent of nucleic acid/percent of radioactivity. At the present time, we favor the view that the mitochondria has a membrane either filled with a soluble protein material or filled with soluble protein plus small particulate material. By either method of disintegration, the protein is released into the solution, and by sonic disintegration the membrane (or membrane plus particulate material) is broken into smaller (but still centrifugable) particles. The radioactivity is associated with this particulate material and/or membrane.

At the present time, we are engaged in other methods, including enzymatic, of studying the binding of stilbamidine in mitochondria and a study of the lipid fraction.

Metabolism of Labeled Purines. Studies with 8-azaguanine-4-C^{14} and 8-azaadenine-4,6-C^{14} have shown that essentially all of these compounds are rapidly excreted in the urine after intraperitoneal injection into C-57 or "A" strain mice. (The 8-azaguanine has been shown to consist essentially entirely of 8-azaxanthine and some 8-azaguanine as shown by coincidence of radioactivity and fluorescent spot visible under ultra-violet light after chromatography on filter paper with synthetic 8-azaxanthine using either butanol-propionic acid-water or butanol-ethylene glycol-hydrochloric acid as the solvent systems.)

8-Azaadenine yields mostly 8-azaisoguanine and unchanged 8-azaadenine in the urine. The amount of 8-azaadenine present in the excreted urine decreased with time, i.e., urine excreted 8-24 hours after injection contains mainly 8-azaisoguanine. This latter compound is also present in large amounts in the kidney and can readily be extracted by cold 10 percent trichloroacetic acid. The identity of these compounds was shown by chromatography in the above named solvents and comparison with the synthetic compound.

The sampe compounds are obtained after injection of 8-azaguanine or 8-azaadenine into Slonaker rats as after injection into mice.

Chicken Feeding Experiments. In the last report the specific activities of the cholesterol, protein, phospholipid and some of the glycerol of the various yolks were given. Since then the activities of the remaining glycerols and of all the fatty acid fractions have been determined (see Table VIII).

TABLE VIII

Activity in Egg Fractions

Yolk No.	Sp. Act. Glycerol $\mu c/mg \times 10^3$	Sp. Act. Fatty Acids $\mu c/mg \times 10^3$	Total Activity All Fractions Microcuries
1	3.00	0.78, 0.74	3.6
2	0.60	3.00, 2.60	12.9
3	1.90	7.86	34.8
4	3.00	14.7	64.6
5		26.8	111.1
6	3.80	26.7	118.5
7		24.1, 26.1	130.6
8		18.3	63.9
9		13.3	27.0
10		6.29	8.2

In the case of the yolk fatty acids, the specific activities have been determined by combusion to CO_2, plating the collected barium carbonate, and counting in a Nucleometer. As had previously been noted, the initial hydrolysis has been re-saponified and the acids thus obtained assayed for activity. The specific activities are in good agreement with those obtained from the first saponification. The yolk specific activities are listed above.

The theoretical activities of all fractions were calculated, based on the percentage of activities given by Romanoff and Romanoff (The Avian Egg, John Wiley and Sons , Inc., N. Y., 1949, p. 338). The percentages used were 33 percent for protein, 3.3 percent for cholesterol, 22 percent for phospholipid 30.2 percent for fatty acids and 9.5 percent for glycerol. The values thus obtained can be plotted to give a smooth curve with the peak at egg 7, as might be expected for continuing accumulation of activity in all fractions. In the initial yolk combustion, the low value for total activity of yolk No. 5 was cause of some concern and was finally attributed to an error in combustion. The data have borne out this conclusion.

Cholesterol Metabolism in Rats. Twenty milligrams of cholesterol (4.6×10^{-2} $\mu c\ C^{14}/mg$) dissolved in 1 ml of Wesson Oil was administered by stomach tube to each of two rats, which had been fasted for 48 hours prior to the experiment. The syringe was rinsed with an additional 0.5 ml of oil. The rats were placed in metabolism cages where their breath, urine and feces were collected. They were given a feed on which p-aminobenzoic acid had been spread (1 g. PAB/14 g. feed). After four days the rats were sacrificed, the liver and adrenals extirpated and the carcass dissolved in 40 percent KOH.

The breath was collected at 30 minute intervals during the first 2-1/2 hours of the experiment, a one hour sample was then taken, and a 1-1/2 hour sample. For each of the next three days, one hour samples were taken twice a day. There was activity in the carbon dioxide in all breath samples. No rate curves have been constructed as yet.

The adrenals were dissolved in 40 percent KOH and the fatty acid and non-saponifiable fractions separated.

Rat A			
Non-Sap.	15.2 mg	4384 dis/min/mg	3.03×10^{-2} μc
Fatty Acid	35.5 mg	1062 dis/min/mg	1.73×10^{-2} μc
Rat B			
Non-Sap.	18.3 mg	2736 dis/min/mg	2.28×10^{-2} μc
Fatty Acid	18.0 mg	2126 dis/min/mg	1.73×10^{-2} μc

The livers were homogenized and lyophylized. The dried livers were extracted with acetone for 24 hours. The acetone extracts were concentrated and extracted with 2 percent aq. sodium carbonate to give the free fatty acids (FFA), the non-extractable material was saponified with 10 percent alc. KOH to give the fatty acid (FA) and non-saponifiable (NS) fractions. The residues

remaining after acetone extraction were extracted with ether for 12 hours to give the phospholipid (PL). The residues were dissolved in 30 percent KOH and the glycogen precipitated with alcohol and saturated sodium sulfate solution. The glycogen was reprecipitated.

TABLE IX

Distribution of Carcass Activity from Labeled Cholesterol

Fraction	mg	dis/min/mg	c x 10^{+2}
Rat A			
FFA	76.6	699	2.43
FA	21.2	2041	1.97
NS	80.3	10783	39.4
PL	7.3	4460	1.45
Glycogen	4140	3.43	0.646
Rat B			
FFA	116.7	698	3.7
FA	24.2	2660	2.93
NS	52.4	718	1.71
PL	4.3	2.54 x 10^4	7.08
Glycogen	970	18.66	0.823

The feces were homogenized with chloroform and then extracted with ether for eight hours. The extracted material was dried, counted and combined for further separation into fractions. The activities are given below. In the case of Rat B, the total activities seem too high. This will be checked upon further fractionation.

The urine samples were made basic to phenolphtalein and extracted with ether for five hours to give neutral material. The urine was then acidified and extracted with ether for 24 hours. The ether extracts were concentrated and the dried, weighed material plated and counted in the Nucleometer.

Metabolism of Propionic Acid in vitro. The study of the metabolism of propionic acid by *in vitro* mouse liver slices has been concluded with positive identification of the one remaining major metabolite β-hydroxyvaleric acid and degradation of this material to show the path of its formation. An authentic sample of β hydroxyvaleric acid was synthesized and run through a silicic acid column with the unknown activity. Coincidence of acidity and activity occurred. The mixture was acetylated and again the acetylated activity coincided with the β acetylvaleric acid on a silicic acid chromatogram. The p-toluidide was prepared and it recrystallized to a constant specific activity.

TABLE X

Activity in Feces from Labeled Cholesterol

Day	Wt. Feces (g.)	Wt. Extract (g.)	Dis/min/mg	μc
Rat A				
1	1.0342	0.3982	3.6×10^5	0.16
2	0.7971	0.2184	8.1×10^4	0.04
3	0.2780	0.0423	1.1×10^5	0.05
4	0.4514	0.2482	2.8×10^4	0.01
Rat B				
1	0.5830	0.2265	4.6×10^5	0.21
2	0.4000	0.1375	4.8×10^5	0.22
3	2.4178	0.2451	2.2×10^5	0.10
4	3.4366	0.1728	7.1×10^5	0.32(?)

TABLE XI

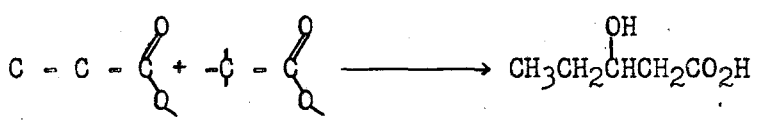
Activity in Urine from Labeled Cholesterol

Day	Wt. (mg)	dis/min/mg	μc x 10^{+3}
Rat A			
1N	102	863	40
1A	52.3	173	4.1
2N	14.9	292	2.0
2A	15.6	204	1.4
3N	11	209	1
3A	9.2	2026	8.47
4N	29.2	576	7.65
4A	20.1	1335	12.2
Rat B			
1N	55.8	3208	81
1A	149	306	21
2N	11	948	4.7
2A	7	437	1.4
3N	5.1	243	0.56
3A	4.0	5100	9.1
4N	30.1	56	0.77
4A	11.6	3759	19.8

Mouse liver incubations were run with α and β labeled propionate to obtain more active β hydroxyvaleric acid for degration. Lactic acid was also isolated and degraded from these incubations. Malonic acid was added as an inhibitor to some of the flasks. In these cases less CO_2 was evolved and less activity was incorporated into water soluble compounds. Paper chromatography showed a slight relative increase in the dicarboxylic acids formed when malonate was present.

The degradation of the lactic acid from propionate-α-C^{14} feedings confirmed previously reported results which showed an equivalence of activity (α, 39 percent; β, 39 percent) between the α and β carbons. The lactic acid from an inhibited incuation with α labeled propionic acid plus 0.005 M malonate appeared to have 49 percent of the activity in the α position and 31 percent in the β position but whether this is actually a significant difference would have to be confirmed.

The degration data on β hydroxyvaleric acid formed from α labeled propionic acid showed oxidation with chromic acid to give some active CO_2 (2-6 percent) and a steam distillable activity (70-80 percent) which was mainly propionic acid. This propionic acid gave CO_2 on treatment with NaN_3 which represented the carboxyl group and contained 2 percent ± 5 percent of the activity. Another portion of the propionic acid was converted to lactic acid and degraded. This showed 105 percent of the activity in the α carbon and 2 percent in the β carbon. These results indicate the propionic acid acts directly as a propylating agent to give β-hydroxyvaleric acid:



Photosynthesis Chemistry

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V. Lynch and W. Stepka

Elucidation of the initial chemical processes of photosynthesis has been approached in a variety of ways in this laboratory. The most fruitful of these approaches has been that of chemical identification and degradation of labeled intermediates followed by a correlation of the accumulated information. Kinetic studies of $C^{14}O_2$ fixation have demonstrated the sequence of formation of the important intermediates. Comparative biochemical studies have demonstrated the ubiquity of several important reactions and groups of intermediates. With the viewpoints engendered by the results we have attempted to choose the most fundamental problems which are soluble with the available methods.

Previous reports have described the techniques developed in this laboratory for these investigations. While more detailed analyses are being continually performed the results of current kinetic data will be summarized.

In photosynthesis at 20°C the earliest intermediates which accumulate in finite concentration are phosphorylated sugars and carboxylic acids. While phosphoglyceric acid and malic acid are the first compounds to accumulate, it is clear that a number of subsequent reactions follow immediately. The rapid production and saturation of labeled sedoheptulose and ribulose phosphate reservoirs from the C_3 and C_4 compounds indicates that they are probably intermediates in CO_2 reduction. The similar structures of these ketoses and their configurational difference from sucrose and starch suggests that sedoheptulose and ribulose are involved in the cycle for the regeneration of the two carbon CO_2 acceptor required in photosynthesis.

The amounts of C^{14} incorporated as a function of time in the phosphorylated reservoirs of these sugars is shown in Fig. 1. While it cannot yet be deduced from these data that sedoheptulose synthesis precedes ribulose or even fructose synthesis it is clear that the formation of the three ketose phosphates precedes saturation of the glucose phosphate reservoirs. It is expected that the results of experiments now in progress will increase our understanding of these concurrent processes.

Isolation of ribulose and Sedoheptulose Phosphates from C^{14} or P^{32}-labeled Plant Extracts. Two dimensional chromatography on acid washed filter paper results in separation of the major products of short photosynthesis. Phsophatase hydrolysis of these isolated compounds or even of partially separated mixtures yields the free sugars which can readily be separated and identified by rechromatography. One major sedoheptulose monophosphate has been isolated in radiochemically pure form and a minor sedoheptulose monophosphate has not been separated from glucose monophosphate. Relatively little evidence exists at present for the biosynthesis of detectable amounts of sedoheptulose diphosphate.

Ribulose results from the phosphatase hydrolysis of two C^{14}-labeled phosphate esters. The relative amounts of the mono-and diphosphate esters

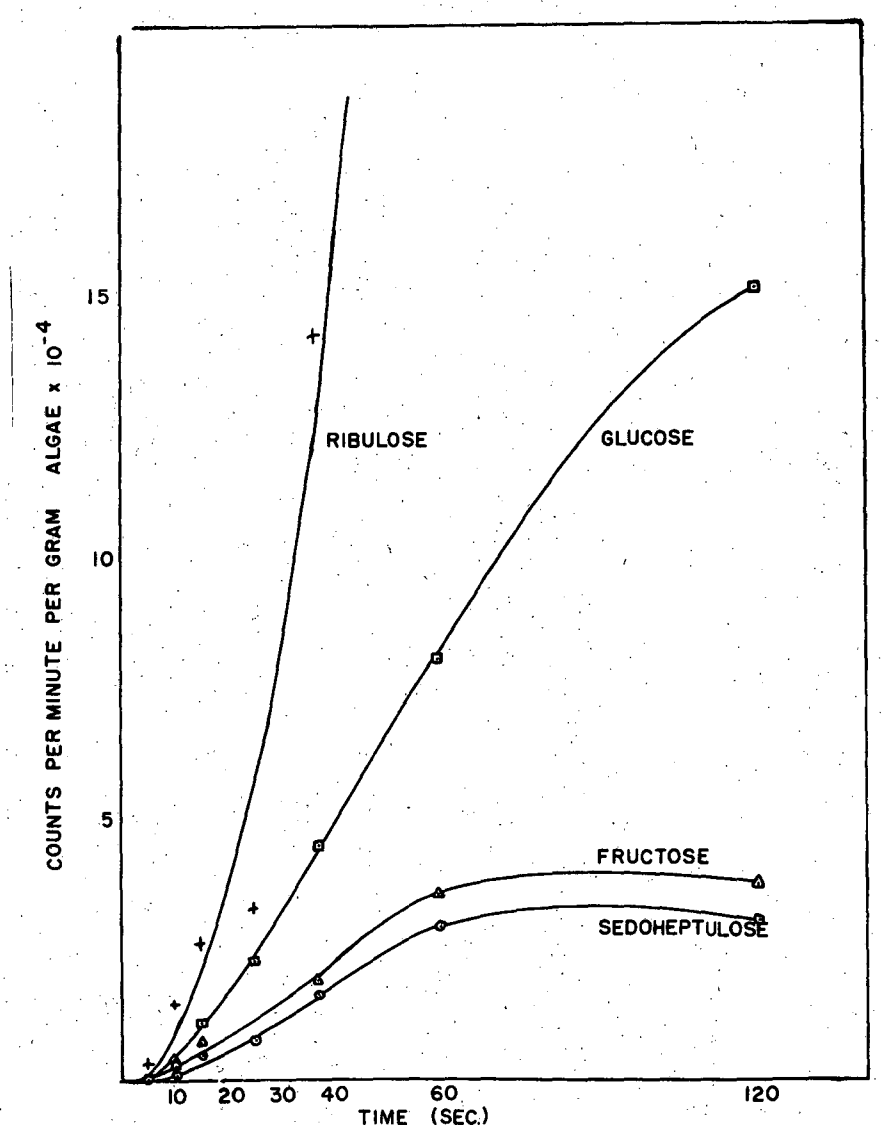

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Fig. 1

Steady State photosynthesis of the major sugar phosphates by Scenedesmus at 20°C and 8000 fc. Ribulose was determined by direct counting of ribulose diphosphate which is almost pure. Glucose, fructose and sedoheptulose were determined by direct counting of phosphatase hydrolysates of the chromatographically isolated esters.

varies with the type of plant or microorganism and varies greatly with age and experimental conditions in Scenedesmus. Scenedesmus is the only plant for which the effect of age has been investigated. Barley, alfalfa and soy bean leaves form ribulose monophosphates to a much larger extent than they do the diphosphate.

No plants have yet been investigated in this laboratory which do not synthesize phosphates of these two sugars. This includes plants from Rhodospirillum rubrum to alfalfa. Experiments with several species of succulent plants have been performed but no unusual accumulation of sedoheptulose monophosphate has been observed.

A general survey of readily available plant material is in progress for the purpose of isolating and classically characterizing these hitherto unknown compounds. Separation of the monophosphate esters will be more readily accomplished if the starting material is rich in the desired compound and poor in difficulty separated impurities. A report of the isolation of ribulose diphosphate by ion exchange separation will be described below.

Identification of Sedoheptulose Monophosphate. The observed separation of this sugar from chromatograms of photosynthetic $C^{14}O_2$ fixation by barley and Rodospirillum rubrum several years ago has lead to its identification as sedoheptulose. Initially it was observed in the free state only when glucose, fructose and glyceric acid appeared in unphosphorylated form. Since the amounts of the free sugars were never found to exceed those found as phosphates in similar experiments, it was deduced that they were probably present in the plant. Subsequent experiments showed that the method of killing affects the amounts of glyceric acid in the extract but since a satisfactory organism for a controlled experiment has not been available a direct proof has not been possible.

A number of experiments were performed with sedoheptulose so isolated. It was shown to have different chromatographic coordinates [R_f (phenol)=0.42; R_f (butanol propionic acid)=0.17] from those of D-mannose and L-sorbose.

A survey of known sugars and glycitols were investigated without finding one with corresponding properties. Simultaneously a chemical investigation of the unknown compound was begun. It was found resistant to bromine oxidation, showing it is a ketose. It did not epimerize rapidly in pyridine nor did it oxidize readily with dilute nitric acid. The major chemical property was its very rapid conversion in hot, dilute mineral acid to another compound, sedoheptulosan. The chromatographic properties of sedoheptulosan [R_f (phenol)=0.69; R_f (butanol-propionic acid)=0.35] differ markedly from any known sugars except that of glyceryl galactoside.

The compound later identified as sedoheptulose was found to resist alkali. Its anhydride contained free hydroxyl groups since it could be acetylated. Neither the acetate nor the anhydride nor the sugar had any basic groups since their distribution constants were unaffected by changes in pH. The sugar itself was unfermentable by Lactobacillus. Its dinitrophenylosazone was found to cocrystallize with that obtained from glucose, but extensive purification was not attempted.

The equilibrium constant of the dehydration to give sedoheptulosan was found to be 4.0 as reported by LaForge and Hudson (J. Biol. Chem., 30 61, (1917). This was determined by elution of both compounds from a chromatogram of an equilibrium mixture and counting aliquots of the eluates. The reversibility of the system was ascertained by heating the isolated anhydride in acid. An approach to the same equilibrium was again observed.

A chemical degration of the compound was then undertaken using periodic acid to obtain fragments characteristic of various structural elements of the compounds. For such an undertaking to be of value it was necessary to degrade uniformly labeled sedoheptulose. It was found that soy bean leaves have a higher steady state concentration of sedoheptulose monophosphate relative to that of the other monophosphates than any of the plants investigated in this laboratory. Available soy bean leaf data indicated that five minutes of $C^{14}O_2$ photosynthesis should suffice to obtain uniformly labeled sugars. The degradation of such sedoheptulose is reported below.

Degradation Studies. Various degradations of C^{14}-labeled products of photosynthesis have been carried out with periodic acid. Originally these studies were made to aid in the identification of the unknown sugars. After the identification of these compounds the studies were continued in order to gain information regarding the sequence of C^{14} introduction into the various carbons of the molecule.

In general the procedure has been to add to the unknown compound, containing a known number of disintegrations per minute, a small amount of periodic acid or sodium periodate and a measured small quantity of unlabeled formic acid, formaldehyde and sometimes carbon dioxide (as $BaCO_3$). After a suitable length of time (conditions for specific compounds will be reported at a later date) the formaldehyde is distilled from the reaction mixture at a neutral pH into a dimedon solution from which formaldimedon is precipitated, recrystallized and counted. The original reaction mixture is then acidified and the formic acid distilled out and into another flask after which it is recrystallized as barium formate and counted. The residue in the reaction flask is taken up in water, an aliquot is counted, and the residual activity may be subjected to chromatography or chemical tests.

The results of several such degrations are indicated in Table XII.

Degradation studies are now in progress with sedoheptulose and sedoheptulosan from shorter periods of photosynthesis in which case the labeling of these compounds is non-uniform.

Catalytic Hydrogenation of Chromatographically Separated Compounds. Another approach to the identification of such unknowns was the study of the products of catalytic hydrogenation of materials on extremely small scale. A summary of this work is given below.

Uniformly labeled fructose eluted from chromatograms of plant extracts was hydrogenated at 1000 to 2000 psi at 150° in a 1 ml stainless steel bomb with Raney nickel catalyst. While mannitol was obtained as the only product a large portion remained adsorbed upon the catalyst. The temperature was

TABLE XII

Degradation of Sugars

Fructose from hydrolysis of sucrose from 5 min. photosynthesis in sugar beet (uniform labeling)

$BaCO_3$	6%	(partial carbon 2)
Ba formate	51%	(carbons 3, 4, 5)
Formal dimedon	22%	(carbon 6, partial carbon 1)
Residue in flask	20%	(partial C_1, C_2 as glycolic acid)

Glucose (5 min. photosynthesis in soy bean, uniform labeling)

$BaCO_3$	0	
Ba formate	83%	(carbons 1, 2, 3, 4 5)
Formal dimedon	16.6%	(carbon 6)
Residue in flask	0	

Sedoheptulose (5 min. photosynthesis in soy bean, uniform labeling)

$BaCO_3$	2%	
Ba formate	55%	(carbons 3, 4, 5, 6)
Formal dimedon	14.4%	(carbon 7)
Residue in flask	25%	(carbon 1, carbon 2)

Sedoheptulosan (5 min. photosynthesis in soy bean)

Ba formate	14.2%	(carbon 4)
Residue in flask	88%	(carbon 1, 2, 3, 5, 6)

reduced to 100°C and it was found that Adams catalyst, platinic oxide gave quantitative yields of the expected products with fructose, rhamnose, L-sorbose and authentic sedoheptulose. The products were chromatographed two dimensionally and detected with methanolic Tollens reagent. This reagent was developed for the purpose of alleviating the difficulties of diffusion of the spots with the usual aqueous reagent. The black silver images were developed at room temperature or at 100°, the former being preferable in some instances. By rinsing the developed chromatogram with water followed by hypo and washing, black spots on white backgrounds were obtained which were well suited to comparison with radiograms.

Labeled sedoheptulose and ribulose were hydrogenated at 120°C, 2000 psi over Adams catalyst in the presence of 100-200γ of fructose carrier. The products were co-chromatographed with sedoheptitol and ribitol respectively. Exact concurrence was observed. Hydrogenated ribulose was co-chromatographed with added arabitol and found to be partially separated. This demonstrated that the unknown compound was not xylulose. This was also demonstrated when the labeled ribulose and added xylulose failed to co-chromatograph.

Identification of Sedoheptitol. Sedoheptitol purified from traces of sorbitol after catalytic hydrogenation was oxidized with periodic acid. The ratio of barium formate to formaldimedon activity was found to be 3.1. The original sedoheptulose had been isolated from a five minute photosynthesis experiment with a soy bean leaf and was expected to be uniformly labeled. The calculated values for linear glycitols are 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0. Since any deviation from uniformity of C^{14} distribution is expected to be in the direction of less active-CH_2OH groups the evidence for an octitol was discounted and that for a heptitol considered possible. No known hexitols had chromatographic positions corresponding to that of the unknown glycitol. Its R_f was less than that of the hexitols and closely approximated that of glucose and sedoheptulose. Finally, co-chromatography of the unknown with sedoheptitol prepared similarly from authentic sedoheptulose demonstrated their chromatographic identity.

Determination of Phosphorus Content of Phosphate Esters by Double Labeling Technique. The experiments described in a previous report have been extended to alleviate the major difficulty encountered. This difficulty was the slowness of saturation of the hexose phosphate reservoirs with C^{14}. While phosphoglycerate was rapidly equilibrated, glucose monophosphate was quite slow. The rate of saturation is a different order of magnitude than the rate of attainment of uniformity of C^{14} distribution within the molecule. This is due to the fact that the equilibria determining saturation of a given hexose phosphate reservoir are quite far separated from those which affect and lead to the distribution in C_3 compounds.

P^{32}-labeled Scenedesmus were prepared by equilibration of 1 gram of cells with 2.0 mc of radiophosphate for 16 hours at moderate light intensity. $C^{14}O_2$ was circulated through an illuminated aqueous suspension of the cells for 35 minutes. The cells were then killed and chromatographed in the usual manner. The separated phosphate esters were eluted and samples counted for both C^{14} and P^{32} at a time when both activities were nearly equal and optimum counting accuracy could be obtained. The absorber used reduced the P^{32} activities by about 3 percent. The results are given in Table XIII.

TABLE XIII

35 Minute Photosynthetic $C^{14}O_2$ fixation by P^{32}-Saturated Scenedesmus

Compound	$C^{14}P^{32}$ Measured	Adjusted $C^{14}P^{32}$
Phosphoglyceric Acid	1.35 1.5	3.0
Fructose + Sedoheptulose Monophosphates*	2.63 2.62	5.8
Glucose Monophosphate	2.35 2.24	5.1
Ribulose Diphosphate	0.84 0.92	2.0

(*) These compounds were not separated chromatographically and exist in almost equal concentrations in this alga under these conditions.

It is readily seen that if one would assume that the glucose monophosphate reservoir were saturated, the ratio for sedoheptulose monophosphate would approximate 7.0 and that for ribulose diphosphate would approach 2.5 which are the calculated values. Experiments of this type are being used for a variety of such measurements as well as for direct determination of specific radioactivities.

Identification of Ribulose Phosphates. A compound observed during the first few seconds of $C^{14}O_2$ photosynthesis in all plants investigated in this laboratory has now been identified as ribulose diphosphate.

The diphosphate ester occupies a paper chromatographic position near that of fructose and glucose-1,6-diphosphates and 2,3-diphosphoglyceric acid. A monophosphate ester which gives the same labeled sugar upon phosphatase (Polidase") hydrolysis occupies a chromatographic position intermediate between triose phosphates and the hexose monophosphates. In young cultures of Scenedesmus the concentration of the diphosphate approaches that of phosphoglycerate.

The chromatographic position of the radioactive sugar [R_f (phenol)=0.60; R_f (butanol-propionic acid-water)=0.27] corresponds exactly to that of ribulose prepared by epimerization of ribose or aribinose in pyridine. No common hexoses or tetroses have such a position.

The radioactive sugar resists bromine but is cleaved by oxygen, particularly under basic conditions such as in diethylamine solutions or on anion exchange resins. Radioactive glycolic, glyceric and polyhydroxy acid (presumably erythronic) are obtained upon air oxidation. The products are those expected from

ribulose oxidation. The labeled diphosphate was found to be oxidized by air in diethylamine solutions to give phosphoglyceric and phosphoglycolic acids as major products. These were identified by chromatography of the hydrolysis products which were found in the expected positions of glyceric acid and glycolic acid. Phosphoglycolic acid observed as a minor radioactive product of $C^{14}O_2$ photosynthesis has been separated and identified. Oxidation during chromatography may provide one source of this phosphoglycolic acid.

Whether the phosphoerythronic acid observed in radiograms of plant extracts arises from oxidation of ribulose diphosphate or whether it is a metabolic intermediate is open to question. Hs apparent concentration in the cell is always lower than that of phosphoglycolic acid. This corresponds to the observed fact that ribulose diphosphate decomposes to give largely phosphoglycolic and phosphoglyceric acids and a smaller amount of phosphoerythronic acid.

The radioactive sugar was epimerized in pyridine. Co-chromatography of the resultant mixture with ribose and arabinose showed identity of the two major radioactive products with the added sugars. The radioactive 2,4-dinitrophenylosazone of D-arabinose was prepared with a tracer quantity of the labeled ribulose. It was found to have the calculated specific activity and this was undiminished by repeated recrystallizations from methyl cellosolve.

The radioactive sugar was catalytically hydrogenated with Adams catalyst and the product was found to co-chromatograph with added ribitol but not with arabitol.

The foregoing observations lead to the conclusion that the radioactive compounds isolated from plants are ribulose 1, 5-diphosphate and monophosphate.

An examination of the kinetics of formation of this compound from $C^{14}O_2$ during steady state photosynthesis is in progress. Although its probable relation to the cycle for generation of two carbon CO_2 acceptors is obvious, present work is designed to supply necessary information for an understanding of its role.

Paper Chromatographic Isolation of Ribuluose Diphosphate. Of all major photosynthetic products in the first several minutes ribulose diphosphate has the lowest R_f value on paper developed with water-saturated phenol. This is the basis for a moderate scale separation of the diphosphate. A concentrated extract of *Scenedesmus* which had photosynthesized in $C^{14}O_2$ for 2.5 minutes was applied *in a strip* on washed Whatman No. 1 filter paper and developed with phenol. A number of suitable organic dyes were used to follow movement of the solvent since it dripped off the serrated edge. After 24 hours separation was satisfactory for elution but since the separation could be improved with longer development, several days to one week was allowed. After a week the ribulose diphosphate had moved 5-6 cm while phosphoglycerate have moved 24 cm. In such unidimensional chromatography the other components of the diphosphate area were not separated.

The diphosphates separated in this way were eluted by extraction of the cut out strips. The paper was well washed in a funnel and the filtrate concentrated *in vacuo*.

Comparison of Photosynthesis in Various Organisms. The path of carbon in photosynthesis by green algae, leaves of higher plants and a photosynthetic purple bacterium has been extensively studied. Another organism, Euglena gracilis, has been added to this list. Euglena gracilis is a unicellular motile chlorophyll-containing organism, claimed by both the botanists and protozoologists. It was grown in the light on a complex organic media containing acetate, tryptone and inorganic salts. After three days growth the cells were removed from the growth medium by centrifugation and resuspended in distilled water. These cells were then exposed to light for one minute in the presence of radioactive carbon dioxide and killed by boiling 80 percent ethanol. Of the total activity fixed 85 percent was soluble in 80 percent ethanol. The soluble fraction was concentrated and the compounds present separated by two dimensional chromatography. Organic phosphates accounted for 93 percent of the soluble activity. When these compounds were treated with polidase the principle products of hydrolysis were glucose, fructose and glyceric acid. Aspartic acid was the other major product of photosynthesis. Free glucose, fructose or sucrose were not found in the extract. Even after five minutes of photosynthesis no soluble carbohydrates were found.

The phosphates formed by Euglena during photosynthesis are similar to those found by other photosynthetic organisms. Although sugar phosphates are found, no free sugars are formed. The insoluble fraction has yet to be investigated for radioactive polysaccharides. The formation of sugars by these organisms during photosynthesis is of interest since they are unable to use carbohydrates for growth.

The path of carbon during photosynthesis by Synechococcus cedorum, a unicellular blue green alga, has also been studied. Again the products formed during five minutes of photosynthesis were very similar to those formed by other photosynthetic organisms. Of the soluble products formed after five minutes of photosynthesis 61 percent of the activity was found in phosphorylated compounds. The amino acids, aspartic acid, glutamic acid, serine, glycine and alanine, were radioactive. No free sugars were found to be radioactive.

These results show that the basic path of carbon in photosynthesis is similar in all of the photosynthetic organisms so far studied.

Dark Carbon Dioxide Fixation. When resting cells of non-photosynthetic organisms are exposed to radioactive carbon dioxide the main radioactive products formed are those involved in the di- and tri-carboxylic acid cycles (see last quarterly report). The fixation of carbon dioxide by photosynthetic organisms in the dark was investigated to see whether the dark CO_2 fixation reactions were the same or different than those for non-photosynthetic organisms. The compounds formed and the percent of the total non-volatile activity fixed are given in Table XIV. The compounds formed are those acids involved in tri- and di-carboxylic acid cycle or amino acids readily derived from them. The distribution of activity is similar to that found in the non-photosynthetic organisms. Therefore the dark fixation of carbon dioxide is the same by both photosynthetic and non-photosynthetic organisms.

The fixation of carbon dioxide by photosynthetic organisms differs strikingly in the light and dark. In the dark over 90 percent of the carbon dioxide fixed is found in compounds closely related to the tri- or di-carboxylic acid cycle and no activity is found in organic phosphates. During a one minute period of photosynthesis the majority of the carbon activity is found in organic phosphates.

TABLE XIV

Radioactive Compounds Formed

% of Total Non-volatile Activity Fixed

Organism	Aspartic	Glutamic	Succinic	Malic	Fumaric	Citric
Scenedesmus	34	44	0.7	7	0.4	14
Chlorella	10	43	7	21	5	4
Synechococcus	15	36	18	16	2	-

II QUARTERLY PROGRESS REPORT Project 48B

W. M. Latimer, Director

A. Metals and High Temperature Thermodynamics

Leo Brewer, LeRoy Bromely, G. Elliott, Russell Edwards, Norman Leroy, and James Robbers.

Oxide Phase Diagrams. Work is proceeding on the study of the Na-O and K-O systems.

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

High Temperature x-ray Studies. Considerable evidence has been obtained for the non-existence of lower oxides of silicon and aluminum in the solid states. A report is being written which will give the details of this work.

Gaseous Oxides. An accurate value of the heat of formation of SiO(g) has been determined from the available data and a detailed report of this work is being written up. Papers on the stability of gaseous oxides of Al and the gaseous alkali and alkaline earths are being published in the August issues of the Journal of the American Chemical Society and the Journal of Chemical Physics. A paper has been sent to the Journal of Metallurgy and Ceramics discussing the purification of NCl with respect to oxide impurities by distillation.

Refractories. The paper summarizing the results of the investigation of refractory boride systems has been published in the June issue of the Journal of the American Ceramic Society. A paper summarizing the fabrication of refractory sulfide crucibles has been published in the April issue of the Journal of the American Ceramic Society. A paper on the preparation and properties of oxide-sulfides of Ce, Zr, Th, and U is to be published in the July issue of the Journal of American Chemical Society.

Stability of Gaseous CN at High Temperatures. This work has been completed and a paper summarizing the work was published in the May issue of the Journal of the American Chemical Society.

Heat Transfer in Forced Convection Film Boiling. A liquid proof enclosure has been constructed around the apparatus. An exhaust system has been installed to decrease the operational hazards, due to the toxicity and the flammable nature of the liquids involved, by the removal of their vapors from the immediate area. An automatic carbon dixode extinguishing system has been installed within the enclosure.

The experimental work has been completed for determining the heat transfer coefficients for the film boiling of ethyl alcohol from 3/8, 1/2, and 5/8 inch diameter graphite heating tubes. The measurements covered a liquid

velocity range from 0. (free convection) to 14.6 ft/sec and a temperature range for the heating surface from 700°F to 1300°F. The results showed overall heat transfer coefficients from 47.6 to 148.1 BTU/HR-FT^2-°F. The values due to convection along were from 42.3 to 138.6 BTU/HR-FT^2-°F respectively. The values of the heat transfer coefficients decrease with increasing tube diameter; they increase with increasing temperature difference and increasing liquid velocity. A new orifice section, designed for measuring small liquid velocities, was installed in the apparatus. A detailed study has been completed for film boiling in the liquid velocity range from 0 to 3 ft/sec. Both ethyl alcohol and benzene have been studied under these conditions. The heat transfer coefficients are smaller for benzene than for alcohol. Work is now in progress for studying benzene at high flow rates.

B. Basic Chemistry, including Metal Chelates

Z. Z. Hugus, William Jolly, Frank Owings, Albin Zielen

Germanium Chemistry. The results presented in the March Quarterly Progress Report led to the suspicion that germanous iodide readily hydrolyzes in water and dilute acids to form hydrous germanous oxide. Consequently a study of the properties of germanous oxide was undertaken. The free energy of germanous oxide is markedly influenced by its degree of hydration; it was necessary to standardize the method of preparation so as to have a uniform product.

The cells GeO, GeO_2, aqueous HCl, HgCl, HG and GeO, GeO_2, aqueous HI, AgI, Ag were studied over wide concentration ranges of HCl and HI. The calculations are not yet complete, but tentatively $E^0 = 0.08\,v$. for

$$GeO(ppt.) + H_2O = GeO_2\ (ppt.) + 2H^+ + 2e^-.$$

Thermodynamics of Rhenium. Calorimetric data of the heats of oxidation of ReO_2 and ReO_3 is being currently taken.

Production of Re(-I) electrolytically has been attempted with negative results to date. It appears that while the hydrogen over-voltage on rhenium is appreciable, formation of a surface layer of hydrogen on rhenium gives a reversible electrode. The matter is being investigated further.

Electron Exchange Rate Between Fe^{2+} and Fe^{3+}. The spectrophotometric determination of iron by measurement of the ferrous bipyridyl ion at 5220Å has been investigated and found to be suitable. The correction for the initial presence of Fe^{3+} is small (corresponding to E 150) and in a period of about 20 minutes, there is negligible change due to the photosensitive reduction of Fe^{3+} . The molar extinction coefficient for the ferrous dipyridyl complex was found to be 8670, which is in agreement with that reported in the literature.

A fresh sample of Fe^{59} was obtained recently. This activity was prepared by the Co^{59}(d, 2n) Fe^{59} reaction in the 60-inch cyclotron. Since considerable uncertainty existed as to the purity of the Fe^{59}, a rather extensive purification procedure has been carried out, using the method given in AECD-2738 (procedure 26-7).

The Hydrolytic Polymerization of Zirconium. The polymerization of zirconium (IV) in 2 M $HClO_4$ solutions has been investigated by means of the aqueous equilibrium between Zr and its first TTA (thenoyltrifluoroacetone) complex:

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

However in the acual experimental work, a two phase benzene-aqueous system was employed. This was done to keep the TTA (aq) concentration low and hence prevent the above reaction from being driven excessively to the right.

In the experimental procedure, equal volumes of benzene and 2 M $HClO_4$, containing known amounts of Zr and TTA, were equilibrated on a mechanical shaker at 25.0°C. Optical density measurements (Beckman Spectrophotometer) were then made on benzene and aqueous phase aliquots. By means of the known molar extinction coefficient of TTA in benzene (11,240 at 3300Å) and the distribution coefficient of TTA between benzene and 2 M $HClO_4$ (35.6), it was then possible to calculate the concentrations first of TTA(b) and then TTA(aq). This last result plus the molar extinction coefficients of TTA (85.8 at 3660 Å) and of ZrK^{+3} (23,400 at 3660Å) in 2 M $HClO_4$ enabled the calculation of the ZrK^{+3} concentration. It was not necessary to correct the final results for Zr chelate, ZrK_4, extracted into the benzene phase. This was kept negligibly small by employing low TTA concentrations.

Knowing now the concentration of ZrK^{+3} and the total uncomplexed Zr concentration, expressed as the summation given below, it is possible to define a complexing coefficient E as:

$$E = (ZrK^{+3})/\sum_{n=1} B(Zr_n)$$

This allows a mathematical treatment entirely analogous to that proposed by Connick and Reas (AECD-2491) and permits a quantitative study of polymer formation as reflected by the decrease of the complexing coefficient with increasing Zr concentration.

Experiments completed to date, covering a range of Zr concentrations from 10^{-4} to 0.3 M, reveal that polymer formation in 2 M $HClO_4$ commences at about 10^{-3} M Zr, which is in agreement with the previous work of Connick and Reas. However a more exhaustive treatment and analysis of the data must await the results of a new series of experiments to be performed in 1 M $HClO_4$ plus 1 M $LiClO_4$ to maintain constant ionic strength.

LMM/7-5-51
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