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UNIVERSITY OF CALIFORNIA

Radiation Laboratory

CHEMISTRY DIVISION QUARTERLY REPORT

December 1955, January, February 1956

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CHEMISTRY DIVISION QUARTERLY REPORT

December 1955, January, February 1956

Radiation Laboratory; Department of Chemistry; and
Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

March 23, 1956

BIO-ORGANIC CHEMISTRY

M. Calvin, Director
Edited by B. M. Tolbert

RADIATION DECOMPOSITION OF HEMIN

Ann Kleerup and Bert Tolbert

Hemin, although a member of the highly radiation-sensitive hemapoietic system, appears to be very stable in the pure dry crystalline state. Samples of crystalline hemin sealed in vacuo in glass tubes have been irradiated in the Co^{60} source with as much as 3×10^8 rep, enough energy to decompose an ordinary organic compound about 98%. These hemin samples indicated on analysis only 3 ± 2% decomposition. Such metal-like stability may be partly due to the ability of the great porphyrin ring with the iron molecules to be excited to high energy levels without breaking. One can imagine that there should be a strong conductance along some axis of the crystalline structure in order to promote such radiation stability.

Experimental Procedure and Results

Hemin was purchased as a fairly pure compound (97%) and recrystallized from a pyridine–glacial acetic acid–NaCl mixture[1] to give a spectrophotometrically pure compound. Anal: Calcd. for $C_{34}H_{32}N_4O_4FeCl$: C, 62.6; H, 4.95; Found: C, 62.39; H, 5.07.

Weighed amounts of the pure dry hemin were sealed in 4-mm o.d. Pyrex tubes under high vacuum. Two samples were given no irradiation. The rest were irradiated in pairs in the Co^{60} source with the amounts of radiation shown in Table I. All except the 3.2×10^8-rep pair were analyzed by quantitative spectra in N/10 NaOH solution. Because of difficulties involved in quantitatively filling the tubes, weighing the samples, sealing the tubes, and transferring the material out of the tubes after irradiation, the experimental error seems to be as much as 2%. Therefore these results indicate that hemin is very stable to irradiation or that the method of analysis is inadequate. Since the shape of the absorption curves did not change with irradiation and tests for iron indicated that no iron was freed

[1] Hans Fischer, in Organic Synthesis, Coll. Vol. III, New York, Wiley, 1955, p. 442.

from the chelate, one might assume that any radiation damage occurring would be such that the porphyrin ring and chelated iron would not be changed. Two very likely possibilities for this type of damage are the loss of carboxyl groups and polymerization through the vinyl groups. Chromatography would show such changes if they existed.

Table I

Irradiation decomposition of hemin by Co^{60} gamma rays

Amount of radiation (rep)	% Decomposition	Analytical Procedure
1.13×10^7	2	Spectra in alkali
1.13×10^7	1.4	" " "
5.17×10^7	1	" " "
5.17×10^7	0.5	" " "
9.77×10^7	0.5	" " "
9.77×10^7	0.6	" " "
3.2×10^8	3.0	Spectra in pyridine
3.2×10^8	2.9	Paper chromatograms

The methods of Nicholas and Rymington[2] and of Eriksen[3] for the chromatography of hemin, using lutidene-water mixtures (1:1 and 5:3), were unsuccessful. Both methods used commercial lutidene mixtures which undoubtedly contained compounds other than lutidene. Pure lutidene is not miscible with water to such an extent. The commercial lutidene in this laboratory saturated with water gave very smeared chromatograms of hemin. After much investigation the following method for paper chromatography of hemin was developed. Hemin was put on Whatman No. 1 filter paper sheets from pyridine solution and the spot dried with a stream of dry nitrogen. The chromatogram was developed with a mixture of equal volumes of water, pyridine, and ethyl acetate. The sample of recrystallized hemin gave one spot, with R_f about 0.8. The original sample of commercial 97% hemin gave three spots.

[2] R.E.H. Nicholas and C. Rymington, Scand. J. Clin. Lab. Invest. 1, 12 (1949).

[3] L. Eriksen, Scand. J. Clin. Lab. Invest. 5, 155 (1953).

The hemin samples irradiated with 3.2×10^8 rep were dissolved in pyridine in order to facilitate chromatography. Quantitative comparison of the spectra of the samples in pyridine with that of a nonirradiated sample indicated about 3% decomposition, with a possible error of 2%. Duplicate chromatograms were made using 50 γ and using 100 γ on each spot. Development with pyridine–water–ethyl acetate (1:1:1) gave only one spot from each origin. The R_f was about 0.9. This difference in R_f was probably due to the fact that the pyridine used here was freshly distilled whereas that in the original chromatograms was not. A mixture of equal volumes of ethyl acetate and water with just enough pyridine to make a clear solution would probably be the best solvent to use.

Since 1% of another colored compound could have been detected, these chromatograms support the original evidence that hemin is extremely stable to radiation. Assuming that the value of 3% decomposition after 3.2×10^8 rep irradiation is true, one can calculate $G_{decomposition} = 0.17$. Benzene, a very stable compound, has a G_{decomp} of about 0.8,[4,5,6] while an ordinary organic compound would have a G_{decomp} of 5 to 10.

Plans for further experimentation include investigation of the radiation decomposition of protoporphyrin (hemin without iron or chlorine), of hemin in aqueous solution, of various phthalocyanines, and of other porphyrins including chlorophyll.

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[4] M. Burton, J. Phys. Chem. 52, 564 (1948).

[5] J. P. Manion and M. Burton, J. Phys. Chem. 56, 560 (1952).

[6] W. N. Patrick and M. Burton, J. Am. Chem. Soc. 76, 2626 (1954).

AN ELECTRON PARAMAGNETIC RESONANCE SPECTROMETER

Power B. Sogo

A recording differentiating electron-spin resonance spectrometer has been designed and built. The unit utilizes a Varian Model V-58 klystron, which is capable of delivering 600 milliwatts of power into a matched load at a frequency of 9.3 kMc. The klystron is isolated from reactive loads by a Uniline and a variable attenuator, and the klystron frequency is stabilized on the cylindrical sample cavity operating in the TE_{011} mode. A transmission system using bolometer detection and magnetic field modulation at 80 cps is employed for the routine investigation of samples for unpaired electrons. Aqueous samples about 2 cm long and 0.2 cm in diameter may be accommodated without a significant decrease in cavity Q. The minimum detectable number of carriers in aqueous solution is about $10^{13}(\Delta H)^2$. The unloaded cavity Q is estimated to be larger than 10^4. Under normal operating conditions, the klystron AFC requires no adjustment for several hours at a time.

At the present time, this apparatus is being remodeled into a "magic-tee" reflection system using the same cavity as above. The extra hole in the cavity will be used for the introduction of light chopped at the rate of about 10 cps. The lock-in amplifier will be synchronized with the light-chopping frequency so that only light-sensitive resonances will be recorded.

EFFECTS OF IONIZING RADIATION ON CHOLINE ANALOGS

Richard M. Lemmon, Margaret A. Parsons, and Peggy Kwong

The great sensitivity of choline chloride to ionizing radiation has been ascribed to the presence of a free-radical chain mechanism of decomposition.[1] One way in which such a chain may be operating is the following:

$$[(CH_3)_3NCH_2CH_2OH]^+ \xrightarrow{-e^-} [(CH_3)_3NCH_2CH_2OH]^{++} \rightarrow (CH_3)_3N^{+\cdot} + {}^+CH_2CH_2OH \qquad (1)$$

$${}^+CH_2CH_2OH \longrightarrow H^+ + CH_2 = CHOH \rightarrow CH_3CHO \qquad (2)$$

[1] Richard M. Lemmon, Margaret A. Parsons, and Doris M. Chin, J. Am. Chem. Soc. 77, 4139 (1955).

$$(CH_3)_3N^{+\cdot} + [(CH_3)_3NCH_2CH_2OH]^+ \longrightarrow$$

$$(CH_3)_3N: + (CH_3)_3N^{+\cdot} + {}^+CH_2CH_2OH \qquad (3)$$

Reactions (3) and (2) might then be repeated until the trimethylamine-radical ion, $(CH_3)_3N^{+\cdot}$, participated in some reaction other than (3), thus breaking the chain.

In order to look for some direct evidence of the operation of a free-radical mechanism in the radiation decomposition of choline chloride, the irradiated compound was examined for paramagnetism in the electron spin resonance equipment described elsewhere in this report. Approximately 50 mg of anhydrous crystalline analytically pure choline chloride was sealed in a 3-mm o.d. Pyrex tube under high vacuum. This sample was then subjected at room temperature to 2.6×10^5 rep of γ radiation from a Co^{60} γ-ray source. During the 45 minutes that elapsed between removal from the source and placing in the resonance equipment, the sample was kept at -196° (liq. N_2). Before the sample tube was placed in the resonant cavity, the upper half of the tube was annealed in a burner flame in order to remove the F-centers (free electrons) in the glass which resulted from the γ-ray irradiation. The upper half was then cooled and the tube inverted so that the sample was now situated in glass that was free of F-centers--only the annealed glass was placed in the e-p-r (electron paramagnetic resonance) spectrometer.

The e-p-r spectrum of the irradiated choline chloride showed a sharp peak at a value for g (electron magnetic moment divided by the spin moment) of approximately 2.0. Free radicals, as well as free electrons, absorb the high-frequency power (9.26 kMc) at this g value. However, the fine structure of the absorption peak makes it quite probable that the energy absorption is due to the presence of nitrogen-containing free radicals. The height of the peak is estimated to be due to the presence of approximately 10^{15} of these free radicals in the sample. Since the sample received 2.6×10^5 rep (= 1.4×10^{-19} ev), the number of free radicals found in the sample amounts to only about 1 for every 10^4 ev of energy expended in the sample. Further work is continuing to determine if longer times of irradiation will achieve a higher concentration of free radicals. This is particularly desirable, as a higher concentration of the free radicals may permit a determination of the nature of the free radical to be made from the hyperfine structure resulting from electron-proton and electron-nitrogen nucleus interactions.

The nitrogen free radical resulting from the choline chloride irradiation decays fairly rapidly, with a half life of something like one hour. There is evidence of a second, long-lived free radical in the irradiated sample. The possibility has not been ruled out, however, that what has been observed is only some free electrons in the glass sample tube.

THE INCORPORATION OF SEVERAL LABELED ADENINES IN C_{57} MICE

Edward L. Bennett, Ruth Deane, and Hilda Karlsson

As discussed in the previous quarterly report, our present knowledge of the biosynthesis of the purine ring suggests that the C-2 or C-8 carbon of the purine ring may be labile and able to equilibrate with the "active formate" of the biological system. Our experiments were designed to test for this possible lability of the adenine ring in mice by use of adenine-2-C^{14}, adenine-4,6-C^{14}, and adenine-8-C^{14}. In the previous quarterly report, the preliminary data from the first experiment were presented,[1] but at that time the adenine RNA*-adenine analyses were not completed. These samples have now been analyzed and the results are given in Table II.

The results of this experiment indicate that no large or consistent differences are found in the specific activity of adenine-C^{14} in 5-AMP*, RNA-adenine or guanine, or DNA*-adenine or guanine from 1 to 28 days after administration of adenine-8-C^{14} or adenine-4,6-C^{14}. Thus, no large lability of the adenine ring is indicated either before incorporation or after incorporation into nucleotides or nucleic acids. It is evident that there is not the sevenfold difference in the utilization of these two isomerically labeled adenines that has been reported for the rat.[2]

The results from this experiment indicated that there may be a difference in the utilization of adenine-2-C^{14} and adenine-4,6-C^{14}, the difference becoming larger with time. Accordingly, a second experiment has been initiated in which six male C_{57} rats were injected with adenine-4,6-C^{14} and six with adenine-2-C^{14}. Three animals from each group (litter mates) were sacrificed 24 hours after intraperitoneal injection of the adenine, and the other three animals from each group were sacrificed 15 days after administration.

The specific activity of the 5-AMP, RNA-adenine and guanine, and DNA-adenine and guanine is being determined from five tissues; carcass, liver, kidney, large intestine, and small intestine. At the present time only the 5-AMP specific activity analyses have been completed. The results are presented in Table III together with the standard error of the means ($\bar{\sigma}$), the standard error of the difference between the means ($\bar{\sigma}_{X_1-X_2}$), and the level of significance (P) calculated from the t values. In no case has the difference between the groups been significant at more than the 0.1 level, and in 8 out of the 10 comparisons, the difference found would have occurred by chance in more than 50% of the cases. It is concluded that there is no significant difference between the amounts of adenine-2-C^{14} and of adenine-4,6-C^{14} incorporated into 5-AMP of mice, either at 1 or 15 days after administration of the adenine.

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[1] Edward L. Bennett, in Chemistry Division Quarterly Report, UCRL-3240, Dec. 1955, pp. 57-58.

[2] M. Gordon, Experientia 11, 183 (1955).

* RNA=ribonucleic acid, AMP=adenosine monophosphate, DNA-desoxynucleic acid

Table II

Specific Activity of 5-Adenylic Acid, RNA-Adenine, and Guanine in C_{57} Mice after Administration of Adenine-2-C^{14}, Adenine-4,6-C^{14}, or Adenine-8-C^{14}*

Time after Injection	Compound	RNA-Adenine s.a.	RNA-Adenine % Ad-4,6	RNA-Guanine s.a.	RNA-Guanine % Ad-4,6	Ratio Ad/Gu
		Small Intestine				
24 hours	Ad-4,6-C^{14}	980		108		9.1
	Ad-8-C^{14}	735	75	78	74	9.4
	Ad-2-C^{14}	946	97	125	116	7.5
8 days	Ad-4,6-C^{14}	83		24.8		3.3
	Ad-8-C^{14}	60	72	16.4	67	3.6
	Ad-2-C^{14}	78	94	23.4	95	3.3
15 days	Ad-4,6-C^{14}	37		14.5		2.5
	Ad-8-C^{14}	38	103	14.0	96	2.7
	Ad-2-C^{14}	30	81	13.1	89	2.3
28 days	Ad-4,6-C^{14}	7.2		5.4		1.3
	Ad-8-C^{14}	10.0	139	6.8	126	1.5
		Large Intestine				
24 hours	Ad-4,6-C^{14}	865		144		6.0
	Ad-8-C^{14}	905	105	88	61	10.3
	Ad-2-C^{14}	1050	121	140	97	7.5
8 days	Ad-4,6-C^{14}	126		39		3.2
	Ad-8-C^{14}	93	74	31	80	3.0
	Ad-2-C^{14}	113	90	42	108	2.7
15 days	Ad-4,6-C^{14}	49		27		1.8
	Ad-8-C^{14}	—	—	—		—
	Ad-2-C^{14}	32	65	16	59	2.0
28 days	Ad-4,6-C^{14}	16.0		8.6		1.9
	Ad-8-C^{14}	19.3	120	12.1	140	1.6
		Liver				
24 hours	Ad-4,6-C^{14}	670		67		10
	Ad-8-C^{14}	432	64	50	75	8.6
	Ad-2-C^{14}	563	84	50	75	11
8 days	Ad-4,6-C^{14}	750		156		4.8
	Ad-8-C^{14}	635	85	122	78	5.2
	Ad-2-C^{14}	450	60	39	57	5.0
15 days	Ad-4,6-C^{14}	342		128		2.7
	Ad-8-C^{14}	380	110	129	101	2.9
	Ad-2-C^{14}	215	63	70	49	3.9
28 days	Ad-4,6-C^{14}	58		36		1.6
	Ad-8-C^{14}	61	105	43	119	1.4
		Carcass				
24 hours	Ad-4,6-C^{14}	253		31		
	Ad-8-C^{14}	236	93	25		9.4
	Ad-2-C^{14}	264	104	35		7.6
8 days	Ad-4,6-C^{14}	208		51		4.1
	Ad-8-C^{14}	183	88	52	102	3.5
	Ad-2-C^{14}	168	81	37	72	4.5
15 days	Ad-4,6-C^{14}	186		54		3.4
	Ad-8-C^{14}	—	—	53	98	—
	Ad-2-C^{14}	128	69	39	72	3.3
28 days	Ad-4,6-C^{14}	130		45		2.9
	Ad-8-C^{14}	139	107	47	104	3.0
		Kidney				
24 hours	Ad-4,6-C^{14}	475		136		3.5
	Ad-8-C^{14}	485	102	133	98	3.6
	Ad-2-C^{14}	407	86	114	84	3.6
8 days	Ad-4,6-C^{14}	620		224		2.75
	Ad-8-C^{14}	640	103	214	96	3.0
	Ad-2-C^{14}	285	46	168	75	1.7
15 days	Ad-4,6-C^{14}	255		157		1.6
	Ad-8-C^{14}	327	128	123	78	2.6
	Ad-2-C^{14}	126	50	92	59	1.4
28 days	Ad-4,6-C^{14}	85		52		1.6
	Ad-8-C^{14}	84	98	61	119	1.4

* The animals were intraperitoneally injected with 1.2 mg of adenine labeled in the indicated position and sacrificed at the appropriate time interval after injection. The specific activity of the adenine injected was 20,000 dis/min/μg. All activities have been calculated in terms of μg equivalent of adenine.

Table III

Specific Activity of 5-AMP of Mice Injected with Adenine-2-C^{14} and Adenine-4,6-C^{14}, and Level of Significance (P Values) for the Differences in Specific Activity*

Time After Injection	Small Intestine		Large Intestine		Liver		Kidney		Carcass	
	Adenine-4,6-C^{14}	Adenine-2-C^{14}	Adenine-4,6-C^{14}	Adenine-2-C^{14}	Adenine-4,6-C^{14}	Adenine-2-C^{14}	Adenine-4,6-C^{14}	Adenine-2-C^{14}	Adenine-4,6-C^{14}	Adenine-2-C^{14}
1 day	1295	1494	1085	1180	690	891	830	923	270	221.4
	1470	1346	1495	1182	1095	1211	1140	1094	261	236.7
	1185	1305	905	1229	1115	932	865	1035	244	270.9
Average	1317	1382	1162	1197	967	1011	945	1017	258	243
σ	144	99	302.5	27.8	239.9	174.1	170	76.8	13.2	25.3
$\sigma_{\bar{X}_1-\bar{X}_2}$	101		175		173		108		16	
t	0.64		0.20		0.26		0.67		0.98	
P	0.5		0.8	0.8	0.8		0.5		0.3	
15 days	16.2	21.1	—	41	68	57	59	75	146	174
	25.0	18.8	45	35	52	70	77	78	155	119
	18.7	16.7	21	37	58	76	67	79	102	88
Average	20	18.4	33	38	59	68	68	77	134	127
σ	4.6	1.7	17	3.2	8.1	9.7	9	2	28.4	42.9
$\sigma_{\bar{X}_1-\bar{X}_2}$	2.8		13		7.3		5.3		29	
t	0.39		0.38		1.14		1.83		0.42	
P	0.7		0.7		0.3		0.1		0.6	

* σ represents the standard error of the mean $= \sqrt{\frac{\Sigma X_1^2}{N} - \frac{(\Sigma X)^2}{N \cdot (N-1)}}$

$\sigma_{\bar{X}_1-\bar{X}_2}$ represents the standard error of the difference between the mean $= \sqrt{\left(\frac{\bar{\sigma}_1}{\sqrt{N}}\right)^2 + \left(\frac{\bar{\sigma}_2}{\sqrt{N}}\right)^2}$

$$t = \frac{\bar{X}_1-\bar{X}_2}{\bar{X}_1-\bar{X}_2}$$

P (Level of Significance) is taken from R. A. Fisher, Statistical Methods for Research Workers, 11th Ed., Edinburgh, Oliver and Boyde, 1950.

PHOSPHORYLATED INTERMEDIATES IN FAT METABOLISM

Rosemarie Ostwald, Richard M. Lemmon, and Samuel Lepkovsky

A project to investigate some aspects of fat metabolism has been undertaken in cooperation with the Poultry Husbandry Department. From previous work it is known that fat is synthesized in the liver of mammals and transported to the adipose tissues for storage. It is also known that adipose tissue *in vitro* can synthesize fat from appropriate precursors such as acetate. The question we would like to resolve is whether adipose tissue *in vivo* can synthesize fat. As an approach to this problem it was decided to inject rats with P^{32} and to look for phosphorylated intermediates of fat metabolism in adipose tissue. If compounds like nucleotides and sugar phosphates (hexose-, pentose-, and triose phosphates) can be found it is reasonable to assume that this tissue not only can but does synthesize fat *in vivo*.

The procedures used were the following: rats, either fasted for 24 hours or fed immediately before the experiments (they had been trained to eat their daily rations in two hours), were injected interperitoneally with an isotonic solution of sodium phosphate-P^{32}. After two hours they were anesthetized with nembutal, decapitated, and bled, and the liver and mesenteric fat were removed and frozen immediately in liquid nitrogen. The tissues were weighed and extracted with an ice-cold solution of 10% trichloroacetic acid (TCA) by grinding the tissue with sand in a chilled mortar or by homogenizing it in a chilled Potter-type homogenizer with a Teflon pestle. The suspensions were centrifuged in a clinical centrifuge, which was chilled with powdered dry ice. The progress of extraction was followed by counting aliquot portions of the solution and (or) by assaying for phosphorus by the method of Umbreit, et al.* The pooled TCA extracts of each tissue were separately extracted with ether for 2 to 4 hours to remove the TCA. This procedure did not remove any appreciable amounts of radioactivity. All manipulations were performed in a 'hot box' to avoid health hazards and contamination of the C^{14} laboratory with P^{32}.

Aliquot portions of the TCA-free extracts were chromatographed on oxalic acid-washed Whatman No. 4 papers with phenol-water and butanol-propionic acid-water as solvents. Duplicate papers were run in these solvent systems. One was chromatographed for about 10 hours in each direction (thus moving each solvent front to the edge of the paper) and one for about 20 hours; the latter treatment moves the sugar phosphate area about one-third the length of the paper. The remainder of the extract was put on a column of charcoal (acid-washed Darco G-60). The column was washed with water until no more radioactivity came off. This effluent contained all phosphorylated compounds except the nucleotides. These were eluted from the column with 10% pyridine in water. Both the effluent and the eluant were lyophilized and then chromatographed by the procedure of E. Bennett and B. Krueckel (Biochim. Biophys. Acta 17, 515 (1955)). A total of four chromatograms were made from the charcoal column effluents. Two were prepared by using the solvents and times described above and two similarly in butanol-propionic acid-water and n-propanel-conc. ammonia-water (60:30:10).

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* W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Manometric Techniques, Minneapolis, Burgess, 1949, p. 190.

X-ray films exposed by these papers were used to detect the radioactive areas, inspection under a UV lamp to detect nucleotide areas, and an ammonium molybdate spray (Hanes and Isherwood, Nature 164, 1107 (1949)) to detect the sugar phosphate areas. For identification, the radioactive spot was cut out, eluted with water, and cochromatographed with an inactive sample of the suspected compound. Coincidence of the radioactivity with the UV-absorbing spot or the color spot was taken as tentative identification.

Results

The first two rats (both fed immediately before the experiment) received 0.17 μc P^{32} per gram. The blood contained 2.8% and 3.3% of the injected dose, the TCA extract of the liver 0.76% and 1.2% of the injected dose per gram of liver, and the TCA extract of the fat 0.06% and 0.05% of the injected dose per gram fat for the two rats respectively. Phosphorus analyses showed that the liver extracts contained 350 γ and 250 γ inorganic phosphorus per gram liver, which means a specific activity of the inorganic P of 2.1 and 3.5 μc per mg P, respectively. The fat extracts contained 106 γ and 93 γ inorganic P per gram fat and 50 γ and 46 γ organic P per gram fat. Paper chromatography of these weakly radioactive samples showed a similar pattern of 3 or 4 major spots for both the liver and the fat extracts. One of these was identified as inorganic P.

A second set of experiments was carried out with a larger amount of P^{32}. Two rats were used, one fasted and one fed, and each received 2.1 μc per gram. The blood contained 2.1% and 1.9% of the injected dose. Only the livers of these animals were used, as we wished to identify some of the phosphorylated compounds among the highly radioactive ones in the liver. The 'fasted' and 'fed' liver extracts contained 1.2% and 1.7% respectively of the injected dose per gram of liver. They contained 190 γ and 270 γ inorganic P per gram liver and 300 γ and 410 γ organic P per gram liver respectively. The specific activities were 12.8 and 10.8 μc per mg inorganic P and 4.6 and 6.0 μc per mg organic phosphorus. The differences between the fasted and fed rat were slight and are probably within the experimental error and the biological variation between animals. The difference between the ratios of the specific activities of the inorganic and organic phosphorus (2.8 and 1.8 respectively) may be significant.

The charcoal separation showed that the nucleotide fraction from the 'fasted' and 'fed' animals contained 20% and 13% respectively of the total radioactivity in the liver extract. Their specific activities were 15.5 and 11.1 μc per mg phosphorus. Paper chromatography showed 4 to 6 major spots and 5 to 8 minor spots. Of these the following have been identified: Inorganic P, AMP, ADP, IMP, and TPN. In addition, ATP, GMP, DPN, and glucose-6-phosphate are strongly suspected. The specific activity of AMP has been determined to be 18 and 16 μc per mg phosphorus for the fasted and fed rat-liver extracts respectively. Muscle-deaminase was used to deaminate AMP to IMP. From the known molar extinction coefficients for AMP and IMP and the measured optical density of the solution before and after the addition of the enzyme, the amount of AMP can be calculated.

There are definite differences in the patterns of the chromatograms from the fasted and the fed rats. Description of these differences has to wait until the affected spots have been identified. Work in progress is concerned with the identification of the other major radioactive compounds, especially the sugar phosphates, of the liver extracts. These patterns can then be compared with fat extracts.

DIMENSIONS OF DISCRIMINATION AND LEVEL OF CHOLINESTERASE ACTIVITY IN THE CEREBRAL CORTEX OF THE RAT

Edward L. Bennett

(In collaboration with Professors David Krech and Mark R. Rosenzweig, Department of Psychology, University of California, Berkeley)

Previously we have presented evidence for a correlation (of the order of 0.28) between hypothesis preference in the rat and the level of cholinesterase (ChE) activity in the cerebral cortex.[1] It was found that the higher the level of ChE activity, the greater the preference for spatial hypotheses in the Krech hypothesis apparatus. The recent work has confirmed the original studies, using a newly developed and, we believe, a more sensitive hypothesis preference test. This new test not only yields much higher correlations (of the order of 0.60), but it also reveals new data which support the theoretical interpretation of the observed correlations.

In both the original studies and the present one, the Krech hypothesis apparatus was used. In the first study the maze was unsolvable, whereas in this study it has been made "progressively solvable." In the unsolvable situation, the pattern of lights and open doors is changed according to a prearranged schedule such that any one cue is associated with the open alley only 50% of the time. Although the rat cannot solve the maze, he typically displays systematic patterns of choices of lighted alleys, of left alleys, etc. From these choices, a Spatial-Visual Preference Score for each animal is calculated in the following manner: the percentage deviation (from 50%) for each systematic pattern is calculated; next the percentage deviations from the visual choices and from the spatial choices are summed, and the difference between these sums is taken as a measure of the animal's visual or spatial preference.

In the progressively solvable situation, the pattern of lights and open doors is so arranged that on the first day any one cue is associated with the open alley only 50% of the time, while on subsequent days one of the cues is associated with the correct alley in increasing proportion, until on the final day, the specified cue is correct 100% of the time. In this test, The Spatial-Visual Preference Score is derived as follows: starting with the second day (the first time that one of the cues is rewarded differentially) we determine the number of choices made of the specified cue, and then determine the number of choices made in accordance with the more preferred responses of the other sensory mode. The difference between these

[1] Science 120, 994 (1954).

two scores is taken as a measure of the animal's Spatial-Visual Preference. To obtain an over-all Spatial-Visual Preference Score, the daily Preference Scores are totaled.

The rationale of our original studies and of the changed procedure can be indicated by a brief discussion of the Preference Score.

(a) In the unsolvable situation, the animal is given a free choice in the sense that neither the visual nor spatial characteristics of the alleys are differentially rewarded. Thus the Preference Score is taken as a quantitative measure of the rat's perceptual preference.

(b) Almost all animals show a light-going preference on the first five or six trials, thus indicating that the lighted alley is the initial dominant stimulus for the animal.

(c) It is next assumed that adaptive behavior is correlated with the ability to "pay attention to" various stimulus aspects of his environment when confronted with a difficult problem.

Achieving a Spatial Preference Score requires that an animal ignore the dominant stimulus and pay attention to the less obvious cues, which permit discrimination of an alley in terms of location in space. From the above four considerations we conclude that an animal that shows a Visual Preference Score cannot readily neglect the one dominant stimulus even though it is not the correct one, whereas the animal that shows a Spatial Preference Score can show readier "shifts in the dimensions of Discrimination" when confronted with a difficult problem, and is, therefore, the more adaptive animal.

It is thought that the progressively solvable maze acts as an "entrapment" procedure, so that the visual score of an animal that has a visual preference is greatly magnified, and therefore the preference differences between the high- and low-ChE animals should be more pronounced. An illustration may clarify this hypothesis. By previous hypothesis and observation, a low-ChE animal would show a light-going preference throughout the first day. On the second day, this response would be rewarded about 63% of the time, thus making the lighted alley an even more dominant cue and, correspondingly, evoking a larger number of lighted-alley choices. On the third day, again the same thing would happen--to an even more pronounced degree. The net result would be that such an animal would earn a high Visual Preference Score. On the other hand, an animal high in ChE would start with a light-going preference, but by the end of the first day such an animal would have dropped, or begun to drop, this light-going response. At the start of the second day, he would begin to try out another hypothesis and thus the slight differential reinforcement of the lighted alley-- a cue which he had already responded to and had dropped-- would presumably have less of an immediate effect on his behavior than on the rat with a low ChE level. Eventually, of course, he would return to his discarded light-going response, but his total Visual Preference Score would be well below the other animal's.

Two groups of animals were used in this test; 29 animals were run in a maze situation in which the lighted alley became progressively more correct, and 24 animals in a maze situation in which the left alley became progressively more correct. The average age of behavioral testing was 96 days, and the animals averaged 116 days at the time of chemical testing. The cholinesterase determinations were made as previously described and the average of the V (visual) and S (somesthetic) areas was computed.

For the behavioral data three basic measures were used:

(a) The choices of the progressively correct alley (i.e., for Group I this would be the number of lighted-alley choices, and for Group II, the number of left-alley choices).

(b) The "other mode" choices (for Group I this would be either the number of left-alley choices or right-alley choices -- whichever was the larger; for Group II, either the lighted-alley choices or the darkened-alley choices).

(c) The Spatial Visual Preference Score; this score is derived by subtracting the value of measure No. 2 above from the value for measure No. 1 above.

The results for the data for the two groups are presented separately.

Group I -- Lighted Alley Progressively Correct

After chemical analysis, the twenty-nine animals of Group I were divided on the basis of their ChE scores into two subgroups: the "High-ChE" group of 15 animals (ChE greater than 64), and the "Low-ChE" group of 14 animals (ChE less than 63). Figure 1 shows the mean number of light-going responses of these two subgroups for the third through seventh day of the maze-testing procedure. The heavy solid line represents the number of times the lighted alley was associated with the opened door. As shown, the high-ChE and low-ChE animals showed remarkably parallel curves, but at each point the low-ChE subgroup shows a higher number of lighted-alley choices than does the high-ChE group. The high-ChE group stays fairly close to the solid curve, where the low-ChE group is well above the base line. During the five days of the maze trial, the number of times the lighted alley led to an open door was 187; the average number of times the high-ChE group chose the lighted alley was 187; and the average number of times the low-ChE group chose the lighted alley was 204.3 times. The difference between the average numbers of choices made by the high- and low-ChE groups is statistically significant ($t = 2.42$ with 27 df). Thus, while the high-ChE group distributed its choices exactly in accordance with the reality situation, the low-ChE group overshot the actual probabilities to a significant extent.

If instead of an analysis of the group averages, an individual analysis is made, the hypothesis appears to be supported even better.

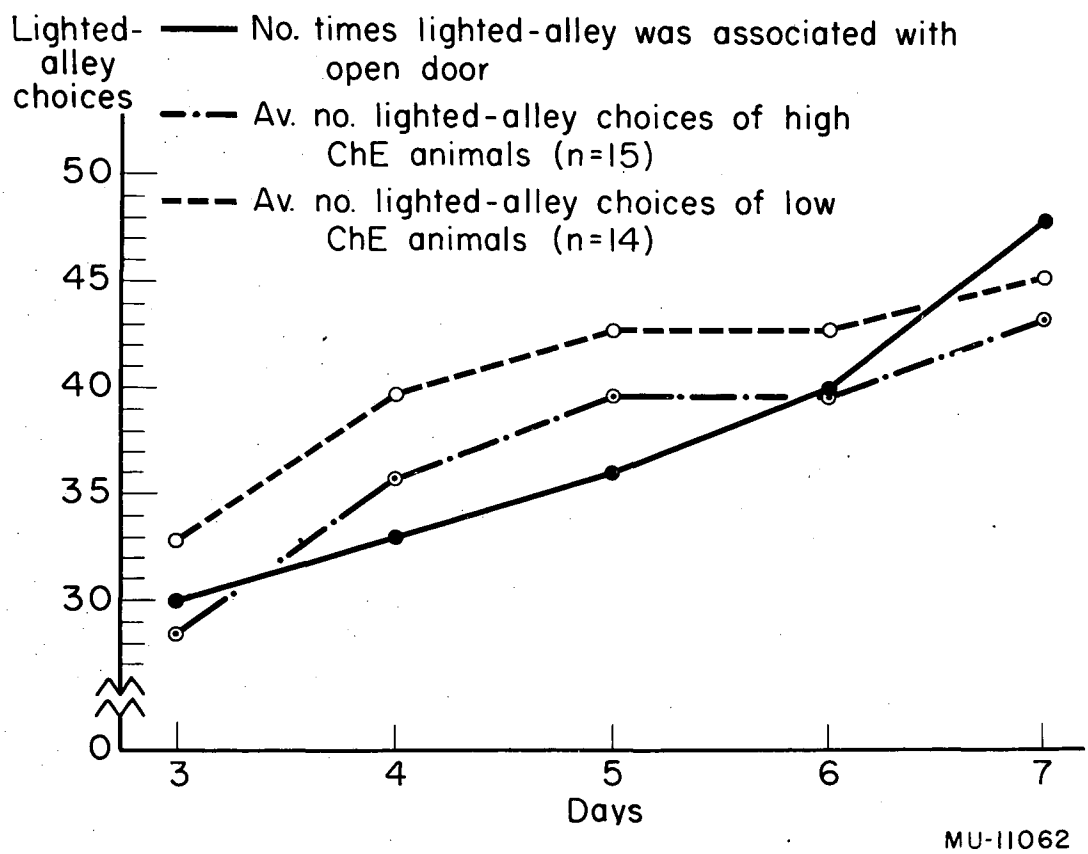

Fig. 1. Responses of high-ChE and low-ChE animals when the lighted alley was made progressively correct (Group I).

Figure 2 presents a plot of the ChE scores against the total Spatial-Visual Preference Scores of all 29 animals of Group I. In accordance with our original hypothesis, a negative correlation is expected, since a high Preference Score means a high visual preference. The correlation found was -0.62($t = 4.10$ with 28 df). Inasmuch as the correlations we were able to obtain in our previous experiments were of the order of 0.28, this correlation of 0.62 lends support to our original observations and to our hypothesis that the progressively solvable situation provides a more sensitive behavioral measure than the unsolvable one.

Group II -- Left Alley Progressively Correct

The experimental situation for this Group differed from that of Group I in that now the spatial response (the initially nondominant cue) became progressively more frequently correct on days 3 through 7. It would therefore be expected that the low-ChE animals of Group II would behave quite differently with respect to the specified cue than did the corresponding animals of Group I. Figure 3 shows the mean number of left-going responses for the high- and low-ChE animals of Group II (the basis of division into high and low ChE being the same as for Group I). The heavy solid line represents the number of times the left alley was associated with the open doors. In general, the high-ChE animals in this situation behaved as did the high-ChE animals of Group I. The low-ChE animals of Group II, on the other hand, differed significantly from the opposite numbers of Group I. Although the average numbers of times the animals of each group chose the left alley were similar, the distribution of their choices over the five test days differed greatly for the two groups. It was found that the lower the ChE activity level of the animal, the lower are his initial left-going choices but the more rapidly the left-going alley becomes the dominant stimulus as it becomes progressively more frequently correct.

The results of these experiments seem to warrant several conclusions. In the first place we have been able to replicate, under different conditions, our previously reported findings of a correlation between Spatial-Visual Preference score and the level of ChE activity in the cerebral cortex of the rat. A revised testing procedure has enabled us to obtain correlations of the order of 0.60 between ChE activity and the observed variance in behavior in our hypothesis apparatus. This more sensitive procedure reveals differences among young rats, sacrificed shortly after their behavioral tests, whereas previously we had found chemical differences between animals with Spatial and Visual preferences only when the animals were sacrificed more than 100 days after their behavioral tests. In the fourth place, the behavior of the high-ChE animals, of both groups, suggests that these animals can maintain a pattern of choices very close to the actual probability situation -- whether the specified stimulus is the lighted alley or the left alley. The animals with a relatively low ChE level, on the other hand, cannot maintain a "probabilistic" response to the same extent, but, instead, overshoot the mark. In addition, we have demonstrated that the behavioral differences between the animals high and those low in ChE activity is not solely a mode-preference difference, but is a more general difference.

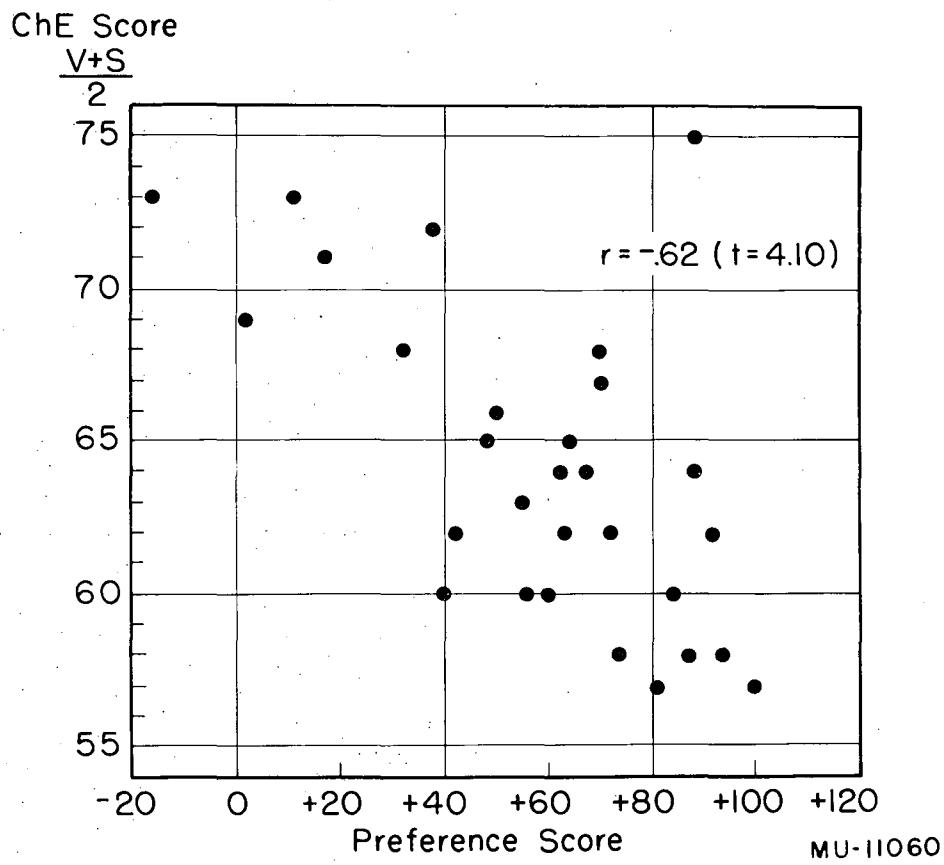

Fig. 2. ChE score versus total Spatial-Visual Preference Score for animals of Group I.

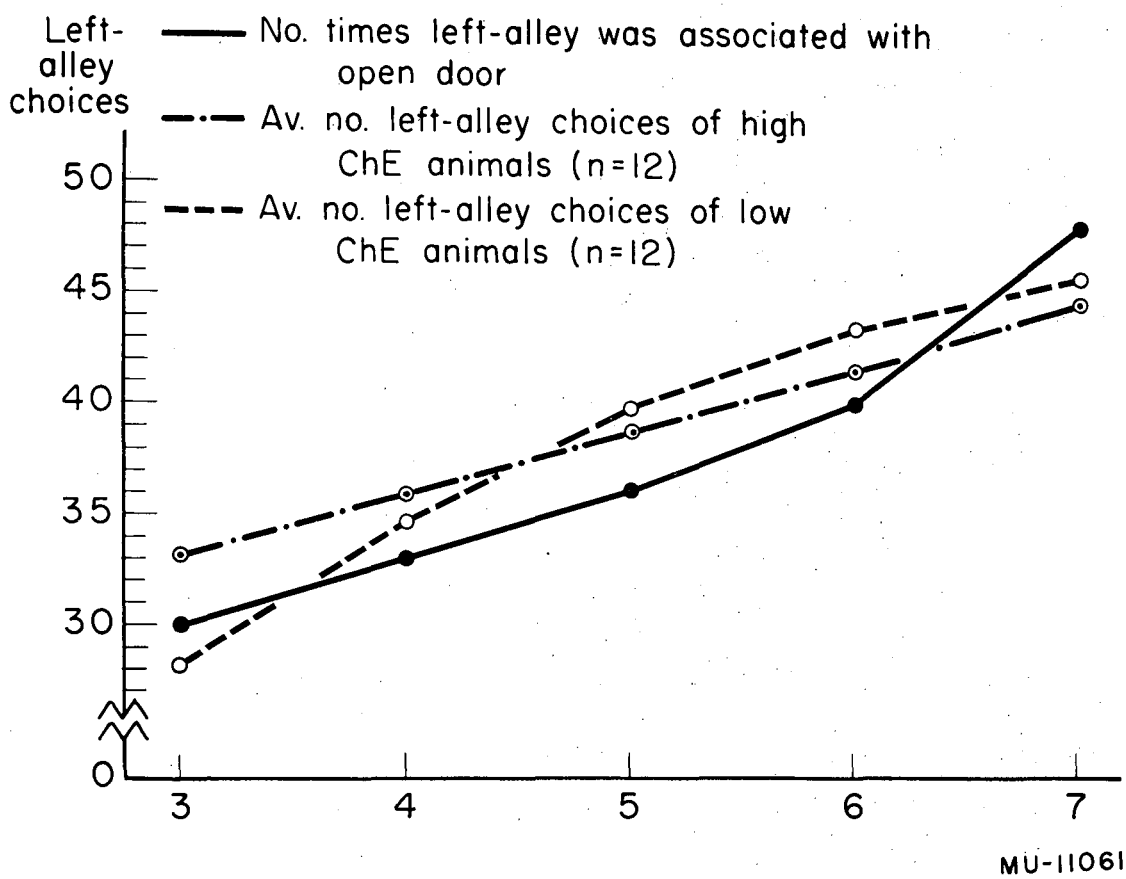

Fig. 3. Responses of high-ChE and low-ChE animals when the left alley was made progressively correct (Group II).

VALINE-4, 4'-C^{14}: MILLIMOLE-SCALE RESOLUTION AND MEASUREMENT OF RESPIRATORY EXCRETION PATTERN

Martha Kirk, Patricia T. Adams, and B. M. Tolbert

Hassan and Greenberg have shown that there is a real difference between the respiratory C^{14} pattern from the D and L isomers of leucine-2-C^{14}. It is of considerable interest to repeat these measurements, using more refined radioactivity-measuring techniques, other amino acids, and other positions of label. The faster rate observed for the oxidation of the abnormal D-isomer would indicate that it is more extensively degraded than is the L-isomer. Important questions include: how far down the carbon chain does this degradation extend; how much utilization can be made of the D-isomer; how do the different amino acids compare; and how toxic are D-amino acid isomers?

In this report, procedures for a five-millimole-scale resolution of DL-valine are described. The labeled resolved valine is then given to rats and the respiratory C^{14} excretion rate measured.

Synthesis

In a previous Quarterly Report (UCRL-3240, page 24) a method for the resolution of valine in small quantities was described. By this method a sample of DL-valine-4, 4'-C^{14} has been separated into the pure optical isomers.

Chloroacetyl DL-valine was prepared as previously described from 5.8 millimoles valine-4, 4'-C^{14} containing 2.2 mc of carbon-14. The acylated amino acid was separated from free DL-valine by ether extraction. Paper chromatography showed it to be free from contamination with valine.

Progress of the enzymatic hydrolysis of the chloroacetyl L-valine was followed by analyzing the digestion mixture for free amino acid by the method of S. Moore and W. H. Stein (J. Biol. Chem. 211, 893, 907 (1954)). Several additions of fresh enzyme were required to maintain an appreciable hydrolysis rate. A total of 60 mg of enzyme and 30 hours' incubation time was necessary to complete hydrolysis of the L-isomer.

Chloroacetyl D-valine was separated from the L-valine by use of a cation-exchange column, and was hydrolyzed in 2 N hydrochloric acid. Both isomers were isolated by precipitation with ethylene oxide from an ethanol solution of their hydrochlorides. Additional amounts of each isomer were recovered by adjusting the pH of the mother liquor to 5.6, evaporating to dryness, and subjecting the residue to sublimation at 190° at 10^{-2} mm. In addition, DL-valine was recovered from the water-soluble residue after chloroacetylation, by sublimation away from the salt residue.

From this preparation, there was recovered 36 mg DL-valine-4, 4'-C^{14}, specific activity 3.1 μc/mg; 182 mg L-valine-4, 4'-C^{14}, specific activity 3.1 μc/mg, α_D: +26; and 214 mg D-valine-4, 4'-C^{14}, specific activity 3.2 μc/mg, α_D: -27. Theory: specific activity 3.2 μc/mg; α_D for L-valine, +27.4, for D-valine, -27.1. The total recovery of labeled valine was 65%.

Rate Curves

The respiratory C^{14} patterns were determined by using ionization chambers in a continuous-flow system (B. M. Tolbert, M. Kirk and E. M. Baker, Continuous $C^{14}O_2$ and CO_2 Excretion Studies in Experimental Animals, UCRL-2941, Aug. 1955). Five animals were measured in each group and typical data for each animal are given in Figs. 4 and 5. In 7 hours about 43% of the D-isomer is oxidized to CO_2, as compared to only 27% of the L-isomer. Furthermore, the peak excretion rate for the D-isomer is slightly delayed. The data have not yet been further analyzed. Similar experiments for leucine are now in progress.

METHIONINE-METHYL-C^{14}: SYNTHESIS AND RESPIRATORY EXCRETION PATTERN

Patricia T. Adams, Martha Kirk, Ann Kleerup, and B. M. Tolbert

Methionine is an important amino acid and a well-known source of methyl groups in biological systems. In order to learn more of the metabolism of this compound we have synthesized L-methionine-methyl-C^{14} and measured the respiratory C^{14}-excretion patterns of this compound, as well as of the labeled DL-isomeric mixture.

L-Methionine-methyl-C^{14}

Methyl-C^{14} iodide (7.8 millimoles, 11.7 mc) was prepared in the usual manner by the reduction of carbon-14 dioxide with lithium aluminum hydride, and subsequent reaction of the methanol with hydriodic acid. The yield was 70% from barium carbonate.[1]

L-homocystine (1.47 g, 11.0 millimoles) was dissolved in 40 ml liquid ammonia. Small pieces of sodium were added to this solution until a blue color persisted for 20 minutes. After this time, the excess sodium was destroyed by the addition of small amounts of ammonium chloride.

The methyl-C^{14} iodide (7.8 millimoles, 11.8 mc) was added to this solution over a period of about 5 minutes. The reaction mixture was stirred for 10 minutes, then 0.20 ml (3.2 millimoles) of unlabeled methyl iodide was added. The ammonia was allowed to evaporate at room temperature.

The nonvolatile residue was dissolved in water and the pH adjusted to 5.74. The water solution was concentrated to 20 ml and filtered, and 75 ml hot ethanol was added. Crystalline methionine was filtered from the cooled solution, washed, and dried in vacuo. The yield of this first crop was 1.04 g, specific activity 7.3 μc/mg (theory 7.2 μc/mg) or 64% yield.

[1] D. E. Pack and B. M. Tolbert, The Reduction of C^{14}-Labeled Carbon Dioxide and Fatty Acids with Lithium Aluminum Hydride, UCRL-1957, Sept. 1952.

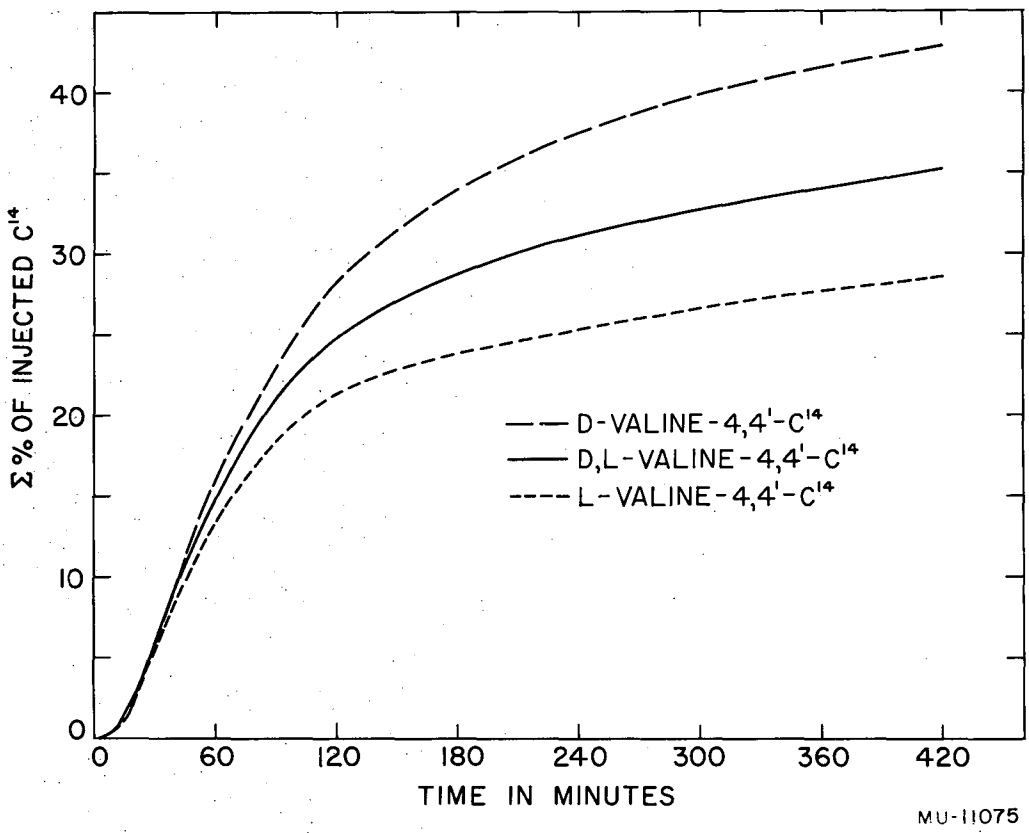

Fig. 4. Cumulative excretion of $C^{14}O_2$ from valine-4, 4'-C^{14} (typical curves).

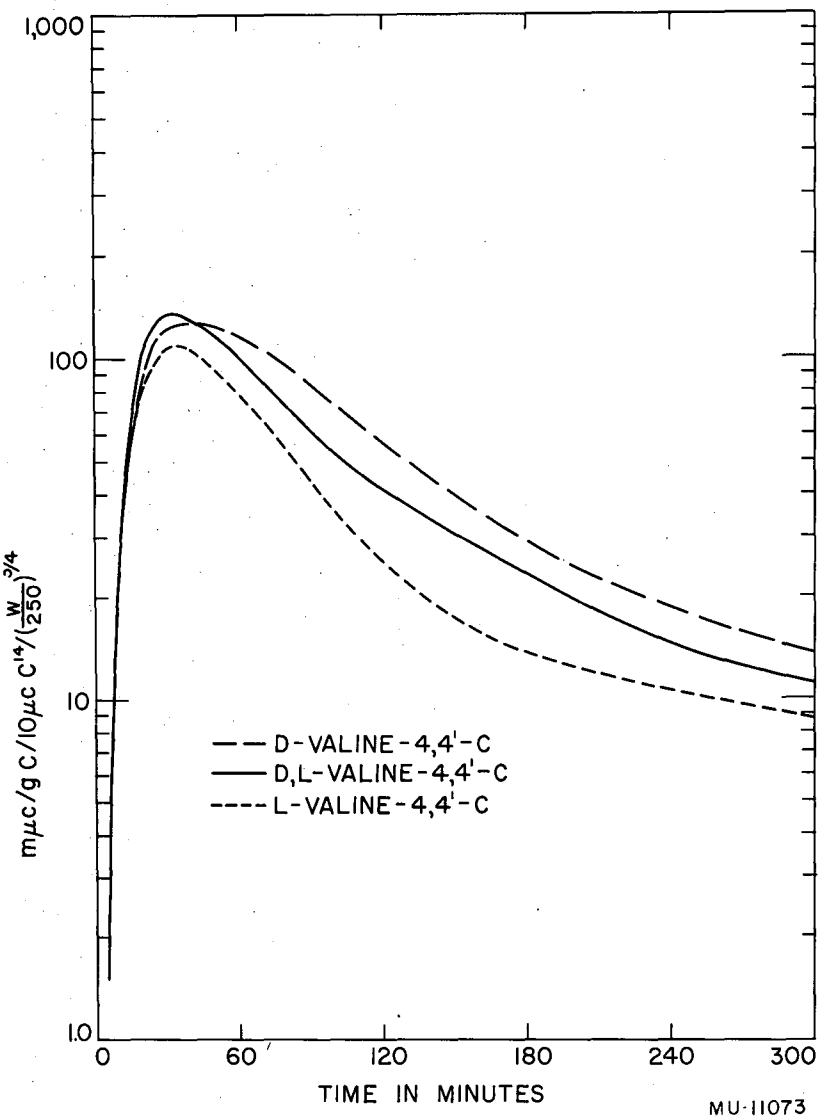

Fig. 5. Specific activity of $C^{14}O_2$ from valine-4, 4'-C^{14}.

Paper chromatographic analysis showed 93% to 97% of the radioactivity coincident with authentic methionine. The remaining activity was methionine sulfoxide, which is formed during the chromatographic process.

Additional methionine was recovered as a second crop, by sublimation from the evaporated mother liquor at 200° and 10^{-2} mm. The sublimate (which contained seven radioactive contaminants totaling about 15% of the total activity) was recrystallized from water-ethanol to give 256 mg of pure L-methionine-methyl-C^{14}, specific activity 7.3 μc/mg, thus raising the total yield to 79%.

Results

In Figs. 6 and 7 the respiratory C^{14} curves for the L- and DL-mixed compounds in rats are presented. After the first 20 minutes the DL-isomer is oxidized at a faster rate than is the L-isomer. This result is in agreement with previously observed results that abnormal optical isomers of amino acids are usually oxidized more extensively and at a faster rate in animal systems than are the naturally occurring isomers.[2,3]

We can infer that the L-isomer may be used directly, in many cases, whereas the D-isomer must be partially degraded before the carbon and nitrogen skeleton can be used in metabolic processes, as distinguished from catabolic processes.

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[2] M. Hassan and D. M. Greenberg, Arch. Biochem. Biophys. 39, 129 (1952).

[3] B.M. Tolbert, J.H. Lawrence, and M. Calvin, International Conference on Peaceful Uses of Atomic Energy, Paper A/CONF.8/P/96, July 1, 1955.

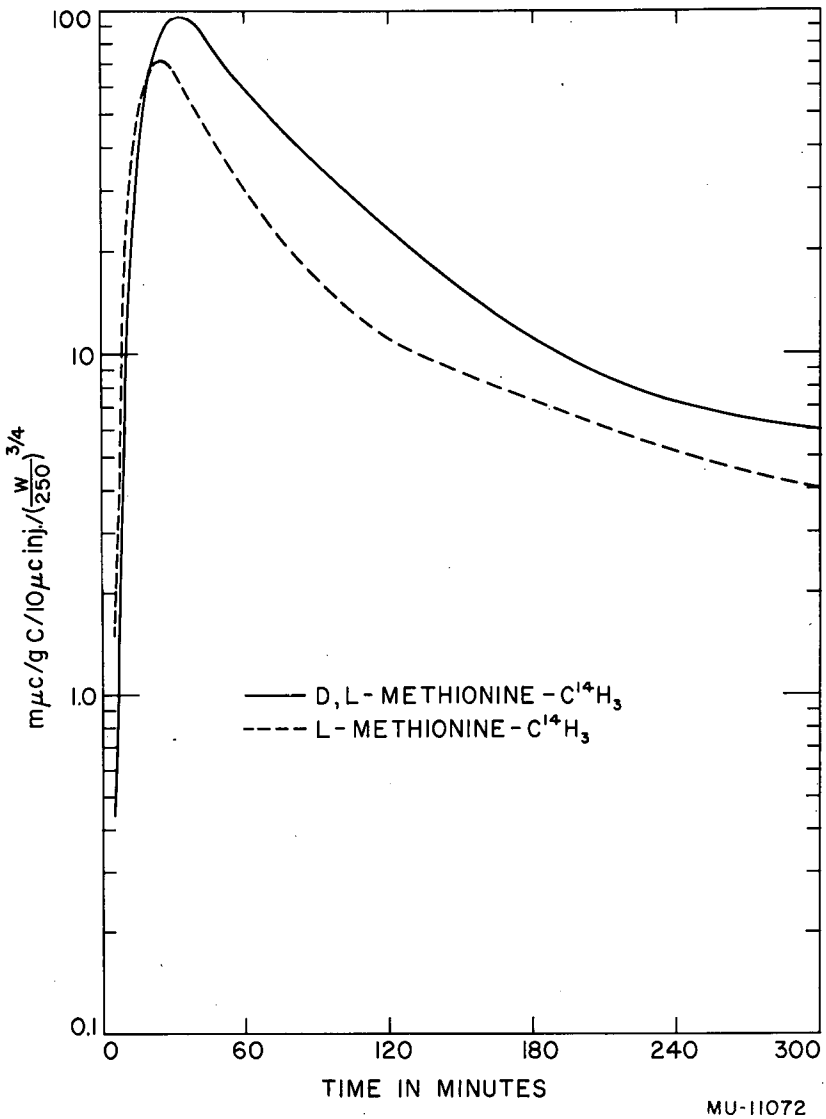

Fig. 6. Specific activity of $C^{14}O_2$ from a rat injected with methionine-methyl-C^{14}.

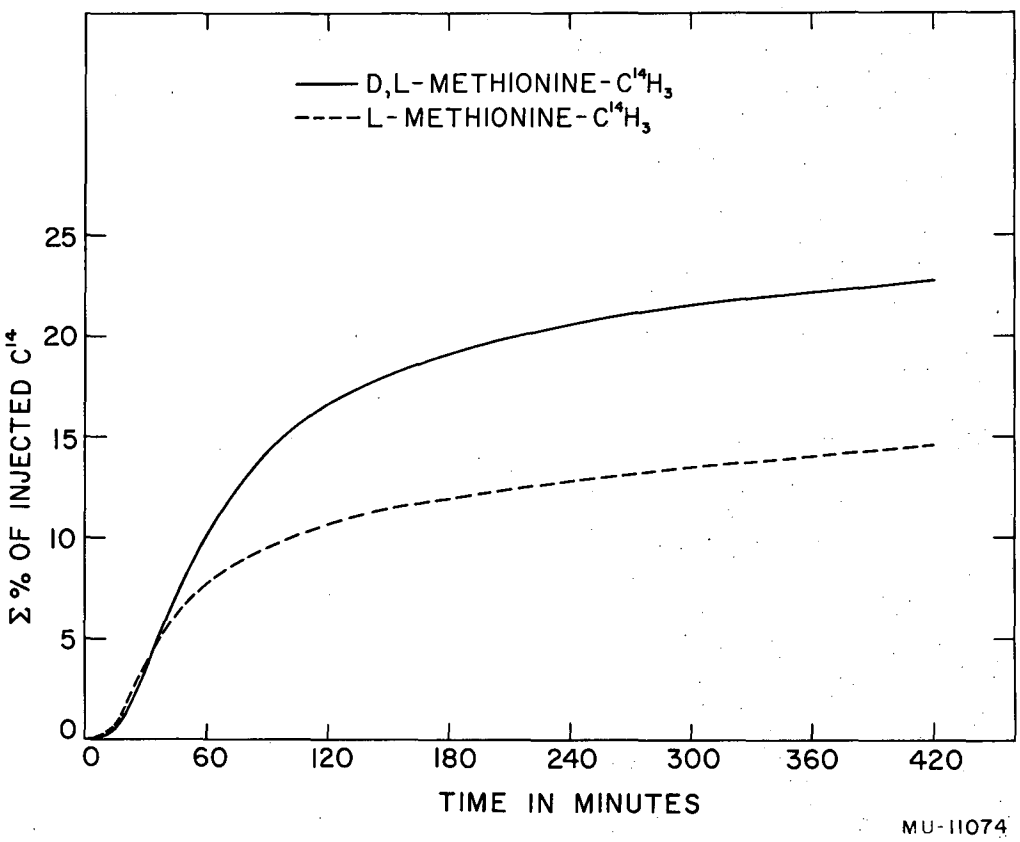

Fig. 7. Cumulative excretion of $C^{14}O_2$ from a rat injected with methionine-methyl-C^{14}.

METABOLISM OF ADENINE-4,6-C^{14} AND TUMOR GROWTH

Martha R. Kirk, B. M. Tolbert, and Edward L. Bennett

The role of adenine in nucleic acid metabolism has been studied in this laboratory (Chemistry Division Quarterly Reports, UCRL-2932, UCRL-3240) as well as at other locations. The present study deals with the effect of tumor development in a rat on the oxidation of adenine-4,6-C^{14} to $C^{14}O_2$.

After the respiratory C^{14} pattern for a normal Curtis-Dunning male rat, had been established, by the method previously described (Tolbert, Kirk, and Baker, Continuous $C^{14}O_2$ and CO_2 Excretion Studies in Experimental Animals, UCRL-2941, June 1955), the rat received a transplant of adenosarcoma tumor tissue. Metabolic changes were then measured by injecting the animal with 2 mg adenine-4,6-C^{14}(6.00 μc) at 4- to 7-day intervals and determining its rate of oxidation to $C^{14}O_2$, using the continuous ion-chamber measurement mentioned above. Results are shown in Table IV. Figure 8 is a graph of the specific activity of the $C^{14}O_2$ produced by the animal and shows the changes that took place as the tumor developed.

It is of considerable interest that the $C^{14}O_2$ respiratory pattern was greatly changed in three runs, yet reverted to near normal in between two of these runs. Although there might be a variable oxidation pattern of adenine in a normal rat, it has not been observed before, and the effect is probably, therefore, due to the disease state. Again, these oxidation changes may here be a secondary effect of the tumor, as starvation, or they may be due to a more direct disturbance from the tumor.

Further studies are being made along this line, since the results obtained indicate some drastic changes in purine metabolism with tumor growth.

Table IV

Cumulative Excretion of $C^{14}O_2$ after the Injection of Adenine-4,6-C^{14}: Changes with Tumor Growth

Days after tumor transplant	Average mg OC_2 per min	Cumulative % of injected adenine-4,6-C^{14} as $C^{14}O_2$ as a function of minutes after injection									
		0-20'	40'	60'	90'	120'	180'	240	300	360	420
Normal	9.55	.16	.46	.71	1.05	1.38	1.91	2.44	3.00	3.51	4.10
8	9.59	.19	1.18	2.71	4.83	6.17	7.88	9.06	10.02	11.01	12.06
15	10.31	.11	.30	.50	.79	1.02	1.47	1.92	2.34	2.76	3.31
22	8.63	.33	2.35	7.16	15.51	22.78	31.39	36.85	40.24	42.97	45.39
28	8.58	.19		.54	.92	1.30	2.05	2.76	3.41	4.09	4.74
32	4.12	.01	.08	.20	.43	.92(died)					

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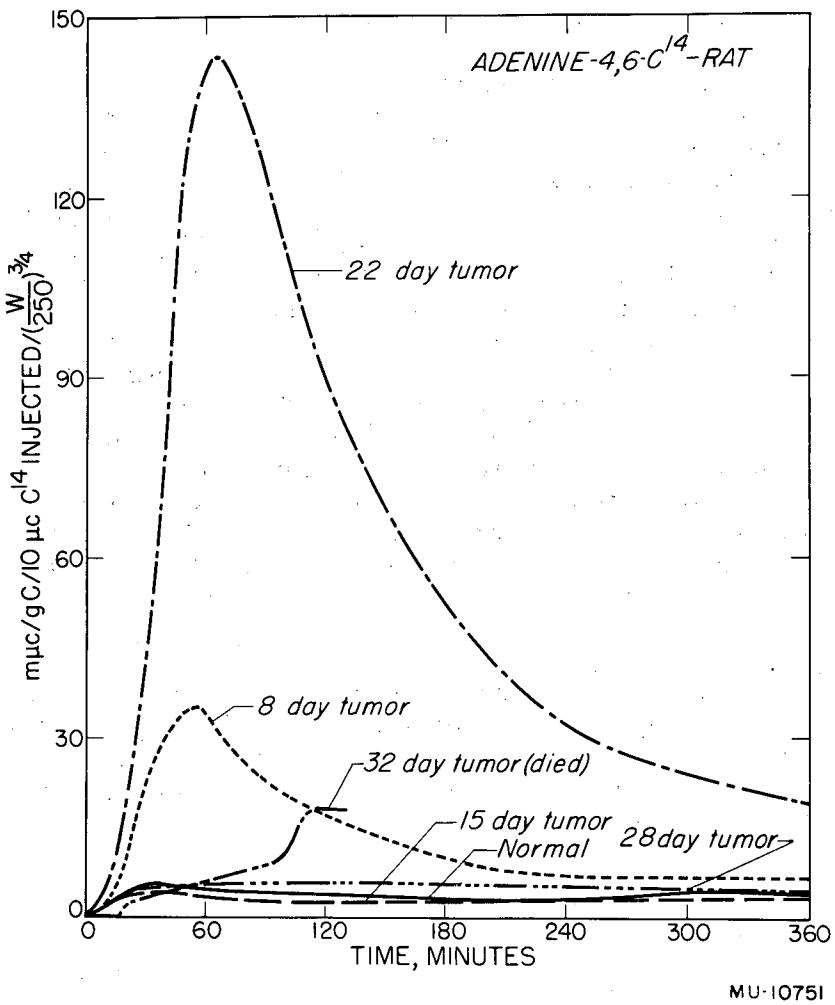

Fig. 8. Specific activity of $C^{14}O_2$ in breath following injection of adenine-4, 6-C^{14}, and the effect of tumor growth thereon.

ACETATE METABOLISM IN EXPERIMENTAL VIRUS-INDUCED DISEASE

Ann M. Hughes, Benjamin V. Siegel, and B. M. Tolbert

Previous studies in this laboratory have investigated the effects of poliomyelitis infection on the oxidative metabolism of glucose in mice.[1] These experiments indicated that the rate and extent of viral multiplication is without apparent effect on the rate and extent of glucose metabolism by the infected intact host. Recently, similar studies have been conducted with sodium acetate used as the substrate instead of glucose.

Webster-strain white mice, 4 to 5 weeks of age, were inoculated intracerebrally with 0.03 ml of a suspension containing 10 LD_{50} (50% lethal dose) of mouse-adapted MEF_1 (Type 2) poliomyelitis virus. In the period 4 to 8 days following inoculation, 20 infected mice were selected for metabolic study. Of these, 12 animals manifested distinct symptoms of involvement of the central nervous system (CNS), marked by excessive irritability and limb paralysis, while 8 animals appeared healthy. These mice, along with 7 normal controls, were injected intraperitoneally with 0.1 ml of a solution containing 1 mg of sodium acetate-2-C^{14} with an activity of 5.0 μc per mg. They were then immediately placed in an apparatus designed to measure respiratory radioactivity excretion. This apparatus consisted of a simple flow system carrying the air from a small glass animal cage through an ionization chamber. The potential of the chamber was amplified by a vibrating-reed electrometer and charted on a continuous recording potentiometer. The $C^{14}O_2$ respired was measured for 7 hours and the resulting curves were analyzed to give the excretion of the $C^{14}O_2$ as a function of time, in terms of both rate and cumulative excretion.

Animals infected with poliomyelitis virus oxidized acetate at a lower rate, and to a lesser extent initially, following administration of the substrate. After 7 hours, the difference in total $C^{14}O_2$ excreted, although small, was significantly greater in mice manifesting paralytic symptoms of CNS involvement.

[1] B. V. Siegel and A. M. Hughes, UCRL-3065.

GLUCOSE DISSIMILATION BY A FREE-CELL NEOPLASM[1]

Karl K. Lonberg-Holm

The purpose of this investigation of Ehrlich's mouse ascites carcinoma cells is to apply paper chromatographic analysis to their contents following exposure to labeled substrates, and by this means to probe into the inner workings of the complex system that is characteristic of mammalian metabolism.

In the past the "salt problem" has been a major obstacle to this type of investigation. No more than about 20 μliters of physiological salt solution or cell suspension may be placed upon the origin of the usual type of two-dimensional chromatogram used in this laboratory if serious salt streaking is to be avoided. We have maximized the amount of cell contents that can be chromatographed by separating the cells from their incubation medium, by centrifugation, before killing with hot alcohol. This procedure precludes the taking of aliquots at short time intervals, and also introduces the question of whether or not the period of centrifugation alters their metabolic pattern. However, this procedure has been used to investigate the compounds elaborated during a one-half-hour or longer exposure of cells to uniformly labeled glucose-C^{14}, lactate-2-C^{14}, lactate-3-C^{14}, and bicarbonate-C^{14}.

In a typical experiment, 1.2 mg glucose-C^{14} (2.6×10^6 cpm or approximately 5.5 μc) was incubated with 60 μliters of cells for 45 min, and the combined cell extracts contained 4.1×10^5 cpm. An aliquot was chromatographed, and showed that 57% of this activity resided in compounds other than lactic acid; the major portion of this activity was in the area of organic phosphates. The identification of these compounds is not yet complete. In this particular experiment glycine, serine, and alanine each contained about 2% of the total activity, while glutamic acid had about a tenth of that. It is interesting to note that labeled lactic acid as substrate gives little if any of its activity to the glycine-serine pool, yet does result in the entry of large quantities of C^{14} into the glutamic acid and aspartic acid pools. Kit has also just reported evidence that an ancillary sequence of reactions leads to serine from the pathway of glucose breakdown to three-carbon products in this same and other tissues.[2]

Ascites cells have been incubated with lactate-2-C^{14} and lactate-3-C^{14}. In a representative experiment 1.7 mg lactate-3-C^{14} (2.8×10^6 cpm) was incubated 46 min with 47 μliters of cells, and the combined cell extracts contained 1.4×10^5 cpm. Both amino acids and organic acids became labeled, but little activity was found in the phosphate area. The percentage of total activity for a few definitely identified compounds (cochromatography with carrier) is given in Experiment I, Table V.

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[1] See also Chemistry Division Quarterly Report, UCRL-3157, Sept. 30, 1955, p. 36.

[2] S. Kit, Cancer Research 15, 715 (1955).

Table V

Percentage of total activity of cell extracts found in several compounds following exposure to lactate-3-C^{14}

Compound	Expt. I	Expt. II
Citrate	4.5	2.3
Aspartate	7.8	5.5
Glutamate	18.5	10.5
Alanine	14.7	13.2
Lactate	(38)[a]	(34)[a]

[a] True results are higher owing to evaporation of lactate from chromatogram.

Experiment II shows the percentage of total activity found when an equal aliquot of cells was handled in an identical manner except that excess glucose was added 5 minutes before harvest of the cells. If equilibration of labeled lactate and unlabeled pyruvate produced from glucose were complete, the large decrease in activity that was observed would not have occurred. Alternatively, the pool sizes of these compounds could have been changed by the addition of glucose.

The incorporation of bicarbonate-C^{14} is being investigated. When 47 μliters of cells were exposed to 1.6×10^7 cpm of bicarbonate (2.6 μmoles) for 50 min, 3.5×10^4 cpm were incorporated into a number of organic acids and into the nucleotide area, provided glucose was present. With the substitution of lactate for glucose only about a third of this activity is found.

The type of study so far reported above can only give information about the static situation obtained after cells have adjusted themselves to a given set of conditions. If the dynamics of change in steady state or of tracer incorporation are to be viewed, then the lengthy procedure of centrifuging the cells must be abandoned. It has been impossible, however, when substrates of the maximum C^{14} activity available until recently have been used, to get enough activity into the intermediates of interest contained in 20 μliters of dilute cell suspension. Desalting was attempted with the hope of being able to increase the size of the aliquot of suspension that could be killed, extracted, and chromatographed.

Conventional methods of electrolytic desalting[3] have proved inadequate to the problem. Electrolytic desalting results in the loss of large amounts of carbon-14, probably as insoluble mercury salts and through electrodialysis. "Desalted" extracts cannot be chromatographed without serious loss of resolution of organic phosphates. Another disadvantage is that inorganic phosphate, which is 0.055 M in the suspending medium, is but slowly removed in comparison with other inorganic ions; this results in a strong acid shift in the sample which may be damaging to labile compounds.

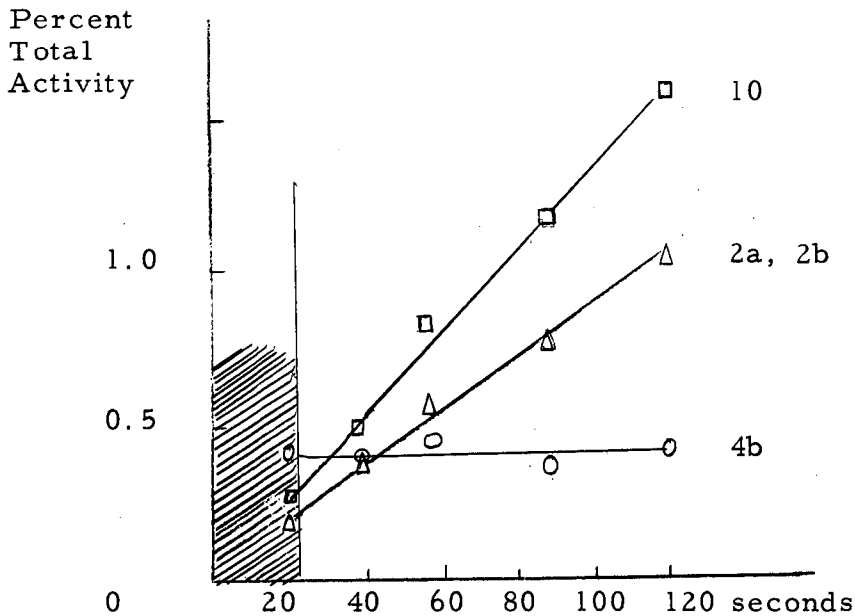
[3] R. Consdon, A. H. Gordon, and A. T. P. Martin, Biochem. J. 41,590 (1947) Calvin

A few attempts have been made to employ paper-strip electrophoresis as a desalting procedure. Although a clean separation of all other inorganic ions is afforded, phosphate remains within the region of carbon-14 activity. (In the hope of attaining resolution, pH was chosen such that the net charge of phosphorylated carboxylic acids was roughly equal to that of free phosphate inasmuch as organic substitution lowers the mobility of phosphoric acid. Ammonium acetate buffer was employed.) This phosphate caused some streaking when the carbon-14 activity was eluted and chromatographed. Electrophoretic desalting still promises to be of use when a modified suspending medium (containing bicarbonate buffer) with low phosphate is employed.

A new buffered phenol solvent for chromatography has been developed. This has been used in the first dimension in the experiments with labeled lactate and bicarbonate described above. It had been expected that addition of salts to the solvent would greatly increase the quantity of salts tolerated in a chromatographed sample. Although the new solvent did not help greatly in this respect and is poor for the resolution of phosphates, it has proved useful for the chromatography of amino acids and of organic acids and compounds formed from labeled lactic acid and bicarbonate. For these purposes it affords better resolution and permits the use of larger quantities that does phenol-water alone. The origin and the paper above it are first treated for a minute with ammonia vapor before development of the chromatogram. The solvent composition is as follows (it must be made fresh just before use):

0.950	g ammonium carbonate (powdered),
150	ml phenol (at about 55°C),
56.5	ml water.

Recent availability in this laboratory of very high activity glucose-C^{14} (224 μc/mg) has permitted direct chromatography of extracts from 15 μliters or less of 10% cell suspension. In a preliminary experiment five aliquots have been removed in the period between 19 and 120 sec following the introduction of enough labeled glucose to last the cell suspension for 4 min. Two minutes before this, enough cold glucose had been given to the cells to last them just 2 min. This latter precaution was taken to insure that the cells would be in an active metabolic state, but does not insure that the intermediary metabolic pools are in a steady state during the experiment. The rapid entry of carbon-14 into a variety of compounds, phosphorylated and unphosphorylated, was seen. The percentage of the total activity for several as yet unidentified compounds is plotted against time in the following graph. The experiment was done at 38°C.



Compound 10 is in the region of alanine, and is rapidly synthesized; 2a, 2b is the sum of two difficultly resolved spots in the nucleotide area; 4b is in the region of PGA and has reached a steady-state activity in less than 20 sec.

A rapid sampling device has been built to permit manual sampling at 5-second intervals following introduction of the substrate; also, the gas-phase composition can be controlled, and can be rapidly changed. Further, a mechanized sampler has been designed by which identical samples may be removed at even shorter intervals. Such devices will almost certainly be of use in further studies with both carbon-14 and phosphorus-32.

ALGAL RESEARCH PROGRAM

Osmund Holm-Hansen

Culturing of Algae for General Laboratory Use

All the algae currently being used are grown in shaker flasks on apparatus that has previously been described.[1] There are now two units in operation, each holding four large flasks. It has been shown that Cyanophyceae (blue-green algae) can be cultured very well on this apparatus, at growth rates almost equal to that of Chlorella or Scendesmus. It is planned to test the growth of other algae from various classes in these flasks, but this will necessitate considerable experimentation with nutrient media and light and carbon dioxide conditions. A special shaker rack (to fit the apparatus now in the laboratory) has been built, which will permit the use of 30 Erlenmeyer flasks at one time, and so allow for easy examination of divers media. Much work remains to be done before we can understand the relations between the growth conditions and metabolic state of the algae, and to set up reliable criteria of growth. The criteria currently employed include cell pack, cell count, chlorophyll content, dry weight, and generation time; in the near future the nitrogen content, the lipid content, and the photosynthetic activity (per unit volume cells) will also be used as growth criteria.

Continuous-Culture Apparatus

An experimental tube (approximately 1 liter capactiy) has been set up to study the feasibility of growing algae in a continuous-flow apparatus designed to maintain the algae in a fairly constant metabolic state. This tube has been described previously,[2] but new electronic control equipment has recently been added which maintains the algal suspension at a constant density in response to either the optical density or the light dispersion. There have been some minor dificulties in the initial trial runs with this equipment, but the electronic control device gives promise of working satisfactorily. Serious consideration must, however, be given the basic design of the tube containing the algae, as it has been shown that the generation time of Scenedesmus is between 15 and 16 hours, which is almost twice that found in the shaker flasks. Work is continuing on testing this equipment, and in trying to improve the design of the tube.

Microelement Studies

Although vitamin B_{12} has been found in a variety of lower plants, the essentiality of cobalt for plant growth has been demonstrated only for blue-green algae.[3] Vitamin B_{12} has been found occasionally in very low concentrations in higher plants, but the possible synthesis of the vitamin

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[1] M. Calvin et al., The Path of Carbon in Photosynthesis. XIV, UCRL-1386, June 1951, p. 9.

[2] Richard E. Norris and Paul M. Hayes, in Chemistry Division Quarterly Report, UCRL-2841, Jan. 1955, p. 5.

[3] O. Holm-Hansen, G.C. Gerloff, and F. Skoog, Physiol. Plantarum 7, 665 (1954).

by soil organisms has not been ruled out.[4] As the use of radioactive Co^{60} could be of valuable aid in settling the question whether or not cobalt is essential for all plants, a project has been undertaken with the following twofold purpose: first, to determine, by means of paper chromatography, if vitamin B_{12} is synthesized by higher plants; and secondly, using Co^{60} as a tracer, to develop efficient methods of removing the cobalt impurities in nutrient salts, in an effort to demonstrate the possible essentiality of cobalt in other plant groups. The equipment necessary for this work has been obtained, and a few preliminary trials have been run with bean plants.

It has been observed in past work that molybdenum and manganese deficiencies in algae have striking results in changing the ratio of phycobilin pigment concentration to chlorophyll concentration. Unfortunately, the salts used in the nutrient solution have so much contaminating Mn and Mo that only Mn deficiency can be demonstrated for the blue-green algae, while no deficiency for Mn or Mo can be shown for Chlorella or Scenedesmus. In Fig. 9 is shown the effect of Mn deficiency on the relative concentration of phycocyanin in Nostoc muscorum. It is seen that in the absence of added Mn, the phycocyanin concentration is very high compared to that of chlorophyll; addition of Mn restores the pigment complement to normal. Work shall be continued in attempting to obtain Mo deficiency by various purification procedures, and then to quantitatively relate the ratio between phycobilin pigment and chlorophyll to different levels of Mo and Mn deficiencies.

[4] W. J. Robbins, A. Hervey, and M. E. Stebbins, Bull. Torrey Bot. Club 77, 423 (1950).

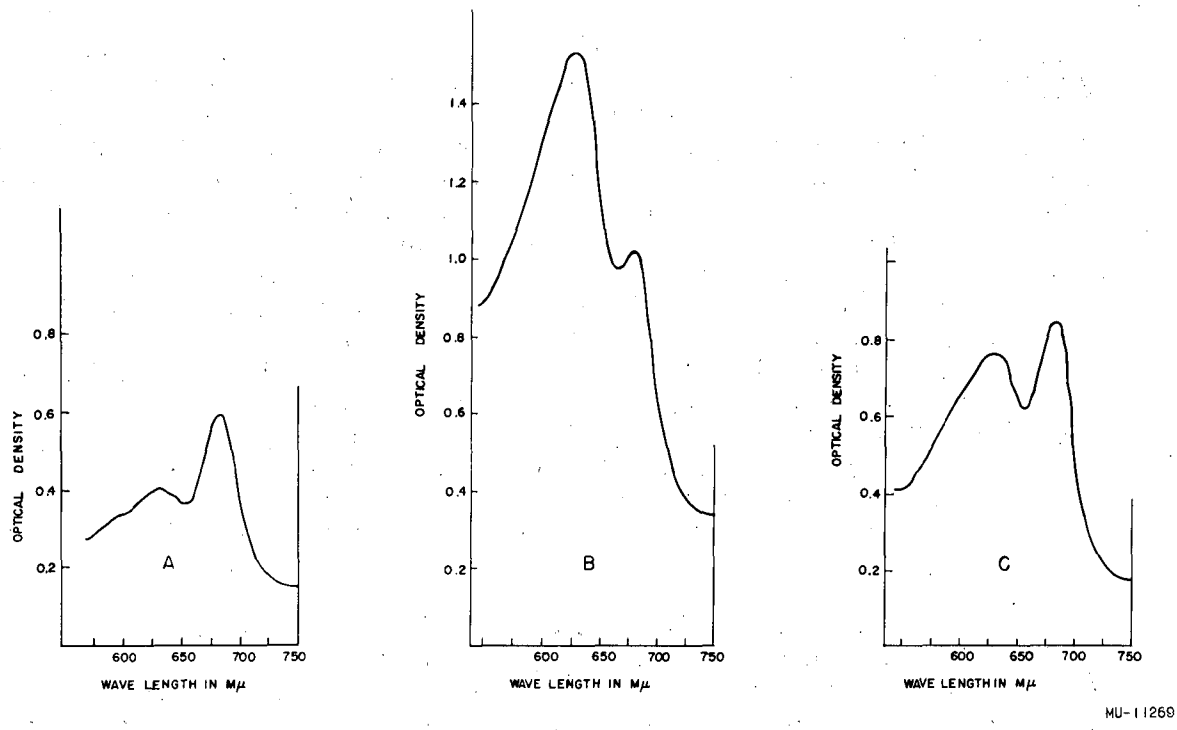


Fig. 9. Graph A. - Absorption spectrum of cells supplied with normal complement of microelements. Graph B. - Absorption spectrum of cells deficient in Mn. Graph C. - Absorption spectrum of cells to which only Mn had been added.

ISOLATION OF AN UNKNOWN COMPOUND FORMED DURING PHOTOSYNTHESIS IN BLUE-GREEN ALGAE

P. Linko and O. Holm-Hansen

Norris et al. have reported that during photosynthesis in blue-green algae an unidentified compound was formed which may have a function in photosynthesis. Our previous quarterly report indicated that this compound could not be found consistently, and -- when it was found -- it did not contain as high a percentage of the total radioactive carbon incorporated into the cellular material as previously reported. Satisfactory experimental conditions have now been established which permit consistent demonstration of this compound. As it is of interest to elucidate the significance of this compound in the metabolism of blue-green algae, the following work has been done to isolate and characterize this compound.

Over a period of 6 to 7 weeks, a total of 150 ml of packed Nostoc muscorum cells was harvested which had been allowed to photosynthesize for 6 min in the presence of radioactive bicarbonate. As it had been shown that this unknown substance behaved like an amino acid, the total ethanol extract was put through an Amberlite IR-120 column, after which the adsorbed compounds were eluted with ammonium hydroxide. This eluate was then put through Amberlite IR-4B anion exchange resin to remove glutamic acid and aspartic acid. The solution was then passed into a Dowex 50 column, from which the adsorbed compounds were differentially eluted by HCl of varying normality. The elution curve is seen in Fig. 10. The fractions containing the unknown were combined and dried in vacuo at 40°C. After addition of water, an inactive precipitate was centrifuged off; further purification was effected by successive evaporation to 0.1 ml and addition of 2.0 ml of absolute ethanol. The ultraviolet absorption spectra showed that the purification removed the peak originally seen at 2350 Å. A yellowish precipitate was finally obtained which weighed 12.1 mg and contained 9.15×10^6 cpm.

It has been shown that though this 12.1 mg does not contain any radioactive contaminant, although there is an impurity which is ninhydrin-positive. Both an infrared absorption spectrum and a titration curve have been obtained, but because of the impurity, these data cannot be relied upon very heavily. The following test results give some idea of the structure of the unknown compound.

1. Catlytic hydrogenation with Adams catalyst showed the absence of any carbon-to-carbon double bond.

2. Deamination with nitrous acid fumes gave evidence for a primary amino group, but ruled out the possibility of a single secondary or tertiary amino group. During this reaction, however, there was an unexplained loss of 40% to 60% of the radioactivity. Treatment with ninhydrin resulted in similar loss of radioactivity. In both cases the lost activity evidently was not in the form of CO_2, an aldehyde or ketone, or an acid or base.

3. With paper electrophoresis at pH 2.5 and 6.7 the unknown behaved like the neutral compounds alanine and glucose.

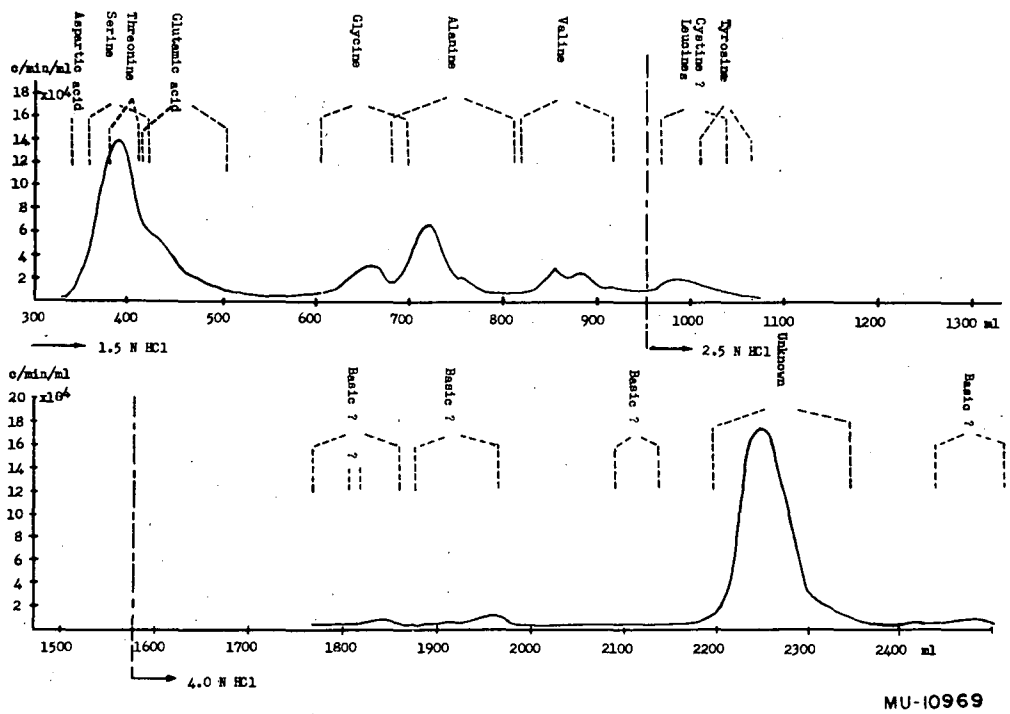


Fig. 10. Elution radioactivity curve for the partially purified ethanol extract from Nostoc muscorum.

4. The unknown has been shown to be stable to hydrolysis at 105° by HCl (1N and 6N) and by acetic acid (2N). The alkaline hydrolysis with saturated barium hydroxide at 105° for 2 hr resulted in considerable decomposition, but the only radioactive compound that could be found after the reaction was the unknown.

5. Spray reagents have been used on paper chromatograms of the compound. The compound gave a violet color reaction with ninhydrin. Aniline hydrogen phthalate, 1,2-napthoquinone-4-sodium sulfonate, p-dimethylaminobenzaldehyde, isatin and p-dimethylaminobenzaldehyde over isatin did not yield any positive evidence, but this may be because the concentrations of the unknown substance were small.

These chemical degradation studies are being continued, and in addition, the occurrence of the unknown substance in different algae and its rate of appearance under different conditions are also being studied.

INTERMEDIATES IN ALGAE METABOLISM

James A. Bassham, S. Alan Barker and V. Carol Quarck

Since the preceding report we have been mainly concerned with laying the foundations for an investigation of the synthesis of pyrimidine and purine nucleotides by Scenedesmus. The progress in the search for tetrose and xylulose phosphates among the photosynthetic products of Scenedesmus is also reported.

Paper Chromatography

The positions of some 30 purines, pyrimidines, nucleosides, nucleotides, and other compounds important in either the investigation of or biosynthesis of nucleotides have been determined on two-way paper chromatograms in phenol-water and butanol-propionic acid (Figs. 11a, 11b, 11c). The positions of some of the nucleoside monophosphates varied according to the pH of the original solutions. Most of the spots were detected by absorption in ultraviolet light or by the perchloric acid-ammonium molybdate spray for phosphates. Deoxyribose was revealed by p-nitroaniline-HCl after treatment with sodium periodate,[1] while ureidosuccinic acid and dihydroorotic acid were detected by spraying successively with 0.5 N NaOH and p-dimethylaminobenzaldehyde-HCl.[2]

Paper Ionophoresis

In a similar manner, the positions of the above compounds were determined (Fig. 12) after paper ionophoresis in a propionic acid-sodium hydroxide electrolyte (see previous report).

[1] J. T. Edwards and D. M. Waldron, J. Chem. Soc. 1952, 3631.

[2] R. M. Fink, R. E. Cline and H. M. G. Koch, Federation Proc. 13, 207 (1954).

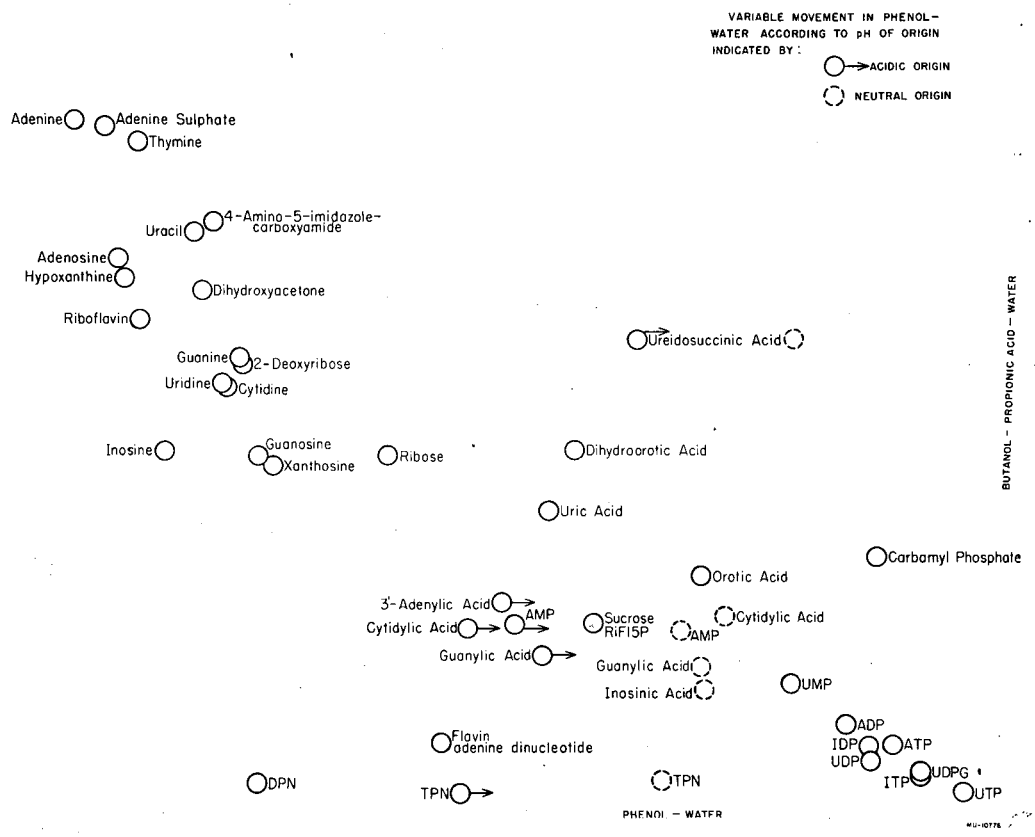


Fig. 11a. Chromatographic map of nucleic acid components.

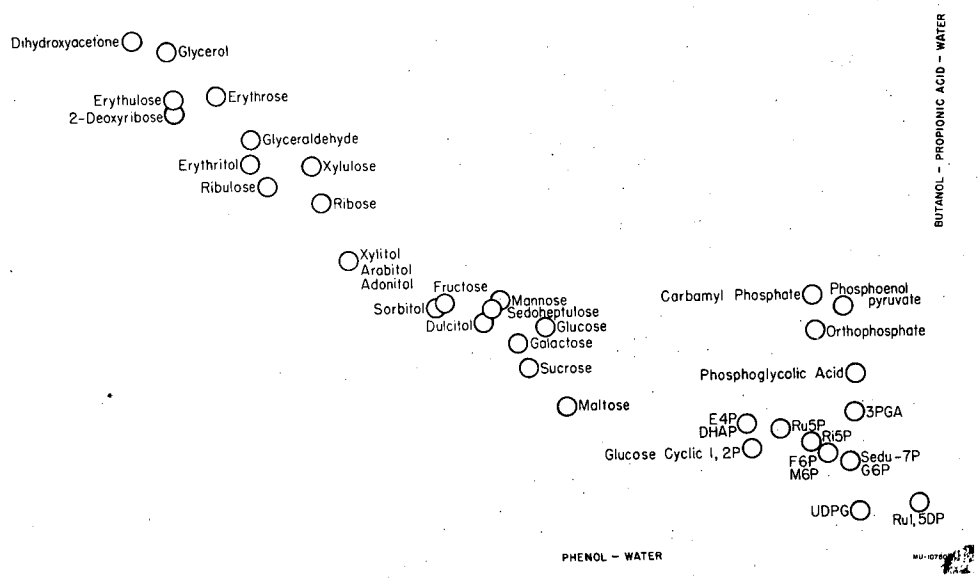


Fig. 11b. Chromatographic map of amino acids, carboxylic acids, and sugar phosphates.

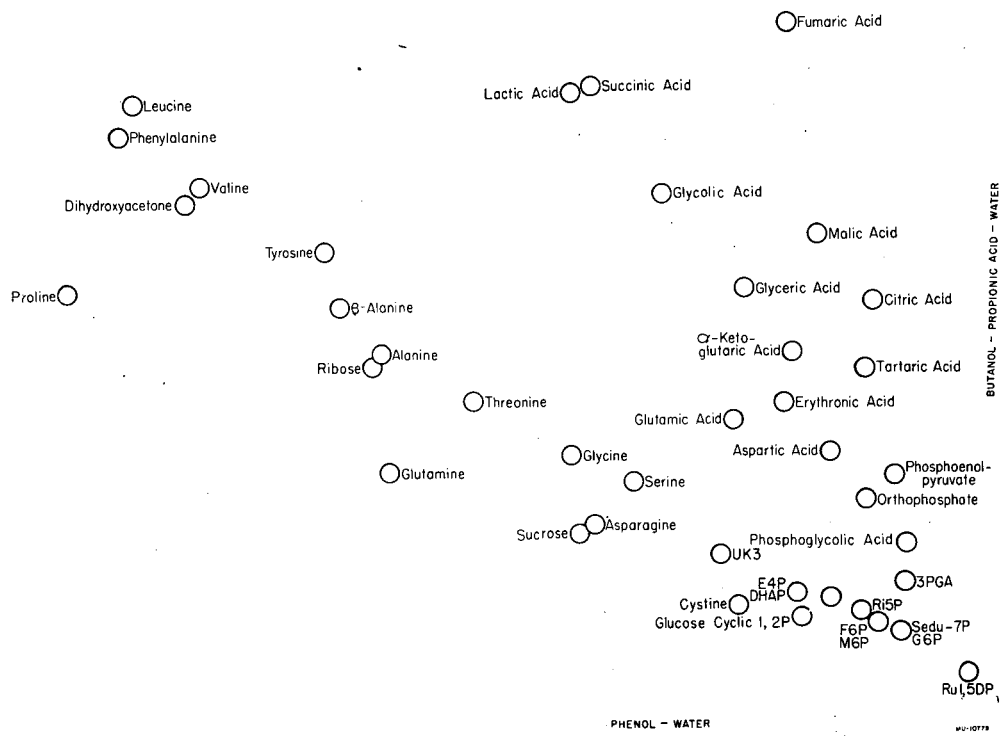


Fig. 11c. Chromatographic map of sugars, sugar alcohols, and sugar phosphate.

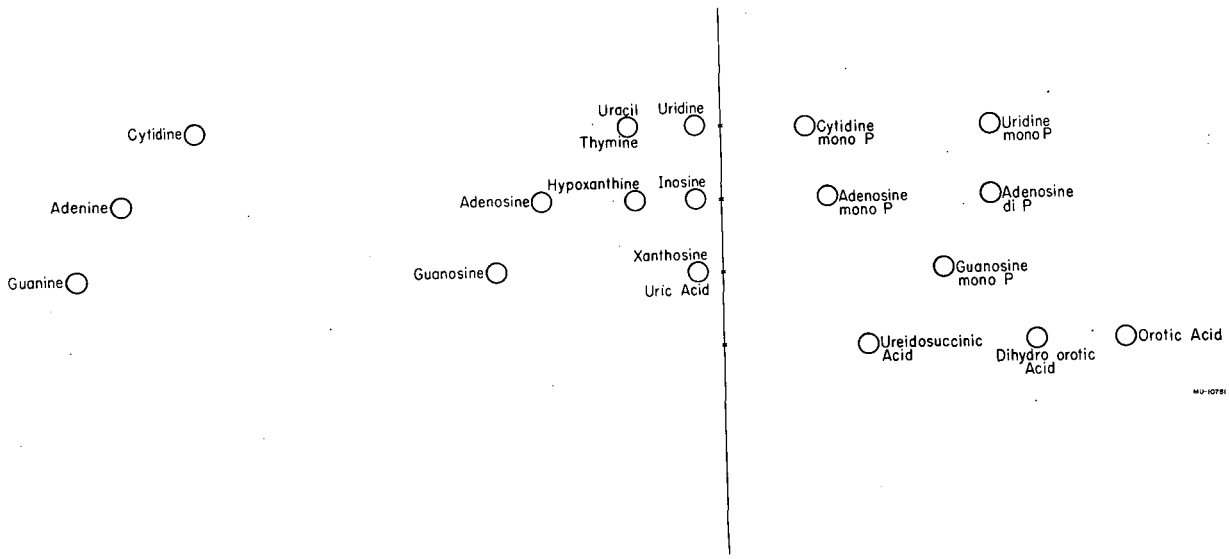


Fig. 12. Ionophoresis map of some nucleic acid components.

Studies on Unidentified Intermediates

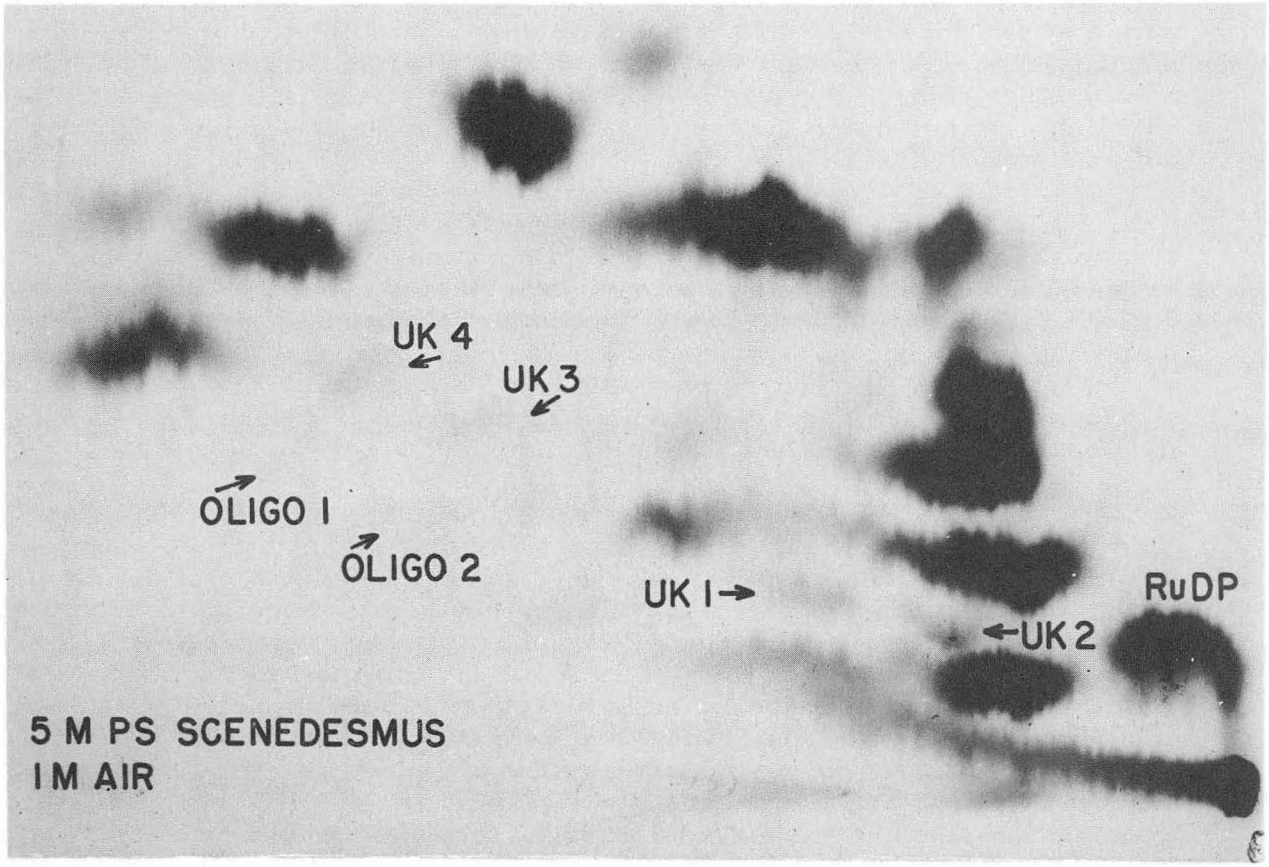
The additional information obtained since the preceding report on the structure of these compounds is as follows:

Unknown 1 (UK 1). - After hydrolysis with N H_2SO_4 at 100° for 20 min the main component (R_f 1.27 > adenosine in butanol-propionic acid) has been identified by cochromatography as adenine. After hydrolysis with 0.01 N H_2SO_4 at 100° for 10 min the main component of the hydrolyzate was identified by cochromatography as adenosine 5'-phosphate. When UK 1 was rerun on a two-way paper chromatogram the main activity cochromatographed with adenosine 5'-diphosphate, with some decomposition to adenosine 5'-phosphate. All evidence so far indicates that UK 1 adenosine diphosphate, with a slight possibility that it could be adenosine diphosphoribose. This doubt is caused by the fact that UK 1 and purified phosphatase gives ribose among other things, but this feature in support of the latter structure is unreliable, since purified phosphatase will also liberate ribose from ATP but not from UDP.

Unknown 2 (UK 2). After hydrolysis with N H_2SO_4 at 100° for 20 min the main component (R_f 1.27 > adenosine in butanol-propionic acid) has been identified by cochromatography as adenine. After hydrolysis with 0.01 N H_2SO_4 at 100° for 10 min the main component of the hydrolyzate was identified by cochromatography as adenosine 5'-phosphate. UK 2 has been shown to have an R_f value in phenol-water identical with the main component of phosphate H (see Fig. 13), which also cochromatographs with ATP. UK 2 is probably ATP or a closely related compound.

Unknown 3 (UK 3). After treatment with purified phosphatase, UK 3 apparently gives only one radioactive component on a two-way paper chromatogram in the position occupied by uracil. No trace of ribose or 2-deoxyribose could be detected. The radioactive product also superimposed on uracil when separated by paper ionophoresis in propionate pH 3 to 4. After hydrolysis with N H_2SO_4 at 100° for 20 min the radioactivity again appeared in the position occupied by uracil on a paper ionophoretogram, and thus ruled out the possibility that a cytosine moiety in UK 3 was deaminated to uracil by an impurity in the phosphatase. However, when uracil, thymine, and the product from phosphatased UK 3 were separated by ionophoresis in 0.12 N NaOH at 600 v for 3 hours on unwashed Whatman No. 4, uracil (14 cm) and thymine (11.5 cm) moved appreciable distances from the origin while the product from phosphatased UK 3 (or the NaOH-decomposed compound) moved only 3 to 5 cm from the origin. UK 3 appears to contain a phosphate moiety, since it cochromatographs with a P^{32}-labeled compound (phosphate R) eluted from a spot midway between phosphates B and L (see Fig. 13). UK 3 does not cochromatograph with uridine 5'-phosphate.

Unknown 4 (UK 4). After hydrolysis with 2 N H_2SO_4 at 100° for 4 hours UK 4 gave ribose, glucose, and a component which ran in the purine-pyrimidine region. Paper ionophoresis of the hydrolyzate showed that the main radioactivity was in the neutral position, with smaller amounts in two positively charged ions and a trace in a negatively charged ion. One



ZN-1451

Fig. 13. Radiogram of extract from Scendesmus after 60 min CO_2 and P^{32} followed by 1 min air.

of the basic components cochromatographed with adenine. The main activity in UK 4 cochromatographs with adenosine 5'-phosphate. The presence of glucose after acid hydrolysis and of glucose and fructose after phosphatase action (see previous report) is attributed to a sucrose impurity in UK 4.

Photosynthesis in the Presence of P^{32}-Labeled Phosphate

In order to determine whether the unknown intermediates discussed above were organic phosphates, a photosynthesis experiment was carried out in the presence of P^{32}-phosphate. The combined 80% ethanol and 20% ethanol extract of Scenedesmus (10 ml of 1% cell suspension), which had been left for 1 hr to photosynthesize with 1% CO_2 in oxygen and sodium phosphate (pH 7.0; approximately 1.2 mc-P^{32}) and then flushed with air (1 min), was distributed on 9 two-way paper chromatograms. Initially only a 2-hr exposure with an X-ray film was necessary.

The phosphates (Fig. 13) were eluted and some of them identified as follows:

A was shown by ionophoresis in sodium propionate as a single component with a mobility similar to phosphoglyceric acid, indicating the presence of at least two acidic functional groups.

B was shown to be adenosine 5'-phosphate by cochromatography on a two-way chromatogram and after ionophoresis in sodium propionate.

C was shown to be triphosphopyridine nucleotide by cochromatography on a two-way chromatogram and after ionophoresis in sodium propionate.

D was a major portion of the activity superimposed on a fructose-1,6-diphosphate spot. Did not react with carboxydismutase -- NOT ribulose-1,5-diphosphate.

E in ionophoresis in sodium propionate, showed same mobility as a sugar diphosphate. Did not react with carboxydismutase -- NOT ribulose-1,5-diphosphate.

F. Unknown.

G. probably was UDPG, since one of the decomposition products on re-chromatographing cochromatographed with uridine-5'-phosphate, and another was probably a pentose phosphate.

H cochromatographed with adenosine triphosphate -- some decomposition to adenosine diphosphate and inorganic phosphate.

I: main activity cochromatographed with glucose-6-phosphate.

J: main activity cochromatographed with fructose-6-phosphate.

K is ribulose-1,5-diphosphate, since it reacted with carboxydismutase to give 3-phosphoglyceric acid (proved by cochromatography).

L: main constituent is uridine-5'-phosphate -- proved by cochromatography on a two-way paper chromatogram and after ionophoresis in sodium propionate.

M is a combination of two phosphates; the main one probably is ribulose-5-phosphate; the weaker spot cochromatographed with ribose-5-phosphate.

N: mobility just less than fructose-6-phosphate after ionophoresis in sodium propionate.

O was shown to be 3-phosphoglyceric acid by cochromatography on a two-way chromatogram and after ionophoresis in sodium propionate.

P: Activity concentrated in 3 small spots, all overlapping erythrose-4-phosphate but none cochromatographing with it.

Q: R_f in phenol-water > adenosine diphosphate.

Search for Tetrose and Xylulose Phosphates

No phosphate identical with erythrose-4-phosphate could be detected in either the "T" phosphate area or its immediate vicinity. The supposed tetrose obtained after phosphatase action is not erythrose, and further work is being carried out to determine whether this compound is erythulose or 2-deoxyribose. The "T" phosphate area is further complicated by the presence of nucleoside monophosphates. The possibility still exists of tetrose's being found as the diphosphate.

ADDITIONAL PROPERTIES OF CARBOXYDISMUTASE

Ning G. Pon

In the reinvestigation of the works of Drs. J. Mayaudon and J. R. Quayle it was found that the carboxydismutase system required a metal ion, Mg^{++}, as a cofactor. It was of interest to proceed further along this line to test for the following effects:

(a) Other metal ions as cofactors.

(b) Organic substances as cofactors.

(c) Presence of cofactor(s) in crude enzyme extracts and in crude ribulose-1,5-diphosphate (RuDP).

(d) Phosphate concentration effect on the activity of the carboxydismutase system.

(e) Electrophoretic studies on the ammonium sulfate purified enzyme. (The major portion of this work was done by K. Lonberg-Holm.)

(f) N-terminal amino acid analysis via the use of 1-fluoro-2,4-dinitrobenzene.

(g) Carboxydismutase activity vs Ni^{++} concentration.

The results are summarized below:

(a) Twenty-five different cations have been tested. Ni^{++}, Mn^{++}, Co^{++}, Mg^{++} and Cr^{++} worked best, and in the order indicated. Particularly interesting is the fact that neither Fe^{++} nor Fe^{+++} had any effect on the activity of carboxydismutase.

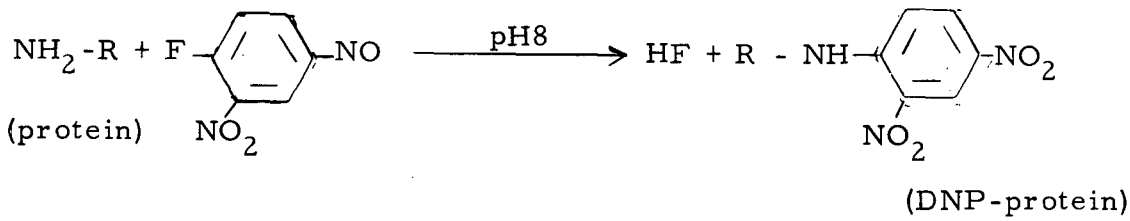
(b) Organic substances that were tested for cofactor functions were biotin, thiamine hydrochloride, thiamine pyrophosphate (TPP), DL-thioctic acid, and some combinations such as Mg^{++} + TPP, Mg^{++} + thioctic acid, Mg^{++} + thioctic acid + TPP, Mg^{++}alone, and thioctic acid + TPP. None of these organic compounds by itself has any effect; none in combination with Mg^{++} has any effect greater than that of the Mg^{++}.

(c) The presence of cofactor(s) in the crude enzyme and crude RuDP was demonstrated. This was done by heat inactivation of enzyme and substrate functions of the crude preparations and then combining them with a complete carboxydismutase system (less any added metal ions). The resulting solution gave enhanced carbon dioxide fixations.

(d) Phosphate concentration has a marked effect on the activity of the enzyme system. For example, an increase of the phosphate concentration from 0.005 M to 0.01 M would inhibit the CO_2 fixation by about 25%. This effect may, however, be purely an effect of increasing ionic strength.

(e) The electrophoretic studies on the purified enzyme were conducted at two different pH's and ionic strengths (pH 6.8, μ = 0.2 phosphate and pH 5.97, μ = 0.1). Both cases showed a single symmetrical peak. The mobilities of these peaks are such that the isoelectric point is estimated to be less than 4.

(f) The N-terminal amino acids of the purified enzyme were determined by the use of the rather specific reagent, 1-fluoro-2, 4-dinitrobenzene. The reaction is as follows:



The protein was hydrolyzed to the constituent amino acids and the dinitrophenyl (DNP)-amino acids. The DNP-amino acids were identified by chromatography, eluted, and determined quantitatively by light absorption at 360 mμ.

The results showed that besides a very large spot of 2, 4-dinitrophenol, the major spot was DNP-glutamic acid. Minor spots were DNP-alanine, -glycine, -serine, and -threonine.

Quantitatively the DNP-glutamic acid was estimated to be approx. 1 μmole per micro-mole of protein of 300,000 molecular weight (previously estimated by ultracentrifugation). However, this result cannot be considered significant, because the amount of protein used for this study was too low to give any accuracy.

(g) The optimum nickel ion concentration for carboxydismutase activity was found to be of the order of 10^{-3} M in the reaction mixture. This was in the absence of phosphate; the bicarbonate was used as both substrate and buffer. It is interesting to note here that the protein concentration in the reaction mixture is about 10^{-6} M, while that of RuDP is about 10^{-3} M.

Further work on this subject is still in progress. At this moment, however, no *Tetragonia expansa* is available, therefore the studies are being carried out on the corresponding protein from *Chlorella*. This project will be reported in a later paper.

IRON-CHLOROPHYLL COMPOUNDS IN GREEN PLANTS

Jean Bourdon

In the preceding report we had shown that if beans were grown in a nutrient solution containing a salt of radioactive iron ($FeCl_3$) and the leaves extracted with acetone, almost all the radioactivity was found in the residual powder and only a very small fraction in the crude acetone solution. The pigments were transferred from acetone to petroleum ether for chromatography, but no measurable radioactivity could be detected in the petroleum ether solution.

According to our hypothesis, it was interesting to look for the presence of copper or manganese which could play the same role as iron in photosynthesis. The radioactive isotopes of copper have a much too short half life to be used conveniently. Two radioactive forms of Mn are possible: Mn^{52}, 6.5 days; and Mn^{54}, 310 days. Several millicuries of Mn^{52} were prepared by cyclotron bombardment of a chromium target on a copper block ($Cr^{52}d$, 2n). The radioactive manganese was extracted from the target and purified according to the method of H. R. Haymond et al.[1] Then beans were grown during two weeks in a nutrient solution containing 20 μc to 400 μc per plant; a harvest of 5.4 g of leaves gave 400,000 counts/min. The acetonic solution after extraction contained 2250 counts, but again the petroleum ether solution after transfer was not measurably radioactive.

The reasonable conclusion is that if there is any iron chlorophyll complex in green plants, it has to be more tightly bound to a protein than chlorophyll itself, and the extraction with solvent used here is not able to break this bond. Therefore it seemed that the only possibility of detecting Fe, Mn, or Cu in chlorophyll complexes would be to look in the chlorophyll-protein compounds that are supposed to exist in chloroplasts. Such a compound, as chlorophyll-lipoprotein complex (CLP), has recently been prepared by Takashima[2] and by Chiba[3] from clover leaves.

The technique used is the following: Fresh clover leaves are ground with sand and phosphate buffer (pH 7), then are centrifuged at 2000 and 3000 rpm. The 2000–3000 fraction is mostly formed of grana and chloroplast fragments, which are dissolved in the phosphate buffer -- α-picoline (1/1) solution -- and dialyzed against the same solution. The CLP crystallizes by addition of dioxane as clusters of green crystals. This compound has been shown to have a molecular weight of 19,000. It is formed of chlorophyll

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[1] H. R. Haymond, W. M. Garrison, and J. G. Hamilton, J. Chem. Phys. 19, 382 (1951).

[2] Shiro Takashima, Nature 169, 182 (1952).

[3] Yasutane Chiba, Arch. Biochem. Biophys. 54, 83 (1955).

a and b (3/1) loosely bound to a lipoprotein.[4] It has been prepared from several different plants and algae.[5]

The present objective of this research is to prepare by the same method this crystalline CLP from plants grown in a nutrient solution containing a salt of radioactive Mn or Fe, then measure the radioactivity of CLP. We have prepared CLP from beans and clover. In subsequent experiments we will make the same preparation in the presence of radioactive iron or manganese.

[4] D. R. Anderson, J. D. Spikes, and R. Lumry, Biochem. Biophys. Acta. 15, 298 (1954).

[5] H. S. Sherrat and W. C. Evans, Nature 173, 540 (1954).

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

FIRST EXCITED STATES OF HEAVY EVEN-EVEN NUCLEI

Royal G. Albridge and Jack M. Hollander

Accurate energy data on the first excited states of some even-even nuclei in the heavy element region are being obtained, by use of the 50-gauss permanent-magnet spectrograph.

The energies of the first excited states of U^{236} and Pu^{236} have been measured from the decay of 22-hour Np^{236}, prepared by the U^{235}(d, n) reaction. The results were: U^{235}, 45.23 kev; Pu^{236}, 44.58 kev. Presently, the value for U^{238} is being sought from decay of the 5×10^5-year parent Pu^{242}.

It is planned also to study the energy levels of U^{235} as populated by the alpha decay of Pu^{239}. It is hoped that some light can be shed on certain anamolies in the present data.

THE ATOMIC BEAM PROJECT

John C. Hubbs

An atomic beam apparatus designed for the investigation of the electronic and nuclear properties of the heavy elements is now nearing completion. We look forward to utilization of the system for the investigation of U^{233} within two months.

The principal difficulty anticipated in the project, the production of a beam of low-vapor-pressure material such as uranium and neptunium,. has now been largely solved through the development of oven systems capable of readily attaining 3000°.

Calculations designed to give approximate values of the hyperfine structure separations in uranium from observed level separations in UI have been initiated. It is hoped that refined versions of the calculation will relate observed hyperfine structure separation intervals to an approximate value of the nuclear magnetic dipole moment and electric quadrupole moment.

MASS SPECTROSCOPY

Frederick L. Reynolds

During this quarter the effort has been devoted mostly toward getting a revamped 20-centimeter-radius 60°-sector spectrometer in operating shape. The entire spectrometer was changed from the previous design of a nearly-all-brass construction using rubber gaskets and oil diffusion pumps to an all-stainless-steel construction using metal gaskets and mercury diffusion pumps. The primary reason for this change was to make this spectrometer a device of higher sensitivity by virtue of higher attainable vacuum and lower background, through the elimination of rubber gaskets and of oil diffusion pumps. The main effort is to make an instrument suitable for running millimicrogram solid samples of the heavy elements for abundance-ratio determination, and to study products obtained from fission or spallation reactions from pile or cyclotron bombardments.

Incorporated in the construction of the instrument are several novel devices. Valves for closing off the diffusion pump section from the trajectory tube used during sample changes depend on an indium seat. This valve was first described by the author at the ASTM conference on mass spectroscopy in May, 1955. The valve consists of a copper block milled as shown in Fig. 1. The top groove is filled with indium and the lower groove outside the vacuum system contains a spiral nichrome heater. To open the valve the heater is used for several minutes to melt the indium (mp 155° C), and then the valve cap can be raised. In closing, the reverse procedure is used. These valves have now been in operation for two months on this equipment and have never failed to give a seal.

Another construction device worth mentioning is the mechanically refrigerated baffles located just above the mercury diffusion pumps. These baffles are cooled to minus 36° C, so that they trap most of the mercury and return it to the pumps by gravity feed. This prevents the bulk of the mercury from collecting on the liquid nitrogen traps. In addition, we trap all forepump lines as well, with liquid nitrogen traps, to prevent any possible back diffusion of oil to the spectrometer from this source.

Preliminary runs on the instrument indicate that we have no trouble in seeing beams of the order of 10^{-18} ampere, and that organic background peaks are at a very low minimum. We are employing a silver-magnesium sixteen-stage multiplier having a total gain of 10^8 at the collector, feeding the output of the multiplier to a vibrating-reed electrometer.

The instrument is equipped with a nuclear magnetic resonance probe, used only for locating accurately the mass position. This device is very useful, especially when very small samples are being run.

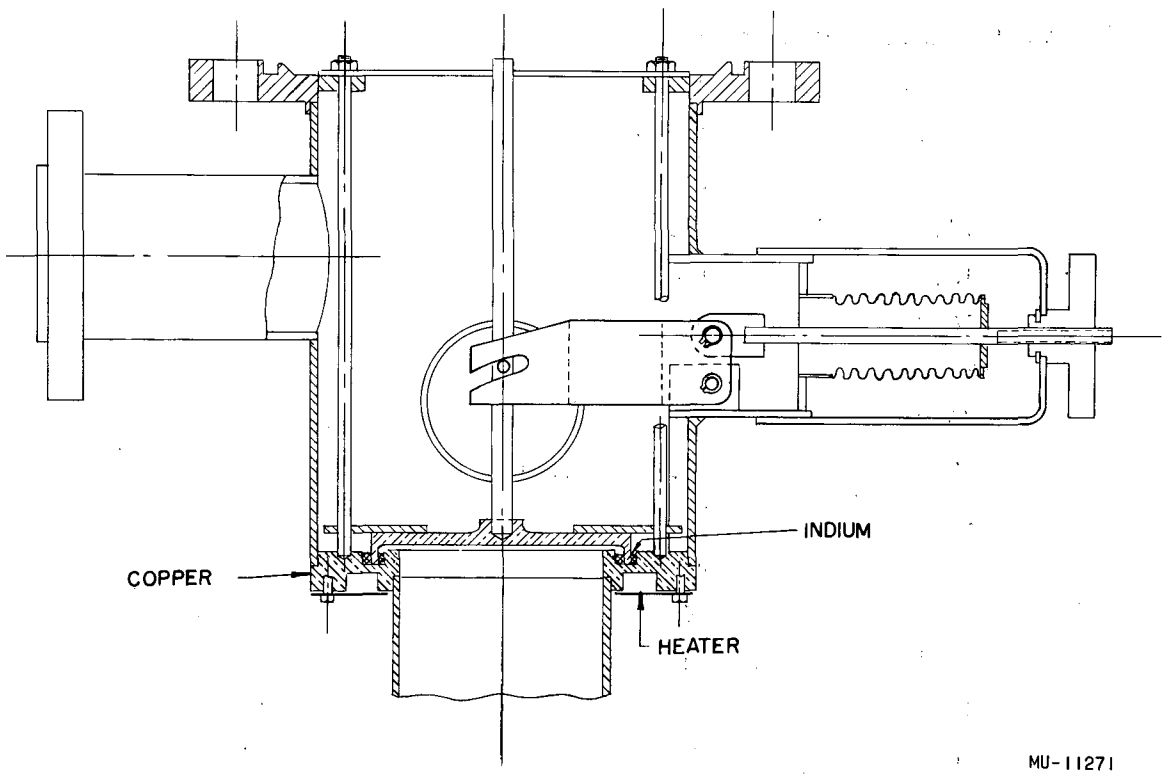

Fig. 1. Valve.

THE DECAY OF THORIUM-227 (RdAc)

R. C. Pilger, Jr., and Isadore Perlman

A more extensive evaluation of the previously obtained electron spectra of Th^{227} has been made, and a provisional decay scheme has been established for the alpha-decay process of this isotope.

The transitions observed in the conversion electron spectra are tabulated below, together with the type and multipolarity of the radiation (when possible), in three groups, reflecting the relative certainty in the electron-line assignments involved. The spectral region investigated was from 10 to 260 kev.

Internal Transitions Observed in the Decay of Thorium-227

I. Well-confirmed transitions:

E (kev)	Type
29.8	M1-E2
31.6	M1
32.6	M1
48.1	E2
50.0	E1
61.3	E2
75.0	M1
100.1	E2 (possibly M1-E2)
113.0	E2
205.0	M1
212.1	M1
230.1	M1
236.1	E1
256.4	E2

II. Reasonably certain assignments:

34.9	(M1 ?)
69.5	
131.0	
168.0	
218.0	
234.9	
250.3	
286.1	
304.5	
312.7	
334.3	

III. Tentative assignments

21.2	
42.3	
53.9	
56.0	
82.6	
86.6	
87.9	
91.1	(E2 ?)
98.5	
99.5	
114.7	
135.2	
166.8	
206.0	
214.6	
247.7	
280.0	
322.5	

A small number of lines in the region 10 to 260 kev remain unassigned. A tentative decay scheme, based upon these observed transitions and upon the alpha-particle spectra previously obtained, is shown in Fig. 2. Three levels not observed in the alpha spectra have been postulated. A curious arrangement of several of the levels in close-lying pairs is noted. Speculative assignments of the transitions not shown have been made, but are regarded as quite tenuous.

Hindrance factors have been calculated for the several alpha-particle groups, as follows:

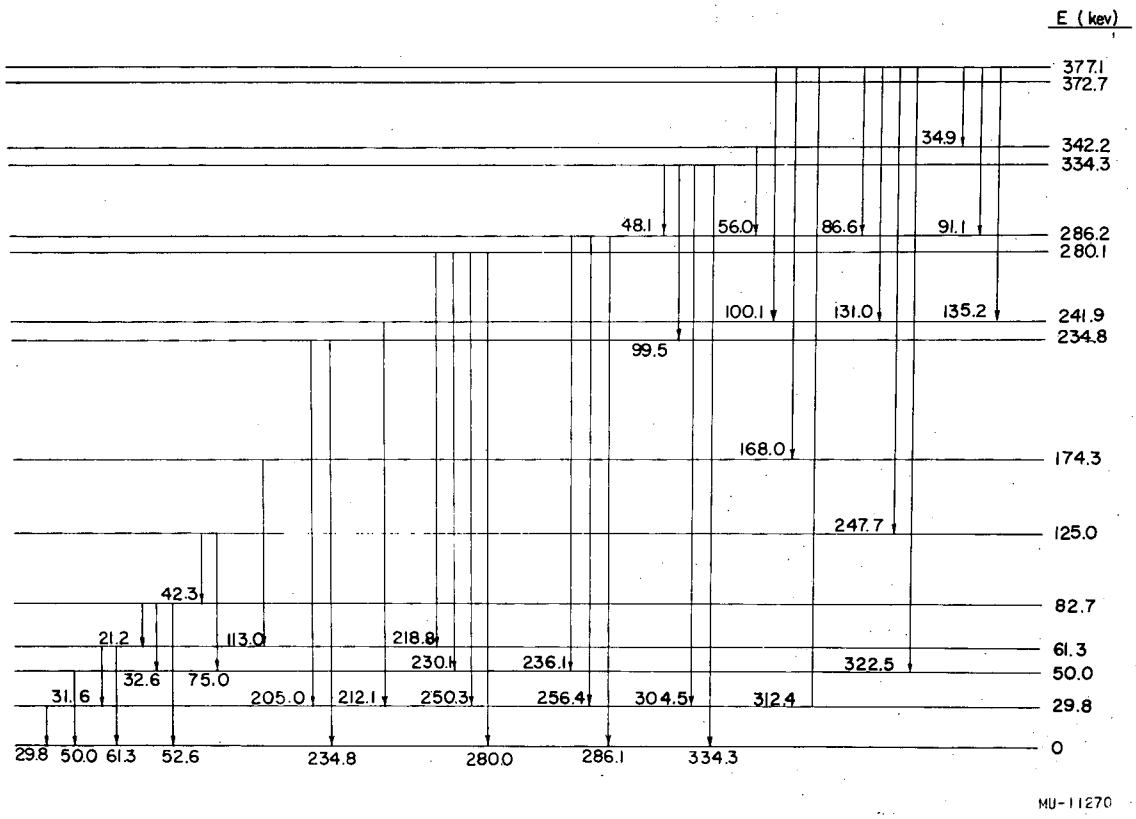

Fig. 2. Level scheme for the transition Th^{227} to Ra^{223}.

ΔE_{Decay}	Group E_{Decay}	Abundance (%)	Half life Measured	Half life Calculated	Hindrance factor
0	6.178	24	77.5d	0.73	106
30	6.148	4	465	0.96	484
61	6.117	25	74.4	1.42	524
80	6.098	3	620	1.64	378
126	6.052	1	1860	2.77	671
175	6.003	3	620	4.60	135
235	5.943	1	1860	9.2	202
243	5.935	0.1	18,600	10.2	1824
287	5.891	23	80.9	16.8	4.82
331	5.847	9	207	29.2	7.09
339	5.839	5	372	30.4	12.2
378	5.800	2	930	46.0	20.2

The 234.9-kev and 236.8-kev transitions discussed in the report for the previous quarter have been subject to reassignment upon re-evaluation of the electron spectra. Only the K-conversion line of the former is observed, and the M1 assignment of Frilley et al.[1] is possible. The latter has now been established by both K and L_1 lines to be 236.1 kev. Our previous determination of the K-conversion coefficient indicated either E2 or E1 character; the appearance of the L_1 line is at variance with E2; hence this transition is now regarded as E1.

[1] Frilley, Rosenblum, Valadares, and Bouissieves, J. Phys. et Radium 16, 378 (1955).

BRANCHING RATIO OF TERBIUM-149

Virginia A. Schultz and John O. Rasmussen

A study of the alpha-to-electron-capture branching ratio of Tb^{149}, begun in the summer of 1955 by Louis Alcorta and John Rasmussen, has just been completed. Special interest in this nuclide and its branching ratio developed as a consequence of its detection in high-energy reactions on various heavy target elements.

A 4.0-Mev alpha group with a half life of 4.1 hours was definitely assigned to Tb^{149} through mass spectrographic studies done by Rasmussen, Reynolds, Thompson, and Ghiorso.[1] Earlier work by Thompson, Ghiorso, Rasmussen, and Seaborg[2] assigned a lower limit of 1% to the alpha-to-electron-capture ratio.

A Tb^{149} sample was prepared by bombarding 50 mg of Eu_2O_3 for 2 hours with 75-Mev alpha particles in the 184-inch cyclotron. The reaction $Eu^{151}(\alpha, 6n)Tb^{149}$ led to the desired product, and other reactions to other neutron-deficient terbium isotopes. The activity due to the other isotopes was such that electron-capture radiation for Tb^{149} could not be resolved from other activities directly; and the electron capture seemed most readily determined by the activity of its daughter, 9-day Gd^{149}, after chemical purification. The target was first freed of most of the unreacted europium through a sodium amalgam reduction. Pure terbium was isolated by twice separating the remaining rare earths on a Dowex-50 column, 3mm x 10 cm, heated to 87° C. The eluting agent was 0.4-M α-hydroxyisobutyric acid buffered to a pH of 3.93 with ammonium hydroxide, and the procedure followed was the same as that used by Thompson et al.[3] After the terbium activity had been left for 16 hours, a column separation was carried out on the terbium fraction to remove all of the Gd^{149} produced in this growth period. The Gd^{149} decay was followed on a platinum plate in a 2π methane-filled windowless proportional counter (nucleometer), and also in a NaI scintillation spectrometer with a 50-channel gamma pulse-height analyzer until the decay curves could be resolved. The alpha branch was measured by following the alpha decay of the terbium sample from the beginning of the growth period.

From the nucleometer data the ratio of α disintegrations to nucleometer counts is 0.086. A counting efficiency of 70% was assumed for the nucleometer, because the complex decay scheme of Gd^{149} is expected to lead to a relatively high counting efficiency. The alpha branching calculated in this way is 6% and the electron capture 94%. From the gamma analyzer data, the ratio of alpha disintegrations to K x-rays is 0.084. If there were one K x-ray per

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[1] Rasmussen, Reynolds, Thompson, and Ghiorso, Phys. Rev. 80, 475 (1950).

[2] Thompson, Ghiorso, Rasmussen, and Seaborg, Phys. Rev. 76, 1406 (1949).

[3] Thompson, Harvey, Choppin, and Seaborg, J. Am. Chem. Soc. 76, 6229 (1954).

disintegration, the alpha branching would be 8% and the electron capture 92%, in reasonable agreement with the nucleometer value, in view of counting efficiency uncertainties. We select 6 ± 2% as the best value.

A second experiment, carried out with the intention of making Tb^{149} free of higher neutron-deficient isotopes, involved a 1.2-hr 120-Mev nitrogen ion bombardment of cerium oxide in the 60-inch cyclotron. The terbium activity was separated from the other rare earths by an ion-exchange method identical with that used in the first experiment. The terbium fraction was counted directly on a platinum plate in a 50-channel alpha pulse-height analyzer and in the above-mentioned nucleometer and gamma pulse-height analyzer. Although interfering terbium activities were present to an extent to make resolving of a 4-hour component in the nucleometer or K x-ray curves uncertain, it was possible to set 10% as a maximum for the Tb^{149} alpha branching. This result is consistent with the 6% result of the previous experiment.

DECAY SCHEMES OF GADOLINIUM-149 AND GADOLINIUM-147

Virginia A. Schultz, Warren G. Smith, and John O. Rasmussen

Gd^{149} was picked for a decay-scheme study partly from a desire to estimate its counting efficiency in connection with the experiments for determination of the α/electron-capture branching ratio of Tb^{149}.

A thick target of Sm_2O_3 was bombarded with 48-Mev alphas in the 60-inch cyclotron to give $Sm^{149}(\alpha, 4n)Gd^{149}$, $Sm^{148}(\alpha, 3n)Gd^{149}$, and $Sm^{147}(\alpha, 2n)Gd^{149}$. Sodium almalgam was used to reduce out most of the unreacted Sm; and a Dowex-50 column, 3 mm x 10 cm, heated to 87° C, was used to separate the remaining rare earths. The eluting agent was 0.4 M α-hydroxyisobutyric acid buffered to a pH of 3.93 with ammonium hydroxide, and the procedure followed was the same as that used by Thompson, et al.[1] Samples of the Gd activity were prepared for study in the double-focusing 0.3%-resolution beta-ray spectrometer, in the anthracene crystal, 3%-resolution lens spectrometer, in the single-channel gamma pulse-height analyzer, and in the 180° 0.15%-resolution permanent-magnet spectrometer.

Twenty-nine transitions with a 9-day half life have been assigned to Gd^{149}: 21.8 kev, 22.9, 77.8, 97.3, 103.2, 106.6, 114.7, 115.5, 119.9, 126.2, 132.1, 150.0, 153.5, 154.6, 174.7, 180.7, 198.9, 244.4, 252.7, 272.8, 298.7, 328.9, 346.6, 460.6, 496.7, 516.9, 584.4, 749.4, 789.8. Twenty-one transitions with a 2.1-day half life have been tentatively assigned to the previously undiscovered Gd^{147}: 69.7 kev, 111.4, 129.9,

[1] Thompson, Harvey, Choppin, and Seaborg, J. Am. Chem. Soc. 76, 6229 (1954).

136.1, 142.1, 147.2, 166.6, 217.3, 226.4, 229.3, 240.9, 249.6, 261.3, 310.1, 348.3, 370.1, 374.5, 390.6, 396.6, 485.2, and 501.8. This isotope could have been made by the $Sm^{147}(\alpha, 4n)Gd^{147}$ reaction, and is being further studied at present. A few longer-lived transitions were found which are probably attributable to europium daughters of higher-mass gadolinium isotopes. Data from a deuteron bombardment on europium to make higher Gd isotopes is being accumulated at present and should help in the assignment of these transitions, as well as making possible a study of the decay schemes of Gd^{151} and Gd^{153}.

Coincidence measurements have been made by Mr. Donald Strominger. Tentative decay schemes incorporating most of the transitions of Gd^{149} have been deduced, but further study is needed to establish any one of them.

ANION-EXCHANGE SEPARATIONS OF MOLYBDENUM AND TECHNETIUM AND OF TUNGSTEN AND RHENIUM

Eugene H. Huffman, Robert L. Oswalt,
and Lawrence A. Williams

Anion-exchange studies of molybdenum, technetium, tungsten and rhenium in hydrochloric acid and of technetium and rhenium in nitric acid have been completed. Convenient separations of these two pairs of elements have been made which allow ready volatilization of the solutions for counting without loss of activity. Figure 3 shows the dependence of the distribution coefficients of these four elements on the hydrochloric acid concentration with Dowex-1, and Fig. 4 shows this dependence on the nitric acid concentration for technetium and rhenium. Figure 5 shows the separation of molybdenum from technetium in 1.0 M HCl, followed by the elution of technetium in 4.0 M HNO_3 and Fig. 6 a similar separation of tungsten from rhenium with 1.5 M HCl.

This work has been reported more fully in UCRL-3324.

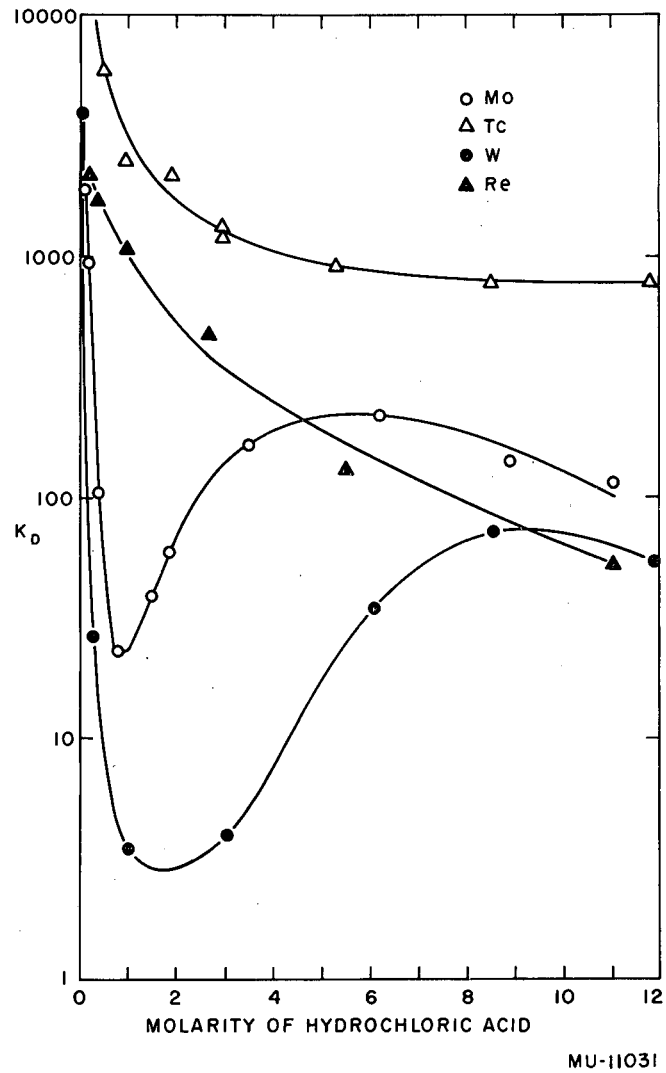

Fig. 3. Dependence of the distribution coefficient at 25° on the hydrochloric acid concentration with Dowex-1.

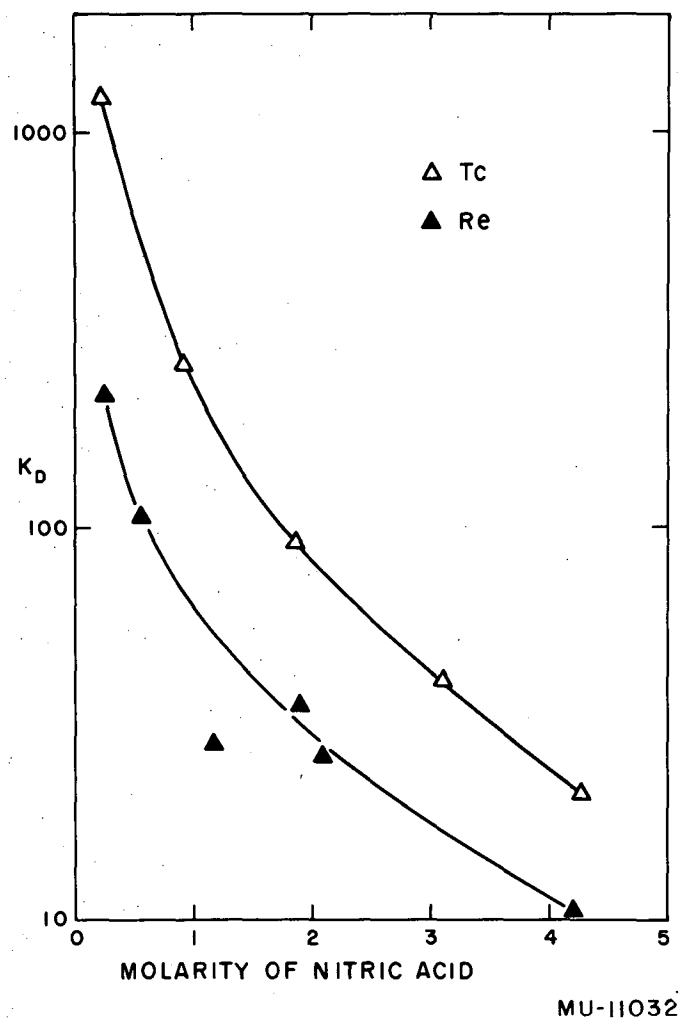

Fig. 4. Dependence of the distribution coefficient at 25° on the nitric acid concentration with Dowex-1.

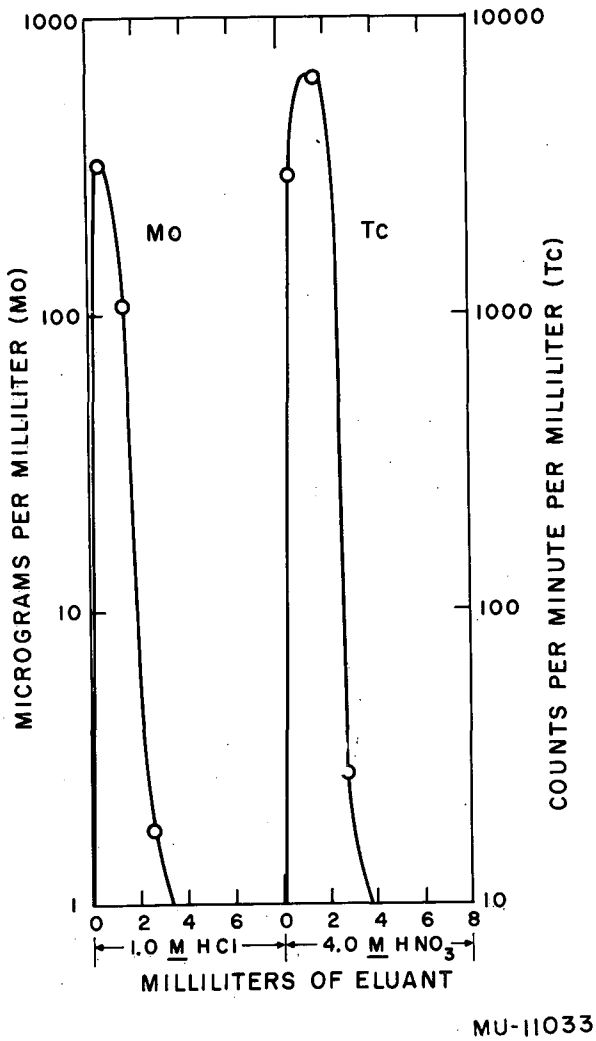

Fig. 5. Separation of molybdenum and technetium on Dowex-1; column 3 mm x 21 mm.

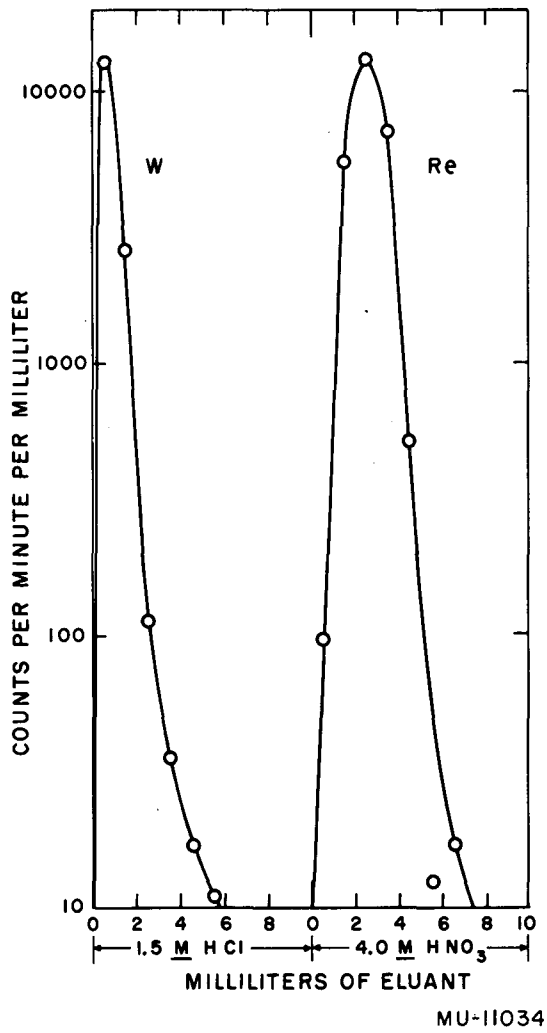

Fig. 6. Separation of tungsten and rhenium on Dowex-1; column 4 mm x 50 mm.

OPTICAL SPECTROSCOPY

John G. Conway and Ralph D. McLaughlin

Fluorescence of Am Cl_3 and $PuCl_3$

(with Dieter M. Gruen and Burris B. Cunningham)

Single crystals of $LaCl_3$ containing 0.04% and 0.2% $AmCl_3$ and 0.2% of $PuCl_3$ have been prepared. All three crystals have a self-luminescence which is the excitation of the fluorescence by the radioactive decay of the americium or the plutonium. The fluorescence is greatly increased by irradiation with ultraviolet light.

$AmCl_3$

The spectra of both the fluorescence and absorption have been photographed. So far 34 fluorescence lines have been found, four of which coincide exactly with four absorption lines.

$PuCl_3$

The plutonium spectra have also been photographed, and to date only two lines in the fluorescence coincide with absorption lines.

DECAY OF BISMUTH-203, BISMUTH-204 AND BISMUTH-205

Arnold R. Fritsch

The decay schemes of Bi^{203}, Bi^{204}, and Bi^{205} are being investigated. A rather large number of transitions have been found in each case. The corresponding Pb isomers have also been studied.

At present the transitions obtained are being analyzed by means of a computer in order to enable one to construct an energy-level diagram for each case.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

NOTES ON WORK IN PROGRESS

The Effect of Pressure on Mass Transfer in the Gas Phase

Robert J. Fallat and C. R. Wilke

Mass transfer rates for sublimation of naphthalene pellets into air have been determined in a series of preliminary experiments covering a range of pressures. Measurements of naphthlene vapor pressures at various temperatures and pressures are in progress to aid in the interpretation of the mass transfer results.

Diffusion in Liquids

Elwood Gift and C. R. Wilke

This work has been temporarily discontinued.

Gas-Liquid Partition Chromatography

Robert H. Houston and C. R. Wilke

Construction of experimental equipment is nearing completion. The initial unit will be a 6-foot-long diatamaceous earth- silicone oil column of 11 mm diameter. Other packing material and nonmobile phase materials will be studied.

Preliminary studies will be on the separation of various mixtures of aromatic hydrocarbons.

Thermal Conductivity of Gases at High Temperatures

Henry Cheung and C. R. Wilke

Apparent temperature differences between the top and bottom of the cell have been shown to be due to errors in temperature measurement caused by conduction along the thermocouple and thermocouple well.

Experimental conductivity measurements are now in progress.

Stability of Perforated-Plate Trays

Robert Brown, Donald N. Hanson, and C. R. Wilke

The new liquid distributor has been found to work satisfactorily.

A blower bypass line has been installed to decrease the effect of pulsations in the gas flow.

Tests are being conducted to compare dry-plate pressure-drop data with those obtained in previous studies.

Correlation of Limiting Current Density at Horizontal Electrodes under Free-Convection Conditions

Eugene J. Fenech and Charles W. Tobias

Limiting current data have been collected for all plate sizes and spacings, for the concentrations 0.8, 0.25, and 0.08 molar copper sulfate and 1.5 molar sulfuric acid, with and without endplates. Correlation of data is now in progress.

Liquid-Liquid Extraction and Agitation

James H. Vanderveen and T. Vermeulen

Further preparations have been made to study the rates of coalescence of the unstable emulsions produced by liquid-liquid agitation. Three experimental measurements are planned for this purpose: (a) rate of approach to steady-state mixing; (b) vertical distribution of drop sizes in the mixing vessel in the steady state; (c) rate of distribution of a component soluble only in one phase.

Vacuum Flow through Circular, Annular, and Rectangular Sections

Walter Dong, Donald N. Hanson, and Leo Bromley

This work has been completed and a thesis is being written which will appear as UCRL-3353.

GENERAL CHEMISTRY

Robert E. Connick in charge

METALS AND HIGH-TEMPERATURE THERMODYNAMICS

Leo Brewer, William Hicks, Geoffrey James, Frank Greene

Absolute f-value determinations

Mechanical modulation seems to be the most practical, and equipment is under construction for light modulation and phase-shift detection. An apparatus for production of the molecular beams to be studied is under construction. A study of arc light sources is in progress.

Ground State of C_2

Preliminary measurements of the relative temperature coefficients of the Swan and Phillips bands of C_2 have established the $^3\pi$ of C_2 as the ground state. The $^1\Sigma$ state is excited by about a volt.

Stability of SiO

Mixtures of Si and SiO_2, using an inductively heated thermal analysis apparatus, have consistently given a heat effect at the melting point of Si. This indicates that solid SiO has no appreciable range of thermodynamic stability.

Stability of Refractory Silicides

This work has been summarized in the paper "Reactions of Refractory Silicides with Carbon and Nitrogen," by Leo Brewer and Oscar Krikorian, J. Electro-chem. Soc. 103, 38-51 (1956). No further experimental work of this type is contemplated, but further analysis of the data in an effort to predict behavior in yet unexplored systems is in progress.

Vaporization Theory for Porous Solids of Low Vaporization Coefficients

A rather complete theory has been developed, which will shortly be written up in a UCRL report.

BASIC CHEMISTRY

Howard Cady, Robert Connick, Claude Coppel, Ladd Griffith, Robert Nickerson, Kenneth Pitzer, Robert Wood, and Earl Worden

Kinetics of Rapid Reactions

At the conclusion of the preceding report period work was being done on measuring the characteristics (firing time, mixing time, etc.) of the new single-baffle plexi-glass mixer. This mixer fired in approximately 5×10^{-3} second and had no premixing characteristics. However, it did present quite a problem with regard to bubble formation. Irrespective of how vigorous an outgassing was carried out, bubble formation was observed. This is believed to be due to the small percentage of orifice area in the turbulator plate. Since the new baffle was also available, further work on the plexi-glass mixer was discontinued for the present.

The new baffle consists simply of a rectangular cell which sits in the mixing chamber on a neoprene gasket. At the bottom of the baffle is a turbulator plate that has approximately 60% orifice area. One reactant is within the rectangular cell, while the other surrounds it. This combination of new baffle with old mixing chamber has been run through a series of tests with the following results:

(a) no premixing when gasket is greased with a thin film of stop-cock grease;

(b) no bubble formation when outgassed thoroughly;

(c) firing time in air: 2×10^{-3} second;

(d) firing time in water: 3×10^{-3} second;

(e) mixing time for approximately complete mixing: 5×10^{-3} second.

We believe that the mixer has good characteristics generally, and now work is being planned to use this mixer in studying some rates of reactions.

Ruthenium Chemistry

The work in the last quarter has been directed toward the preparation of Ru (II) and Ru (III) solutions in perchloric acid. It is hoped that the Ru will not be complexed as it was in trifluoroacetic acid. Preliminary experiments indicate that it is possible to prepare Ru (II) by chemical reduction with titanous ion. The Ru (II) is more stable than the Ti^{+3} in perchloric acid solutions. The solutions of Ru (III) in perchloric acid mentioned in the preceding quarterly report showed definite signs of oxidation after 1.5 months.

Determination of the Molecular Structure of Aluminum Hydride

Various methods have been investigated for adapting the available Varian Nuclear Magnetic Resonance equipment for use at low temperatures (from room temperature down to the boiling point of liquid hydrogen). The method decided upon consists of placing, in a specially designed dewar a coil into which a sample container may be inserted. The coil is part of a balanced bridge utilizing the transmitter and receiver of the Varian equipment. The coil and sample are cooled by passing previously cooled nitrogen gas around the sample and coil. The temperature is measured with a thermocouple inserted in the sample.

Heat of Formation of $FeO_4^{=}$ Ion

A heat of formation for the $FeO_4^{=}$ ion of 70 kcal has been determined calorimetrically. Errors caused by impurities in the ferrate and inaccuracies in the heats of dilution are being reduced.

Acetaldehyde t_1

The acetaldehyde, t_1, found to have a specific concentration of tritium too high for counting was diluted several times until a sample suitable for counting was obtained. Some preliminary thermal decompositions with pure normal acetaldehyde are being made.

The ultraviolet spectrum of pure acetaldehyde is being studied in preparation for a study of the isotope effect in the photochemical decomposition of acetaldehyde.

Radiation-Induced Reactions in Nonaqueous Solutions

The study of the photolysis of α-α-diphenyl-β-picryl-hydrazyl (DPPH) in solution has been extended to the aromatic solvents benzene and toluene. Light of wave length 3130 A is isolated by the use of appropriate filters. The source is a Hanovia medium-pressure mercury arc, Type 16,200.

Preliminary results indicate that the disappearance of DPPH is not a process involving the reaction of DPPH molecules excited by the incident radiation, but rather a process involving the reaction of an excited solvent molecule with the DPPH.

Experiments are in progress to confirm these preliminary results.

Composition of Carbide Vapors

Stanley G. Davis

Assembly has continued of the system for heating metal carbides by RF induction, effusing the vapors, and collecting them on a cold target. Cooperation has been given to Engineering Research in planning apparatus for measuring vapor molecular weights by effusion from a torsion cell heated by radiation. Initial measurements, by effusion and weight loss, of the vapor pressure above SiC have been made; the vessel is being revised to decrease condensation on its lid.