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UNIVERSITY OF CALIFORNIA

Radiation Laboratory

CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August 1955

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CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August 1955

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

September 30, 1955

BIO-ORGANIC CHEMISTRY

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

THE FREE ENERGIES OF REACTIONS OF THE CARBON-REDUCTION CYCLE

J. A. Bassham

It is of interest to calculate the free energies of every step in the carbon-reduction cycle in order to ascertain that each postulated step is energetically possible and in order to see how energy, supplied to the cycle by energy-rich compounds such as adenosine triphosphate (ATP) and reduced triphosphopyridine nucleotide (TPNH), is used to keep the cycle going at a rapid pace.

Since the condition of photosynthesis in an active green plant is a condition of dynamic steady state, rather than an equilibrium, there is a certain excess of energy supplied to the cycle over that stored in the form of reduced carbon compounds, which are the end products of the operation of the cycle. This excess of free energy is required to keep the cycle turning with a rapid net flow of material from one intermediate compound to the next in the forward direction.

The calculations that follow show that the excess free energy, which we will call E for each carbon atom reduced, is distributed among various reactions of the carbon-reduction cycle in such a way as to keep the cycle running smoothly and rapidly in the forward direction, without vanishingly small concentrations of any of the postulated intermediates.

It seems unnecessarily long to go through the details of these calculations in this report. Instead, the assumptions and results are summarized.*

---

* The following abbreviating symbols are used: E4P, erythrose-4-phosphate; DHAP, dihydroxyacetone phosphate; SDP, sedoheptulose diphosphate; GA13P, glyceraldehyde-3-phosphate; FDP, fructose diphosphate; S7P, sedoheptulose-7-phosphate; F6P, fructose-6-phosphate; RuDP, ribulose diphosphate; Ru5P, ribulose-5-phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Ri5P, ribose-5-phosphate; TPNH, reduced triphosphopyridine nucleotide; TPN, triphosphonucleotide; G6P, glucose-6-phosphate.

1. The calculations of reaction energies of glycolytic steps from glucose to pyruvic acid are based on the published calculations of Burton and Wilson[1] and of Burton and Krebs.[2]

2. Following their system, free energies were calculated for all compounds from glucose to pyruvate at concentrations of 10^{-2} M, aq., pH = λ, O_2 = 0.21 atmos, CO_2 = 0.05 atmos.

3. After free-energy values for glucose and fructose had been obtained from these calculations, the free energies of these hexoses in their straight-chain form were calculated, based on published data indicating that 0.024% of glucose in solution is in straight-chain form, and on a corresponding value of 0.28% for fructose, which was measured here. These straight-chain values plus free-energy values for glyceraldehyde, dihydroxyacetone, and glycerol--all in solution--were used in setting up an empirical calculation of the free energies of ribose, ribulose, sedoheptulose, and erythrose.

4. The ring stabilization of sedoheptulose was assumed to be the same as that of fructose; the ring stabilization of ribose was calculated from published data on the amount of straight-chain form in solution.

5. The free energies of formation of erythrose-4-phosphate, sedoheptulose-7-phosphate, and ribose-5-phosphate from their respective free sugars and inorganic phosphate were all assumed to be the same.

6. Other reactions were assumed to have the same energies as analogous reactions. These included

$$E4P + DHAP \longrightarrow SDP,$$

assumed to be the same as

$$GA13P^{-2} + DHAP^{-2} \longrightarrow FDP^{-4}.$$

$$SDP^{-4} + H_2O \longrightarrow HPO_4^{-2} + S7P^{-2},$$

assumed to be the same as

$$FDP^{-2} + H_2O \longrightarrow HPO_4^{-2} + F6P^{-2}.$$

The free energies of formation of all intermediates in the carbon-reduction cycle are given in Table I. In this table, ΔF values are calculated both for dissolved reductants at 10^{-2} M, aq. and for the actual concentrations measured by kinetic studies during photosynthesis.

---

[1] K. Burton and T. H. Wilson, Biochem. J. 54, 86 (1953).
[2] K. Burton and H. A. Krebs, Biochem. J. 54, 94 (1953).

The free energies of the various steps in the carbon-reduction cycle follow:

$HCO_3^- + RuDP^{-4} \longrightarrow 2\ PGA^{-3} + H^+$ $\Delta F' = -9.5$ kcal (1)

$Ru5P^{-2} + ATP^{-4} \longrightarrow RuDP^{-4} + ADP^{-3} + H^+$ $\Delta F' = -3.2$ kcal (2)

$Ri5P^{-2} \longrightarrow Ru5P^{-2}$ $\Delta F' = +0.4$ kcal (3)

$S7P^{-2} + GA13P^{-2} \longrightarrow Ri5P^{-2} + Ru5P^{-2}$ $\Delta F' = +1.4$ kcal (4)

$SDP^{-4} + H_2O \longrightarrow S7P^{-2} + HOP_4^{-2}$ $\Delta F' = -4.6$ kcal (5)

$E4P^{-2} + GA13P^{-2} \longrightarrow SDP^{-4}$ $\Delta F' = -4.2$ kcal (6)

$F6P^{-2} + GA13P^{-2} \longrightarrow Ru5P^{-2} + E4P^{-2}$ $\Delta F' = +3.2$ kcal (7)

$FDP^{-4} + H_2O \longrightarrow F6P^{-2} + HPO_4^{-2}$ $\Delta F' = -4.8$ kcal (8)

$GA13P^{-2} + DHAP^{-2} \longrightarrow FDP^{-4}$ $\Delta F' = -3.2$ kcal (9)

$GA13P^{-2} \longrightarrow DHAP^{-2}$ $\Delta F' = -1.3$ kcal (10)

$PGA^{-3} + ATP^{-4} + TPNH + H^+ \longrightarrow GA13P^{-2} + TPN^+ + ADP^{-3} + HPO_4^{-2}$

$\Delta F' = -1.5$ kcal (11)

Table I

Free Energies of Formation of Intermediates in the Carbon-Reduction Cycle

Compound	ΔF (10^{-4} M aq.)	Conc in chloroplast (1% $CO_2 \times 10^{-2}$ M)	$\Delta F'$ at PS conc
PGA	(P) -157.0	1.63	(P) -156.7
GA13P	(P) -102.2	0.05 (est.)	(P) -104.0
DHAP	(P) -104.0	0.11 (est.)	(P) -105.3
FDP	2(P) -209.0	0.004	2(P) -212.5
F6P	(P) -216.0	0.12	(P) -217.3
G6P	(P) -216.5	0.33	(P) -217.2
E4P	(P) -138.6	0.05 (est.)	(P) -140.4
SDP	2(P) -245.6	0.006	2(P) -248.6
S7P	2(P) -245.6	0.25	(P) -253.2
Ri5P	(P) -176.7	0.10 (est.)	(P) -178.1
Ru5P	(P) -176.1	0.07 (est.)	(P) -177.7
RuDP	2(P) -169.9	0.51	2(P) -169.6
HCO_3^-	-142.9	0.187	-143.9

For each complete turn of the carbon-reduction cycle, three molecules of CO_2 are taken up, 6 molecules of PGA produced, and the various steps in the cycle take place the number of times indicated below. The free-energy change of each step per cycle can therefore be calculated.

Step No.	$\Delta F'$	No. of times N	$N\Delta F'$
1	-9.5	3	-28.5
2	-3.2	3	- 9.6
3	+0.4	1	+ 0.4
4	+1.4	1	+ 1.4
5	-4.6	1	- 4.6
6	-4.2	1	- 4.2
7	+3.2	1	+ 3.2
8	-4.8	1 1/2 (1 + 1/2*)	- 7.2
9	-3.2	1 1/2 (1 + 1/2*)	- 4.8
10	-1.3	2 1/2 (2 + 1/2*)	- 3.3
11	-1.5	6	- 9.0
Total			-66.2

* These amounts needed for noncyclic formations of end product, 3 $[CH_2O]$

These reactions bring the three carbon atoms to the level of F6P. To get to the level of glucose we need two more steps,

$$F6P^{-2} \longrightarrow G6P^{-2}, \qquad \Delta F' = +0.1 \qquad (12)$$

and

$$G6P^{-2} + H_2O \longrightarrow \text{Glucose} + HPO_4^{-2} \qquad \Delta F' = -4.9 \qquad (13)$$

Each of these steps occurs 1/2 time per turn of the cycle (3 carbon atoms), so to -66.2 we must add

$$1/2\,(-4.9 + 0.1) + -2.4$$

to give a total of 68.6 kcal. For each HCO_3^- molecule reduced this is E = -68.6/3 = -22.9 kcal. This represents the excess of energy supplied to the carbon-reduction cycle over that stored in the form of reduced carbon. The energy stored for the reduction of one HCO_3^- molecule is given by

$$HCO_3^- + H^+ \longrightarrow [CH_2O] + O_2 \qquad \Delta F' = +115.6 \text{ kcal} \qquad (14)$$

The energy supplied to the cycle for each HCO_3^- reduced may be calculated as that expended by the hydrolysis of three molecules of ATP and the oxidation of 2 molecules of TPNH.

The oxidation of TPNH by O_2 is given by the sum of

$$TPNH \longrightarrow TPN^+ + H^+ + 2e^- \qquad \Delta F = -14.9 \qquad (15)$$

$$1/2\ O_2 + 2e^- + 2\ H^+ \longrightarrow H_2O \qquad \Delta F = -36.7 \qquad (16)$$

which is

$$TPNH + 1/2\ O_2 + H^+ \longrightarrow H_2O + TPN^+ \quad (\Delta F = -51.6 \text{ kcal})$$

The sum of

oxidation of 2 TPNH is 2(51)	$\Delta F = -103.2$
and hydrolysis of 3(ATP) 3(2)	$\Delta F = -33.9$
which is	$\Delta F = -137.1$

The energy given out by 2(51) + 3(2) differs from the energy stored in Reaction (13) by -137.1 + 115.6 = 21.5 kcal. This represents (E), the energy expended in keeping the cycle running at a dynamic steady state. At equilibrium, E would be zero.

METABOLISM OF THIOCTIC ACID IN ALGAE

Hans Grisebach

Since a previous report (UCRL-2932, March 1955), a more detailed study of the distribution of the 6-thioctic acid (6T) inside the cells has been undertaken. After 3 and 4 hours' contact of Chlorella with the $6T\text{-}S^{35}{}_2$, the algae were centrifuged, washed with distilled water, suspended in water plus phosphate buffer, and ruptured by 10-minute treatment in a 9-kc oscillator. Plastid material containing chlorophyll was isolated by ultracentrifugation. This plastid material was then extracted with hot 80% ethanol and the extract was chromatographed with butanol/0.5 N NH_3. Table II shows the distribution of the radioactivity in the cells. Table III shows the distribution of the radioactivity in the plastid extract. The insoluble material that remains after the extraction of the plastids with hot 80% ethanol was hydrolyzed with 6 N HCl in a steam bath for 12 hours. The hydrolysis products were chromatographed two-dimensionally (phenol-water; butanol-propionic acid). The hydrolysis product was 6T besides a very weak (~2% of total radioactivity) spot with lower R_f value which did not correspond to any of the cochromatographed sulfur-containing amino acids (methionine, glutathione, cystine, cysteine, homocystine).

Table II

Radioactivity distribution in Chlorella

	3 hours metabolized	4 hours metabolized
plastids	7.3%	6.3%
supernatant	16.8%	24.6%
insoluble	76.0%	70.0%

Table III

Radioactivity distribution % of total S^{35} on chromatogram of plastid extract

origin	8.2%
6T sulfoxide	7.7%
6T	24.0%
chromatogram front (lipids)	60.0%

In another experiment the soluble proteins in the supernatant were precipitated by adding ammonium sulfate up to 90% of saturation. The precipitated proteins were redissolved in 0.05 M phosphate buffer and then dialyzed against 10^{-5} M phosphate buffer. The dialyzed protein solution contained 49% of the radioactivity of the total supernatant. The proteins were hydrolyzed and chromatographed in the same way as the insoluble material. The hydrolysis product was 6T besides a very weak spot with lower R_f value which is probably the same compound as from the hydrolysis of the insoluble.

The distribution of the 6T in the cells is now quite clear. Approximately 4% of the total 6T, which is taken up by the cells in 3 to 4 hours, is in the form of a more lipid-soluble compound in the plastids. This lipids is the major metabolic compound in the plastids. The bulk of the 6T is in the insoluble fraction, which contains the insoluble proteins, glucoproteins, starch, etc. A considerable amount is also bound to or strongly absorbed by the soluble proteins. One cannot decide yet if the 6T is actually bound to or only strongly absorbed by the soluble proteins and the insoluble fraction.

The amount of the metabolic products of 6T varies with the time of the contact of the 6T with the cells. Scenedesmus was metabolized with 6T. After various lengths of time, small samples of cell suspension were withdrawn and filtered rapidly, washed, and extracted with hot aqueous ethanol. The concentrated extracts were chromatographed in one dimension with butanol-0.5 N ammonia. Table IV shows the distribution of the radioactivity after various lengths of time. The amount of the 6T lipid at first increases with time,

Table IV

Radioactivity distribution % of total S^{35} on chromatogram

Time	Origin		6T sulfoxide		6T		Chromatogram front (lipids)	
	extract	medium	extract	medium	extract	medium	extract	medium
1 min	14.0	4.7	79.5	24.0	4.8	73.0	2.0	0
10 min	19.5	5.0	68.3	37.0	8.2	56.0	4.0	0
30 min	22.0	5.5	57.0	27.0	10.0	39.0	12.0	0
1 hr	24.0	11.0	37.0	35.0	13.5	53.0	25.0	0
4 hr from other experiment	59.2	--	13.6	--	8.9	--	14.5	--
1 hr with dead algae	16.5	--	50.9		27.5		1.5	
control (6T extracted together with algae)	5.3		4.8		85.0		2.0	

but the relative amount seems to decrease after a longer time because the amount of the insoluble material increases considerably.

One can see clearly that the 6T lipid is formed only inside the cells. The medium does not contain any detectable amount of the 6T lipid. Also, dead algae (killed by boiling) do not form any considerable amount of the 6T lipid, which is a further proof of its true metabolic character. Whether or not the rapid increase of the 6T sulfoxide after a very short time in the cells has any biological significance is difficult to decide, because the factors that might catalyze the formation of the 6T sulfoxide are still unknown.

In order to get more information about the nature of the 6T lipid the compound (eluted from the front of the chromatograms) was hydrolyzed with N/10 HCl and various lipases. The results of the hydrolysis with N/10 HCl at 110° C at various lengths of time are shown in Table V. Furthermore, the 6T lipid was treated with lipase (steapsin commercial product, Nutritional Biochemicals Corp.) in 0.1 N acetic acid for 24 hr at 37° C. The results in Table VI show that the 6T lipid is hydrolyzed by the lipase. It was hoped that more information could be obtained about the type of ester (glycerol ester or ester with higher alcohol) by using a more specific lipase. For this purpose, pig pancreas lipase was used, which was stated by other workers to be specific for triglycerides. The lipid was split by this lipase, but when the specificity of the lipase was checked it was found that n-amylacetate is also hydrolyzed under the same conditions. This experiment therefore has not significance for the type of the ester. The hydrolytic behavior, with respect to both acid and enzyme, pretty well established the 6T lipid as esterified at the carboxyl group.

On the normal paper chromatograms, the 6T lipid runs always with the solvent front together with the chlorophyl and the other plant pigments. On paper impregnated with "Quilone"[1] and absolute methanol as the solvent, the 6T lipid separated into two spots with R_f values of 0.7 and 0.8, and some radioactivity was spread out on the front of the chromatogram. There was no definite separation of the 6T lipid from the chlorophyll.

Experiments were therefore undertaken to separate the 6T lipid from the pigments. On an Al_2O_3 column with the solvents petroleum ether, petroleum ether + 3% benzene, and petroleum ether + 3% absolute ethanol no separation of the radioactivity from the chlorophyl band could be obtained. The recovery of the activity was only 39%. The rest of the activity was strongly absorbed on top of the column. On powdered sugar (C and H commercial product, dried for 12 hr at 90° C), a separation of the bulk of the activity from the chlorophyll fraction could be obtained. Two typical experiments are shown in Tables VII and VIII. Fractions No. 3 and No. 4 from Table VII and fraction No. 3 from Table VIII appeared as gray bands on the sugar columns. According to the literature[2] this seems to be oxidized or "allomerized" chlorophyll. Fraction No. 3 from Table VIII was rechromatographed on powdered sugar and developed with ligroin benzene 1:1. The bulk of the radioactivity could be separated in this way from the gray band and the solution showed only very weak absorption in the visible range of the spectrum. It seems from these experiments that the 6T lipid is not bound to any of the plant pigments.

---

[1] D. Kritchevsky and M. Calvin, J. Am. Chem. Soc. 72, 4330 (1950).
[2] E. Rabinowitch, "Photosynthesis II," Interscience Publishers, 1951, p 613.

Table V

Hydrolysis of 6T lipid with N/10 HCl at 110° C

Time	% hydrolysis
10 min	3.5
30 min	19.5
1 hr	43.5
3 hr	68.5

Table VI

Radioactivity distribution of 6T lipid hydrolyzed with lipase commercial

	lipid + lipase	Control (lipid + 0.1 N CH_3COOH without lipase)
Origin	16%	4%
6T sulfoxide	55%	
6T	11%	
Front	19%	96%

Table VII

Liquid chromatogram of the 6T lipid on powdered sugar; % distribution of the radioactivity in the different fractions

1)	Benzene-ligroin (bp 65°–110°)	1:8	6.2%
2)	" "	1:4	2.9%
3)	" "	1:1	24.0%
4)	" "	4:1	9.0%
5)	Ether-benzene	400:1	4.0%
6)	"	40:1	
	(a) before chlorophyll band		3.0%
	(b) chlorophyll band		1.6%

Recover 51%; the rest of the activity stays on top of the column and is probably polymerized product.

Calvin

Table VIII

Liquid chromatogram of the 6T lipid on powdered sugar; % Distribution of the radioactivity in the different fractions. The chromatogram was developed first with benzene-ligroin (bp 65°–110°) 1:8 and then with benzene-ligroin 1:1.

Fraction 1	strong yellow	11%
Fraction 2	weak yellow	6.5%
Fraction 3	weak green	13.4%
Fraction 4	strong yellow	19.6%
Fraction 5	chlorophyll a	1.4%
Fraction 6	chlorophyll b	5.2%
Recovery 57%		

Experiments for the preparation of lipothiamid

According to work of Reed and DeBusk[3] one of the biologically active forms of 6T is the so-called lipothiamid (LTD), and amide of 6T with vitamin B_1. Previous work had been done only with very impure preparations of 6T (~50% pure). We therefore tried to prepare LTD synthetically with crystalline 6T as a starting material. Reed claims to have prepared LTD by reacting 6-thioctic acid chloride with free thiamine in formamide. In order to prepare the 6T acid chloride he treated 6T with oxalylchloride in a sealed tube at 60°–70° C for 90 min. We repeated this experiment with crystalline 6T, and found that even at room temperature the characteristic peak of the 6T at 330 mμ disappeared in benzene solution in the presence of oxalylchloride. This shows that oxalylchloride destroys the 6T even under very mild conditions. With thionylchloride in benzene solution the 6T is destroyed immediately. In another experiment we attempted to synthesize LTD by reaction of 6T and thiamin in the presence of dicyclohexylcarbodiimid which readily forms peptide bonds.[4] As yet, no compounds other than 6T and 6T anhydride have been detected on the chromatogram (butanol, 0.5 N ammonia) of the reaction mixture in aqueous tetrahydrofuran.

[3] L. G. Reed and B. G. DeBusk, J. Am. Chem. Soc. 74, 3457 (1952); J. Biol. Chem. 199, 881 (1953).
[4] J. C. Sheehan, J. Am. Chem. Soc. 77, 1067 (1955).

ULTRAVIOLET ABSORPTION SPECTRA OF RIBULOSE DIPHOSPHATE

E. M. Thain

In an unpublished report of J. R. Quayle of the laboratory on the preparation and properties of ribulose diphosphate (RuDP), it was mentioned that enzymatically prepared samples of this compound possessed a weak absorption peak in the ultraviolet, ϵ_{mol} 494 at 260 mμ. This report deals with a continuation of the work, particularly to determine whether the absorption was due to RuDP or to an impurity contaminating the sample. For trail experiments fructose diphosphate (FDP) was used, since it also had been observed to have a similar, but weaker, absorption in the ultraviolet. It was found that brucine was preferable to acridine in the preparation of a crystalline derivative. FDP regenerated from a twice crystallized sample of the brucine salt possessed no absorption peak in the ultraviolet. Similar experiments with RuDP gave products with no peak at the original wave length, 260 mμ, but a weaker peak at 280 mμ.

Experimental

Dowex 50 was freed from ultraviolet-absorbing material by repeated switching from the acid to neutral form with 6 N HCl and 2 N NaOH at 90°, until the washings gave no absorption in the ultraviolet. Count was lost of changes required, but it seemed a large number. With columns of this material acridine hydrochloride and brucine hydrochloride gave acid eluates with no absorption in the uv, proving the efficiency of the column.

Tribrucine salt and barium salt. RuDP (70 mg barium salt) was freed from the barium by Dowex 50 [H^+] treatment, and to the acid eluate (1 to 2 ml) was added brucine (0.14 g, anhydrous, 3 mols) in acetone (2 ml). The brucine salt crystallized spontaneously from the clear solution. It was recrystallized twice from aqueous acetone before decomposition with Dowex 50 [H^+], as before. Barium hydroxide was added to the acid eluate to pH 6, followed by a few drops of barium acetate solution (1 M) and ethanol (4 vols). After standing at 0° for 3 hrs, the precipitate was gathered by centrifugation, washed with 100% ethanol, and dried in a vacuum desiccator over phosphorus pentoxide at room temperature; weight of barium salt, 30 mg. Analysis of this material by paper chromatography showed it to be identical with the original RuDP sample but lacking in some impurities present in the original. The purified material served as substrate for carboxydismutase as well as or better than the original sample.

The absorption spectra of the purified and of the original material, as determined by the Cary recording spectrophotometer, are shown in Fig. 1. The purified material has a molar extinction coefficient of $\epsilon = 80$ at $\lambda_{max} = 279$ mμ.

It is concluded that the peak observed at 260 mμ in the original sample is due to an impurity.

It is difficult to decide whether the peak, now observed at 279 mμ, existed in the original RuDP absorbing at 260 mμ. Should it be considered that it would have shown up, at least as an inflection, then it must be concluded that the peak at 279 mμ is due to an artifact, introduced in the brucine salt

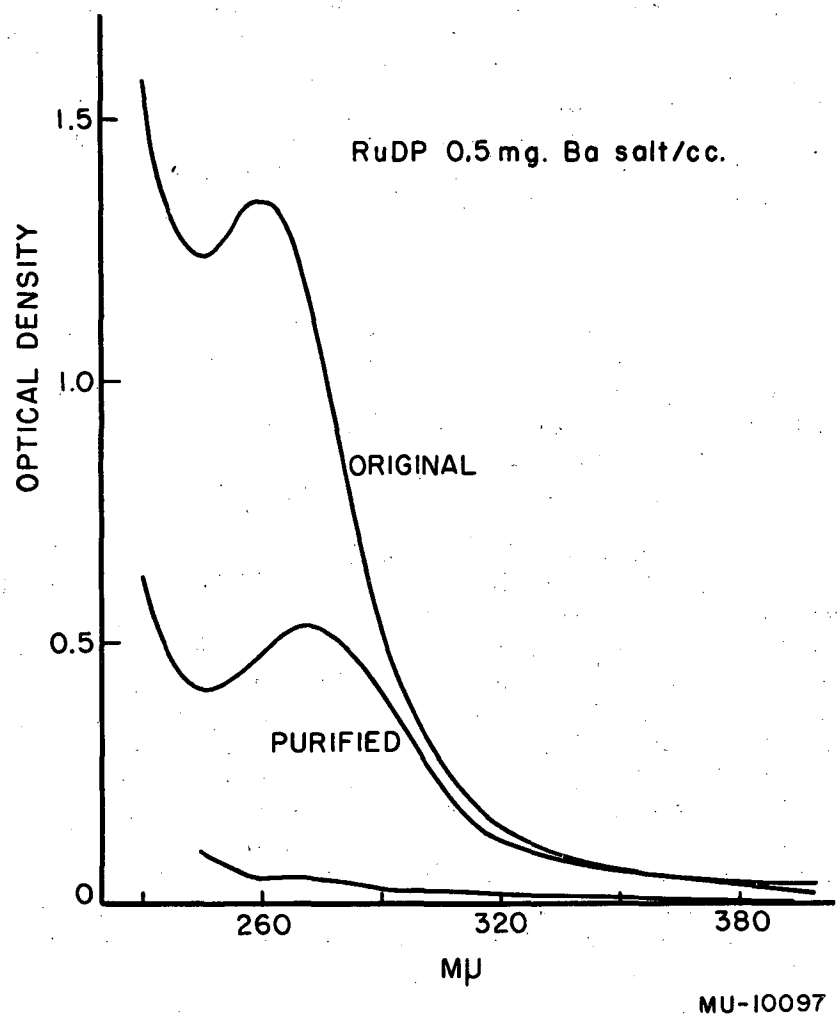

Fig. 1. Absorption spectra of ribulose diphosphate.

formation or crystallization. Otherwise, one of two alternatives can be assumed. First, that the peak is due to an intensely absorbing chromophore present in small amount. A conjugated ketone, e.g., furfural, λ_{max} 278, log ϵ_{max} 4, would fit into this category, although the peak actually found in RuDP is rather broad for this type of compound. Secondly, that the peak is actually due to RuDP and is due to the free ketonic group in the molecule. The λ_{max} = 279 mμ and the somewhat broad shape of the spectrum are in keeping with the ketonic forbidden transition at that wave length; cf. acetone λ_{max} = 280 mμ, ϵ_{max} = 14. It is, however, difficult to understand the intensity of the absorption ϵ_{max} = 80 in what is regarded as the purest sample. It seems that this value is too high for the ketonic group in these surroundings, since dihydroxyacetone phosphate gives a peak λ_{max} = 262 mμ and ϵ_{max} 23 (unpublished report of J. R. Quayle).

STUDIES ON THE INITIAL STEPS OF REACTIONS IN PHOTOSYNTHESIS WITH INTERMITTENT LIGHT

James A. Bassham and Kazuo Shibata

It has been shown that the efficiency of photosynthesis changes with different periods of intermittent light. This type of experiment was first carried out by Emerson and Arnold[1] for studying the nature of the initial steps of reactions in photosynthesis. From their results, the theory of a "photosynthetic unit"[2, 3] was proposed. Later on, Tamiya and Chiba[4] repeated the same experiment more precisely, paying particular attention to the temperature dependence of the maximum yield in flashing light with changing light intensity. They pointed out that the maximum yield becomes temperature-dependent when the light intensity is very high, which contradicts the theory of a "photosynthetic unit." They proposed a new mechanism which includes second-order decay of the excited chlorophyll. Recently Strehler, Arnold, and Davidson[5, 6, 7] observed chemiluminescence by algae, which decays with the order of about 1.5. If excited chlorophyll is responsible for chemiluminescence, and reacts with a limited amount of substance as in enzymatic reactions with a deactivation process of second order, the apparent order of the decay of chemiluminescence would be about 1.5. Also, both the change of yield of photosynthesis by flashing light and the decay of chemiluminescence occur in a time of the order of 0.01 sec, so that there is no doubt about the existence of a reaction with this rate under the condition of strong light intensity, which is quite different from the decay of chlorophyll *in vitro* observed by Livingston et al.[8]

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[1] R. Emerson and W. Arnold, J. Gen. Physiol. 15, 391 (1932); 16, 191 (1933).
[2] H. Gaffron and K. Wohl, Naturwiss. 24, 81, 103 (1936).
[3] K. Wohl, Zwitschr. Physik. Chem. B, 37, 105, 122, 169, 186, 209 (1937); New Phytologist 39, 33 (1940); 40, 34 (1941).
[4] H. Tamiya and Y. Chiba, Studies from the Tokugawa Institute, 6, No. 2 (1949).
[5] B. Strehler, Arch. Biochem. Biophys. 34, 239 (1951).
[6] B. Strehler and W. Arnold, J. Gen. Physiol. 34, 809 (1951).
[7] W. Arnold and J. B. Davidson, J. Gen. Physiol. 37, 677 (1954).
[8] R. Livingston, G. Porter and M. Windsor, Nature, 173, 485 (1954).

Recently, Bradley and Calvin[9] found that thioctic acid stimulated the Hill reaction with flashing light. The purpose of this experiment is first to study in more detail the features of the initial steps of photosynthesis with flashing light, by changing not only the dark period between flashes but also the light period; and secondly, to observe the effect of chemicals, expecially thioctic acid, in the system of actually living organisms. In this report, only the preliminary work for this study is described.

Methods

The system used in this experiment is the same as the system for our quantum-requirement experiment,[10] although the total volume was changed to increase the sensitivity. The photosynthetic rate was measured by the change of oxygen content in the system, by use of the paramagnetic property of O_2. At the same time, the change of CO_2 was observed as a reference by an infrared CO_2 analyzer. A 400-W photospot illuminated the suspension of algae through two sheets of infrared absorbing glass with a water cooling system. In front of the cell, which contained 70 ml of algal suspension, a sector was rotated. Two kinds of sectors were used as a preliminary study; one had 4 holes and the other had 8 holes, of a fan shape in a disk 40 cm in diameter. The solid and open sectors were made equal so that equal dark and light periods were obtained. Keeping the relative rotational speed of the 4-hole sector at twice the speed of the 8-hole sector, we can give the same length of light time with two different dark periods. From this set of data, we can calculate the change of the photosynthetic rate per flash with two different dark periods. By changing the absolute rotational speeds of two sectors, but in each case keeping the relative speeds at a 2:1 ratio so as to maintain the light period the same for the two sectors, one can obtain the change of the yield per flash for many different dark periods. From these data the curve for the change of yield per flash as a function of dark period can be calculated, provided the light intensity is strong enough to saturate the photosynthetic process in the light period. Under the condition of flashing light used, the light intensity was found to be strong enough to achieve saturation. The dark period was changed from 1/400 sec to 3/40 sec. The concentration of the suspension of Scenedesmus was 0.1% in packed cell volume units.

Results

Table IX shows the change (ΔG) in relative yield per flash for different dark periods. The longer the dark period, the greater the yield per flash. At about 3/40 sec, the curve for ΔG against dark period levels off. Therefore, about 1/40 = 0.025 sec is the lifetime of the excited state of some molecules (these may be chlorophyll), although the lifetime cannot precisely specify the decay reaction, since the over-all deactivation processes are not exactly first-order. The result obtained is similar to the data that have been obtained under a strong light illumination. Further study with different kinds of sectors may disclose not only the features of the deactivation process but also the activation process, from which we will be able to see the whole mechanism of the initial steps of photosynthesis leading to the photochemical reaction.

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[9] D. F. Bradley and M. Calvin, UCRL-2993 (1955).
[10] J. A. Bassham, K. Shibata and M. Calvin, Biochim. Biophys. Acta 17, 332 (1955).

Table IX

Change of relative photosynthetic yield per flash	
Dark period (in sec)	ΔG
1/400-3/400	0.096
1/400-3/200	0.204
1/400-1/40	0.331
1/400-3/80	0.488
1/400-3/40	0.640

FURTHER STUDIES ON CARBOXYDISMUTASE

Ning G. Pon

This is a continuation of published and unpublished work done by Drs. M. Calvin[1] and Jacques Mayaudon and J. R. Quayle of this laboratory. The main problem is concerned with the re-examination of the over-all reaction that is catalyzed by carboxydismutase,

$$\text{RuDP} + CO_2 \xrightarrow{\text{pH } 7} 2\ \text{PGA},$$

where RuDP represents ribulose-1, 5-diphosphate and PGA represents 3-phosphoglyceric acid. The only thing different here is the RuDP used as substrate. In this case, the RuDP was derived from ribulose-5-phosphate via the isomerase-kinase system. In the work of Mayaudon, RuDP was derived from the alga, Scenedesmus.[2] The above reaction can be followed by determining the amount of $C^{14}O_2$ fixed.

Carboxydismutase is prepared from New Zealand spinach (Tetragonia expansa); all operations are carried out near 0°C. Fifty grams of wet leaves, with mid ribs partially removed, are ground in a blendor for 90 sec with 50 ml of pH 6.8 0.1 M phosphate buffer. The filtrate obtained from the homogenate is centrifuged at 40,000 rpm for 10 min. The yellow-brown supernatant (designated as "total") is made to 35% saturated ammonium sulfate (SAS), and the pH of the suspension is adjusted to 7.0 with 2 N NH_4OH. (Saturated ammonium sulfate is considered to be 68.5 g $(NH_4)_2SO_4$ for each 100 ml initial volume of solution.) After centrifugation at 20,000 rpm for 5 min, the precipitate

[1] M. Calvin, The Photosynthetic Cycle, UCRL-2924, March 21, 1955, p. 27.
[2] A. A. Benson and M. Calvin, Intermediates of Photosynthesis: Isolation and Degradation Methods, UCRL-2682, August 1954, p. 17.

is dissolved in 3 to 4 ml of 0.05 M pH 6.8 phosphate buffer (designated as "0%–35% SAS ppt.") and the supernatant is made to 39% SAS. The operation is repeated until six fractions are obtained: (a) "Total," (b) 0%–35% SAS ppt., (c) 35%–39% SAS ppt., (d) 39%–50% SAS ppt., (e) 50%–90% SAS ppt., and (f) 90% SAS supernatant.

The CO_2-fixing activity of each fraction was tested by using the above reaction. Some of these fractions were dialyzed against 10^{-4} M NaOAc or 10^{-4} M Na-EDTA at pH 7, 0°C; others were not dialyzed.

Experiments were conducted to test the following effects:

(1) Reproducibility of activity by fractions derived from spinach grown in different localities.

(2) Comparison of activity of these enzyme preparations with the enzyme preparations previously prepared by Mayaudon.

(3) Comparison of different substrates: RuDP (Scenedesmus) vs RuDP (R. Quayle).

(4) Location of activity among the different $(NH_4)_2SO_4$ fractions.

(5) Effect of dialysis on the activity of the enzyme.

(6) Effect of added reducing agents on the enzyme system.

(7) Effect of added Mg^{++} on the enzyme system.

Summary of results

1. The activity of enzyme preparations from spinach grown in different localities varies considerably. Even when spinach is obtained from one source (private hot house) the activity ranges over a fifteenfold variation.

2. In general, the CO_2-fixing activity of present enzyme systems is lower than that of Dr. Mayaudon. Only recently has spinach been obtained that can once again yield enzyme preparations having very high carboxydismutase activity.

3. When RuDP (Scenedesmus) is used as substrate, two to three times as much CO_2 is fixed as with RuDP (R. Quayle). This suggests the presence of a cofactor(s).

4. In subsequent experiments with RuDP (R. Quayle) little or no activity was found in any of the $(NH_4)_2SO_4$ fractions, although the "total" fraction had CO_2-fixing activity. Even when all of the $(NH_4)_2SO_4$-precipitated fractions were recombined with the 90% SAS supernatant, no activity was obtained.

5. Dialysis of the "total" and the 35%–39% SAS ppt. showed no improvement as far as CO_2 fixation is concerned. However, the rate of inactivation (demonstrated in later experiments) was substantially decreased by dialysis.

6. Addition of reducing agents such as cysteine and ascorbic acid at 10^{-3} M concentrations completely destroyed the activity of the enzyme. Glutathione (10^{-5} M) and cysteine (10^{-4} M) showed little or no effect.

7. When Mg^{++} at 10^{-5} M concentration was added to undialyzed fractions no effect was observed. However, when 10^{-4} M Mg^{++} was added to a dialyzed fraction (35%–39% SAS ppt.) that had been allowed to become nearly inactive the preparation was reactivated. Mg^{++} added to a fresh preparation of enzyme

not only increased the activity of the enzyme, but also preserved the activity for a long period. (Some ammonium-sulfate-precipitated fractions exhibited no activity until Mg^{++} was added.) Combination of added cysteine and Mg^{++} showed no better results than those obtained with Mg^{++} alone. Activity as a function of the concentration of added Mg^{++} has been studied and certain patterns have been observed. The results are not definitive, however, and hence no conclusions can be drawn.

Some interesting aspects of this study are the breaking of the cofactor(s)-enzyme complex by ammonium sulfate, the cofactor(s) present in RuDP derived from Scenedesmus, the effect of other metal ions, and the destruction of activity of the enzyme with reducing agents. Mayaudon has found that known inhibitors of -SH groups destroy the activity, but that cysteine reactivates the enzyme. Further work is in progress to clarify these points.

THE CONCENTRATIONS OF METABOLIC INTERMEDIATES IN ALGAE

J. A. Bassham, K. Shibata, and J. Bourdon

In the most recent report* "Further Photosynthetic Kinetic Studies with Carbon-14" were described. As a result of continued work in determining the concentrations of compounds formed during that experiment by methods previously reported, it is now possible to tabulate the steady-state light and steady-state dark concentrations of a number of metabolic intermediates in Scenedesmus.

These concentrations are believed to represent amounts of compounds inside the chloroplasts, the compounds outside the chloroplasts being labeled to a much lesser extent during the course of the experiment. The reason for this belief is that very little radioactivity finds its way into citric acid and glutamic acid while the light is on despite the fact that respiration is known to occur in the light at a rate comparable to that observed in the dark.[1] Thus if PGA, for example, were labeled in the space outside the chloroplast, it would be converted by normal respiration via pyruvic acid and acetyl CoA to citric acid. Moreover, in a previous quarterly report[2] it was seen that citric acid labeling in Scenedesmus, which rises sharply when the light is turned off, drops to about its original level when the light is turned on again. Glutamic acid labeling, on the other hand, rose in the dark and then remained at a higher level when the light was turned on. In the most recent report it was seen that PGA and RuDP labeling go up and down respectively, in the dark and then return to their original levels in the light. These various results are best explained by assuming that most photosynthetic carbon-reduction cycle intermediates as well as some respiratory intermediates in the Krebs cycle are retained within the chloroplasts and diffuse but slowly into the cellular spaces outside the chloroplasts. The latter spaces would have their own reservoirs of glycolytic and respiratory intermediates, which do not become rapidly labeled although slow labeling via the Wood-Werkman reaction and malic acid must certainly occur.

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*UCRL-3068, July 6, 1955.
[1] A.H. Brown, Am. J. Bot. 40, 719 (1953).
[2] J. A. Bassham and A. O. M. Stoppani, UCRL-2647, July 8, 1954, p. 35.

Respiration outside the chloroplast must continue at a constant rate in light and in dark, unaffected by the concentrations of reducing agents inside the chloroplast in the light. However, long periods of photosynthesis would produce sucrose and other products of photosynthesis, which diffuse out of the chloroplasts. Respiration within the chloroplasts, a small fraction of the total respiration of the cell, would appear to be strongly inhibited in the light; this inhibition, according to Calvin and Massini,[3] is due to the lack of oxidized thioctic acid in the light and consequent absence of pyruvic acid oxidation.

If the assumption is correct that total amounts of photosynthetic intermediates as determined by the kinetic carbon-labeling method represent the amounts within the chloroplasts only, then these concentrations are obviously higher than those calculated on the basis of total cell volume. Nonetheless, since the ratio of chloroplast volume to cell volume is not known in this experiment, concentrations are expressed in Table X in terms of amount of uniformly labeled intermediate compound per unit volume of packed cells. The actual concentration at the point where enzymatic conversion takes place is, for the reasons given above, undoubtedly much higher.

Table X

Steady-state concentrations of Photosynthetic intermediates and related hexose phosphates in moles/cc. of algae

Compound	Light	Dark	Change
PGA	1.63	2.42	+0.79
RuDP	0.51	0.0005	-0.51
Ru5P + Ri5P	0.17	0.08	-0.09
SDP	0.006	0.006	0
FDP	0.004	0.001	-0.003
F6P	0.12	0.12	0
GDP	0.003	0.003	0
G6P	0.33	0.37	+0.04
DHAP + GAl3P	0.21	0.12	-0.09

[3] M. Calvin and P. Massini, Exper. 8, 445 (1952).

SPECTROPHOTOMETRIC MEASUREMENTS AT LOW OR HIGH TEMPERATURES WITH THE CARY RECORDING SPECTROPHOTOMETER

Ernst Fischer

Spectrophotometric measurements in the temperature range +180° to -180°C with the Beckman DU spectrophotometer have been reported previously.[1] This report deals with the design of similar equipment for use with the Cary recording spectrophotometer.*,**

Principles

The optical cells are either immersed or fused into a small Dewar vessel with optical windows. The considerable reduction of heat losses achieved thereby makes it possible to heat (cool) the optical cell by heated (cooled) air flowing around the cell containing the solution. The quality of the results obtained thus depends mainly on the quality of the glass or quartz surfaces which the light has to traverse during the measurement of absorption.

Sources of hot or cold air

Air from a compressor or a cylinder is passed over an electric heating coil having a low heat capacity. At a constant rate of air flow the temperature in the solution cell is controlled by the voltage applied to the heating coil from a variable transformer. The coil is made by winding nichrome wire on a piece of metal sheet 5 by 1/2 by 1/8 inches. When the coil is taken off the sheet, it forms a haze of wire which, when put in a glass tube, offers a large surface to air passing through the tube. With a stable line voltage the temperature in the solution cell reaches a constant temperature after about 5 min and maintains it within 1° to 2°. Temperatures of up to 180°C are easily maintained with this arrangement.

Cold nitrogen was generated by boiling liquid nitrogen in a 25-liter metal Dewar container of the conventional type. An electric immersion heater (nichrome wire wound on a glass tube) supplied the heat required to keep the liquid nitrogen boiling. The voltage applied to the immersion heater controls the rate of boiling of the nitrogen and thereby the amount of cooling achieved in the optical Dewar and the temperature in the solution cell. The cold nitrogen is transferred from the storage container to the optical Dewar with only small heat losses by means of two Dewar tubes connected by a piece of rubbing tubing, to ensure mechanical flexibility of the system. Temperatures down to -160°C can be kept in the solution cell, constant within about 2°C, by controlling the voltage supplied to the immersion heater.

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[1] Y. Hirshberg and E. Fischer, J. Chem. Soc. 1953, 629.

* Cary Model 11 recording spectrophotometer, Applied Physics Corporation, Pasadena, California

** The development of the methods outlined above was made possible by the ability and patience of Mr. Harry Powell, head of the Glass Workshop, who made the absorption cells.

Optical cells

The cell compartment in the Cary spectrophotometer forms approximately a cube with an edge of about 5 inches, with the light beam passing about 2.5 inches above the bottom of the compartment. Ample space is thus available for rather large cells and Dewar vessels. Moreover, this space is closed on all sides but the top, and the air in it is therefore easily displaced by the cold dry nitrogen overflowing from the optical Dewar during operation at low temperatures. Condensation of moisture from the air on the windows of the Dewar is thereby eliminated even when these windows reach rather low temperatures (e.g., if measurements are made at temperatures close to -180° C). Two cell designs were used. In the first design a regular 10-by-10-mm square-section optical cell was equipped with a 5-inch-long glass tube and hung into a Pyrex Dewar vessel having a rectangular cross section. The walls of this Dewar were made of optical quality Pyrex sheet. The clearance between the walls of the cell and the inner walls of the Dewar, in the direction of the light beam, was about 0.5 mm. A 0.4-mm copper wire wound around the upper and lower parts of the cell kept this clearance to about 0.1 mm, ensuring the parallelism between these surfaces. This arrangement of cell and Dewar gave satisfactory results at wave lengths of 350 mμ and longer (Fig. 2). For work in the ultraviolet region, however, the cell and the Dewar obviously have to be made of fused silica. Moreover, because of the more pronounced diffraction and dispersion of light in this region, the number of surfaces light beam has to traverse should be kept to a minimum. For use within the whole range of wave lengths available in the Cary spectrophotometer quartz cells of the fused-in type described previously[1] were therefore constructed (Fig. 3), and showed satisfactory performance down to 220 mμ (Fig. 4). Masks have to be used with both types of absorption cells in order to confine the light beam to the part of the cell filled with the solution being investigated.

Applications

These include such measurements as the temperature dependence of absorption spectra (e.g., lower temperatures often result in narrower bands and improved resolution), photochemical reactions at low temperatures which can be followed spectrophotometrically, and the temperature dependence of certain equilibria in solutions. The last application mentioned is illustrated by the results described in Fig. 2, obtained with a Pyrex cell immersed in a Pyrex rectangular Dewar flask. Here the absorption spectrum of a mixture of piperidine and Universal Indicator dissolved in ethanol-methanol (2:1) was measured between +60° and -160° C. With decreasing temperature the equilibrium shifts in favor of the basis (anion) form of the dye, characterized by the band at 625 mμ. Under the conditions used this shift is virtually complete at about -70°, when the 625-mμ peak reaches about the same height as in the presence of excess piperidine at room temperature. The band at 410 mμ is almost unaffected by temperature or excess piperidine. The curves shown in Fig. 2 are corrected for the dependence of the volume concentration on the temperature, but not for the zero line shown.

Figure 5 illustrates a case where less overlapping and better resolution are obtained with a copper chelate compound, in which a weak visible band is situated close to a very strong band in the ultraviolet. The Pyrex Dewar and cell were again used.

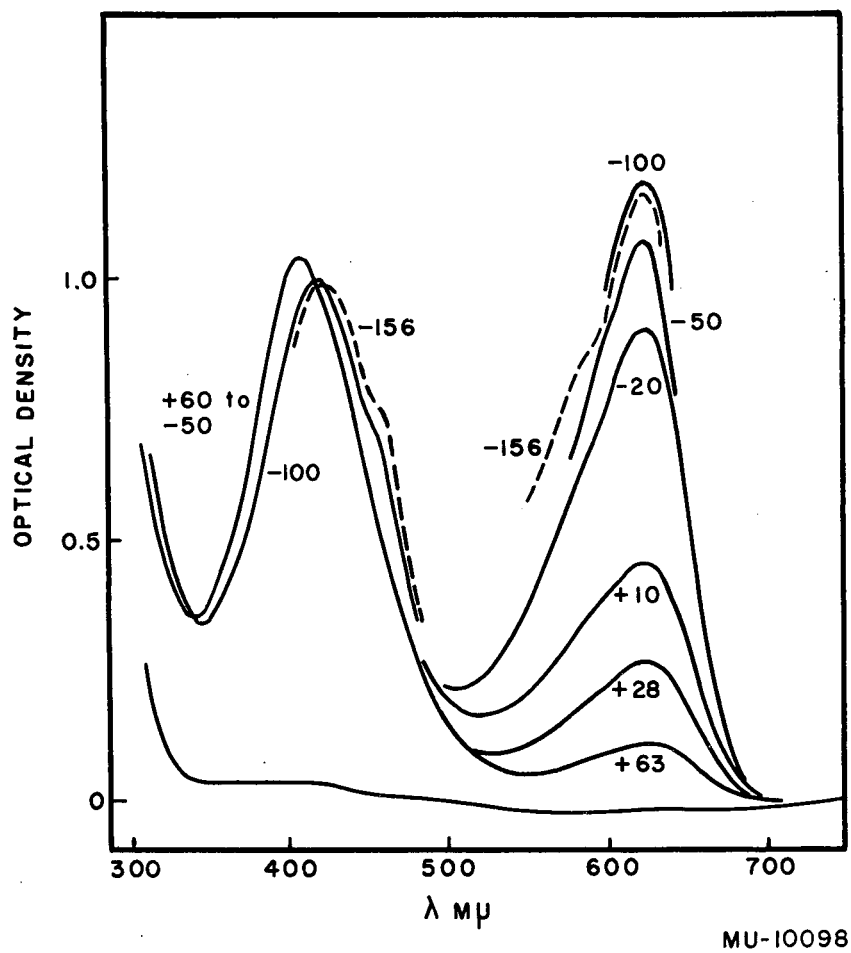
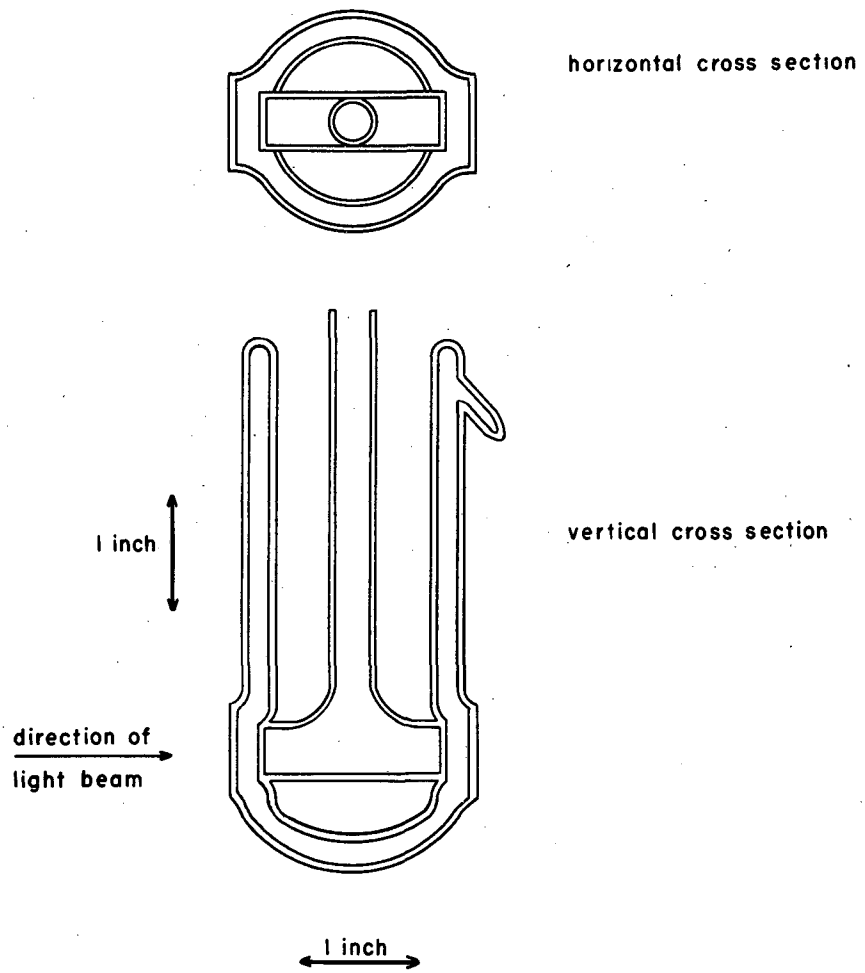


Fig. 2. Absorption curves at various temperatures of a solution of "Universal Indicator" and piperidine in ethanol-methanol (2:1). Bottom curve is the base line.



DEWAR-TYPE QUARTZ CELL

MU-10099

Fig. 3

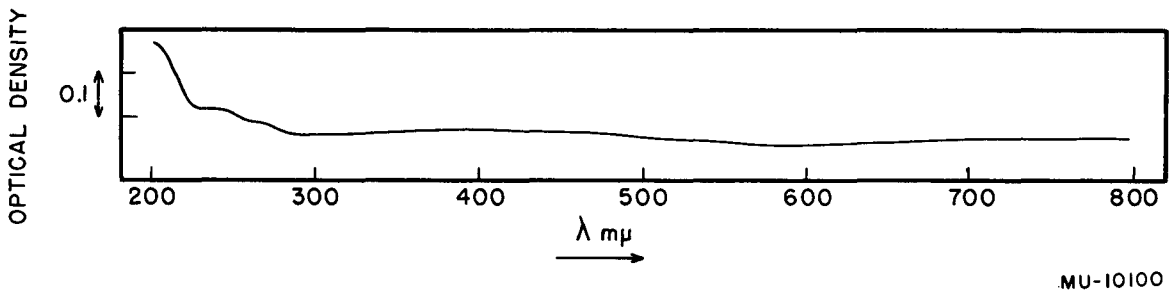


Fig. 4. Zero line of fused-in quartz cell.

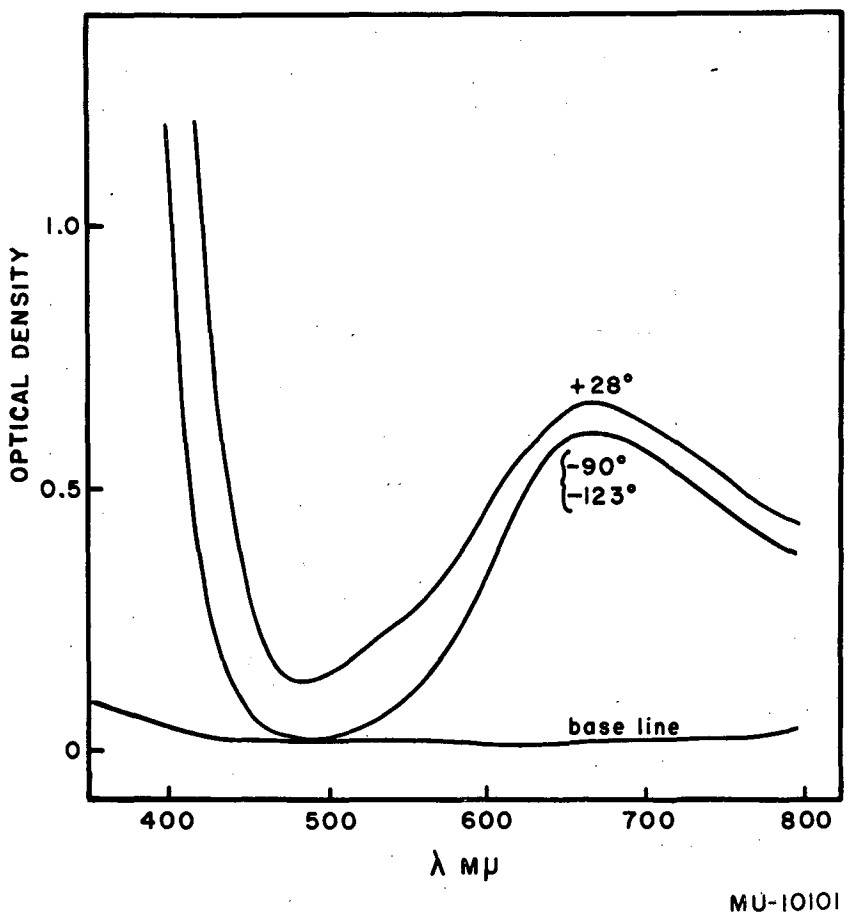


Fig. 5. Absorption curves, at various temperatures, of a copper chelate in ethanol-methanol (2:1). (Cu-diethylacetoacetate, 7 g/l at 28° C.)

Measurements with the Cary Model 14 spectrophotometer recently acquired by this laboratory show that absorption measurements can be extended to wave lengths up to 2500 mμ.

BRAIN CHEMISTRY AND ADAPTIVE BEHAVIOR*

Edward L. Bennett, Barbara Krueckel, and Alvin Halevy

(In cooperation with Professors Mark R. Rosenzweig and David Krech and Mr. James McGaugh, Dept. of Psychology, University of California, Berkeley; supported in part by a grant from the National Science Foundation.)

In previous quarterly reports[1] a significant correlation in rats was reported between a behavioral measure, the "hypothesis" behavior in the Krech unsolvable maze, and a chemical measure, the cholinesterase activity in homogenates of selected areas of the rat brain cortex. These preliminary results encouraged us to repeat and extend our initial experiments. With the increased number of experimental animals, our initial observations have been confirmed and new statistically significant observations have been noted.

The behavioral testing procedure is beyond the scope of this report, but it should be emphasized that the conditions for "hypothesis" testing must be as carefully controlled as those for a fairly complex chemical analysis. The behavioral test used was one in which the rats showed large and reliable individual differences that could be quantified. The differences reflected stable characteristics of the individual animal rather than ephemeral situational influences. The behavior tested has adaptive significance in the normal life experiences of the animal and the neurological basis is already understood, at least in part. Rather similar requirements were desired for the chemical variable; i.e., it must discriminate reliably among different animals; reflect stable characteristics of the animal, be significant in normal neural functioning; and be a substance that, on the basis of already existing knowledge or theory, plays a role in interneural functioning. The enzyme cholinesterase was chosen on the basis of the above considerations.

As has been previously described, samples of cortex were removed from the visual (V), somesthetic (S), and motor (M) areas of both hemispheres (Fig. 6), weighed, and homogenized in 0.9% NaCl. The rate of hydrolysis of acetyl choline perchlorate was determined under carefully standarized conditions with an automatic recording titrator which maintained the pH constant and recorded the amount of standard NaOH added as a function of time. After subtraction of the "blank" the results were calculated on the basis of the moles of acetyl choline hydrolyzed per mg of tissue used for each analysis.

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[1] E. L. Bennett and B. Krueckel, UCRL-2709, Sept. 30, 1954, p. 4; UCRL-2841, Jan. 10, 1955, p. 13.

*A more complete report of this work has been presented at a symposium, "Interdisciplinary Research in the Behavioral, Biological, and Biochemical Sciences," University of Wisconsin, Aug. 28-Sept. 2, 1955, and will be published, together with other papers presented, by the University of Wisconsin Press.

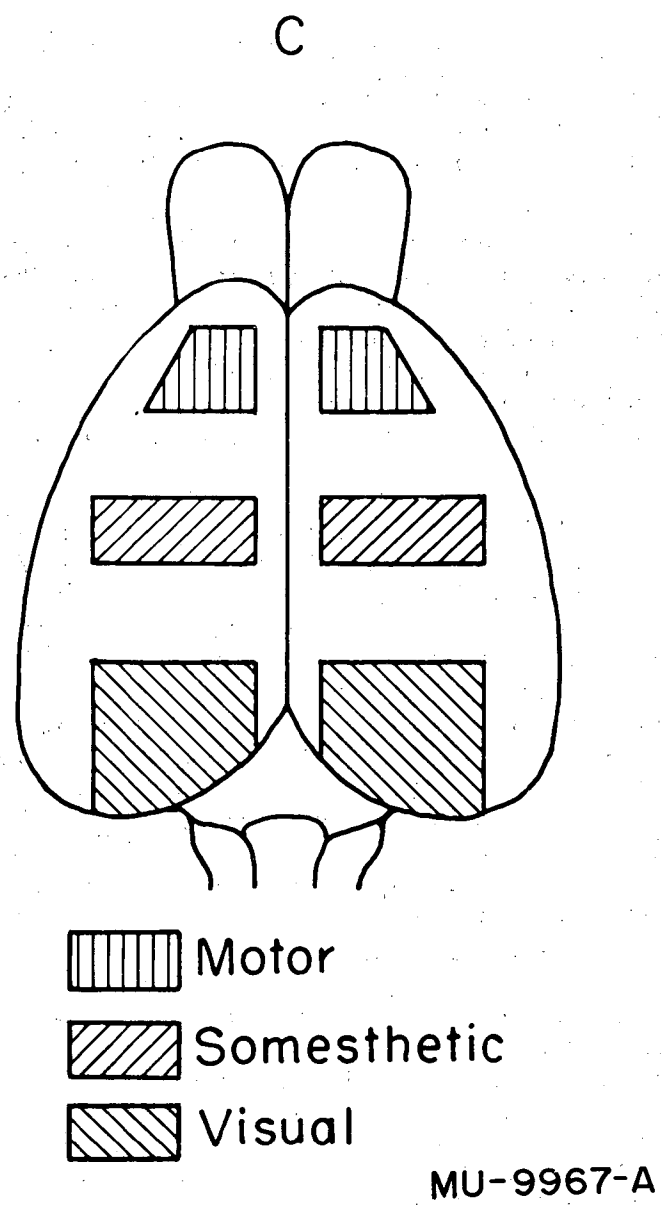

Fig. 6. Diagrammatic representation of brain areas of the rat used in this study.

Results

Differences Among Cortical Areas in Cholinesterase Activity

Localization of function within the cerebral cortex has been demonstrated by a number of techniques--surgical, electrophysiological, and histological. It is to be expected that biochemical differences will be found to be correlated with functional differences among areas of the cortex. For cholinesterase (ChE), "activity" has been shown to vary widely from one level of the nervous system to another, but there has been little evidence that it varies significantly from area to area within the cerebral cortex. In our own work, we have measured ChE activity in 129 rats of seven strains, of both sexes, and of several ages. For every group tested, our data show consistent and significant differences between the ChE activities of the motor, somesthetic, and visual areas of the cortex.

The values of ChE activity for the two hemispheres of an animal usually agreed closely. For 95 male rats, the mean ChE activity was 53.94 in the visual area of the left hemisphere and 54.77 in the visual area of the right hemisphere; 64.05 in the left somesthetic area and 64.59 in the right somesthetic area; 68.63 in the left motor area and 69.50 in the right motor area. For the female rats the interhemisphere agreement was equally good. None of these small interhemispheric differences approached statistical significance. Because of this, it was decided to combine the readings of both hemispheres for any one area and for any one animal. In the rest of this paper, the values reported for each cortical area will be the mean of the values obtained at the two hemispheres.

The ChE values thus obtained, for each of the three cortical areas investigated, are presented in Table XI. Separate values are given for male rats of five strains and for female rats of two strains (each subgroup being homogeneous in age). In each of these seven subgroups the very same pattern of ChE activity was found, i.e., ChE activity level increased from the visual area (V) to the somesthetic area (S) to the motor area (M). For all male animals combined, ChE is 18% greater in S than in V, 29% greater in M than in V, and 9% greater in M than in S. For the female animals, the corresponding percentages are 17, 24, and 6.

Differences

Another way to test the generality of this patterning was to tabulate the number of individual rats (of all strains and ages and of both sexes) who showed the progressive increase from V to S to M. Of the 129 animals, 104 fitted this order exactly, and 122 animals showed greater ChE activity in S than in V.

Not only does ChE activity differ among these areas, but as the age of the animal increases, the values of ChE activity decrease. The top curve in Fig. 7 shows the ChE values for male animals of the TB, TC, and TD strains with a mean age of 108 days. The next curve, for another group of male animals whose mean age was 156 days, shows somewhat lower ChE values. The bottom curve is for a third group whose mean age was considerably greater--401 days; in this curve the ChE values are again lower. All three curves are generally similar in shape, but they are closer together for V than for M.

Table XI

Cholinesterase activity in three cortical areas for animals of five strains and both sexes

Strain*	Sex	Mean Age (days)	No.	Means		
				V**	S	M
TB	M	104	18	58.94	69.28	74.94
TC	M	109	12	53.92	67.00	68.92
TD	M	113	12	54.67	65.25	72.83
GI	M	154	6	54.67	58.50	66.50
GB	M	118	5	59.20	67.20	78.00
All Males			53	56.38	66.43	72.43
TB	F	132	13	58.38	66.92	72.00
TC	F	146	5	60.40	74.60	75.80
All Females			18	58.94	69.06	73.06

* Our TB strain are Tryon's[2] maze-bright animals: the TD strain are Tryon's maze-dull animals. The TC strain is a cross between the TB and TD strains. The GI and GB strains are two highly inbred strains obtained from the University of California Genetics Laboratory.

** V, S, and M refer to the visual, somesthetic, and motor areas, respectively, of the rat cortex. The results are expressed as moles of acetyl choline (ACh) x 10^{10} hydrolyzed per minute per milligram of tissue under the conditions of the assay procedure.

[2] R. C. Tryon, University of California, (Berkeley) Publ. Psychol. 4 (1929).

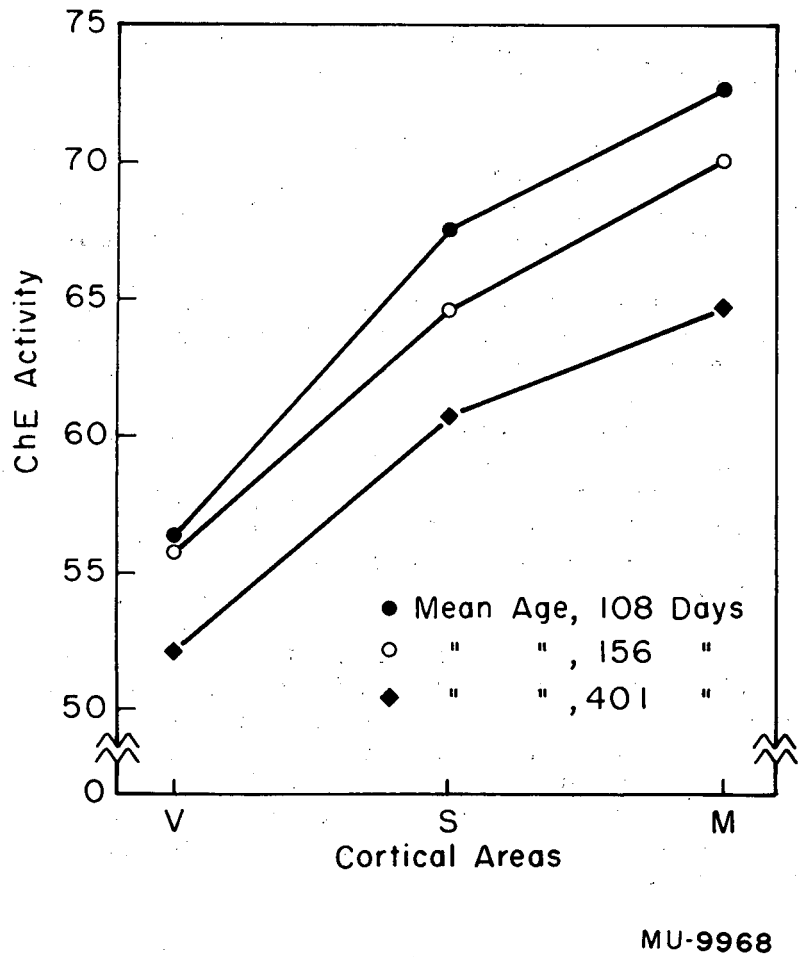

Fig. 7. ChE activity in areas V, S, and M. (Age is the parameter.)

We have found very few previous studies that we can use to check upon our results correlating age with changes in ChE activity. Changes in ChE activity with age have mainly been studied with fetal or immature animals. Some data for older rats are reported by Metzler and Humm.[3] Using whole brain, they have reported a drop in ChE activity between 75 and 120 days and no decline thereafter--or even a slight rise--in adult rats. For ages 75 days, 120 days, and "adult" they apparently made a total of only eight determinations. It will be remembered that our data showed a consistent drop in ChE activity after the age of 120 days. The difference between our results and those of Metzler and Humm may be because our determinations were made on cortical tissue and theirs were made on whole brain. More likely, the difference is due to the inadequacy of their data. For the "adult" age, they had only two cases.

Relationship Between Individual Differences in Hypothesis Behavior and in Cortical Cholinesterase Activity

Our main objective in all these studies is to relate biochemical activities in the cortex to adaptive behavior patterns, and we now turn to an analysis of the relationship between hypothesis behavior and ChE activity--based on the records of 95 male rats of five different age groups.

The basic question we wish to examine is the possible relationship between hypothesis preference (spatial or visual) and ChE activity level. However, since age has a significant effect on ChE activity level, and since our animals were assayed for the ChE activity at various ages, it becomes necessary in making comparisons between the spatial and visual animals to keep age constant. Figure 8 presents the summary data for the present section of our report. The curves represent the change with age in ChE activity* for the spatial rats and visual rats separately. This permits us not only to compare the spatial and visual rats at various age levels, but also to note some very interesting differences in the shapes of the curve.

Regarding first the shapes of the curves, we see that in the spatial animals (the solid curve) ChE activity declines slowly and almost linearly with age. Over a span of 450 days, there is a drop of only 5.25 units. The visual animals, however, (dashed curve), show a very different pattern; ChE activity declines very rapidly between 110 and 230 days, and thereafter levels off.** It appears as if the spatial animals had not yet reached their "floor" at age 550 days, whereas the visual animals had reached their minimum as early as age 230 days.

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[3] C. J. Metzler and D. G. Humm, Science 113, 382 (1951).

* Throughout this section we use, for our index of ChE activity level, the average of the ChE activities in the two sensory areas, V and S, i.e., V + S/2. This is the same index as we used in our preliminary report (D. Krech, M. R Rosenzweig, E. L. Bennett, and B. Krueckel,) Science 120, 994 (1954).

** In drawing the curves, we have not connected the points for the 3 spatial animals and the 8 visual animals of the 390-day-old group. Their data were aberrant in several respects. In this sense the curves are "smoothed" curves. However, in testing for statistical significance, the data of these eleven animals are included. In other words, we preferred to have these curves represent our best estimate of the general trends of the data, but in the quantitative analysis, we have included all the data.

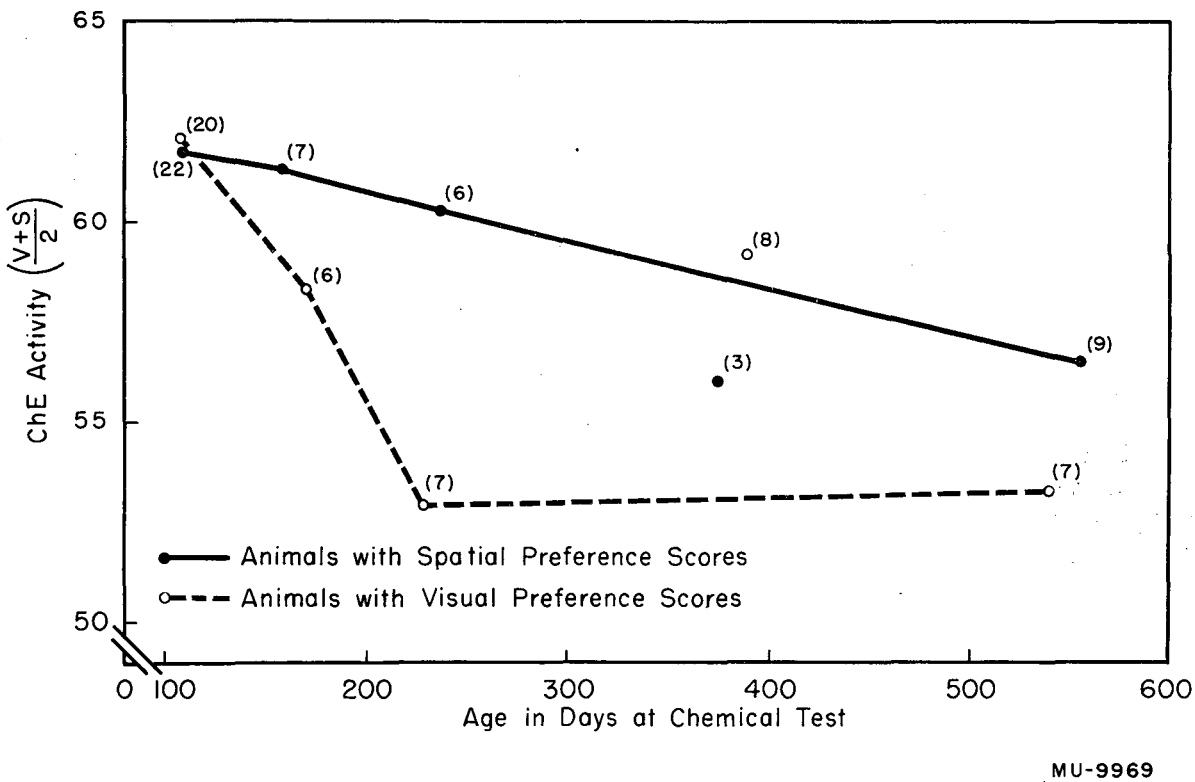

Fig. 8. Comparison between spatial and visual animals for ChE activity at various ages. (The number of animals in each group is given in parenthesis.)

The second generalization that these curves seem to support is that after age 100 days, the spatial animals have higher ChE activity levels than do their age mates among the visual animals.

We have already noted that the chemical differentiation between the spatial and visual animals appears only after age 110 days. At that age, and probably prior to that age, there is no detectable difference between the two groups of rats. We will reserve for a later discussion some possible reasons for the failure of these young animals to show differences in ChE activity, but this empirical finding (whatever its causes) suggests that the most favorable test for a chemical difference between the spatial and visual rats should be made with animals older than 125 days.

The following general conclusion seems warranted: Individual differences in hypothesis behavior are correlated in a systematic fashion with the level of cortical ChE activity and with progressive change in such levels as a function of age.

Our findings of a significant relationship between the behavior measured by our test and brain chemistry leads us to speculate about the mechanism involved. This necessitates interpretations of the functional significance of both variables, ChE activity and hypothesis behavior.

Acetylcholine (ACh) metabolism is importantly involved in transmission in the central nervous system. We would now suggest that animals with higher rates of ACh metabolism also show readier transmission of nerve impulses. No causal relationship is specified here--we are merely positing a correlation between the two. We would next accept the common assumption that an animal whose nervous system is capable of relatively efficient transmission would tend to manage new problem situations more effectively than an animal whose transmission of nerve impulses is less efficient. Such an assumption would also involve, of course, limiting factors. It is conceivable, for example, that too ready transmission of nerve impulses may interfere with effective behavioral adjustment, and we intend soon to put this to experimental test. In any event, the reasoning indicated here would assume that animals with higher rates of ACh metabolism (within limits) would tend to show more effective behavioral adjustment than animals with lower ACh metabolism.

On the behavioral side, we believe that animals who show a spatial preference score are more adaptive than animals who show a visual preference score. We make this interpretation for the following reasons: (a) our test is designed to measure the animal's perceptual selectivity; (b) we next assume that adaptive behavior is correlated with the ability of an animal to "pay attention to" various stimulus aspects of his environment when confronted with a problem. Achieving a spatial preference score therefore requires that an animal ignore the dominant illumination cue and pay attention to the less obvious cue of location in space. In other words, an animal that develops a spatial preference shows readier "shifts in the dimension of discrimination" than an animal whose behavior remains controlled by the visual cues.

These two proposed interpretations of the functional significance of ACh metabolism and hypothesis behavior suggest, in most general terms, a rationale for the observed correlation between ChE activity and hypothesis behavior. ChE activity level provides a measure of the readiness of nerve impulse

transmission in the central nervous system; the relative ease of nerve impulse transmission is correlated with capacity for more adaptive behavior; a spatial preference score in our maze is indicative of a more adaptive animal; therefore one might expect a positive correlation between level of ChE activity and a spatial preference score.

GLUCOSE DISSIMILATION IN A FREE-CELL NEOPLASM

K. K. Lonberg-Holm

Ehrlich's mouse ascites carcinoma cells have been chosen as a future subject for intensive study by methods heretofore employed extensively only with photosynthesizing plant cells.[1] These free cells are resistant to mechanical damage in pipetting and washing by centrifugation of their suspensions in physiological buffer, and can also be kept respiring in the presence of substrate for many hours at 38° without decline.[2] Approximately 5×10^8 cells are obtained one week after inoculation of 10^7 cells intraperitoneally into young C-57 black mice. The tumor is passed in two sublines with at least three hosts in each; only nonhemorrhagic and well-developed tumor is used.

Aliquots of twice-washed cells containing about 10^7 cells each are added to the incubation medium, and after a period of preincubation labeled substrate is introduced. Until now, phosphate Lockes buffer[3] has been employed as the incubation medium, inasmuch as previous studies[4] have shown that alteration of the concentration of its components, of its tonicity, or the pH results in poorer lactate oxidation as measured over a period a several hours. For future work a bicarbonate buffer is being developed for short periods of aerobic glucose dissimilation.

Preliminary experiments show that C^{14} from evenly labeled glucose is found in more than twenty chromatographically isolatable spots after ten or more minutes of incubation. Roughly 15% of the glucose utilized appears in compounds other than lactic acid, the major product of glucose dissimilation. Some of these have been tentatively identified as hexose and pentose phosphates and monophosphates, triose phosphate, PGA, aspartic acid, alanine, glycine, serine, and Krebs-cycle intermediates.

Until now the cell suspension has been removed for analysis and centrifuged to separate the supernatant and its salts from the small volume of packed cells, thus permitting direct paper chromatography of the alcohol-water cell extracts, using the usual solvents employed in this laboratory. It is hoped that the use of electrolytic desalting will permit elimination of this time-consuming step before the alcohol killing of the metabolizing tissue. In this way it should be possible to study incorporation within several-second intervals. The over-all goal of this project is to obtain kinetic verification for the existence of several postulated metabolic systems and in what way these systems are interrelated and controlled within the cell itself.

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[1] See for example, M. Calvin, UCRL-2924, March 21, 1955.
[2] R. W. McKee, K. K. Lonberg-Holm, and J. Jehl, Cancer Res. 13, 537 (1953).
[3] R. W. McKee and A. J. Walker, Science 118, 133 (1953).
[4] K. K. Lonberg-Holm, Undergraduate Thesis, Harvard College, 1953.

RESPIRATORY CARBON-14 PATTERNS AND TURNOVER RATES OF RAPIDLY METABOLIZED COMPOUNDS

Martha R. Kirk and Bert M. Tolbert

The interpretation of respiratory excretion patterns as a measure of the turnover rate of labeled metabolites is complicated by the bicarbonate excretion time of the body. By the use of $C^{14}O_2$ excretion rates and total body bicarbonate burden, it can be calculated that the half time for CO_2 turnover in a rat should be between 10 and 15 minutes. This has been approximately confirmed by Gould et al.[1] We have repeated and extended this work, using the ratio recorder described in previous quarterly reports of this group. By using a 10^{11}-ohm grounding resistor, a 250-cc ion chamber, and a 1000-cc/min flow rate, we achieve a half time for response of the instrument of about 5 sec.[2]

Additional experiments in which rats were injected with $NaHC^{14}O_3$, either intraperitoneally (i.p.) or intravenously (i.v.), have substantiated the results reported in the Quarterly Report for March, April, and May 1955.[2] Details of these results are shown in Table XII.

Whether the first two half time rates have any physiological or biochemical basis is hard to say. They may be artifacts of our calculation procedure. The 18-minute half time, however, seems to be related to total body bicarbonate turnover time.

If simple carboxyl-C^{14}-labeled intermediates are similarly injected in the tail vein and their excretion curves measured, we see in each case a rise and peak in $C^{14}O_2$ specific activity, then apparently a decrease by a series of rate processes whose half times correspond approximately to those seen after bicarbonate injection. This would indicate that the fraction of the compound that is going to be directly oxidized to $C^{14}O_2$ is rapidly oxidized compared to the excretion rate of the bicarbonate. On the basis of this assumption, it should then be possible to separate the excretion rate of $C^{14}O_2$ from the oxidation rate of the labeled compounds. We are doing this at present.

In Figs. 9 through 13 we have presented comparative excretion curves for several of these compounds, and be several modes of injection. Notice how similar the excretion slopes for most of these substances are after the first half hour. These curves have been plotted with arbitrary units for the ordinates, and no correction has been made for variation in amount of radioactivity injected.

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[1] R. Gould et al., J. Bio. Chem. 177, 295 (1949).
[2] B. M. Tolbert and M. Kirk, UCRL-3068, July 6, 1955, p. 16.

Table XII

Bicarbonate turnover rates in rats

Compound	Injection method	Time of maximum specific activity	First-order rates: half times		
$NaHC^{14}O_3$	i.v.	20 sec after inj.	30 sec	2.7 min	18.5 min
	i.v.	35 sec after inj.	38	5.2	18
	i.v.	15 sec after inj.	30	4.1	16
	i.p.	4.75 min after inj.		5.7	16.25
	i.p.	5.0 min after inj.		8	22.5
	i.p.	3.5 min after inj.		5.8	18.5

RESPIRATORY CARBON-14 PATTERNS IN RATS GIVEN LABELED ACETATE

Ann M. Hughes and Bert M. Tolbert

Previous quarterly reports[1] have presented the respiratory patterns of rats given lactic acid or propionic acid labeled separately in each carbon atom. The work has been expanded to include studies on each of the two acetic acids.

As in the previous work about 5μc (2 mg) of the labeled compound was injected i.p. in young adult rats. The curves for cumulative excretion and rate of excretion of $C^{14}O_2$ are shown in Figs. 14 and 15. Each of these curves respresents the average of about 12 animals. The half time for response of the machines was about one minute, and very fast excretion changes would be integrated out of the rate curve.

The most interesting observation to be made from these curves is the relatively low rate of $C^{14}O_2$ excretion after injection of acetate-1-C^{14}, as compared to lactate-1-C^{14} or propionate-1-C^{14}. The relative specific activity of the $C^{14}O_2$ is about 2/3 that observed in the lactate and propionate experiments. The 7-hr cumulative $C^{14}O_2$ excretion from acetate-1-C^{14} is about 80%; from lactate-1-C^{14} and propionate-1-C^{14} it is about 95%.

If lactate is primarily metabolized through pyruvate to acetate, direct decarboxylation could produce this difference. Direct decarboxylation of propionate is also indicated by our data. It will be interesting to see if butyrate or caproate is similarly metabolized, or if the β-oxidation of the fatty acid holds instead.

[1] A. M. Hughes, J. Weaver and B. M. Tolbert, UCRL-2932, March 30, 1955, p. 12; A. M. Hughes and B. M. Tolbert, UCRL-3068, July 6, 1955, p. 17.

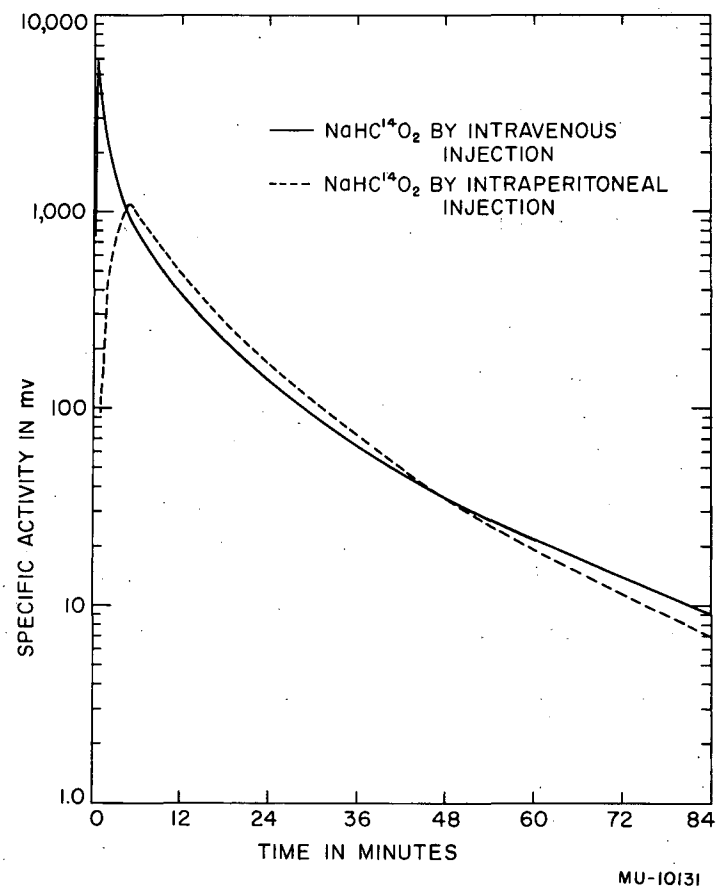


Fig. 9. Specific activity of $C^{14}O_2$ from rats injected with $NaHC^{14}O_2$.

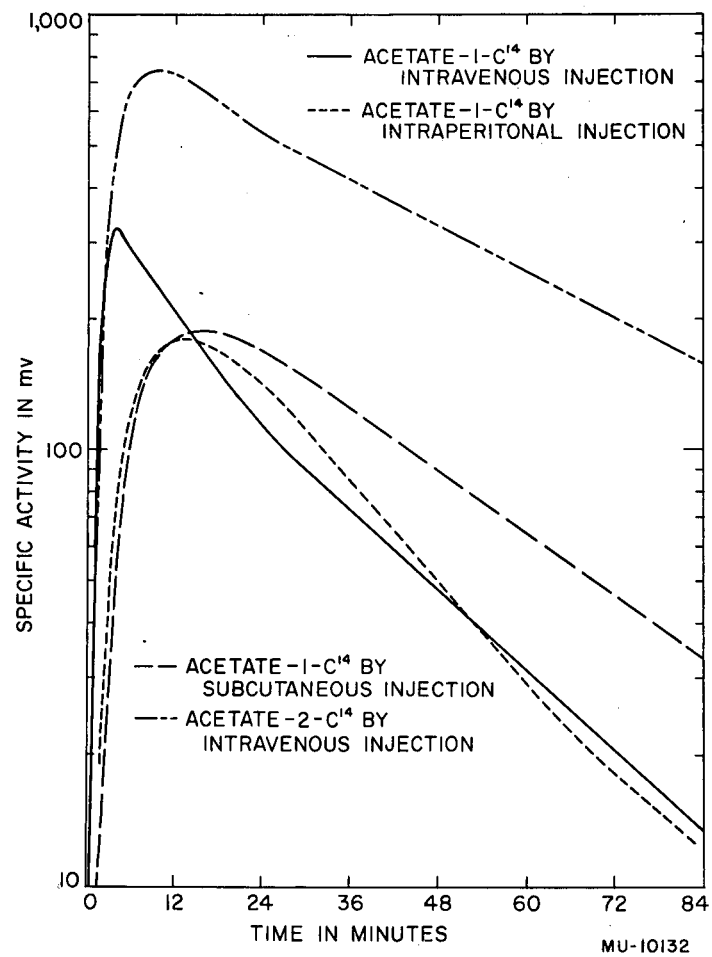


Fig. 10. Specific activity of $C^{14}O_2$ from rats injected with acetate-1-C^{14} or acetate-2-C^{14}.

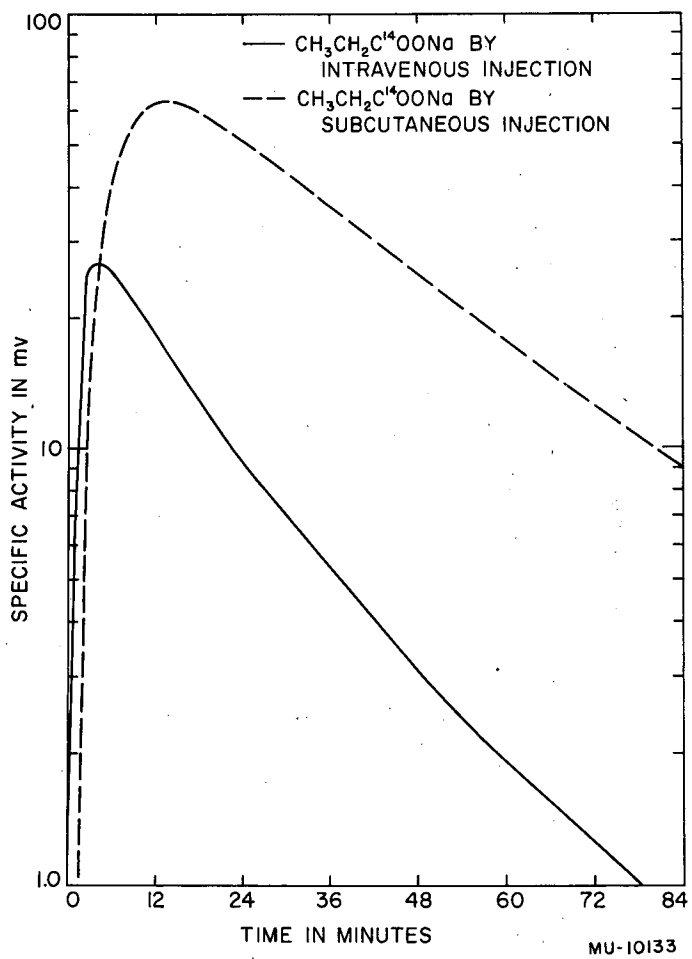


Fig. 11. Specific activity of $C^{14}O_2$ from rats injected with sodium propionate-1-C^{14}.

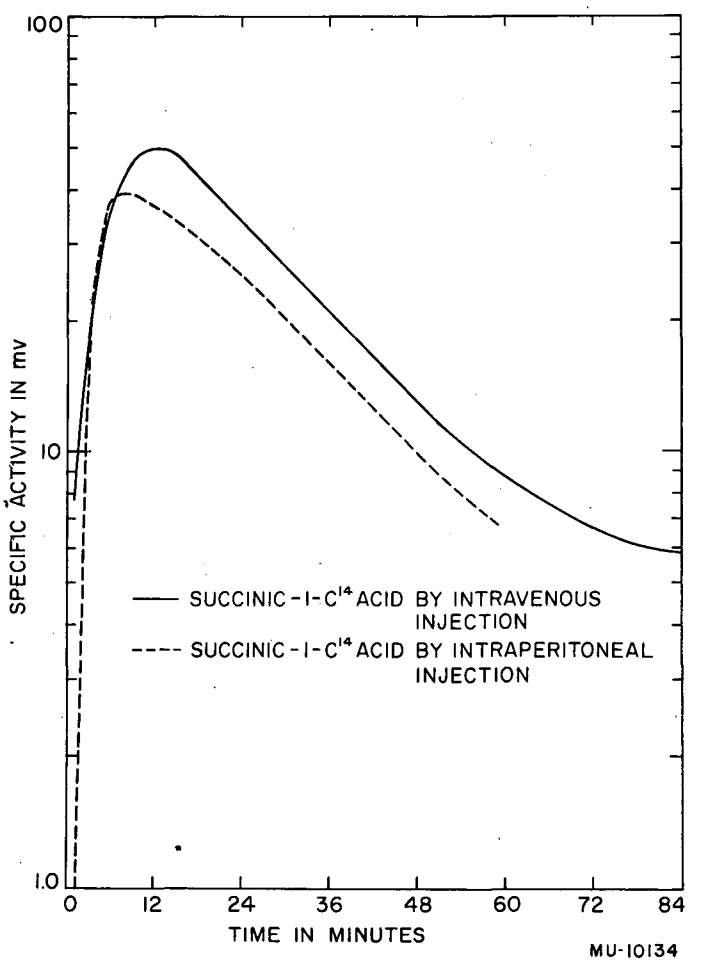


Fig. 12. Specific activity of $C^{14}O_2$ from rats injected with succinic-1-C^{14} acid.

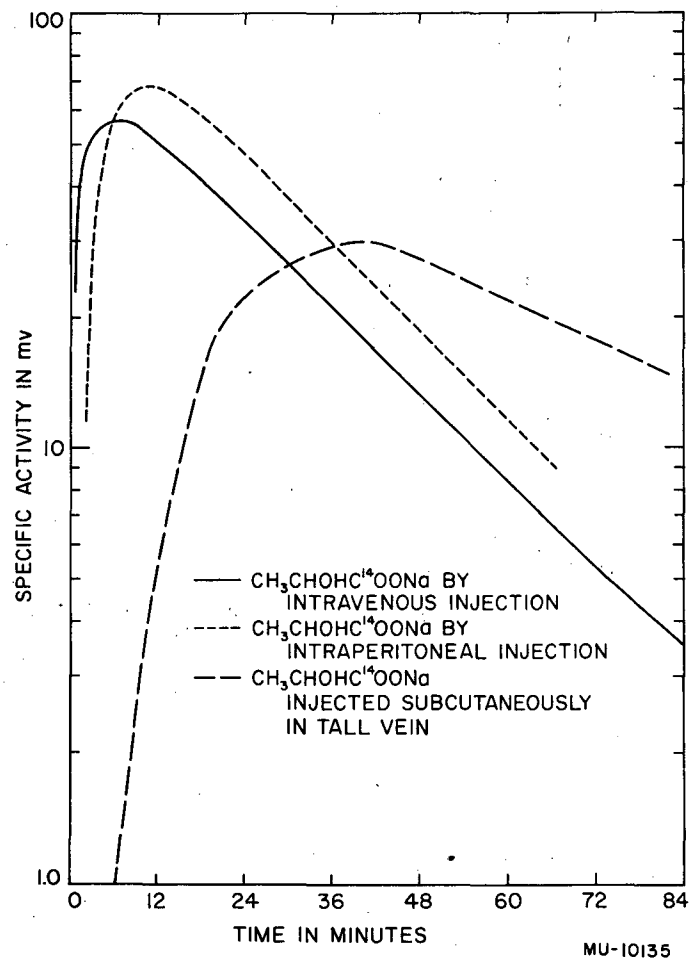


Fig. 13. Specific activity of $C^{14}O_2$ from rats injected with sodium lactate-1-C^{14}.

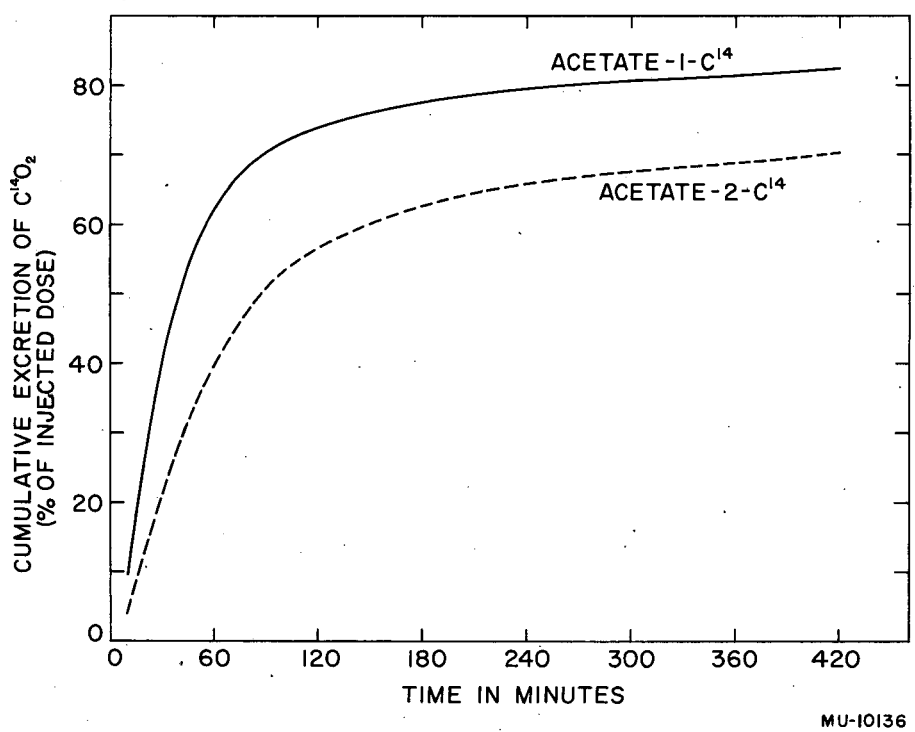


Fig. 14. Cumulative excretion of sodium acetate-C^{14} labeled in the 1 and 2 positions.

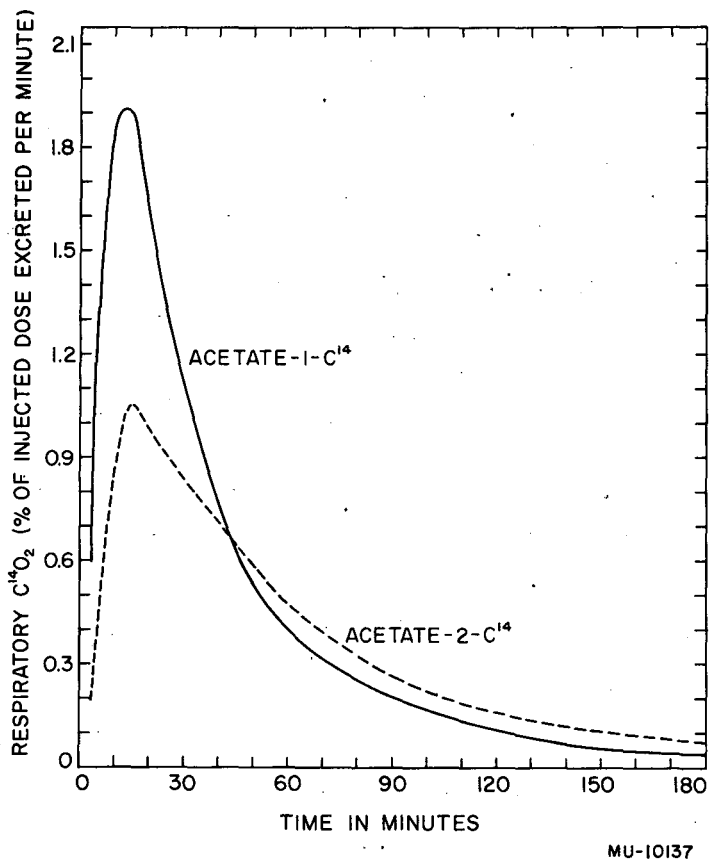


Fig. 15. Rate of excretion of sodium acetate-C^{14} labeled in the 1 and 2 positions.

Table XIII compares the $C^{14}O_2$ excretion for eight compounds. For the sake of comparison, they are divided into three groups. These excretion data agree with postulated biochemical theory.

Table XIII

$C^{14}O_2$ Excretion in Rats

Substrate	Peak rate*	Cumulative %
Group I		
Lactate-1-C^{14}	2.6	97.5
Propionate-1-C^{14}	3.1	98
Group II		
Lactate-2-C^{14}	1.3	84
Acetate-1-C^{14}	1.9	82.5
Propionate-2-C^{14}	1.3	79
Propionate-3-C^{14}	1.2	77
Group III		
Lactate-3-C^{14}	0.6	67.5
Acetate-2-C^{14}	1.1	70

*Rate units are % of injected dose excreted per minute.

KINETICS OF THE ALKALINE HYDROLYSIS OF N-TRIFLUOROACETYLAMINO ACIDS

Rosemarie Ostwald

As mentioned in a previous report,[1] it seemed of interest to study the kinetics of the alkaline hydrolysis of N-trifluoroacetylamino acids because of their value as intermediates in peptide synthesis.

An automatic recording titrator[2] was used to perform and follow the course of hydrolysis. Three ml of an aqueous solution of N-trifluoroacetylamino acid of known concentration was placed in a thermally regulated cell. The cell was equipped with standard Beckmann pH-meter electrodes, stirrer, and gas inlet and outlet holes. The system was kept under a stream of nitrogen during the course of the reaction. The solution was brought to the desired pH as quickly as possible by the addition of a known amount of base.

[1] R. Ostwald, UCRL-2932, March 30, 1955, p. 31.
[2] J. B Neilands and M. D Cannon, Anal. Chem. 27, 29 (1955).

After the titration motor had been turned on, the amount of base (0.1-0.5 N KOH was used), added from a syringe to the solution, necessary to maintain the given pH was recorded against time. The N-trifluoroacetylamino acids used in this study were prepared by E. Schallenberg.[3] Except for N-trifluoroacetylamino glycine they are insoluble in water and were dissolved by addition of one equivalent of base.

Results

The reaction for the hydrolysis of N-trifluoroacetylglycine, -valine, -norleucine, -phenylalanine, tryptophane, and -ε-aminocaproic acid is second-order. This is shown by:

(a) The linearity of a plot of the logarithm of the fraction of N-trifluoroacetylamino acid remaining unhydrolyzed (1-f) vs time at a given pH as expected from the expression

$$\frac{dcA}{dt} = k \left[OH\right] c_A = k' c_A ,$$

where c_A is the concentration of substrate at the time t, k is the rate constant and k' the apparent rate constant.

(b) The comparisons of the plots of log (1-f) vs time for different initial concentrations of N-trifluoroacetylamino acid, which shows that the rate of hydrolysis is proportional to the first order of the concentration, since the slopes of the lines are the same.

(c) The linearity of the plots of log k' vs pH with a slope of 1 at each temperature as expected from the equation.

$$\log k' = \log k - 14 + pH.$$

(d) The linearity of the plots of log k' vs 1/T, as expected from the equations

$$k = Ae - E/RT,$$

$$\log k' = \log A - 0.4343\ E/RT - 14 + pH.$$

The activation energies obtained from these plots and the relevant data for k' and k are given in Table XIV.

The hydrolysis of N-trifluoroacetyllysine is an exception. It is first-order in respect to N-trifluoroacetyllysine, but of an apparent order less than one in respect to hydroxyl ion, (the slope of the plot of log k' vs pH is less than one). One possible mechanism of the hydrolysis, which would account for these results, can be expressed as follows:

[3] E. E. Schallenberg and M. Calvin, J. Am. Chem. Soc. 77, 2779 (1955).

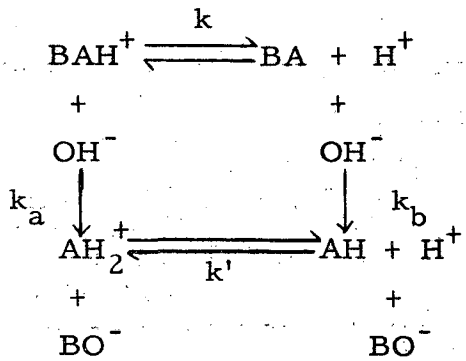
Table XIV

Hydrolysis of N-trifluoroacetylamino Acid

TFA	t	pH	$k' \times 10^{-4}$	k	E kcal/deg./mole	most prob. error, 50% significant[a]	2 x std. deviation of mean, 25% significant[a]
Glycine	40	10.23	1.62	0.952	21	0.54 kcal	1.6 kcal
		11.01	12.0	1.17			
		11.53	46.1	1.36			
	30	10.99	3.73	0.39			
		11.51	10.2	0.31			
		12.0	40.6	0.40			
	20	11.0	1.14	0.114			
		11.87	11.0	0.15			
Norleucine	50	11.54[b]	28.4[b]	0.820[b]	23.1	5.5	16.3
	40	11.54[b]	7.69[b]	0.221[b]			
Phenyl-alanine	50	11.47[b]	4.09[b]	0.137[b]	20.8	3.0	8.9
	40	11.47[b]	1.46[b]	0.049[b]			
Trypto-phane	50	11.15[b]	2.23[b]	0.158[b]	30.4	4.7	14.0
		11.42	4.06	0.154			
	40	11.42[b]	0.89[b]	0.034[b]			
		11.69	1.42	0.039			
Valine	40	11.56[b]	9.59[b]	0.265[b]	31.2	10.2	30.2
	30	11.56	1.70	0.047			
-Amino-caproic acid	50	10.76	24.1	4.2	31.8	1.68	5.0
		11.07	41.2	3.5			
	40	10.77	6.01	1.06			
		11.35	17.4	0.76			
Lysine	50	11.47	69.1	--	21.1	0.54	1.6
	40	11.47	19.3	--			
	30	11.47	6.45	--			

t = °C
k' = apparent rate constant = slope of curve of log (1-f) vs time x $\frac{2.303}{60}$
k = absolute rate constant = $k'/[OH^-]$ l $mol^{-1} sec^{-1}$.
E = activation energy in kcal/deg./mole.
a = these values were calculated according to Ref. 4 below.
b = average values from a number of experiments.

[4] R. O. Fischer, "Statistical Methods for Research Workers," Oliver and Boyd Publ., 1950.



BAH^+ = $COCF_3NH(CH_2)_4CH(COOH)NH_3^+$
BA = ----------NH_2
AH_2^+ = $NH_2(CH_2)_4CH(COOH)NH_3^+$
AH = ----------NH_2
BO^- = CF_3COO^{--}

A comparison of the data for the activation energies shows that they all fall into the same range and no influence of the R-group in RCH $(NHCOCF_3)COOH$ on the rate of hydrolysis can be demonstrated.

EFFECTS OF IONIZING RADIATION ON CHOLINE ANALOGS

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

The following table summaries the work to date on determining the effects of changes in the choline molecule on its radiation sensitivity. Eighteen compounds have been irradiated and their relative sensitivities have been determined and recorded as G values (or molecules permanently altered per 100 electron volts of energy expended in the compound). All compounds have been irradiated as anhydrous crystals maintained under high vacuum. As has been described in previous quarterly reports, the amount of a compound remaining after irradiation has been determined by (a) spectrophotometric determination of the reineckate precipitate, (b) gravimetric determination of the tetraphenylboron precipitate, and (c) paper chromatographic determination of the C^{14}-labeled compound. Owing to the uncertainties introduced by the presence of varying amounts of decomposition products produced by the radiation, and to uncertainties in dosage measurements and analytical procedures, the G values recorded may be in error by ± 50%. With three exceptions (noted in the table) all the G values are averages of at least two experiments; some of the G values are averages of as many as eight or nine experiments.

The data of Table XV clearly show that clorine chloride is in a unique position as regards radiation sensitivity. All of the changes that have been made in the molecule have, without exception, led to a great increase in radiation stability. This fact indicates that there may be a unique factor in the crystal structure of choline chloride that gives rise to its extraordinary radiation sensitivity. The crystal structure of choline chloride is now under investigation by x-ray diffraction techniques in the University of California Chemistry Department.

In addition to the 18 compounds listed in Table XV, choline fluoride, choline acetate, and tetrakis (2-hydroxyethyl) ammonium chloride are being prepared and their radiation sensitivities will also be investigated.

Table XV

Radiation Decomposition of Choline Analogs

Compound	Radiation	G
$[(CH_3)_3NCH_2CH_2OH]^+ Cl^-$	e^-	113
	γ	204
$[(CH_3)_3NCH_2CH_2OH]^+ Br^-$	e^-	35
	γ	89
$[(CH_3)_3NCH_2CH_2OH]^+ I^-$	e^-	1.6
	γ	3.3
$[(CH_3)_3NCH_2CH_2OH]^+ NO_3^-$	e^-	1.4
	γ	4.9
$[(CH_3)_3NCH_2CH_2OH]_2^+ SO_4^=$	e^-	15
	γ	28
$[(CH_3)_3NCH_2CH_2OCOCH_3]^+ Cl^-$	e^-	1.8
	γ	2.4
$[(CH_3)_3NCH_2CH_2Cl]^+ Cl^-$	e^-	3.3
	γ	12
$[(CH_3)_3NCH_2CH_3]^+ Cl^-$	e^-	1.6
	γ	2.3
$[(CH_3)_3NCH_2CH_2CH_2OH]^+ Cl^-$	e^-	2.1
	γ	4.5
$[(CH_3)_3NCH_2CH(OH)CH_3]^+ Cl^-$	e^-	7*
	γ	8
$[(CH_3)_3NCH_2COOH]^+ Cl^-$	e^-	16
	γ	13

*G values determined from one experiment only.

Table XV, cont.

Compound	Radiation	G
$[(CH_3)_3NCH_2CH(OH)CH_2COOH]^+ Cl^-$	e^-	18
	γ	33
$[(C_2H_5)(CH_3)_2NCH_2CH_2OH]^+ Cl^-$	e^-	11
	γ	24
$[(C_2H_5)_2CH_3NCH_2CH_2OH]^+ Cl^-$	e^-	6
	γ	19
$[(C_2H_5)_3NCH_2CH_2OH]^+ Cl^-$	e^-	15
	γ	17
$[(C_6H_5)(CH_3)_2NCH_2CH_2OH]^+ Cl^-$	e^-	8
	γ	4.5
$[(CH_3)_2N(CH_2CH_2OH)_2]^+ Cl^-$	e^-	10*
	γ	12
$[(CH_3)_3PCH_2CH_2OH]^+ Cl^-$	e^-	2.4*
	γ	4.1

*G values determined from one experiment only.

SCINTILLATION COUNTING

Elton M. Baker

Studies are continuing in this laboratory to obtain satisfactory methods of counting radioactive samples emitting soft β-particles in water solutions. A system described previously, using two photomultiplier tubes in coincidence and sealing a sample in a small thin flat-sloped flask, is being used. The electronic system approaches 0.1 microsecond in resolving time, with a minimum of after-pulsing at the operating voltage. The operating voltage is not the same for C^{14} samples and for tritium.

A previously described study using a solvent system of n-propylalcohol-toluene and water containing both C^{14}- and tritium-labeled benzoic acid has been tried at -14°C. The quantities in the sample of the carboxyl compound are less than 10 gamma, so that a very small effect of quenching due to the amount and structure of the compound is observed.

Present work is being carried on to determine the effect of methyl, ethyl, n-propyl, and isopropyl alcohols and dioxane in the liquid scintillator, which consists of toluene and diphenyloxazole as the phosphor. Quenching effects of alcohol on the detection of the radiation energy are in the increasing order dioxane, isopropyl, n-propyl, ethyl, and methyl alcohol for samples of C^{14} with no water present. The presence of water makes little significant difference in the quenching; the effects are within the allowable error of preparing different samples. This is in agreement with our studies indicating that the degradation of energy that is available for light emission is more severe for high-energy β-particles from C^{14} than for lower-energy particles, but that the total number of events that can be detected remains more or less constant.

In one experiment we have successfully counted C^{14} as dissolving aqueous bicarbonate in a toluene-alcohol scintillation mixture. The counting efficiency was very high at first, 95% or better. An interesting observation was that the micro amounts of carbonate did not stay in solution but were rather completely absorbed on the glass walls in 5 to 6 hr.

Further studies will continue using water containing radioactive carbonate samples and with water-soluble compounds. The use of tritium samples in organic compounds and in water will be tried in alcohol-toluene mixtures.

PREPARATION OF CALCIUM GLYCOLATE FOR RADIATION SENSITIVITY STUDIES

I. M. Whittemore and B. M. Tolbert

It has been noted by S. Weinhouse[1] that C^{14}-labeled calcium glycolate does not appear to be stable when stored over a period of years. H. Grisebach, on the other hand, found only ordinary radiation sensitivity for this compound when it was subjected to Co^{60} γ radiation and to β radiation from a linear accelerator.[2] He suggested that the decomposition noted earlier by Weinhouse may have been due to factors other than radiation.

Work is now being carried out to prepare calcium glycolate-1-C^{-14} on a millimole scale with a specific activity of about 1/mc/mM. The method of preparation is that which has been previously described.[3]

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[1] See B. M. Tolbert et al., J. Am. Chem. Soc. 75, 1867 (1953).
[2] H. Grisebach, UCRL-3068, July 6, 1955.
[3] D. M. Hughes and B. M. Tolbert, UCRL-256 (1948).

The purity of the calcium glycolate made in this preparation will be carefully checked to insure its freedom from radioactive and other impurities. Small quantities of it will be sealed in various ways and stored for periods of a year or longer. The purity will then be checked for possible decomposition products such as formic and oxalic acids.

It is hoped that the relative purity of the samples after storage will indicate whether or not calcium glycolate is subject to more than ordinary self-decomposition.

THE SYNTHESIS OF THIOCTIC-$S^{35}{}_2$ ACID

Patricia T. Adams

In a separate report* the synthesis of thioctic-$S^{35}{}_2$ acid was described. Material of high specific activity was prepared but could not be isolated in the crystalline state. A new synthesis, using lower specific activity and some modifications in the purification and isolation of the intermediate dibenzylmercaptooctanoic acid, has now been completed. Crystalline product of good purity was obtained.

Dibenzylmercapto-$S^{35}{}_2$-octanoic Acid (DBMO)

A solution of sulfur-35 (1.3 mg, 100 mc ± 20%) in benzene was added to 320 mg of amorphous sulfur in 10 ml dry benzene. An ethereal solution containing 14 millimoles of benzylmagnesium chloride was added with stirring, and the mixture was allowed to stand overnight. α-Toluenethiol was isolated as before in a dry ethereal solution in 90% yield. After evaporation of the ether, the thiol was allowed to react in 15 ml of alcohol solution with 205 mg (8.9 millimoles) of sodium and 1.32 g (4.0 mM) of ethyl 6,8-dibromooctanoate. This mixture was heated under reflux for 4 hr. Potassium hydroxide (600 mg) was added, and the solution was stirred at room temperature for 16 hr.

The reaction mixture was diluted with 75 ml of water and extracted with ether. The ether-soluble, nonacidic fraction contained 4 mc of sulfur-35. The aqueous layer was acidified and extracted with ether. The ethereal solution contained the DBMO and acidic contaminants of unknown identity. It was extracted with two small portions of cold 5% sodium bicarbonate to remove these contaminants (6 mc of S^{35}), washed with water, dried, and evaporated to dryness.

The crude, solid DBMO was dissolved in 4 ml of dry benzene. Warm hexane (12 ml) was added gradually. The clear solution was cooled in the refrigerator for 2 days. The white crystals were filtered, washed with cold hexane, and dried in vacuo. The yield was 902 mg (2.3 mM, 52% from toluenethiol). The specific activity was 33 μc/mg. Chromatographic analysis showed the sample to be free from radioactive contaminants.

*Patricia T. Adams, "Thioctic-$S^{35}{}_2$ Acid: Synthesis and Radiation Decomposition," UCRL-2949, April 8, 1955.

Because thioctic acid is quite sensitive to decomposition from light, heat, and air oxidation over long periods of storage, most of the product from this synthesis is being stored as the DBMO intermediate. The conversion to thioctic acid is made on a very small scale according to the procedure below, whenever the thioctic acid is needed for use in the biological experiments.

Thioctic-$S^{35}{}_2$ Acid

A solution of 70 mg (0.18 mM) of DBMO in 3 ml of ether was added to a solution of sodium in 8 ml of liquid ammonia. After 1/2 hour's stirring, the excess sodium was decomposed with ammonium chloride and the ammonia was allowed to evaporate. The residue was dissolved in 10 ml of water and the pH adjusted to 9. Several drops of 1% ferric chloride were added and oxygen was bubbled through the solution for 20 min. The basic solution was extracted once with ether to remove small amounts of insoluble material. After acidification, the aqueous solution was extracted with chloroform. The organic layer was dried and evaporated to dryness under a nitrogen stream at room temperature in a small sublimation apparatus. The yellow residue was sublimed onto a dry-ice-cooled cold finger at 90°-100°C for 4 hr.

The crystalline sublimate was rinsed into a weighing tube with dry benzene and evaporated to dryness. The residue, when dried, was yellow needle-like crystals weighing 11 mg (0.05 mM, 30% yield). The specific activity was 58 μc/mg (calc: 61 μc/mb). Spectrophotometric analysis showed the product to be 85% thioctic acid. Paper chromatographic analysis shows the remaining material to be thioctic acid monosulfoxide and the linear polymer of thioctic acid, with no other radioactive impurities.

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

GAMMA-GAMMA ANGULAR CORRELATIONS

Frank S. Stephens, Frank Asaro, and Isadore Perlman

It is well established that throughout the entire periodic table almost all the first excited states of even-even nuclei have spin to and even parity. In the region well above the closed shell of 82 protons and 126 neutrons, additional low-lying states are observed which together with the ground (0+) and 2+ states are generally considered to be members of a rotational band according to the Bohr-Mottelson unified collective and individual particle nuclear model.[1] In members of such a band, the spins of these levels are expected to be 0+, 2+, 4+, 6+, ... Although the spin of the 2+ level has been proven for many nuclei in this region from both conversion-coefficient and angular-correlation arguments, this has not yet been done conclusively for the spins of the higher levels. Valladas et al.[2] have shown that spin and parity assignments of 4+ for the second excited state of Ra^{226} are consistent with their alpha-gamma angular correlation measurements. They were not able, however, to eliminate the possibility of the state's having other spins if it de-excited by mixed multipole order transitions. The work reported here represents an attempt to prove unambiguously the 4+ assignment for this state.

The technique we used was gamma-gamma angular correlations between the 142-kev photon (second excited state to 2+ state transition) and the 68-kev photon (2+ to 0+ transition). The levels of Ra^{226} were selected for several reasons. Its parent, Th^{230}, could be obtained and easily purified to an extent where radiations from impurities were extremely small. Also, owing to the long half lives of Th^{230} and Ra^{226}, there was no problem either with decay of the parent or with a growth of daughter activity. In addition, the alpha population to the second excited state is considerably larger than is found for higher elements such as plutonium or curium. The final advantage is that the level spacings in this region are wider than found for higher elements, which makes the gamma-ray transitions of higher energy, and correspondingly makes the conversion coefficients much smaller.

The sample consisted of about 2×10^8 alpha disintegrations per minute of Th^{230} (5 milligrams), which was mounted in a glass capsule 0.9 cm in diameter. The gamma-ray detectors were NaI crystals, 1.5 inches in diameter by 1 inch thick. These were placed 7 cm from the source, and the angular distribution of coincidences was examined by rotating one of the detectors. The movable detector was set to accept only pulses from the 68-kev photon, and these pulses were counted in order to avoid the problem of reproducing the exact geometry. The coincidences were then normalized to the

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[1] A. Bohr and B. R. Mottelson, Dan. Mat. Fys. Medd. 27, no. 16 (1953).
[2] G. Valladas, J. Teillac, P. Falk-Vairant, P. Benoist, private communication. P. Falk-Vairant, J. Teillac, G. Valladas, P. Benoist, Compt. rend. 238, 1409 (1954).

same number of 68-kev single counts. The spectrum observed by the fixed counter was examined on a 50-channel pulse-height analyzer which was gated only when a coincidence occurred between one of these pulses and one of the 68-kev pulses from the movable detector. The coincidence circuit had a resolving time of about 3×10^{-7} second, which was not sufficiently small to make the chance coincident rate negligible. The chance rate was therefore determined by recording a comparable number of gate counts from an external Am^{241} source (60-kev photon).

Data were taken at three angles, 90°, 135°, and 180°. Each run took about 1.5 days, and in all, 9 runs were made. The coincidences at each angle were then totaled, normalized, and used in the following equation to calculate the angular anisotropies:

$$Ax = \frac{N_x - N_{90^{\circ}}}{N_{90^{\circ}} - N_{ch}} ,$$

where Ax is the anisotropy at either 180° or 135°, N_x is the normalized count at angle x, and N_{ch} is the normalized chance coincident count. Corrections for finite acceptance angle were made from the data of Rose.[3] There is some ambiguity as to what the exact corrections should be. Compared with the limits of error, however, such differences are not large.

The result of this treatment is shown in Table I together with the correlations for all the possible spin sequences. Since the level is populated by alpha particles, only the even-spin even-parity, and odd-spin odd-parity designations are possible. In cases where mixed transitions can exist, the mixtures were selected which gave the observed anisotropy at 180°. The limits of error on the experimental points are the standard deviations to be expected from the total number of counts recorded.

Because the gamma rays in other nuclei--presumably analogous to the 142-kev gamma in this case--have been shown to be at least predominantly E2, the first three spin sequences in Table I are the most probable. The last two represent rather unlikely mixtures of E1 and M2 radiations, but were considered both for the sake of completeness and because extremely slow E1 transitions are known to exist in the odd-mass nuclei of this region.

The experimental correlation is seen to be in excellent agreement with that expected for 4+ assignment of the state. It is not possible, however, to rule out on this basis alone two other assignments: that of a 2+ spin with predominantly M1 radiation, and that of a 3-spin with predominantly E1 photons. In order to rule out these possibilities it is necessary to consider the K-conversion coefficients to be expected of such mixtures; these are shown in the bottom row of Table I. The theoretical K-conversion coefficients are extrapolated from the tables of Rose et al.[4] The experimental limit comes from integrating the peak of about 85 kev also seen in the coincident spectra

[3] M. E. Rose, Phys. Rev. 91, 610 (1953).
[4] M. E. Rose, G. H. Goertzel, and C. L. Perry, ORNL-1023 (1951).

Table I

Spin Assignment of the Second Excited State of Ra^{226}

Spin Sequence	$4+ \xrightarrow{E2} 2+ \xrightarrow{E2} 0+$	$0+ \xrightarrow{E2} 2+ \xrightarrow{E2} 0+$	$2+ \xrightarrow{M1, E2} 2+ \xrightarrow{E2} 0+$		$1- \xrightarrow{E1, M2} 2+ \xrightarrow{E2} 0+$		$3 \xrightarrow{-E1, M2} 2+ \xrightarrow{E2} 0+$		Experimental Data
Photon Comp.	Pure E2	Pure E2	99%E2 1 %M1	96%M1 4 %E2	90%E1 10%M2	97%M2 3 %E1	94%E1 6 %M2	81%M2 19%E1	
A_{180}(%)	+17	+100	(+17)	(+17)	(+17)	(+17)	(+17)	(+17)	+17 ± 5
A_{135}(%)	+7	-50	-24	+7	+17	+150	+9	+16	+6 ± 4
α_K	0.35	0.35	0.4	6.5	2.4	22	1.6	18	<0.7

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and comparing it with the 142-kev peak. Since there were large amounts of lead in the vicinity of the detector, it is quite likely that a part of this peak arises from lead K x-rays, and hence it represents only an upper limit to the K-conversion coefficient. Nevertheless the 2+ assignment is eliminated by a factor of almost 10, and the 3- assignment by a factor of more than two.

In the course of this work the angular correlation of another gamma ray in the coincident spectrum was observed. This gamma ray of 184 kev arises from a state at 253 kev (presumed for reasons described elsewhere[5] to have spin one, odd parity) and terminates at the 2+ level. The only other gamma ray of appreciable abundance present (at 253 kev) is assumed to go to the ground state, and, in agreement with this, did not appear in the spectrum coincident with the 68-kev photon. The 184-kev gamma ray is considerably less abundant than the one at 142 kev, and so the standard deviations obtained in this case are considerably larger than those in Table I. The anisotropies observed, corrected for finite solid angle, were: $A_{180^\circ} = -0.27 \pm 0.10$ and $A_{135^\circ} = -0.16 \pm 0.09$. These are in good agreement with those expected of a $1- \xrightarrow{E1} 2+ \xrightarrow{E2} 0+$ sequence, which are $A_{180^\circ} = -0.33$ and $A_{135^\circ} = -0.17$. Since the 253-kev level decays to the ground state also, the only other reasonable assignment of this level is 2+. In this case negative anisotropies at 180° of about this magnitude are possible for a wide range of photon compositions centering roughly around equal amounts of E2 and M1 radiation. Throughout this region, however, the anisotropy at 135° is expected to be negative and at least as large as that at 180°. Although this does not seem to be the case from the experimental data, the limits of error are so large that it cannot be excluded. The expenditure of undue amounts of time to reduce the limits of error did not seem worth while, since the spin of the state can be very easily and conclusively proved by alpha-gamma angular correlations with the 253-kev photon. Such an experiment is presently in progress.

In concluding it should be added that measurements similar to these have been made on Ra^{222} obtained from the decay of Th^{226}. In this case, for the sequence assumed to be $4+ \longrightarrow 2+ \longrightarrow 0+$, the following anisotropies were obtained: $A_{180^\circ} = -0.14 \pm 0.05$ and $A_{135^\circ} = -0.05 \pm 0.05$. These values are in good agreement with the expectations for such a sequence; however, a good K-conversion coefficient is not available, so that the exclusion of all spins except 4+ is not possible.

STUDIES OF GAMMA-RAY SPECTRA

Richard C. Pilger, Jr., Frank S. Stephens, Frank Asaro and Isadore Perlman

Thorium-227

The gamma-ray spectrum and gamma-gamma coincidence spectra of Th^{227} (RdAc) were investigated. Several gamma rays not reported in previous work in this laboratory[a] were observed. Those observed in this work are:

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[5] Stephens, Asaro, and Perlman, Phys. Rev. 96, 1568 (1954).
[a] E.K. Hyde, Phys. Rev. 94, 1221 (1954).

Approximate Energy (kev)	Estimated Abundance (photons/alpha particle)
49	(0.15)
137	0.12
207	0.04
237	0.3
257	---
295	0.09
325	0.09

The abundances are estimated, based on the value determined for the 49-kev gamma ray by Hyde.[a] In addition, indications of gamma rays of about 80 kev and 420 kev are noted.

The 50-kev and 237-kev radiations are distinctly in coincidence, but coincidence abundance measurements are inconclusive at present. K x-rays also appear in coincidence with the 50-kev emission, and upon the assumption that all of these arise from conversion of the 237-kev transition, we can calculate the K-conversion coefficient for this transition to be 0.1 electron/photon, a value that is in agreement with the predicted value for an electric quadrupole (E2) transition,[b] although the possibility of electric dipole (E1) cannot be entirely eliminated.

The 237-kev photons do not appear to be in coincidence with K x-rays, although the 137-, 207-, and 257-kev clearly are. Coincidence of the 295- and 325-kev radiations with K x-rays is uncertain.

Emanation-219 and Polonium-215

A preliminary study of the gamma-ray spectrum of Em^{219} and its daughter Po^{215} in equilibrium has been made. A gas-flow technique was employed to obtain separation of the 4-second-half life Em^{219} from its parent Ra^{223}. The parent activity was placed in an evacuated chamber shielded from the scintillation spectrometer equipment. The emanation was allowed to pass through the shield in an arm of the evacuated system, where it was presented to the spectrometer. After a suitable exposure, the gas flow was terminated and the Em^{219} permitted to die out. Then a background determination was made, allowing all contribution of the 30-minute activity Pb^{211} and its daughters to be subtracted from the spectrum.

By this means, prominent gamma rays of about 270 and 395 kev were observed. Indications of radiations at 140 to 150, 190, and 240 kev were noted but not confirmed. A radiation of 590 kev reported by other workers[c] was not observed, yet these workers did not report the 390-kev radiation seen in this work.

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[b] Rose, Goertzel, and Perry, ORNL-1023 (1951).

[c] Frilley, J. Phys. et radium 1, 34 (1940) (as quoted in Table of Isotopes, J. M. Hollander, I. Perlman, and G. T. Seaborg, Revs. Modern Phys. 25, 469-651 (1953)

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ENERGY OF THE GAMMA RADIATION EMITTED BY THE ISOMERIC STATE OF YTTRIUM-88

Lester Winsberg, M. Frances Gallagher, Earl K. Hyde, and Warren G. Smith

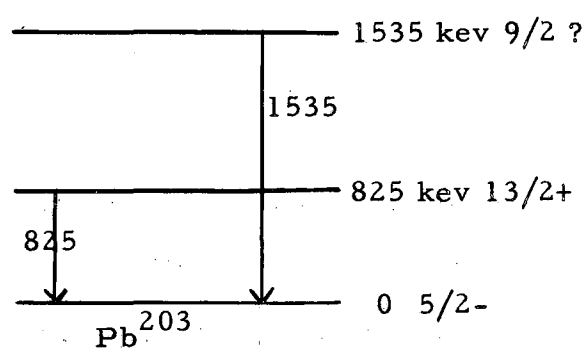
Zirconium-88 has been shown to decay by electron capture to an isomeric state of yttrium-88 with a half life of 85 days.[1] The latter de-excites by emission of gamma radiation. Since its energy has been variously reported as 387, 395, and 406 kev,[1] we have redetermined the energy on a permanent-magnet electron spectrograph.

Niobium was irradiated at the 184-inch cyclotron and allowed to cool for two months. Zirconium was separated carrier-free by the procedure of Hyde and O'Kelley[1] and was finally dissolved in 0.1 M ammonium sulfate solution brought to a pH of 3.6 by the addition of dilute sulfuric acid. The zirconium was then electroplated on a 10-mil platinum wire. The wire was mounted in the spectrograph and a film was exposed for five days. A K- and an L-conversion line, the latter probably a combination of L_I, L_{II}, and L_{III}, were present. The energy of the gamma transition was calculated to be 393.7 ± 1.0 kev. Because of the greater accuracy of the permanent-magnet spectrograph, this value supersedes the three previously reported values.

THE DECAY SCHEMES FOR BISMUTH-203, BISMUTH-204, AND BISMUTH-205

Arnold R. Fritsch and Isadore Perlman

In Bi^{203} and Bi^{205} a search was made for short-lived isomers of the M4 type such as in the 1050-kev transition in Bi^{207}. A 7.2±.4-second isomer was found in the Bi^{203}, resulting from 825-kev transition, which had nothing in coincidence with it. Previously a 1535-kev gamma ray had been seen, also, so that a tentative decay scheme to date would be:



[1] E. K. Hyde and G. D. O'Kelley, Phys. Rev. 82, 944 (1951);
E. K. Hyde, Phys. Rev. 92, 927 (1953);
E. K. Hyde, M. G. Florence, and A. E. Larsh, Phys. Rev. 97, 1255 (1955)

The ground state of Pb^{203} is $f_{5/2}$ according to Prescott,[1] and therefore some assignments can be made for Pb^{203m}. This isomer is undoubtedly the 5.6-second one of Hopkins,[2] which was assigned tentatively to Pb^{203} by Wapstra and Bergstrom after Pb^{202m} had been found.[3]

The same sort of M4 isomer was sought in Bi^{205}, where accurate gamma-ray energies had been reported by Pryce and Alburger earlier.[4] No short-lived isomer was seen, and limits could be set, since the Pb^{207m} resulting from a small amount of Bi^{207} could be measured. On this basis the transition, if it has a half life of 0.3 second or more, cannot occur in greater than 0.02% abundance.

The investigation of Bi^{204} has been continued by means of the permanent-magnet spectrographs, which have a resolution of 0.1%, and the results of the lines seen are as follows:

Lines seen in permanent-magnet spectrographs of Bi^{204} decay

Energy (kev)	Type of Results	Energy (kev)	Type of Results	Energy (kev)	Type of Results
78.63	1	374.9	1	725.5	2
97.68	1	421.9	2	759.1	2
140.82	1	438.8	2	765.2	2
142.55	3	454.0	2	792.1	2
152.45	1	468.1	3	847.5	3
168.60	1	501.7	1	889.3	1
176.25	1	522.2	1	911.8	1
216.21	1	532.6	3	918.5	1
219.69	2	548.5	2	1044.1	2
222.45	2	568.3	3	1056.7	2
273.9	2	590.7	2	1092.1	2
289.47	1	661.2	1	1139.8	2
321.13	3	670.4	1	1204.7	2
348.9	3	710.4	1	1211.5	2
				1226.0	2

Type of results:

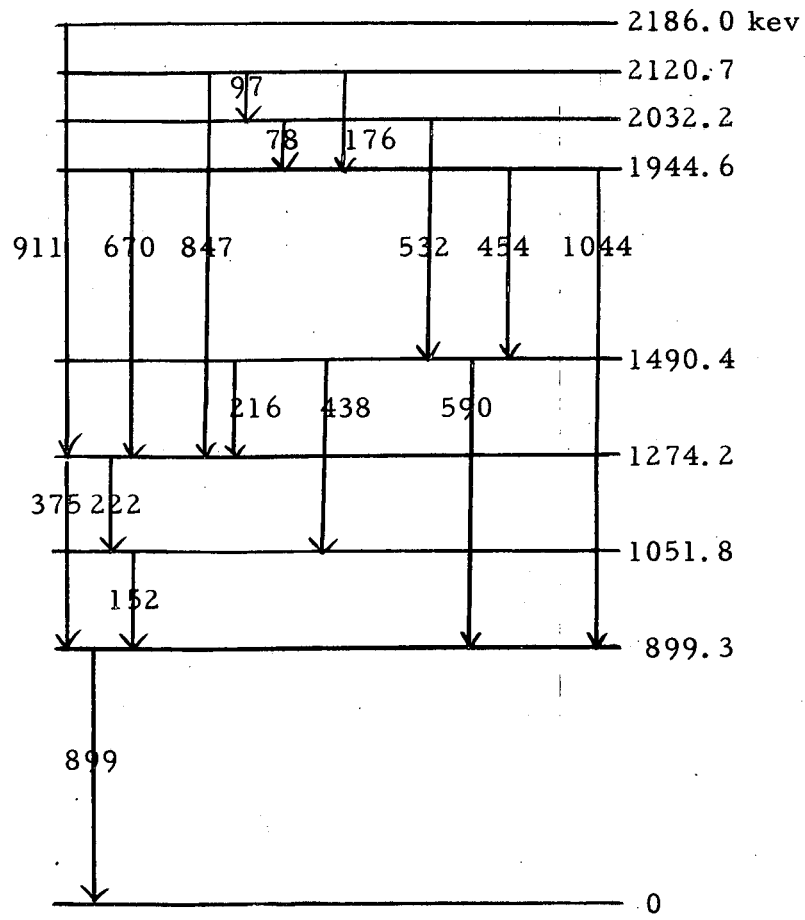
1 = three or more electron lines seen, intensities agree,
2 = two lines seen, intensities agree,
3 = one line seen, or intensities questionable.

Note: From 1044.1 on out only one line was seen in most cases; however, K/L ratios are such that L line is not expected to be seen here.

[1] J. R. Prescott, Proc. Phys. Soc. A 67, 254 (1954)
[2] J. N. Hopkins, Phys. Rev. 88, 680 (1952)
[3] Wapstra and Bergstrom, Phil. Mag. 46, 61 (1955)
[4] Pryce and Alburger, Phys. Rev. 95, 1482 (1954)

Earlier work on a high-transmission beta spectrograph has shown that there are numerous high-energy lines also, which to date have not been seen with the permanent-magnet spectrograph. A partial--as yet very tentative--decay scheme based on sums that yield self-consistent energy levels is:

68-min Pb^{204m}



Work is continuing on all three nuclides. At the present Bi^{203} and Bi^{205} are being studied with the permanent-magnet spectrographs, and electron-electron coincidences measurements are being planned for Bi^{205} and possibly for Bi^{204} and Bi^{203}.

DECAY OF RHENIUM-183

Sigvard Thulin, John O. Rasmussen, Warren G. Smith, and Jack M. Hollander

The electron-capture decay scheme of 120-day Re^{183} is being studied, principally by use of the high-resolution 180°-focusing permanent-magnet spectrographs. The activity was prepared by intensive alpha-particle irradiation of tantalum foils in a special probe in the internal beam of the 60-inch cyclotron. Essentially weightless deposits of the activity on platinum

wires were obtained by cathodic electrodeposition following initial chemical purification by distillation of volatide oxybromides of rhenium.

The conversion electron spectra we obtained are quite consistent with the level scheme for W^{183} proposed by Murray et al.[1] from study of Ta^{183} beta decay. However, we find a considerably different pattern in the direct population of the various excited levels. Ta^{183} beta decay mainly populates a 7/2-spin level at 453.08 kev, while Re^{183} electron capture mainly populates the 3/2-spin level at 208.81 kev, with a small population of the 5/2 level at 291.71 and a very small population of the 7/2 level at 453.08.

By means of L-subshell conversion ratios we have made or confirmed the multipolarity assignments of a large number of the gamma transitions.

Preliminary attempts to assign Bohr-Mottelson K-quantum numbers were made, particularly for the 208.81-kev level, which emits gamma to three members of the ground rotational band. It is not possible to explain the relative intensities of these gamma rays if K is assumed to be a good quantum number.

COINCIDENCE WORK ON RHENIUM-184

Donald Strominger, John O. Rasmussen, and Sigvard Thulin

A sample of mixed radioactive isotopes Re^{183} and Re^{184} was studied with the fast-coincidence counter. Wilkinson[a] had reported conversion lines corresponding to gamma energies of 159, 206, 244, 784, and 890 kev. Scintillation spectroscopy on the 50-channel pulse-height analyzer in this laboratory, using sodium iodide (TlI) crystals, showed gamma peaks at 790 and 890 kev. We saw gamma peaks at other energies but all of these could be attributed to the Re^{183} in the sample.

Coincidence work showed that the 790- and 890-kev gamma rays were not in coincidence. The 890-kev gamma ray is in coincidence with gamma transitions of 110 and 250 kev. The 790-kev gamma ray is in coincidence with gamma radiation of 110 kev. From the relative counting rates for the 790 to 110 kev coincidence and the 890 to 110 kev coincidence it can be shown that the 790-kev gamma must be in coincidence with at least two 110-kev gamma rays.

A delay curve was also run on the sample to look for metastable states. The 110-kev gamma ray was used for the gate and the 790- and 890-kev coincidences were observed on the 50-channel pulse-height analyzer. The results of this experiment showed that there is a delayed 110-kev gamma ray with a half life of about one millimicrosecond. Both the 890- and 790-kev gamma transitions decay to this metastable state either directly or via other levels.

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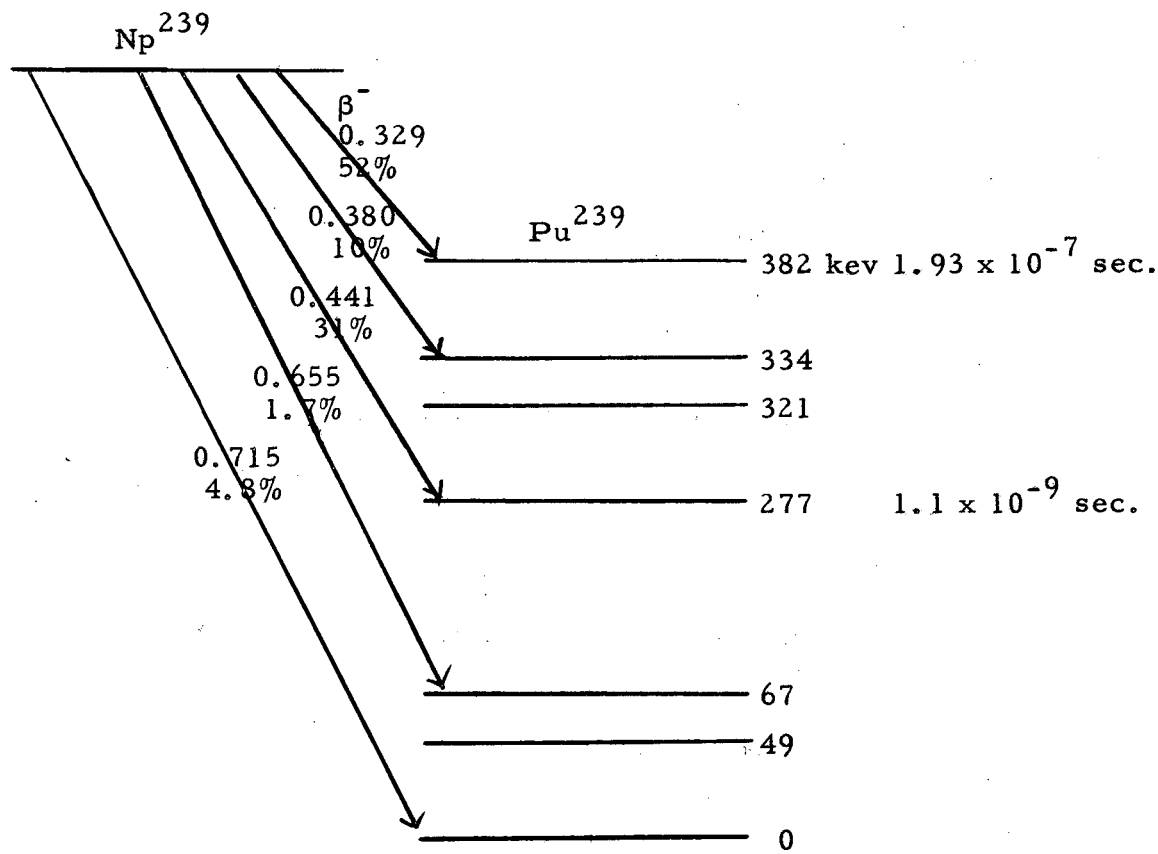
[1] Murray, Boehm, Marmier, and DuMond, Phys. Rev. 97, 1007 (1955)
[a] As reported in "Table of Isotopes," J. M. Hollander, I. Perlman and G. T. Seaborg, Revs. Modern Phys. 25, 469-651 (1953)

ENERGY LEVELS OF PLUTONIUM-239 POPULATED BY THE BETA DECAY OF NEPTUNIUM-239

John W. Mihelich*, Jack M. Hollander and Warren G. Smith

We have been studying the energy levels of Pu^{239} populated by the beta decay of Np^{239}. The beta decay of 2.3-day Np^{239} has been studied by several groups,[1,2,3,4] and the most recently proposed energy-level scheme[4] is shown below:



In addition, the alpha decay of Cm^{243} has been investigated by Asaro, Thompson, and Perlman.[5] The results of their experiments also define several of the Pu^{239} levels.

The Np^{239} samples were prepared by irradiation of approximately 1-gram amounts of U^{238} for eight hours in the Livermore reactor.

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*Now at Notre Dame University

[1] Graham and Bell, Phys. Rev. 83, 223 (1951) (A)

[2] Tomlinson, Fulbright, and Howland, Phys. Rev. 83, 223 (1951) (A)

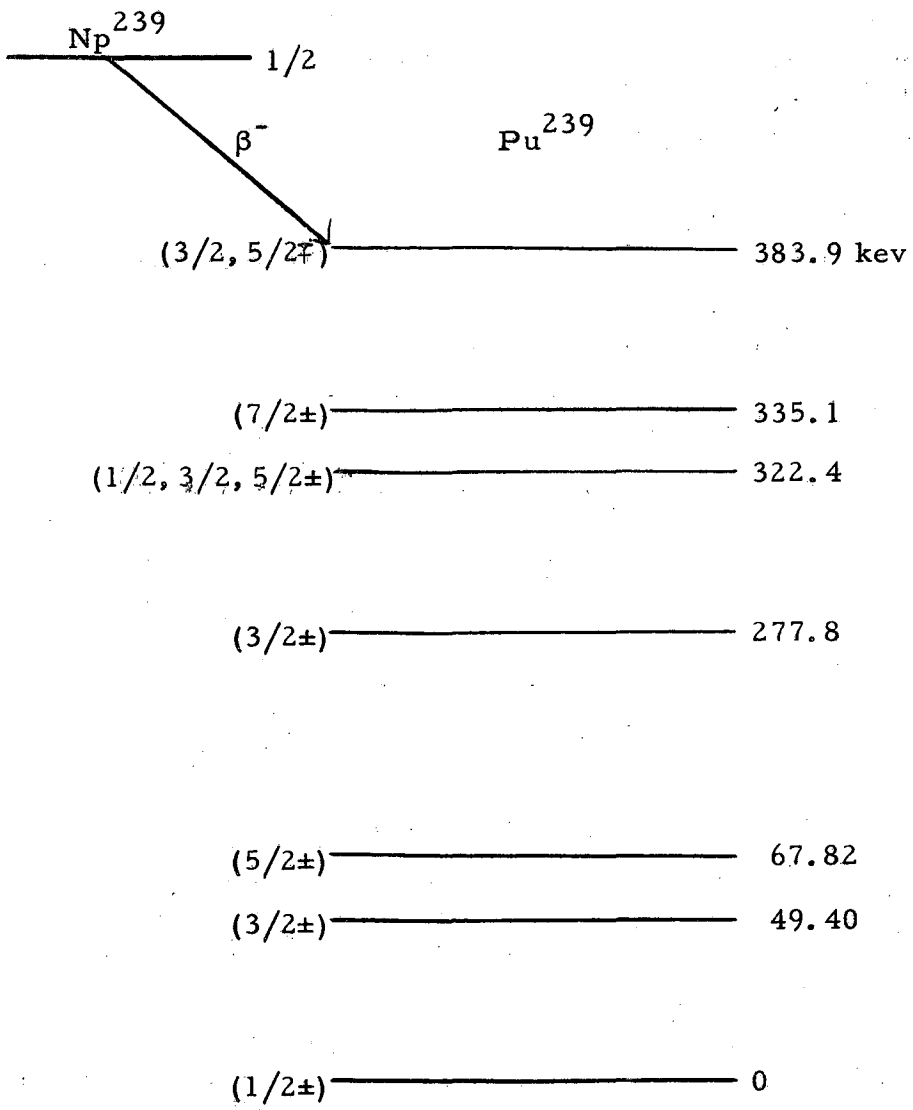
[3] Freedman, Wagner, Engelkemeir, Huizenga, and Magnusson, private communications (Nov. 1952)

[4] Engelkemeir and Magnusson, Phys. Rev. 99, 135 (1955)

[5] Asaro, Thompson, and Perlman, Phys. Rev. 92, 694 (1953) and unpublished data.

Three flat 180° permanent-magnet spectrographs, which record electron lines with energies up to ~800 kev, were used in the present study.

The most prominent transitions, with multipolarities where known, that were observed from the internal conversion electrons are as follows: 44.64 kev (M1+E2), 49.40 (M1+E2), 57.25 (E2), 61.4 (E1), 67.82 (E2), 106.1 (E1), 209.9 (M1), 228.4 (M1), and 277.8 (M1). On the basis of transition-energy sums and multipolarities our present level scheme was constructed (the spin of Pu^{239} was determined to be 1/2 by Bleaney, et al.[6])



This study is being continued at the present time.

[6] Bleaney, Llewellyn, Pryce, and Hall, Phil. Mag. 45, 773, 991 (1954)

STUDIES USING THE FAST-COINCIDENCE COUNTING APPARATUS

Donald Strominger and John O. Rasmussen

The fast-coincidence counting apparatus has been used to study the possibility that metastable states exist in several heavy nuclides. This apparatus has been described in previous quarterly reports.[1] In the work to be reported NaI (TlI activated) crystals were used in all cases to detect and analyze gamma transitions.

Uranium-230 Family

There are a number of gamma transitions in the U^{230} family from 70 kev to 330 kev that are in coincidence with alpha particles.[2] A stilbene crystal was used to detect alpha particles. Upper limits for the lifetimes of these gamma transitions have been set as shown in the following table:

Lifetimes of gamma transitions in U^{230} family

α Energy (kev)	Half Life (mμsec)	Parent	Multipolarity
330	<0.8	Ra^{222}	E2
230	<1.2	U^{230}	E1
240		Th^{236}	E1
190	<1.5	Th^{226}	E2
160	<1.5	U^{230}	E1
110	<1.4	Th^{226}	E2
70	<1.5	U^{230}	E2

The parent and multipolarity assignments were made by Stephens.[2] We are grateful to Frank Asaro for the preparation and loan of the sample.

Polonium-208 and Polonium-209

Following the electron-capture decays of Po^{208} and Po^{209} there are gamma transitions of energies, 290, 600, and 900 kev.[3] A sodium iodide (TlI) crystal was used to detect the K x-rays at 80 kev. All three

[1] J. O. Rasmussen, M. I. Kalkstein, and D. Strominger, Quarterly Reports (1953-1955)
[2] Frank S. Stephens, University of California Radiation Laboratory Report No. UCRL-2970 (1955)
[3] F. Asaro, unpublished data (1955)

gamma transitions were seen in coincidence with the K x-rays, and all three were shown to have a half life of less than one millimicrosecond.

We are grateful to Frank Asaro for the preparation and loan of this sample.

Americium-243

Following the alpha decay of Am^{243} there is a 75-kev E1 gamma transition.[2] Previously Kalkstein and Rasmussen[4] had set an upper limit of 2 millimicroseconds for the half life of this gamma transition. We have now set a new upper limit of 1.6 millimicroseconds for the half life of the level giving rise to this transition. A potassium iodide (TlI) crystal was used to analyze and detect alpha particles.

We are grateful to Bernard Harvey, Gregory Choppin, and others of the heavy-element research team for the preparation and loan of this sample.

ALPHA-PARTICLE SPECTROSCOPY

John P. Hummel, Frank Asaro, and Isadore Perlman

Astatine-209

We have measured the energy of the main alpha group of At^{209} as 5.642 Mev, using Po^{210} α_0 (5.299 Mev) and At^{210} α_0 (5.519 Mev) as standards. This is to be compared with the earlier ion chamber measurement of 5.65 Mev.[a] An upper limit of 2.5% was set on the abundance of any other alpha group of At^{209} in the regions 4.98→5.26, 5.37→5.42, 5.45→5.50, and 5.53→5.61 Mev.

The spectrograph samples were prepared by Dr. Richard Hoff and by Allan W. Stoner.

Thorium-230

A study of the alpha-complex structure of Ionium (Th^{230}) showed two main alpha groups separated by 66.6 kev. The resulting gamma energy after correction for the difference in recoil energy is 67.8 kev. The highest-energy group has an abundance of 75% and is probably the ground-state transition. The group populating the 67.8-kev level has an abundance of 25%. No lower-energy alpha groups were observed. Since the limit of detection for these groups was 0.2%, the low-intensity groups reported by Rosenblum[b] are not inconsistent with this work. The energy separation and abundances of the main groups are in good agreement with Rosenblum's work.

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[4] M.I. Kalkstein and J. O. Rasmussen, unpublished data (1952).
[a] Barton, Ghiorso, and Perlman, Phys. Rev. 82, 13 (1951).
[b] Rosenblum, Valadares, Blandes-Vial, and Bernas, Compt. rend. 238, 1496 (1954).

Protactinium-231

We have continued the investigation of the alpha decay of Pa^{231}, and we have now seen three alpha groups not observed in our previous studies. These groups are in relatively low abundance and populate levels 75, 126, and 358 kev above the ground state of Ac^{227}. The alpha spectrum of Pa^{231} is now known to have eleven alpha groups with the following energies (in Mev) and abundance:

5.046(11%),	5.018(22%),	5.001(25%),	4.973(1.5%),
4.938(23%),	4.922(3%),	4.838(1.5%),	4.724(10%),
4.694(1%),	4.665(1.5%), and 4.624(0.2%).		

The energy standard used here is the energy of the 4.938-Mev group (23%) as determined by Rosenblum.[c] The energies and abundances of most of the above groups are in good agreement with the results of Goldin, Tretyakov, and Novikova.[d] The groups at 4.922 and 4.624 Mev, however, were not reported by the Russian workers.

Plutonium-242

A mixture of plutonium isotopes has been examined with an alpha-particle spectrograph. Knowing the isotopic abundance and relative alpha activities of Pu^{242} and Pu^{240}, we calculate the half life of Pu^{242} to be 3.6×10^5 years. This is in good agreement with a recently reported value of 3.76×10^5 years.[e]

THE REACTION PRODUCTS OF TANTALUM WITH 5.7-Bev PROTONS

James R. Grover and Glenn T. Seaborg

The yields for several nuclides that appear as products in the reaction of tantalum with 5.7-Bev protons have been measured. The following table lists the results to date. All these cross sections are based on a cross section of 8.3 millibarns[1] for the reaction Al^{27} (p, 3pn) Na^{24} in an aluminum monitor foil, which was included in each bombardment.

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[c] Rosenblum, Cotton, and Bouissieres, Compt. rend. 229, 825 (1949).
[d] L. I. Goldin, E. F. Tretyakov, and G. I. Novikova, Series from the Academy of Science, USSR, for the Peaceful Uses of Atomic Energy (July 1-5, 1955)--Conference: Subdivision on Physical-Mathematical Sciences, p. 226-250. (In Russian).
[e] Mech, Stevens, Diamond, Studier, Fields, and Hüizenga, Argonne National Laboratory Report No. ANL-5348 (1954).
[1] Rex H. Shudde, private communication.

Cross sections for the formation of nuclides in the bombardment of tantalum with ~5.7-Bev protons

Nuclide	Cross sections in millibarns	Energy (Bev)	Type of yield
Ce^{141}	~ 1	5.7	C
Ce^{134}	10	5.8	C
Ce^{133}	11[a]	5.8	C
Ba^{129}	12[c a]	5.8	C
Ba^{128}	5.8[c]	5.8	C
Te^{127}	~ 0.3	5.7	I
Te^{118}	5.6[b]	5.7	C
Te^{117}	4.6[b]	5.7	C
Sb^{122}	~ 0.01	5.7	I
Sb^{120}	0.3[a]	5.7	I
Sb^{119}	0.6	5.7	I
Sb^{118}	0.8[a]	5.7	I
Sb^{117}	3.4[a]	5.7	C
Cd^{115}	~ 0.02	5.7	C
Cd^{107}	2.8	5.7	C
Ag^{103}	4.7	6.1	C
Pd^{101}	4.2	5.7	C
Mo^{99}	0.10	5.7	C
Cu^{67}	0.31[c]	5.7	C
Cu^{64}	1.8[c]	5.7	I
Cu^{61}	0.56	5.7	C
Ni^{66}	0.014	5.7	C
Ni^{65}	0.058	5.7	C

[a]These yields were based on the total K x-ray count, corrected for the fluorescence yield, but not corrected for the x-ray contributions from γ rays in the decay schemes of the nuclides involved.

[b]These nuclides were assumed to have a counting efficiency of 100% for positrons.

[c]These values are the averages of two determinations; all the other reported yields are based on one determination.

[C]This designates cross sections that are--or most probably are--cumulative chain yields.

[I]This designates independent yields.

In addition, several other elements have been taken out, and repeat determinations have been made for cadmium, silver, palladium, and molybdenum.

In the course of the work, branching ratios were measured in the decay of some nuclides.

Branching Ratios (K vacancies to positrons)

Nuclide	K vacancies : positrons
Ba^{129}	67 : 33
Cs^{128}	~18 : 82
Ce^{133}	~81 : 19
La^{133}	~89 : 11

Lindner[2] has reported a ratio of electron capture to positron emission of 29 : 71 for Cs^{128}.

FISSION-SPALLATION COMPETITION IN HEAVY ELEMENTS

Cross Sections for Deuteron-Induced Reactions of Plutonium-239

Walter M. Gibson, Richard A. Glass, and Glenn T. Seaborg

Descriptions of the fission-spallation studies, including preliminary cross-section values, have been given in the two preceding quarterly reports. The present report contains extended spallation-excitation functions for reactions of three heavy-element target isotopes under investigation: Pu^{239} (deuterons), Pu^{240} (deuterons), and U^{233} (alpha particles).

Spallation-excitation functions for deuteron-induced reactions of Pu^{239} are presented in Fig. 1. They are based on the tentative electron-capture counting efficiencies of 91% for Am^{240} (d, n), 17% for Am^{239} (d, 2n), and 17% for Am^{238} (d, 3n).

Cross Sections for Deuteron-Induced Reactions of Plutonium-240

E. Victor Luoma, Richard A. Glass, and Glenn T. Seaborg

Spallation-excitation functions for deuteron-induced reactions of Pu^{240} are presented in Fig. 2. They are based on the tentative electron-capture counting efficiencies of 91% for Am^{240} (d, 2n), and 17% for Am^{239} (d, 3n). Corrections have been made for the Pu^{239} content of the target.

[2] Private communication to W. E. Nervik (cited in UCRL-2542, 1954).

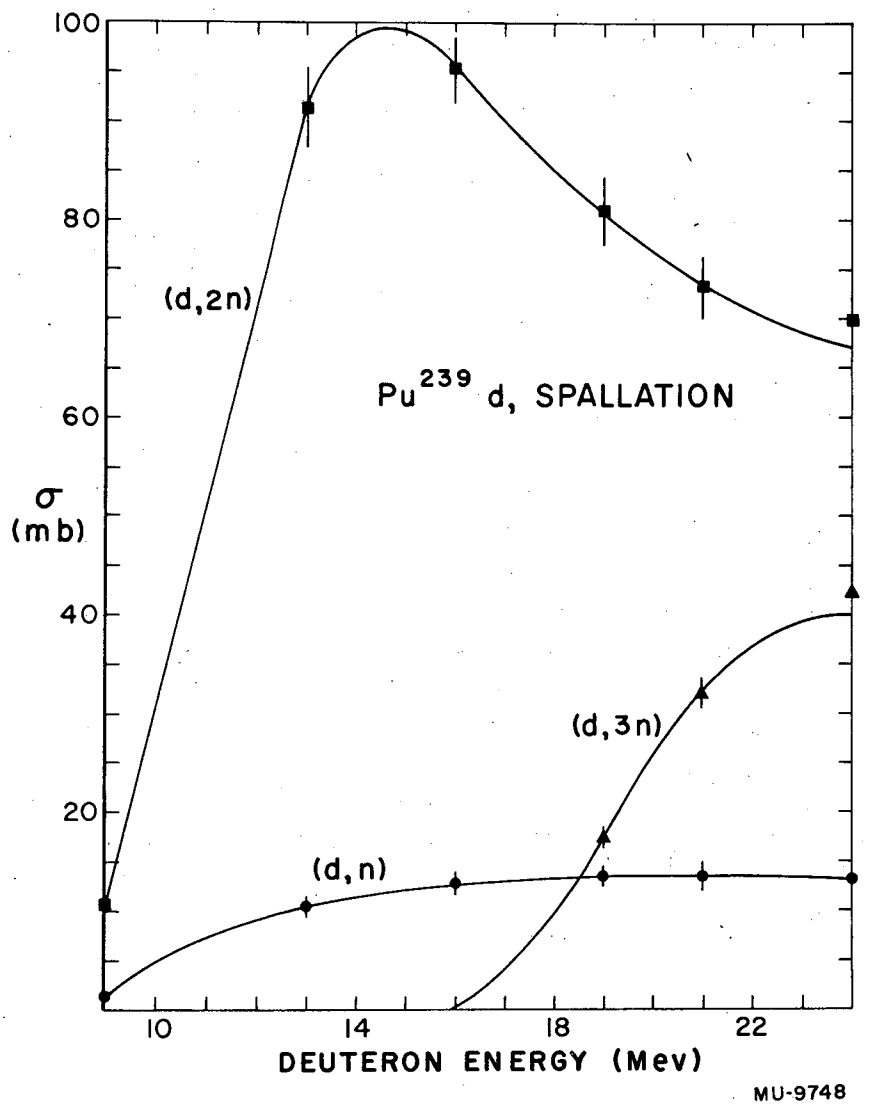
100
80
60
40
20
σ
(mb)
(d,2n)
Pu239 d, SPALLATION
(d,3n)
(d,n)
10
14
18
22
DEUTERON ENERGY (Mev)
MU-9748

Fig. 1.

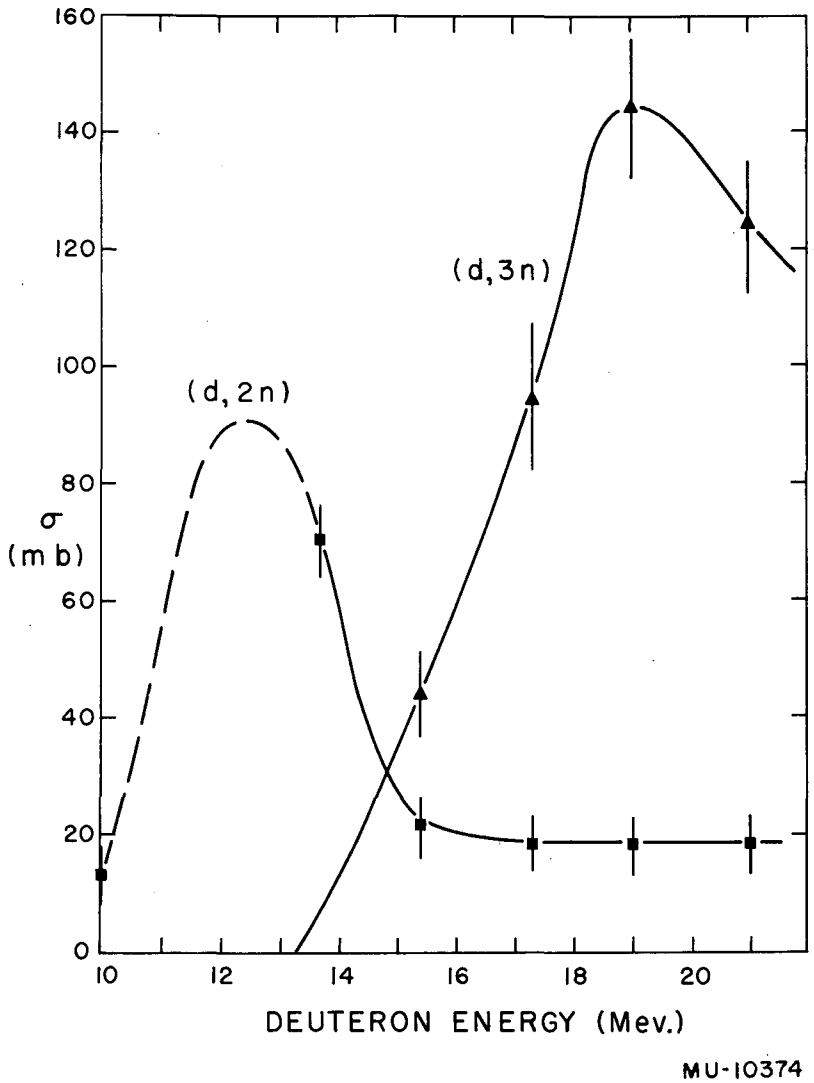

Fig. 2. $Pu^{240}d$, Spallation

Cross Sections for Alpha-Particle-Induced Reactions of Uranium-233

T. Darrah Thomas, Richard A. Glass, and Glenn T. Seaborg

Spallation-excitation functions for alpha-particle-induced reactions of U^{233} are presented in Fig. 3. They are based on assumed electron-capture counting efficiencies, to be investigated at a later time, of 50% for all isotopes.

SPALLATION OF COPPER AT 5.7 Bev

Donald W. Barr and Earl K. Hyde

Since the initiation of this program in January 1955 the formation cross sections of 25 radioactive nuclides formed in the spallation-fission of natural copper (69% Cu^{63}, 31% Cu^{65}) have been measured at maximum energy of the Bevatron. These nuclides have mass numbers ranging from 7 (Be) to 67 (Ga), with the majority, however, in the immediate region of the target nuclei. Some of the figures obtained are the averages of as many as four separate determinations, with the agreement quite good in view of the inherent difficulties in accurate reproduction of results in a study of this sort. The nuclides are characterized by half-life determinations and various properties of their decay schemes in Geiger-Muller and proportional counters along with occasional gamma pulse-height analysis where necessary to differentiate isotopes of similar half life.

The results to date do not seem to differ significantly from those of a similar study conducted at 2.2 Bev in the Cosmotron at Brookhaven National Laboratory last year, except for a definite discrepancy for the copper isotopes themselves and the hint of an increased yield for secondary reactions (Ga, Ge, As). A more thorough investigation of the latter two exceptions may be of more significance in strengthening the high-energy nuclear picture than a rigid survey of the lower-mass-number nuclides formed, and is planned for the future.

THE STRUCTURE OF $BaTi_4O_9$

Carol H. Dauben and David H. Templeton

Weissenberg diagrams obtained from single crystals of this material have shown $BaTi_4O_9$ to be orthorhombic, with the probable space group Pmmn and

$$a = 14.56 \text{ Å}$$
$$b = 3.80 \text{ Å}$$
$$c = 6.33 \text{ Å}.$$

With two molecules per unit cell, $\rho x = 4.5$ and $\rho m = 4.6$. Work is in progress on determining the atomic positions.

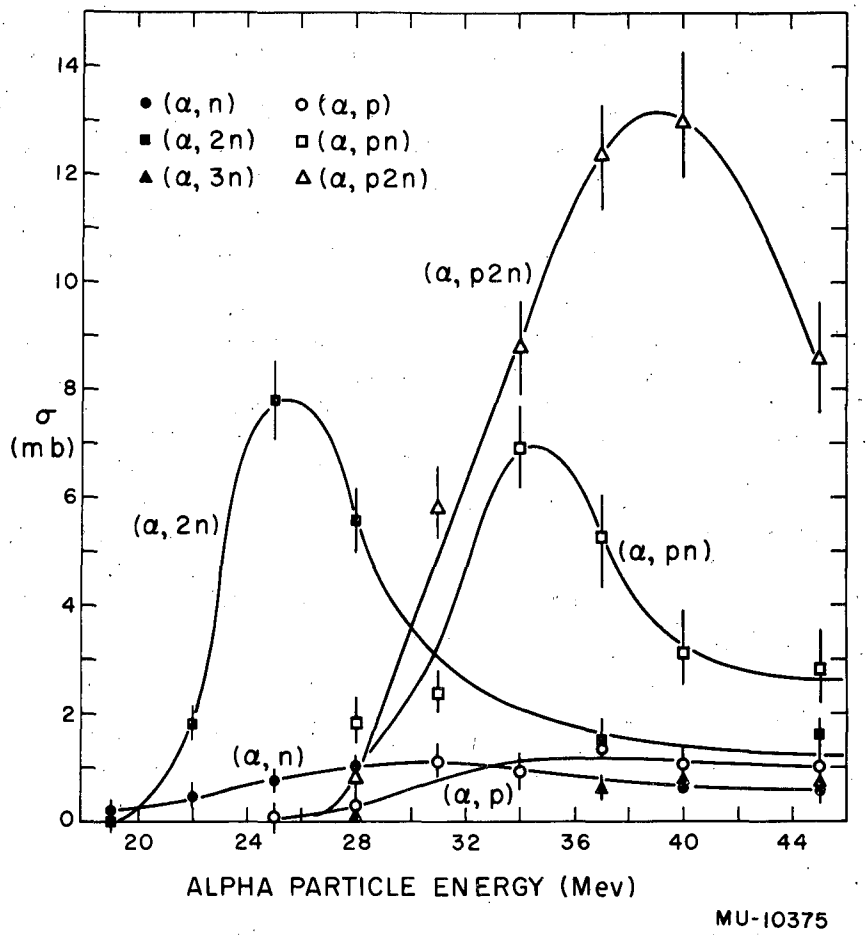

Fig. 3. U^{233} α, Spallation

MODIFICATION AND TESTING OF DOUBLE-FOCUSING BETA-RAY SPECTROMETER

Sigvard Thulin, Frederick L. Canavan, and John O. Rasmussen

The double-focusing spectrometer previously described[1] and modified[2] has been further modified in the course of its assembly in the new Nuclear Chemistry Building.

The modifications included the installation of a set of externally controlled vertical shutters to define the portion of the emitted electrons accepted for focusing, the installation of an externally controlled slit in front of the detector, and the installation of a new flux-measuring probe and associated magnetometer circuit. In the course of installing the instrument in its new location, the magnet current supply was redesigned and now seems stable to about one part in a thousand. A new-type side-window Geiger counter was designed for use as the detector, and the counter-filling arrangements were improved.

Systematic tests are being conducted on the effect of shutter openings, slit width, and source width on the transmission and resolution of the instrument. The highest resolution so far achieved is 0.15% with a source 11 by 0.5 mm and a 0.5-mm detector slit opening. At this resolution the transmission is of the order of 0.10%.

Of interest is the failure of the instrument to behave in accordance with the design predictions. The magnetic field was designed to give independence of resolution on the horizontal acceptance angle. It has been found that the resolution is strongly dependent on the horizontal shutter opening and practically independent of the vertical shutter opening.

EMPIRICAL FORMULA FOR CALCULATING YIELDS IN RADIOACTIVE DECAY PROCESSES

Herman P. Robinson

A useful formula is proposed for calculating the growth of successive members in a chain of nuclear transformations. Let the chain be represented by

$$A \xrightarrow{\lambda_1} B \xrightarrow{\lambda_2} \text{----} N \xrightarrow{\lambda_n} ,$$

where λ_i is the disintegration constant for the ith member of the chain. Then over a certain range of time the growth of N is given by

[1] G. D. O'Kelley, The Spectrometric Determination of Some Beta Particle and Conversion Electron Energies (thesis), UCRL-1243, June 1951.
[2] T. O. Passell, Internal Conversion of Gamma Radiation in the L Subshells (thesis), UCRL-2528, March 1954.

$$N = \frac{B(n)}{\lambda_n t}\left(1-e^{-\frac{2\lambda_1 t}{n}}\right)\left(1-e^{-\frac{2\lambda_2 t}{n}}\right)\text{----}\left(1-e^{-\frac{2\lambda_n t}{n}}\right),$$

where $B(n) = \frac{n^n}{2^n(n-1)!}$.

This formula can be evaluated with a slide rule and becomes exact as t approaches zero. It begins to fail when more than about two-thirds or three-fourths of the terms $(1-e^{-2\lambda_i t/n})$ have values approaching 1. It may fail completely when N has reached its maximum, giving results much too high. A great advantage of this formula is that each λ except the last appears only once, so that the equation can be solved explicitly for any λ except λ_n. This facilitates determination of an unknown λ when the other λ's and the yield are known.

The formula is particularly useful in calculating yields of elements produced by pile bombardments. For example, in the irradiation (at a neutron flux of 1.5×10^{14} per second) of Pu^{239} to give Cf^{250}, in which there are twelve members in the chain, the yield of Cf^{250} calculated by the formula is less than 2% high after 25 years, and is high by a factor of only about 2.5 at 50 years. On the basis of the cross sections used, Cf^{250} reaches a maximum in 60 years, at which point the approximate equation gives a result that is too high by a factor of 3.

THEORETICAL CALCULATIONS OF NUCLEAR PROPERTIES BY USE OF SPHEROIDAL WAVE FUNCTIONS

John O. Rasmussen and Donald Strominger

S. G. Nilsson[1] has computed nucleon eigenvalues and wave functions for an anisotropic harmonic oscillator potential including a spin-orbit interaction. These wave functions make possible further application and testing of the Bohr-Mottelson model for nuclei in the regions of large deformation and rotational band spectra.

We have been carrying out three types of calculations with these wave functions: (a) magnetic moments, (b) electric dipole transition probabilities, and (c) K mixing of wave functions by inclusion of Bohr's[2] off-diagonal matrix elements designated U_1.

The preliminary results indicate that magnetic-moment calculations may be of much value in the assignment of intrinsic nuclear states, but that exact agreement is not generally to be expected. One suspected source of magnetic-moment deviation from these calculations is K mixing of neighboring intrinsic states. We would assign the ground state of Np^{237} even parity and the 60-kev excited state odd. The ground state (I = 5/2) we believe to have mainly K = 1/2 character but with appreciable K mixing (see below).

[1] S. Nilsson, Dan. Mat. Fys. Medd. (in press).
[2] A. Bohr, Dan. Mat. Fys. Medd. 26, No. 14, p. 29 (1952).

For various possible pairs of intrinsic states in the 82-to-126 proton shell we have calculated E1 transition probabilities. In the limit of small spheroidal deformation these transition probabilities, of course, vanish by j-forbiddenness. Interestingly enough, the present calculations show that transition probabilities never become greater than about 0.001 of the Weisskopf lifetime value even for the largest deformations. The generally slow E1 transitions (Np^{237}, Pa^{231}, Pa^{233}, Ac^{227}) may thus have a rather simple explanation. Since the lifetimes depend rather sensitively on the near cancellation of large terms in the matrix element, the rather erratic fluctuations in E1 transition rates from isotope to isotope are qualitatively understandable.

Usually neglected in Bohr-Mottelson states are the effects of off-diagonal matrix elements U_1, which are a measure of the failure of an intrinsic nucleonic wave function in the spheroidal well to follow adiabatically the rotation of that well. These matrix elements become larger, with greater speed of rotation of the well. The matrix elements connect states of like spin and parity differing in K and Ω by one. We have used Nilsson's wave functions to calculate at largest deformation ($n = \partial$) the effects of including U_1 for the even-parity states of the 82-to-126 shell. The rotational band patterns are somewhat perturbed and the K mixing is appreciable. For a single nucleon occupying an even-parity state in the Np^{237} nucleus a spin 5/2 level lies lowest. Inclusion of U_1 depresses the level by about 30 kev; the resultant state has 95% K = 1/2 character, 5% K = 3/2, and 0.03% K = 5/2. Such amounts of K admixtures may well have an important influence on magnetic-moment and transition-probability calculations.

EXPRESSIONS FOR CALCULATING THE ENERGIES OF IONIC CRYSTALS ACCORDING TO THE METHOD OF BERTAUT

Robert Jones and David H. Templeton

The expressions for calculating the energies of ionic crystals have been studied. In particular the method of Bertaut for calculating the energies of charge and dipole arrays has been considered.

The equation for charge-charge interaction energy involves evaluation of an infinite series. The error due to using only a finite number of terms has been estimated for a number of possible forms of the Bertaut equation.

For calculation of energy terms due to the dipoles and charges, programs of calculation using the IBM 650 have been written. The first class of crystals to be considered will be those having the TlI structure. It will be possible to itemize the energies of the charge-charge, charge-dipole, and dipole-dipole terms. By varying the structural parameters of these crystals it should be possible to determine the theoretically most stable configuration and compare it to the observed structure. It is hoped that calculations of this sort will show the reason for the stability of this particular structure.

To calculate the dipoles in InI (one of the crystals having the TlI structure) a value of the polarizability of InI must be determined. If possible, this will be determined from the indices of refraction of crystalline InI.

COLOR CENTERS AND PHOSPHORESCENCE IN EUROPIUM CHLORIDE AND LANTHANUM CHLORIDE

John G. Conway, Ralph D. McLaughlin, and Dieter M. Gruen

A blue color is produced in single crystals of lanthanum chloride containing 1% or 10% of europium dichloride. The blue color is due to three color centers which have absorption peaks at 525, 650, and 790 mμ. The color centers may be produced by ultraviolet light or gamma rays. The material also phosphoresces for several minutes following an irradiation with ultraviolet light. A 1% mix of $EuCl_3$ does not show color center or phosphorescence. A 1% mix of $EuCl_2$ does have color center and phosphorescence. Additional work is being done on the pure dichloride.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

NOTES ON WORK IN PROGRESS

Vacuum Flow through Circular, Annular, and Rectangular Sections

Walter Dong and Donald N. Hanson

In the experimental work on flow through the rectangular section, additional screens were inserted at the inlet of the test section to better distribute the inflow of the gas, and modifications were made on the pressure taps to give more representative pressure readings. No noticeable differences were observed between the data taken after modifying the equipment and the previous data.

The rough draft of the thesis on this work is now being written.

Stability of Perforated-Plate Trays

Robert S. Brown, Donald N. Hanson, and Charles R. Wilke

Construction of the equipment for this study is nearing completion. Preliminary operating tests in the gas-circulation system have indicated satisfactory performance.

Gas-Liquid Partition Chromatography

Robert H. Houston and Charles R. Wilke

Theories of gas-liquid partition chromatography have been reviewed. Experimental equipment is being designed for testing of various theoretical developments. The general objective is the development of engineering principles for design of equipment for such separations.

Liquid-Liquid Extraction and Agitation

Robert H. Houston and Theodore Vermeulen

Studies of the impeller geometry and speed needed to produce bulk homogeneity of mixing have been continued. Measurements on water in mineral oil and on mineral oil in water confirm the previously developed correlation. Experiments are now being carried out to ascertain the physical factors that determine which phase of a two-phase mixture will be dispersed. Equipment is being designed for experimental study of coalescence rates as a separate factor in controlling the mean drop diameter in unstable emulsions.

Previous work on pulse-column extraction of uranyl nitrate–nitric acid mixtures has been reviewed. A detailed method of calculating

transfer units for multicomponent systems in countercurrent towers has been developed. For use in this method, activity plots have been prepared for the system uranyl nitrate-nitric acid-pentaether-water. Preliminary results are given in UCRL-2983.

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

Eugene J. Fenech and Charles W. Tobias

The change in concentration of the electrolyte during a run was found to be an important factor. In order to reduce the concentration change during a run, duration of electrolysis has been cut from 12 to about 3 minutes and freshly analyzed solution is used for each run.

Because of the small changes in the limiting current with change in the electrode width and the change of the distance between diaphragm and cathode, further experiments will be carried out with smaller electrodes and larger cathode-to-diaphragm distances.

The measurement of limiting current densities is being continued over a wide range of pertinent geometrical and physical variables. The possibility of theoretical interpretation and correlation is also being investigated.

The Effect of Fluid Properties on the Mass Transfer Rate in the Gas Phase

Robert J. Fallat and Charles R. Wilke

High-pressure equipment for the study of HTU_G correlations has been built and tested. Operation of the equipment is about to begin. It is hoped that the data collected will clarify the use of $\sqrt{\rho u^2}$ as a correlating parameter for HTU_G in the flow of gases through packed towers.

Thermal Conductivity of Gases at High Temperatures

Henry Cheung and Charles R. Wilke

The piping and pumping systems for the thermal-conductivity cell have been completely redesigned and reconstructed. All thermocouples have recalibrated with NBS pure metals. The equipment is nearly ready for operation.

Diffusion in Liquids

Elwood Gift and Charles R. Wilke

An apparatus employing unsteady-state diffusion in a porous glass diaphragm has been constructed along the lines employed by Wall,

Grieger, and Childers (J. Am. Chem. Soc. 74, 3562 (1952)). Preliminary experiments indicate that the apparatus will give satisfactory results. A program of measurements will be conducted to serve as a basis for improvement of existing correlations for diffusion coefficients in liquids.

Gas-Phase Mass Transfer Studies

Loren J. Hov and Charles R. Wilke

This work has been completed and a report is in the process of being written.

GENERAL CHEMISTRY

METALS AND HIGH-TEMPERATURE THERMODYNAMICS

Leo Brewer, William Hicks, Neilen Hultgren

Determination of Absolute f Values

The phase-shift circuit for these measurements is under construction.

Study of C_2

Measurements of the temperature coefficients of the Phillips and Swan bands of C_2 are continuing in an effort to fix accurately the heats of formation of the singlet and triplet states.

Molybdenum Gaseous Oxyhalides

Work on this system has been completed with the demonstration that MoO_2Cl_2 is a chemically important gaseous molecule and that it has been the interfering molecule in the attempt to study $MoCl_4$. A UCRL report will be written shortly summarizing this work.

Oxide Reaction Studies

The study of the reaction of oxygen with urania-thoria solid solutions was completed by Dr. Roberts and will be written up by him.

Ternary Boride-Carbon Systems

This work has been suspended until John Engelke completes his service with the Army.

Vaporization and Condensation Coefficients of Red Phosphorus

This problem has been completed and will be written up for publication shortly.

Ge-GeO_2 and Si-SiO_2 Phase Diagrams

The Ge-GeO_2 work has been completed and the results have been written up for a UCRL report. (UCRL-3116) The Si-SiO_2 work is being suspended temporarily.

BASIC CHEMISTRY, INCLUDING METAL CHELATES

Howard Cady, Robert E. Connick, Claude Coppel, Ladd Griffith, William Hicks, Howard Mel, Robert Wood, and Earl Worden

Radiation-Induced Reactions in Nonaqueous Solutions

The study of the radiation chemistry and photochemistry of diphenylpicrylhydrazyl (DPPH) in nonaqueous solvents is being continued. Some results have been obtained from the photolysis and radiolysis of DPPH in chloroform.

Values of the extinction coefficient of DPPH at 5300 Å are as follows:

from the literature, $\epsilon = 14,500$,[1]
$\epsilon = 6,500$,[2]
$\epsilon = 11,500$;[3]
from this research, $\epsilon = 13,100$.[4]

It was found that DPPH, prepared by the method of Bennington,[1] formed a very stable 1:1 compound with benzene. This 1:1 benzene-DPPH compound remained stable when subjected to pressures reduced to 10^{-3} mm of Hg and temperatures increased to 56°C for several hours.

Likewise it was found that DPPH, prepared by the method of Goldschmidt,[2] retained a significant amount of solvated chloroform, although not in a 1:1 mole ratio.

The extinction coefficients for the 1:1 benzene-DPPH compound in chloroform and DPPH with a known amount of solvated chloroform have been shown to have the same value.

The source used in the photolysis of DPPH in chloroform is a Hanovia medium-pressure mercury arc, Type 16,200. Light of wave lengths 2537 A and 2650 A was isolated by the use of two cm of saturated nickel sulfate solution, 1 cm of Cation X (0.2 g per liter), and a Corning 9863 filter 2 mm thick.

The intensity of the radiation reaching the reaction cell was about 1.4×10^6 quanta per second per liter. The quantum yield for the disappearance of DPPH under these conditions and in the presence of air has been shown to be about 0.12. The disappearance of the DPPH is linear with time within experimental error.

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[1] F. Benington, E. Kahler, and R. Poirier, J. Org. Chem. 17, 1437 (1952).
[2] S. Goldschmidt, and K. Renn, Ber. deut. chem. Ges. 55, 628 (1922).
[3] Prevost-Bernes et al., Discussions of Faraday Soc., p. 98 (1952).
[4] This research.

A two-compartment cell arrangement was used to determine the amount of light absorbed by the DPPH in $ChCl_3$. The potassium ferrioxalate actinometer described by Parker[5] was found suitable for the direct measurement of the light intensities.

No postirradiation reactions have been observed in the photolysis of dilute solutions of DPPH in chloroform, i.e., 2×10^{-5} to 10×10^{-5} mole per 1000 cc. Postirradiation reactions are observed in the x-ray irradiation of solutions of DPPH in chloroform in this concentration range.

The results just presented are only preliminary ones, and the complete study of this photolysis is now in progress.

Silicic and chromatographic columns are being employed to effect the separation of the various products formed both in the photolysis and in the radiolysis of DPPH in chloroform. Four products have been separated from the solutions irradiated by 45-kev x-rays, and an attempt to identify them by their respective visible, ultraviolet, and infrared spectra is in progress.

Preparation of Acetaldehyde-t_1

A small amount of acetaldehyde-t_1 has been prepared by the following reactions:

$$H^*_2O + Ac_2O = 2H^*Ac,$$

$$4H^*Ac + 4Na + R\text{-}O\text{-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-}CH_3 = ROH^* + 4NaAc + CH_3CH^*_2OH^*,$$

$$3CH_3CH^*_2OH^* + Cr_2O_7^{=} + 8H^+ = 3CH_3CH^*O + 2Cr^{+3} + 7H_2O.$$

H^* represents a hydrogen or tritium atom, since very dilute T_2O was used in the initial reaction. R represents the n-butyl group. These reactions were used in order to prepare the compound with the tritium attached to the aldehyde carbon alone. An apparatus is now being constructed in order to study the isotope effect on the photolysis and pyrolysis of acetaldehyde-t_1.

Ruthenium Chemistry

Excess Ti^{+3} will react with RuO_4 in trifluoroacetic acid to form Ru^{+2}. Titrations of Ti^{+3} with RuO_4 are being studied optically and potentiometrically.

Solutions of Ru^{+3} are being studied by means of ion-exchange columns. A difficulty has been encountered which complicates the work. The

[5] Parker, C. A., Proc. of the Royal Society, A, 220, 104 (1953).

Dowex-50 ion-exchange resin appears to be slightly soluble in trifluoroacetic acid. As yet no good way has been found to study the optical properties of Ru^{+3} in these solutions.

The Entropy of the Selenide Ion in Aqueous Solutions

The determination of the second acid constant of H_2Se is progressing although hampered by the irreproduceability of glass electrodes in highly alkaline solutions. The calorimetric determination of the heat of formation of potassium ferrate is still in the preliminary stage.

Thermodynamics of Open Systems

Work has continued on various aspects of thermodynamics of open systems, with particular consideration for systems far removed from equilibrium. For a particular chemical reaction with autocatalytic kinetics it was shown that the function entropy production per unit time does not even retain its qualitative aspects when very far from the steady state (i.e., it is a "force function" for evolution of near-equilibrium systems). This is even true for an equilibrium steady state. For this same case, another function called the LaGrangian acts as a well-behaved thermodynamic potential.

A paper entitled "The Thermodynamics of Thiosulfate Ion," by Howard Mel, Wendell M. Latimer and Z. Zimmerman Hugus, has been submitted for publication in the J. Am. Chem Soc. (see UCRL-2330).

The Kinetics of Rapid Reactions

The work being done presently is a continuation of the work done here recently by John F. Below.

Recent work has consisted of some kinetic experiments using the mixing device designed and used by Below. These experiments have a twofold purpose; first, to add additional data to the rate law (Ferric Thiocyanate Reaction) studied by Below; and, secondly, to give experience in using the above-mentioned mixer. No actual data were obtained from these experiments; however, several changes in experimental technique were developed.

Also, a great deal of work has been done on calculations of several problems closely related to the mixing techniques. From the diffusion laws the force required for complete mixing, as a function of mixing time and shape of mixer, was calculated. Another calculation made was that for the bubble-formation problem. In this work an attempt is being made to see if it may be possible to eliminate bubble formation in the rapid-mixing device without previous outgassing. This would remove several severe limitations on the reactions, and they can now be studied.

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