UCRL 3629

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

CHEMISTRY DIVISION QUARTERLY REPORT

September, October, November 1956

BERKELEY, CALIFORNIA

DISCLAIMER

UCRL-3629
Chemistry Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

CHEMISTRY DIVISION QUARTERLY REPORT

September, October, November 1956

January 2, 1957

Printed for the U. S. Atomic Energy Commission

CHEMISTRY DIVISION QUARTERLY REPORT

September, October, November 1956

Contents

* Preceding Quarterly Reports UCRL-3595, UCRL-3415

NUCLEAR CHEMISTRY

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

Notes on Work in Progress

GENERAL CHEMISTRY

Metals and High-Temperature Thermodynamics

CHEMISTRY DIVISION QUARTERLY REPORT

September, October, November 1956

Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

January 2, 1957

BIO-ORGANIC CHEMISTRY

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

BONDING AND ABSORPTION SPECTRA OF VANADYL CHELATES

Robert Feltham

The first report in the literature of the preparation of vanadyl bis-acetylacetonate was by Morgan and Moss.[1] They report the formation of the monohydrate as well. The most recent and complete studies were done by Jones.[2] He reports the following method of preparation to be satisfactory: dissolve the ligand in ethanol, add this to a solution of vanadyl sulfate in dilute sulfuric acid, and neutralize with 10% Na_2CO_3. Generally the chelate settles out as an oil which then solidifies.

Pfeiffer et al.[3] have made a thorough study of vanadyl complexes with aldimines. They also report the formation of vanadium bissalicylaldehyde-ethylenediimine dichloride.

The method of Jones was used for preparing all the following chelates: vanadyl bisacetylacetonate, vanadyl bistrifluoroacetylacetonate, vanadyl bishexafluoroacetylacetonate, vanadyl bisbenzoylacetonate, vanadyl bisdibenzoylmethanate, vanadyl bissalicylaldehyde, vanadyl bissalicylaldehyde-ethylenediiminate, and vanadium^{+4} bissalicylaldehydeethylenediimine diacetate. In all cases the yields were about 65%, except for the hexafluoro compound, which was 40% of theoretical yield. The vanadium bissalicylaldehydeethylene-diimine diacetate was prepared by dissolving the vanadyl bissalicylaldehyde-ethylenediimine in acetic anhydride.

The visible absorption spectra were obtained using a Cary Model 14 recording spectrophotometer. All the solvents were spectral grade except pyridine and dioxane, which were redistilled Baker's C.P. Analyzed Reagent Grade. Unless otherwise specified, the spectra are plotted in units of molar extinction and angstroms.

---

1 G. T. Morgan and H. W. Moss, J. Chem. Soc. 103, 78 (1914).

2 M. Jones, J. Am. Chem. Soc. 76, 5995 (1954).

3 P. Pfeiffer et al., J. Prakt. Chem. 259, 217 (1937).

Figures 1 through 4 give some typical absorption spectra of the vanadyl chelates. The first important characteristic of all these spectra is that there are three weak absorptions in the visible region whose molar extinctions range from 20 to 350. These transitions are identifiable as d-d transitions. This is in agreement with the assumed tetragonal configuration of the complexes. In tetragonal symmetry the ligand field splits the five degenerate d wave functions into four levels, to which these transitions are assigned. In changing the basicity of the solvent, one finds that the positions of these absorptions are varying in a regular manner. The absorption at approximately 4000 Å, called u_1, shifts to lower energies. The next absorption encountered on moving to the visible is u_2, and it moves to higher energies with increasing solvent basicity. The last absorption in the visible, u_3, moves to lower energies. It is apparent that pyridine and methanol do not follow this scheme exactly. Table I summarizes the spectra of all these compounds and includes that of the VO^{++} $(solvent)_5$, and V^{+4} salicylaldehyde-ethylenediimine diacetate.

Using these data we would like to identify the electronic states involved in these transitions in order to decide what bonding scheme will best describe the vanadium^{+4}-ligand system.

There are two extreme ways of describing this system. First, we can consider the bonds as purely electrostatic in nature. This is the view taken by Penny[4] and Van Vleck,[5] and more recently and specifically by Orgel[6] and Hartmann.[7] Secondly, we might consider the bonds to be best described in terms of "covalent bonds." We have chosen to use molecular orbitals for this latter type of description.

Let us consider for a moment what determines the geometrical arrangement of atoms in a metal chelate. First, since usually these ligands are all good electron donors, the ligands repel one another. Secondly, if covalent bonding is not important the d electrons, if present, orient themselves in such a manner as to minimize the electrostatic repulsions between the ligands and themselves. We assume here that the ligand electrostatic field is the most important energy effect in the formation of the V^{+4}-ligand bond.

---

4 W. G. Penny and R. Schlapp, Phys. Rev. 42, 666 (1932).

5 J. H. Van Vleck, J. Chem. Phys. 7, 61 (1939).

6 L. E. Orgel, J. Chem. Soc. 1954, 332.

7 H. Hartmann, Theorie Der Chemischen Bindung (Springer-Verlag, Berlin, 1954), p. 221.

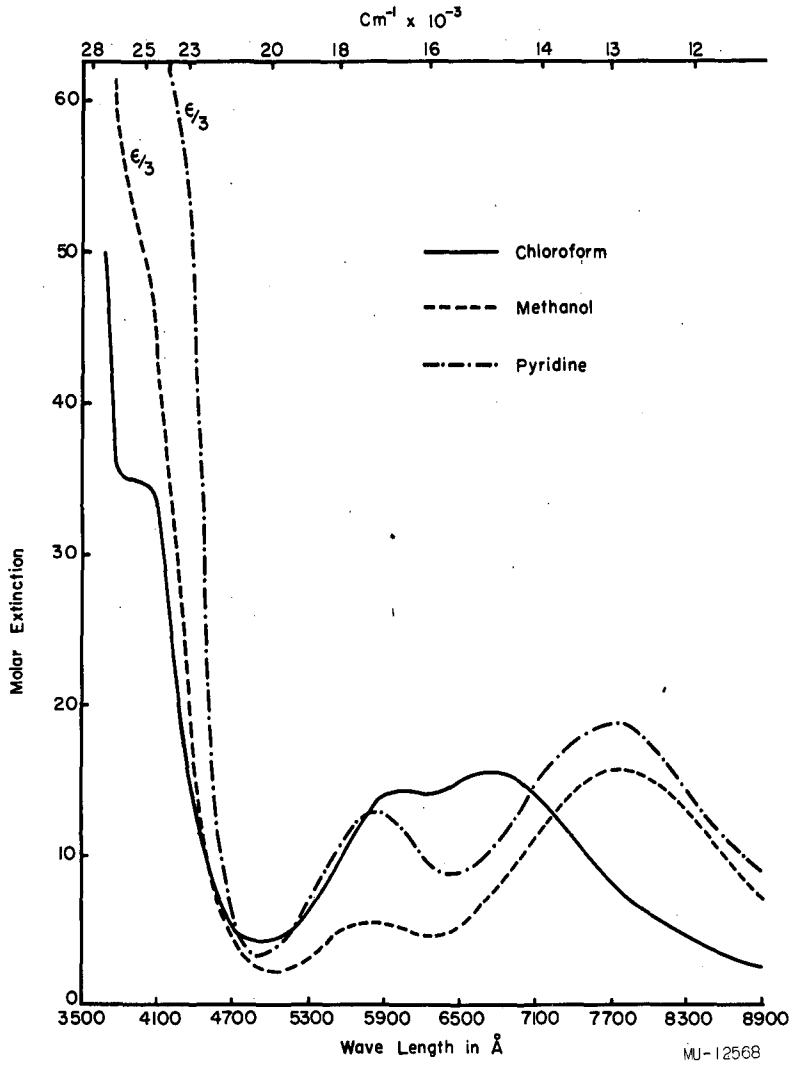


Fig. 1. Effect of solvent on vanadyl bisacetylacetonate.

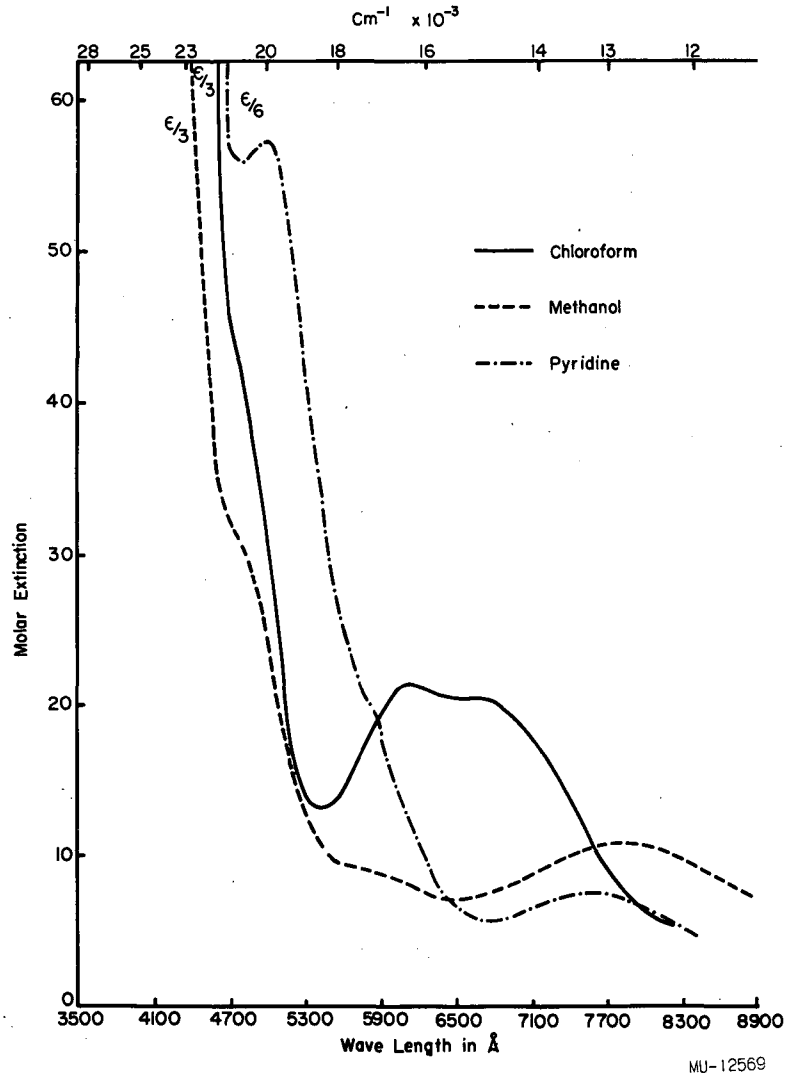


Fig. 2. Effect of solvent on vanadyl bisdibenzoylmethanate.

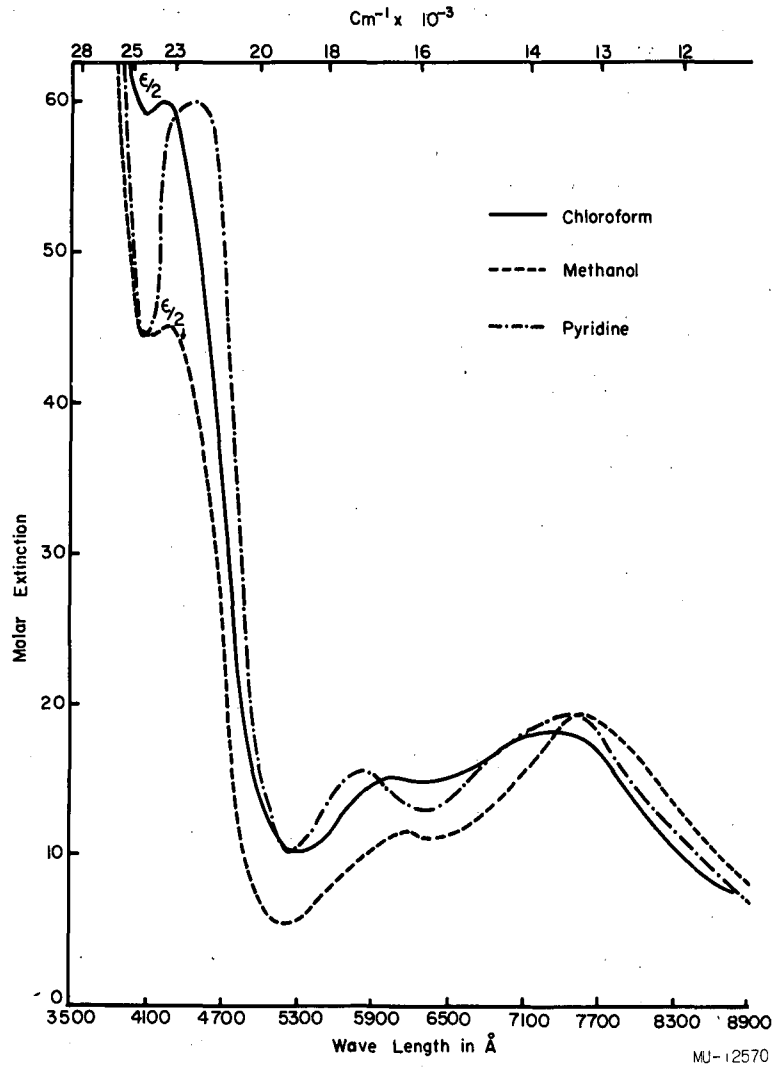


Fig. 3. Effect of solvent on vanadyl bistrifluoroacetylacetonate.

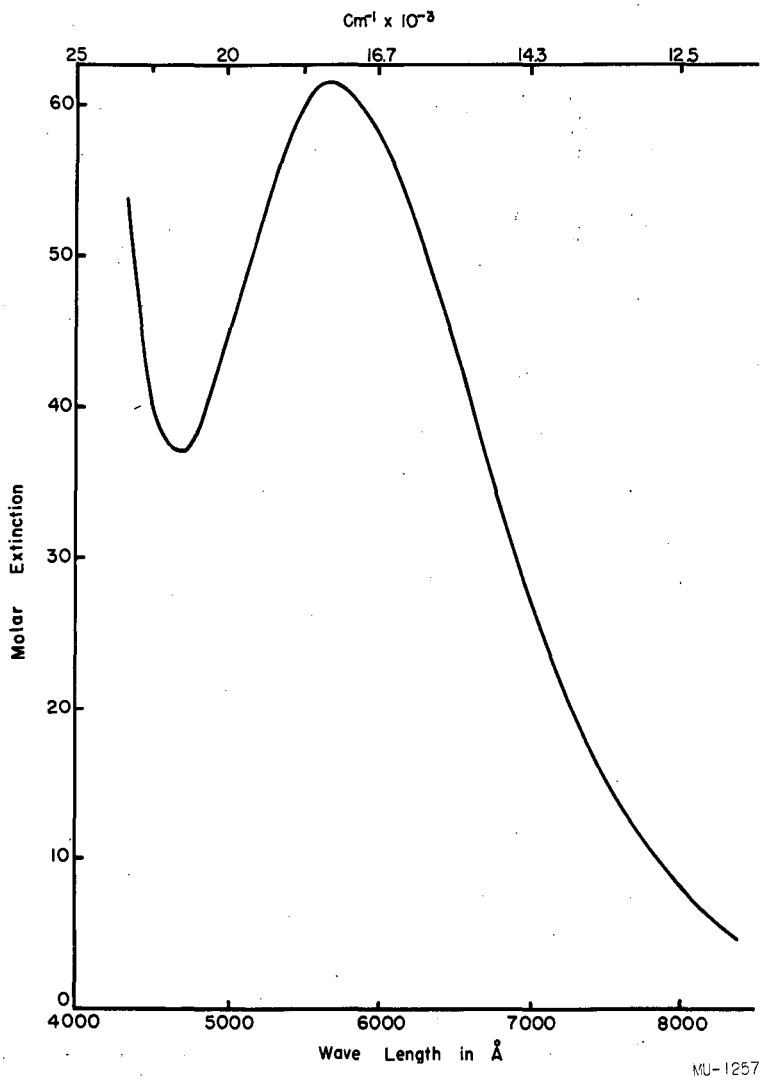


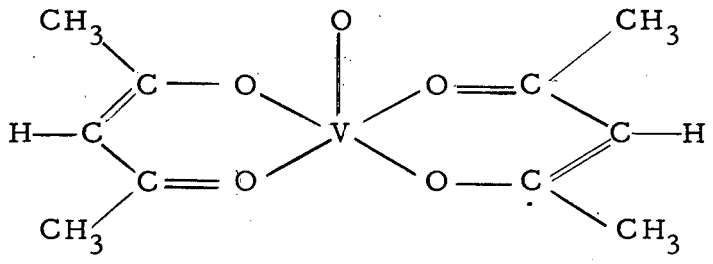
Fig. 4. Spectrum of vanadium bissalicylaldehydeethylenediimine diacetate.

Table I

Summary of spectra of some vanadyl chelates

Compound	Solvent	u_1 (Å)	ϵ	u_2 (Å)	ϵ	u_3 (Å)	ϵ
Vanadyl bisacetyl-acetonate	Chloroform	4000	40	5950	34	6750	38
	Methanol	4000	30	5750	10	7550	32
	Pyridine	4200	100	5800	37	7700	56
Vanadyl bisbenzoyl-acetonate	Chloroform	4550	30	6075	48	6700	48
	Methanol	4500	50	5850	19	7800	40
	Pyridine	--	--	5725	36	7625	43
Vanadyl bisdibenzoyl-methanate	Chloroform	4750	75	6150	64	6650	60
	Methanol	4800	60	5700	26	7820	32
	Pyridine	5000	340	5800	50	7600	47
Vanadyl bistrifluoro-acetylacetonate	Chloroform	4250	60	6250	30	7300	36
	Methanol	4300	70	6100	24	7550	40
	Pyridine	4550	100	5850	32	7400	39
Vanadyl bis-hexafluoroacetyl-acetonate	Chloroform	4850	112	6150	28	7340	29
	Methanol	4825	120	6000	21	7200	29
	Pyridine	4800	70	6200	47	7300	47
Vanadyl bissalicyl-aldehydeethyl-enediiminate	Chloroform	4700	10	6000	24	7100	10
	Methanol	4600	40	5700	58	7600	75
	Pyridine	4800	50	5950	140	7600	50
Vanadyl^{+2} solvate	Water	--	--	6300	6	7675	15
	Pyridine	(3525)	300	5900	34	7750	52
Vanadium^{+4} salicyl-aldehydeethylene-diimine diacetate	Acetic anhydride	One band only at 5700 Å					

In order to decide how the d orbitals are arranged energywise, we need only know the symmetry of the molecule. Dodge[8] has carried out a preliminary x-ray examination of vanadyl bisacetylacetonate, and finds that it is triclinic with two molecules per unit cell. The two chelate rings are almost certainly coplanar. Therefore, we have assumed that the molecule has the following structure:



The symmetry group for such a tetragonal pyramid is C_{2v}.

Let us assume that the ligand electrons appear as an average negative potential field, so far as the central electrons are concerned, and express this potential in spherical harmonics:[9]

$$V' = \sum_{\ell=o}^{\infty} \ell C_\ell X^\ell + \sum_{n=o}^{\infty} C_n Y^n + \sum_{m=o}^{\infty} C_m Z^m.$$

To find the energies of these levels, one needs only to calculate the integrals

$$\int \psi_i^* \, V' \, \psi_j d\tau.$$

This has been done by Holmes[9] and Belford.[10] If we use the atomic radial wave functions and perform the integrations, we find that we do not obtain the correct energies. However, it is convenient to express the data in terms of the potential parameters, Dq, Ap, and Qq.

---

[8] R. Dodge, private discussions.

[9] Owen Holmes, Thesis, University of California, Berkeley, 1955.

[10] R. Linn Belford, Bonding and Spectra of Metal Chelates: Ultraviolet, Visible, Infrared, and Electron Resonance Absorption. Near-Infrared Spectra of Alcohols (Thesis), UCRL-3051, June 1955.

One can calculate the relative order of the d levels for several extreme models. This was done for three model molecular arrangements: square planar, octahedral, and linear triatomic. From these models we can predict how the spectra of a given molecule will change with solvent basicity and ligand basicity, if we assume that base strength is a measure of the average negative field of ligand electrons. Figure 5 shows these levels for one 3d electron.

We may tentatively identify the three transitions in the visible region as:

$$u_{x^2-y^2},\ \sim 4000;\ u_{z^2},\ \sim 5800;\ u_{xy},\ \sim 7000;$$

the ground state is $u_{xz,yz}$. In assigning the transitions in this order we find that the electrostatic field is somewhere between square planar and octahedral, which is a most reasonable type of field on the basis of our assumed tetragonal pyramidal structure. Table II lists the field parameters calculated on the basis of this assignment of the visible spectra. If indeed this assignment is correct, then we may calculate the g values, using these d wave functions. In order to calculate this parameter we must use second-order perturbation theory, and $\lambda L \cdot S$ as the perturbing term in the Hamiltonian. The unperturbed wave functions are:

$$d_{xy}\alpha,\ d_{xy}\beta \quad \text{or} \quad 1/\sqrt{2}\left[\,|+2\rangle - |-2\rangle\,\right]\alpha,\beta;$$

$$d_{xz}\alpha,\ d_{xz}\beta \quad \text{or} \quad 1/\sqrt{2}\left[\,|+1\rangle + |-1\rangle\,\right]\alpha,\beta;$$

$$d_{yz}\alpha,\ d_{yz}\beta \quad \text{or} \quad 1/\sqrt{2}\left[\,|+1\rangle - |-1\rangle\,\right]\alpha,\beta;$$

$$d_{z^2}\alpha,\ d_{z^2}\beta \quad \text{or} \quad |0\rangle\,\alpha,\beta;$$

$$d_{x^2-y^2}\alpha,\ d_{x^2-y^2}\beta \quad \text{or} \quad 1/\sqrt{2}\left[\,|+2\rangle + |-2\rangle\,\right]\alpha,\beta.$$

In order to simplify this calculation we may write the matrix elements as: $\langle L\rangle \cdot \langle S\rangle$. Table III lists the matrix elements for the operators Lx, Ly, Lz, σx, σy, and σz. These matrix elements are listed in Condon and Shortley.[11] Schiff[12] has developed the formulas for evaluating the coefficients of the unperturbed wave functions for a second-order perturbation.

---

[11] E. U. Condon and G. H. Shortley, The Theory of Atomic Spectra, (Cambridge University Press, 1953,) p. 48.

[12] L. Schiff, Quantum Mechanics, (McGraw-Hill New York, 1949,) p. 151.

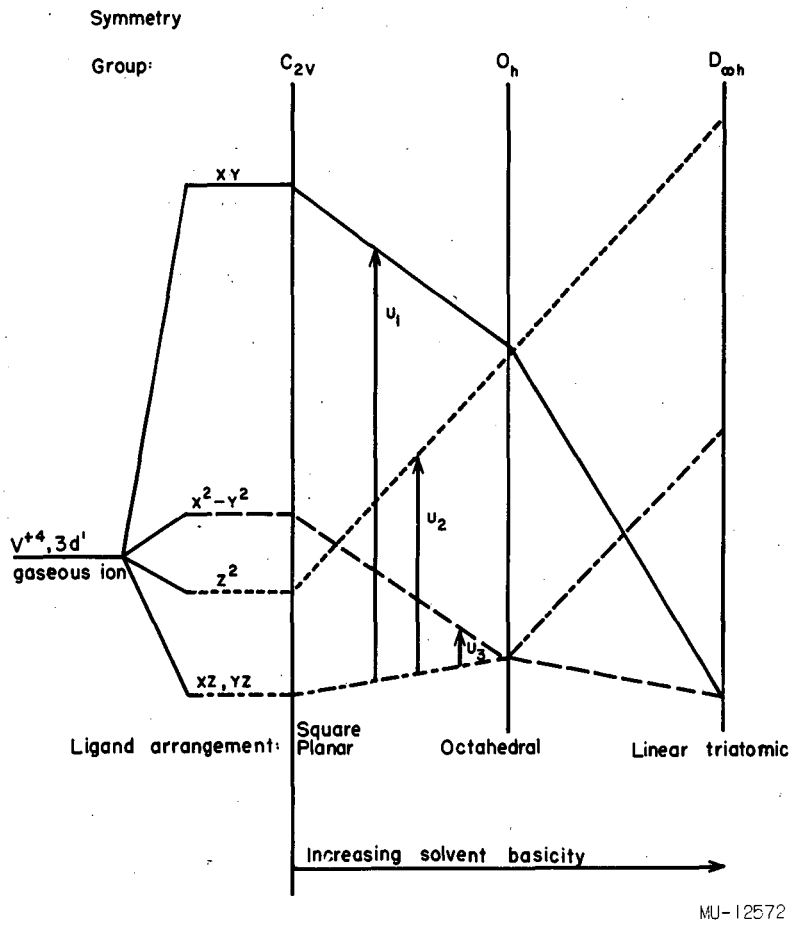


Fig. 5. Electronic levels for a 3d electron in order of various symmetries.

Table II

Field parameters calculated on the basis of assignment of visible spectra

Compound	Dq	Ap	Qq
Vanadyl bisacetyl-acetonate	1069	109.6	247.6
Vanadyl bisbenzoyl-acetonate	1037.4	97.1	309.1
Vanadyl bisdibenzoyl-methanate	1034.9	94.4	326.9
Vanadyl bistrifluoro-acetylacetonate	987.5	101.0	230.0
Vanadyl^{+2} pyridinate	992.3	115.8	123.8

Table III

Matrix elements for vanadyl chelates

$\lambda \langle j | L_i | xy \rangle$

	x	y	z
xz	$-\lambda$	0	0
yz	0	$i\lambda$	0
x^2-y^2	0	0	2λ
z^2	0	0	0

$\langle s | \sigma | \alpha \rangle$

	α	β
x	0	1
y	0	-i
z	1	0

$\lambda \langle j | L_i | x^2-y^2 \rangle$

	x	y	z
xz	0	$-i\lambda$	0
yz	λ	0	0
xy	0	0	-2λ
z^2	0	0	0

$\langle s | \sigma | \beta \rangle$

	α	β
x	1	0
y	-i	0
z	0	-1

z quantization

$\lambda \langle j | L_i | -1 \rangle$

	x	y	z
+1	0	0	0
x^2-y^2	$\frac{\lambda}{\sqrt{2}}$	$\frac{i\lambda}{\sqrt{2}}$	0
xy	$\frac{\lambda}{\sqrt{2}}$	$\frac{i\lambda}{\sqrt{2}}$	0
z^2	$\frac{\sqrt{6}\lambda}{2}$	$\frac{-i\sqrt{6}\lambda}{2}$	0

$\langle s | \sigma | \alpha \rangle$

	α	β
x	0	1
y	1	0
z	0	-i

$\lambda \langle j | L_{i-} | 0 \rangle$

	x	y	z
+1	$\frac{-\sqrt{+6}\lambda}{2}$	$\frac{+i\sqrt{6}\lambda}{2}$	0
-1	$\frac{\lambda\sqrt{+6}}{2}$	$\frac{+i\sqrt{6}\lambda}{2}$	0

$\langle s | \sigma | \beta \rangle$

	α	β
x	1	0
y	0	-1
z	i	0

y (or x) quantization

Our perturbed eigenfunctions are:

$$\left|z^2,\ \alpha\right\rangle = d_{z^2}\alpha, \quad \left|z^2,\ \beta\right\rangle = d_{z^2}\beta,$$

$$\left|xy,\ \alpha\right\rangle = d_{xy}\alpha + \frac{\lambda\beta}{2\Delta_{xz,\,xy}}(d_{yx} - d_{xz}) + \frac{\lambda\alpha d_{x^2-y^2}}{\Delta_{xy,\ x^2-y^2}},$$

$$\left|xy,\ \beta\right\rangle = d_{xy}\beta + \frac{\lambda\alpha}{2\Delta_{xz,\,yz}}(d_{yz} - d_{xz}) - \frac{\lambda\beta d_{x^2-y^2}}{\Delta_{xy,\ x^2-y^2}},$$

$$\left|x^2-y^2,\ \alpha\right\rangle = d_{x^2-y^2}\alpha + \frac{\lambda\beta}{2\Delta_{xz,\,yz}}(d_{yz} - d_{xz}) - \frac{\lambda\alpha d_{xy}}{\Delta_{xy,\,x^2-y^2}},$$

$$\left|x^2-y^2,\ \beta\right\rangle = d_{x^2-y^2}\beta + \frac{\lambda\beta}{2\Delta_{xz,\,yz}}(d_{yz} - d_{xz}) + \frac{\lambda\beta d_{x^2-y^2}}{\Delta_{xy,\,x^2-y^2}},$$

$$\left|-1,\ \alpha\right\rangle = \left|-1\right\rangle\alpha + \frac{\beta\lambda}{\sqrt{2}}\left(\frac{d_{x^2-y^2}}{\Delta_{x^2-y^2,\,\left|-1\right\rangle}} + \frac{d_{xy}}{\Delta_{xy,\ \left|-1\right\rangle}}\right),$$

$$\left|-1,\ \beta\right\rangle = \left|-1\right\rangle\beta + \frac{\alpha\lambda}{\sqrt{2}}\left(\frac{d_{x^2-y^2}}{\Delta_{x^2-y^2,\,\left|-1\right\rangle}} + \frac{d_{xy}}{\Delta_{xy,\ \left|-1\right\rangle}}\right),$$

$$\left|+1,\ \alpha\right\rangle = \left|+1\right\rangle\alpha - \frac{\beta\lambda}{\sqrt{2}}\left(\frac{d_{x^2-y^2}}{\Delta_{x^2-y^2,\,\left|+1\right\rangle}} + \frac{d_{xy}}{\Delta_{xy,\ \left|-1\right\rangle}}\right),$$

$$\left|+1,\ \beta\right\rangle = \left|+1\right\rangle\beta - \frac{\alpha\lambda}{\sqrt{2}}\left(\frac{d_{x^2-y^2}}{\Delta_{x^2-y^2,\,\left|+1\right\rangle}} + \frac{d_{xy}}{\Delta_{xy,\ \left|-1\right\rangle}}\right),$$

where Δ represents the energy separation of the d orbitals.

Calvin

In order to have the g values deviate from the free-spin value of 2.0023 we must have some orbital contribution to the angular momentum. To find this contribution we need only find the matrix elements,

$$\langle m | L_z | m \rangle \quad \text{and} \quad \langle m | L_{x \text{ or } y} | m \rangle \ ,$$

which depend on the angle between direction of the magnetic field and the molecular axis. Table IV lists these matrix elements. We see that if our ground state is $|-1\rangle$, then we have $g_{\parallel} = 2.0023$, and $g_{\perp} = 1.9756$. We have measured the g values of a single crystal of vanadyl bisacetylacetonate with the electron resonance spectrometer build in this laboratory by Dr. Power B. Sogo. These are found to be $g_{\parallel} = 2.00$, and $g_{\perp} = 1.950$. Since the method of mounting the single crystal is rather crude and the resonance is broad, the absolute values are not very accurate. Comparison of these values is shown in Table V.

From these data we see that the ligand field theory provides a convenient and qualitative way of describing the electrons of the cation involved in the bonding to ligands.

Table IV

Matrix elements for $\langle m | L_z | m \rangle$ and $\langle m | L_{x \text{ or } y} | m \rangle$

$\lvert m \rangle$	L_z (or $\Delta g_{\parallel}$)	$L_{x \text{ or } y}$ (or $\Delta g_{\perp}$)
$\lvert xy \rangle$	$\dfrac{-8\lambda}{\Delta_{x^2 - y^2}}$	$\dfrac{-2\lambda}{\Delta_{xz,\ yz}}$
$\lvert x^2 - y^2 \rangle$	$\dfrac{8\lambda}{\Delta_{xy}}$	$\dfrac{+2\lambda}{\Delta_{xz,\ yz}}$
$\lvert -1 \rangle$	0	$\left(\dfrac{1}{\Delta_{x^2-y^2}} + \dfrac{1}{\Delta_{xy}}\right)\lambda$
$\lvert 0 \rangle$	0	0

Calvin

Table V

Observed and calculated g values for vanadyl bisacetylacetonate

	$g_{average}$	$g_{\parallel}$	$g_{\perp}$
Solution	1.9859	--	--
Single crystal	1.990	2.00	1.950
Theoretical	1.993	2.0023	1.9756

ISOTOPE EFFECTS IN THE REVERSIBLE OXYGENATION OF COBALT SALICYLALDEHYDE ETHYLENEDIIMINE

Ann K. Coombs and B. M. Tolbert

Certain metallo-organic chelates have the ability to form stable peroxides which upon heating or evacuation decompose reversibly to form the original chelate and gaseous oxygen. Calvin and others reported that the oxygenation probably proceeds by several steps and the X-ray analysis showed "holes" in the crystal lattice of active forms of the chelates but no such holes in the inactive forms.[1] A large isotope effect between O^{16} and O^{18} may exist in the reversible oxygenation of these chelates. One of them, cobalt salicylaldehyde ethylenediimine, has been examined for an isotope effect.

The sample used had been pressed into small pellets and had absorbed 3.9% by weight of oxygen, 79% of the theoretical absorption of this chelate. The chelate was placed in a vacuum-line system of known volume and could be heated by means of heating tape wrapped around that part of the system which held the chelate. Temperatures were determined by a thermocouple, and pressures by a mercury manometer in the system. The content of O^{18} in the samples was analyzed mass-spectrographically (see Fig. 6).

For the first set of samples the chelate was deoxygenated at 110°C under vacuum and then oxygenated at room temperature by passing O_2 from a tank over the chelate for 1/2 hour at a rate of 50 cc/min. Oxygenation was considered complete when the manometer indicated no more oxygen uptake in a closed system. Excess O_2 was rapidly removed by 15-second evacuation. Then the temperature was raised to 52°C. When apparent pressure equilibrium had been reached (i.e., no observable pressure change over 15 minutes), a 50-cc sample of the gas over the chelate was collected and the system was then evacuated for about 2 seconds. This sampling was repeated until the equilibrium pressure was too low to collect a sample for the mass spectrograph. The last sample of the gas was forced off the chelate by heating to 78°C. Three of the nine samples collected were analyzed for O^{18} (Table VI).

---

[1] M. Calvin with many others, J. Am. Chem. Soc. 68, 2254, 2257, 2263, 2267, 2273, 2612 (1946).

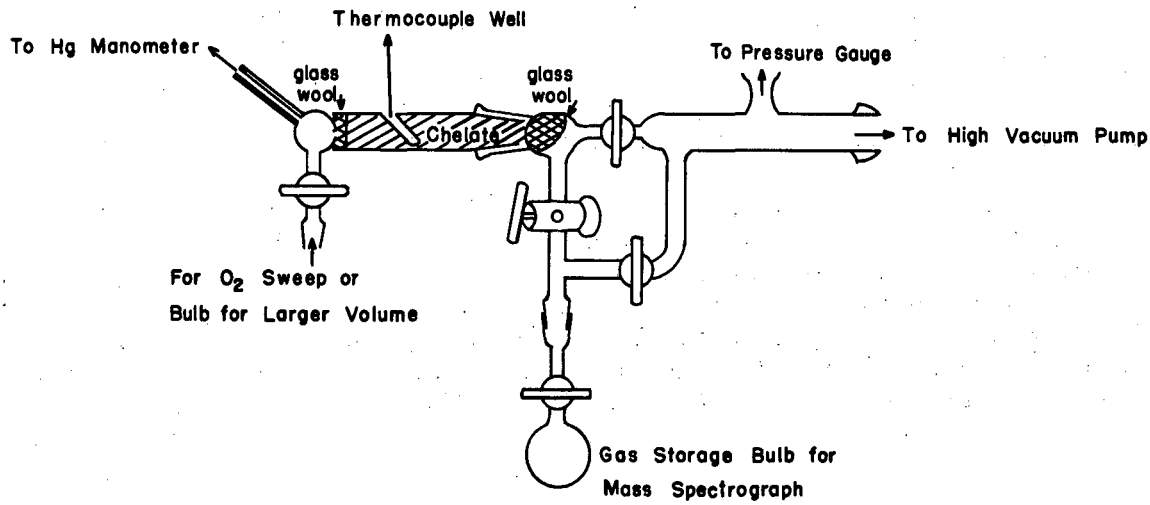


Fig. 6. Apparatus for measurement of isotope effect in oxygen absorption with some cobalt chelates. The body of the chelate-containing tube is wrapped with a heating tape.

Table VI

Analysis of oxygen samples rapidly removed from a cobalt chelate

Sample No.	Size (mmoles)	O left on chelate (%)	O^{18} in sample (%)
1	0.94	75	0.206 ± .002
5	0.29	28	0.207 ± .002
9	0.09	5	0.200 ± .002

Another experiment was done by collecting samples after equal lengths of time rather than after the gas had reached equilibrium. The chelate was oxygenated in the same way as in the first experiment. Samples were removed every 1/2 hour. Nineteen samples were collected at 38°C. The twentieth was collected at 68°C. For the twenty-first sample the chelate was heated to 96°C and the sample bulb immersed in liquid nitrogen to get the last of the oxygen (Table VII).

Table VII

Analysis of oxygen samples slowly removed from a cobalt chelate

Sample No.	Size (mmoles)	O left on chelate (%)	O^{18} in sample (%)
1	0.52	91	0.202 ± .002
19	0.15	6	0.207 ± .002
20	0.10	4	0.198 ± .002
21	0.25	-	0.191 ± .006

Mass spectrographic analysis of the starting oxygen showed 0.205% O^{18}. However, the sample was not very pure. In later experiments, therefore, the oxygen was purified by distilling a quantity of liquid oxygen and using the center fraction.

Calvin

The first two experiments indicated the trend of an isotope effect; that is, that the O^{18} was coming off the chelate more easily than O^{16}. To verify the effect, a set of three identical experiments was undertaken. Since the isotope effect should be more pronounced in a kinetic experiment than in an equilibrium one, these experiments were done as rapidly as possible, and only the very last of the oxygen, where the isotope effect would be the greatest, was collected and analyzed. The chelate was oxygenated for 18 hours at room temperature under about 700 mm pressure of purified oxygen. A 50-cc sample of the starting oxygen and a 50-cc sample of the oxygen left above the chelate after oxygenation were collected for analysis. The chelate was heated to 36°C and evacuated until about 2% of the total oxygen was left on the chelate. With the system closed, the temperature was raised to 78°C and the evolved oxygen was collected. These last samples contained decomposition products from the chelate, which were removed in order to eliminate large probable errors in the O^{18} analysis. This was done by shaking the sample with a small amount of degassed 8 N KOH to remove CO_2 and then distilling at -75°C, collecting the oxygen at liquid nitrogen temperature (-190°) and leaving impurities behind. The results of this experiment are shown in Table VIII.

Table VIII

O^{18} Isotope analysis of oxygen samples slowly removed from a cobalt chelate

Run No.	O_2 standard	O_2 in equilibrium with oxygenated chelate (%)	O_2 in last 2% of oxygen desorbed
1	0.203 ± .002%	0.203 ± .002	0.204 ± .002%
2	0.204 ± .002	0.206 ± .002	0.208% (bad peaks, 0.205 probably a better figure)
3	0.205 ± .002	0.205 ± .002	0.204 ± .003

There seems to be an isotope effect in the slow deoxygenation of this chelate, but no isotope effect in the rapid deoxygenation. Work to be undertaken includes affirmation of the original experiments and also a study of isotope effect in the reversible oxygenation of the 3-fluoro derivative of cobalt salicylaldehyde ethylenediimine, which appears to be oxygenated by a second-order reaction rather than by a first-order oxygenation like that of the parent compound.

ELECTRON SPIN RESONANCE OF CHOLINE CHLORIDE AND RELATED COMPOUNDS

Robert Lindblom and Richard M. Lemmon

The high G value (molecules destroyed per hundred electron volts) of radiation-damaged choline chloride has been discussed in a summary in the preceding quarterly report.[1] Electron spin resonance appears to be the only promised method for studying the radical responsible for this G value, as the high G value occurs only in the crystalline solid. The best resolution of the spin resonance curves occurs when the sample is irradiated and preserved at -196°C until its spin resonance is observed at room temperature. The resulting curve is similar to the first derivative of a Gaussian curve with each peak split into a triplet. This curve has not yet been interpreted.

To gain some experience in interpreting such curves, some compounds related to choline chloride have been irradiated and the spin resonance absorption observed. Ammonium chloride, the four methyl-substituted ammonium chlorides, ethyl ammonium chloride, ethanol ammonium chloride, and meta-acetaldehyde have been tried. Of these, the ammonium chloride and ethyl ammonium chloride gave no signals. These were colored yellow at -196°C but became colorless in a few seconds at room temperature. This suggests that these samples must be observed at a lower temperature when their radicals are more stable. The other compounds all gave complex, as yet uninterpretable, spectra except for methyl ammonium chloride. This gave a distinct quadruplet spread over about 67 gauss, and the peak heights follow a binomial distribution law (1:3:3:1). This type of hyperfine pattern results from an unpaired electron interacting with three protons, e.g., in a methyl radical or an ammonia (NH_3) radical ion. A further hyperfine interaction with the nitrogen would also be expected from ammonia ion; since this is not observed, we probably have a methyl radical. It is planned to confirm this by using methyl ammonium chloride separately deuterated on the nitrogen and on the carbon. The deuterated radical should show seven peaks instead of four, and, in this way, it can be determined whether the radical in question is methyl or ammonia.

The irradiated trimethyl ammonium chloride gave ten peaks in approximately a binomial distribution. This is the spectrum one would expect from the presence of nine protons in the radical. The ten peaks were split into triplets, which were not equal but shifted in their relative height through the spectrum. Nitrogen hyperfine interaction should give splitting to peaks of equal height.

The various radicals observed from ammonium chloride have half lives that vary from 1 to 4 hours at 25°C except for the tri- and tetramethyl ammonium chlorides. These showed no change over two weeks at 25°C. Trimethyl ammonium chloride radical disappears rapidly at 100°C.

---

[1] Richard M. Lemmon, Margaret A. Parsons, and Franco Mazzetti, in UCRL-3595, Sept. 1956, p. 10.

Calvin

CARBON-14-ION IRRADIATIONS

Richard M. Lemmon, Frederick L. Reynolds,
Ann Coombs, and Melvin Look

In our preceding quarterly report the C^{14} labeling of benzene by means of a C^{14}-ion beam in a mass spectrometer was described.[1] In the meantime, such irradiations have been performed on another benzene sample, on β-carotene, and on morphine. Each of these experiments will be described in turn.

Benzene

The benzene irradiation was carried out in the same manner as was described in the earlier report. The purification by vapor-phase chromatography differed only in that the irradiated benzene was passed first through a carbowax, instead of a silicone, column. The radioactivity determinations were again made by means of liquid scintillation. Results are given in Table IX. The fore and back parts of sample 5, Table IX, were collected separately. Within the limits of measurement, both parts of the fraction had the same specific activity.

Before the irradiated benzene was passed through the first carbowax column, small amounts of toluene and heptane were added. The toluene was collected separately and, after passage through the carbowax, it was found to have 1.9% of the total activity of the C^{14} ion beam. After a succeeding passage through a silicone column, this value was measured as 2.0%. No further purification of the toluene was carried out.

The heptane fraction, which emerges from the carbowax column before benzene, was found to contain 1.3% of the total activity of the C^{14} ion beam. At the present time, nothing further has been done with this fraction.

The benzene experiments clearly indicate that when this compound is irradiated with a beam of 2-kv C^{14} ions, some 1% to 5% of those ions are incorporated into C^{14}-labeled benzene molecules.

---

[1] Richard M. Lemmon, Fred L. Reynolds, and Franco Mazzetti, in UCRL-3595, Sept. 1956, p. 5.

Calvin

Table IX

Carbon-14 Ion[a] irradiation of Benzene: analysis of purified benzene fractions

Sample	Treatment	Activity (dis/min)	% of total ion beam
1	$C^{14}O_2$-free	27,500	44.4
2	(1) plus carbowax chromatography	923	3.4[b]
3	(2) plus silicone chromatography	530[c]	1.9
4	(3) plus carbowax chromatography	810	2.9[b]
5	(4) plus carbowax chromatography	863	3.2[b]

[a] Ion beam: approximately 2×10^{-9} ampere (this value is not known within a factor of 3); time of irradiation: 3 hours; total number of C^{14} ions striking benzene: 2.6×10^{14}.

[b] Errors in taking aliquot portions are such that these values can be considered identical.

[c] The lower activity here is probably in error.

β-Carotene

β-Carotene was selected for a further experiment in C^{14}-ion irradiations because it is a natural product of fairly high molecular weight (537), it is a hydrocarbon, and an excellent method for purification (column chromatography) is available.

Approximately 180 mg of β-carotene (Nutritional Biochemicals Corp.) was evaporated from benzene solution onto the target area of the mass spectrometer. The carotene was maintained at -80° during the irradiation. It was irradiated for 10 hours with a C^{14} ion beam of approximately 3×10^{-10} ampere. The amount of radioactivity in the total beam amounts to 17,400 dis/min.

The target area was removed from the spectrometer and the carotene was dissolved in acetone. The solution was evaporated to dryness and the residue was crystallized from benzene-methanol, giving 130 mg of β-carotene crystals; mp 180°. The specific activity was determined on a gas-flow proportional counter (Nuclear-Chicago) and was found to contain 7 dis/min/mg. This amounts to 1260 dis/min in the 180 mg of carotene, and is equal to 7.2% of the calculated (but not known to better than a factor of 3) number of C^{14} ions striking the carotene target.

The carotene was then chromatographed on a column consisting of one part MgO and two parts of Celite. The column was developed with a solution of 1% acetone in hexane. The column was extruded and the band of all-trans-β-carotene was cut out and eluted with acetone, the solvent was evaporated, and the carotene crystallized from benzene-methanol. Considerable difficulty was encountered in the crystallization, and a large fraction of the carotene was lost through oxidation and thermal decomposition. Consequently, the chromatography and crystallization were repeated, this time under milder conditions and with essentially no losses. The specific activity was determined as before and was found to be 11 dis/min/mg. The increase in specific activity from 7 to 11 dis/min/mg can only be explained by some radioactive contamination during the purification or counting processes.

The chromatographic purification of the carotene was repeated and the specific activity found to be 1 dis/min/mg. This amounts to 180 dis/min in the total irradiated carotene, and represents approximately 1% of the total C^{14} ion beam. The chromatographic purification is being repeated, and the specific activity of the cis-isomers, as well as that of the all-trans compound, will be determined.

Morphine

Approximately 50 mg of morphine hydrate was irradiated in the mass spectrometer in a manner very similar to that employed for the β-carotene; the morphine was also kept at -80°. It was irradiated for 19.5 hours with an average C^{14} ion current of 4×10^{-11} ampere. The amount of radioactivity in the total ion beam amounts to 8400 dis/min.

Calvin

The morphine was removed from the target area by solution in methanol. After the solvent was removed, the residue was dissolved in 3 ml of 0.1 N HCl. After the solution was filtered, addition of 0.25 ml of ethanol and 0.1 ml of conc. NH_4OH produced needles of morphine; 29 mg was recovered. The specific activity (also determined on the gas-flow proportional counter) was 28 dis/min/mg, or 1400 dis/min in the total sample of morphine.

Further purification of the morphine was effected by sublimation at 12 to 15 microns pressure and a temperature of 150° to 180°. The sublimate was recrystallized from methanol-water and was found to have a specific activity of 20 dis/min/mg. Purification of the morphine by these methods is being continued in order to reach a point of constant specific activity.

Calvin

PRECISION ASSAY FOR CARBON-14 IN ORGANIC COMPOUNDS

Irville Whittemore, Bert M. Tolbert, and Ann Ludwigsen

Pregl microcombustion methods and ion-chamber measurements have been combined to achieve an analytical method that is rapid and gives very reproducible results when used to determine C^{14} in organic compounds.[1]

A combustion train is set up with an oxygen-CO_2 tank, pressure regulator, combustion tube and furnace, drying tube, flowmeter, and evacuated ionization chamber, in that order (see UCRL-3595). The combustion proceeds while the ionization chamber is allowed to fill with the combustion gases. The flow rate and size of the ionization chamber are so chosen that there is ample time for the combustion to be completed and the train to be swept out before the chamber has filled (a 250-cc chamber used in conjunction with a flow rate of 10 to 12 cc of oxygen per min has proved successful in our work). When the chamber has filled to atmospheric pressure with the O_2-CO_2 mixture from the tank, as indicated by ceasing of the flow in the flowmeter, the ionization-chamber valve is closed and the activity in the chamber is measured by means of a vibrating-reed electrometer.[2] Either the high-resistance-leak method or the rate-of-drift technique may be used.[3,4] The results in this report were obtained by the high-resistance-leak method, using a 10^{11}-ohm resistor.

Table X shows the results obtained by the method as finally worked out. The results are expressed as the number of microcuries of C^{14} necessary to produce a deflection of 1 millivolt in the electrometer when a 10^{11}-ohm resistor is used. Three values not listed were rejected by Chauvenet's Criterion.

The method is quite rapid and uses only enough active material to give a reliable weighing. A 2- or 3-mg sample containing 0.05 μC or more of C^{14} activity is enough for an assay. Even smaller weights of sample may be used if precise enough weighing methods are available, although in such cases the addition of some inactive carrier material might be recommended. The combustion and measurement of a weighed sample does not usually take longer than 3/4 to 1 hour to finish.

Complete calibration of the analytical method is obtained by burning samples of known activity.

---

[1] I. M Whittemore, Ann E. Ludwigsen, and B. M. Tolbert, in UCRL-3595, Sept. 1956, p. 12.

[2] Applied Physics Corp. Model 30, Pasadena, California.

[3] B. M. Tolbert, Ion Chamber Assay of Radioactive Gases, UCRL-3499, Aug. 1956.

[4] G. L. Brownell and H. S. Lockhart, Nucleonics 10, No. 2, 26 (1952).

Table X

Assay of a sample of benzoic-C^{14} acid by the high-resistance-leak method with a 10^{11}-ohm resistor

Chamber No. 1 $\mu C\ C^{14}/mv$	Chamber No. 2 $\mu C\ C^{14}/mv$
1.871×10^{-3}	1.877×10^{-3}
1.867	1.870
1.867	1.871
1.874	1.874
1.869	1.880
1.869	1.875
1.877	1.881
1.875	1.871
1.874	1.882
1.878	1.885
1.869	1.878
	1.881
	1.873
Av. = 1.872	Av. = 1.877
Av. dev. = 3×10^{-6}	Av. dev. = 4×10^{-6}
Standard dev. = 4×10^{-6}	Standard dev. = 5×10^{-6}
% Standard dev. = 0.2%	% Standard dev. = 0.3%

The following points of technique seemed to help achieve more precise results. Use of a mixture of 5% CO_2 in oxygen as the combustion gas resulted in reducing the number of low results. The usual CuO in the combustion tube was replaced by broken quartz (24- to 48-mesh) in order to avoid the $C^{14}O_2$ memory effect associated with CuO. A furnace temperature of 700°C was found to be satisfactory. We found it helpful to use two burners while igniting the sample, one stationary near the oxygen inlet to prevent back-diffusion of the sample and another to volatize the sample so it would be carried into the furnace. Establishment of a regular schedule of weighing, burning, and measuring improved the precision greatly.

GROWTH OF ALGAE IN MEDIA CONTAINING D_2O

Osmund Holm-Hansen, Vivian Moses, and Edgar Yarberry

Hydrogen plays a considerable role in cellular organization. This role is particularly important in a consideration of substances whose molecular configuration is largely determined by hydrogen bonding. It would be of great theoretical interest to replace most or--if possible-- all of the cellular hydrogen with deuterium and to investigate the effects of such a replacement on cellular organization and metabolism. Furthermore, such plant material could be used in special nuclear or electron paramagnetic resonance studies.

Algae represent particularly suitable organisms with which to investigate this problem, since the whole of their hydrogen requirements can be met by water.

I. Scendesmus

Erlenmeyer flasks (250 ml capacity) containing 50 ml of Myer's medium and a D_2O concentration of 0% to 90% at 10% intervals were inoculated with 2 ml of Scendesmus suspension. The flasks were shaken and aerated with air containing 4% CO_2 for 48 hours. Measurements were then made of the packed cell volume and dry weight of the cells in each flask (see Table XI). The inoculum contained 0.0156 ml of packed cells.

Table XI

Growth of algae as a function of D_2O concentration in the medium

D_2O concentration (%)	Packed cell volume (ml)	Dry weight (g)
0	0.50	0.1032
10	0.53	0.1233
20	0.56	0.1019
30	0.39	0.0783
40	0.38	0.0761
50	0.23	0.0495
60	0.18	
70	0.16	not recorded
80	0.13	
90	0.12	

Photosynthesis

The packed Scenedesmus cells were resuspended in 10 ml of distilled water containing the same concentration of D_2O as that in which they were grown. Each sample, contained in a flattened test tube, was adapted to light for 25 min, during which time air plus 4% CO_2 was bubbled through each tube. After 25 min the tubes were flushed with air for 1 min, and 0.15 ml of 0.025 N $NaHC^{14}O_3$ (60 μC) was added to each sample, which was shaken in the light for 6 min. They were then poured into 40 ml of boiling ethanol. The samples were concentrated to 6 to 7 ml of which 400 λ was used for chromatography. Cell counts were performed before the experiment and the total quantity of $C^{14}O_2$ fixed by the cells, as well as the activities obtained in alanine, sucrose, and aspartic acid, were recorded. (See Table XII.)

Table XII

Photosynthesis in Scenedesmus as a function of D_2O concentration

D_2O conc. during growth (%)	Total No. of cells used in expt. ($\times 10^{-8}$)	Total $C^{14}O_2$ fixed (cpm $\times 10^{-6}$)	Activity in alanine (cpm)	Activity in sucrose (cpm)	Activity in aspartate (cpm)	$C^{14}O_2$ fixed per 10^8 cells (cpm)
0	9.77	7.2	27,100	46,000	237,400	736,600
60	4.71	4.1	49,000	29,600	111,000	870,000
70	4.41	3.9	58,000	22,500	113,000	883,800
80	1.35	4.2	87,900	42,200	85,000	3111,100
90	1.27	5.2	109,700	16,500	68,000	4078,400

Cells grown in 80% and 90% D_2O were much larger than cells grown in the lower concentrations, and there were many single cells and comparatively few autospores. As cells grown in high concentrations of D_2O were large, the small increase in packed cell volume represents, to a considerable extent, the enlargement of cell volume rather than increase in cell numbers.

II. Chlorella

A growth experiment similar to that performed with Scenedesmus was carried out with Chlorella. Inoculum (0.5 ml) was added to 49.5 ml of medium containing graded concentrations of D_2O from 0 to 99%. Samples of the medium were withdrawn before and after autoclaving and also after growth, for determination of the D_2O content. The cells were grown for 96 hours at 25°C, and were shaken and aerated with air plus 4% CO_2 during this period. At the end of the experiment, measurements were made of the dry weight, packed cell volume, optical density and cell number. The volume of 10 million cells was calculated.

The inoculum (0.5 ml) contained 37×10^6 cells, having a dry weight of 0.55 mg and a packed cell volume of 0.0029 ml. The optical density when diluted to 500 ml was zero. The results of the growth experiment are given in Table XIII.

Table XIII

Growth of Chlorella as a function of D_2O concentration in the medium

D_2O conc. before autoclaving (%)	D_2O conc. after autoclaving (%)	D_2O conc. after growth (%)	Packed cell volume (ml)	Total No. of cells ($\times 10^{-6}$)	Dry weight (mg)	Optical density 1:10 dilution	Cell volume $\mu l/10^7$ cells
0	0	0.9	0.87	7925	179.4	0.87	110
11.1	11.3	12.4	0.58	3344	142.8	0.67	176
22.2	22.5	23.2	0.63	5306	138.3	0.77	120
33.3	35.2	35.6	0.53	3988	121.8	0.62	135
44.4	45.3	47.0	0.15	294	28.5	0.08	511
55.5	53.9	58.9	0.05	54	8.9	0.02	930
66.6	70.6	73.2	0.03	33	6.0	0.00	1169
77.7	78.1	83.7	-	35	-	0.00	-
88.8	84.1	87.8	0.02	38	4.3	0.00	667[a]
99.9	98.2	100.0	0.01	41	2.3	0.00	320[a]

[a] Large experimental error due to low readings

This experiment shows that the growth of Chlorella, although not markedly inhibited by D_2O up to a concentration of 33%, is very strongly inhibited by higher concentrations. This also shows that at increasingly high D_2O concentrations increase of packed cell volume during the incubation period is largely a result of an increase of cell size, rather than of cell number.

Determinations of D_2O content of samples of media before and after autoclaving have shown that there is very little change in D_2O concentration during this process, even at the higher D_2O levels. There was similarly only a slight rise in D_2O content during the aeration period due to preflushing the gas mixture through 100% D_2O. The growth flasks were aerated in series, the air passing first through a preflush of 100% D_2O and then through the growth flasks, entering at the flask containing 100% D_2O and leaving the system via that containing only H_2O.

Work is in progress to attempt to adapt Chlorella to grow well at high D_2O concentrations. By initially growing the cells in a medium containing 35% D_2O and subculturing into the media of higher D_2O concentrations, it is possible to obtain good growth in 60% D_2O. Growth to a lesser extent has also been obtained up to 77% D_2O. It is hoped in this way to adapt Chlorella to grow in very high concentrations of D_2O, possibly as high as 100%.

COMPARISON OF THE CAROTENOID CONCENTRATIONS IN ALGAE IN LIGHT AND DARK

Ulrich Blass

A method has been developed to study the role of carotenoids in photosynthesis. The first experiments of this kind attempted to compare the carotenoid concentrations of algae in light with those in the dark. The present report describes the experimental conditions.

Fifty ml of a 100-ml 1% suspension of algae (Scenedesmus or Chlorella) in a nutrient solution was exposed to light in a lollipop-shaped flask for 30 min; at the same time the remaining 50-ml suspension was exposed to the dark for 30 min. A gentle bubbling stream of air containing 3.7% carbon dioxide was passed through both of them. The algae were then killed with 0.5 ml of aqueous 2 N KCN (per 50 ml suspension) and centrifuged. The cell residues were extracted twice with 10-ml boiling methanol and separated by centrifugation.

Column chromatography was used for the separation of the pigments, with magnesium oxide, cellulose, and polyethylene powder as adsorbents. As columns, glass tubes with long capillary ends were used. The capillaries were bent up into S-shaped syphon-type overflows which prevented the chromatograms from running dry. The adsorbents, freshly mixed with solvent in an electric blender, were filled in as slurry. The columns were then washed under the pressure of ~50 cm of the desired solvent (applied through a capillary).

Calvin

Magnesium oxide and cellulose powder columns were started with ligroin (petroleum ether, bp 65°–110°) as solvent, which was gradually reinforced with toluene and later on with ether. The methanol fractions were extracted into ligroin by mixing these aliquots with ligroin and aqueous sodium chloride solution.

Polyethylene powder columns were started with 80% aqueous methanol as solvent, which was gradually reinforced to pure methanol. Additions of ether were used to wash out the final carotene fraction. The methanolic algae extracts were diluted on top of the column to 80% aqueous methanol solutions, which caused some precipitation of pigments. This precipitate dissolved during the development of the chromatogram.

Determination of carotenoid concentrations

Fractions of 10 to 15 ml elution were collected in which we determined the light absorption in a Cary spectrophotometer, Model 11. The calculation of the carotenoid concentrations, c, was made by means of Formula 1:

$$c = \frac{(\log I_0/I)\ (V)}{(\epsilon)\ \ (d)}, \qquad (1)$$

where

I_0 is light intensity passing the reference cell,

I is light intensity passing the fraction,

V is volume of the fraction in liters,

ϵ is molar light-absorption coefficient,

and d is length of cell.

We used 1.3×10^5 throughout as the value of the molar light-absorption coefficient ϵ at the wave length of maximal absorption for all carotenoids. This value is in agreement within 7% with every reliable determination of ϵ of any pure carotenoid occurring in our extracts. Data are given in Table XIV. The comparisons of the concentrations of these carotenoids in algae showed that in the dark all the carotenoid concentrations are higher than in the light, especially in the case of the violaxanthin.

Table XIV

Carotenoid absorption from algae

Carotenoid in order of elution from polyethylene columns	Wave lengths of maximal light absorption (mμ)			Wave lengths of maximal absorption after rearrangement with 1% of 0.1 N HCl (mμ)			
1. Violaxanthin[a]	416	438	468	380	400	424	ether
	415	436	468	375	398	421	80% methanol
2. Xanthophyll-epoxide	411	435	464	400	421	448	ether
	411	435	465	396	420	448	80% methanol
3. Mixture of lutein and zeaxanthin	422	442	472				90% methanol
4. Green pigments							
5. Mixture of β-carotene and α-carotene	425	446	474				ether

[a] Blue color reaction with conc. hydrochloric acid–water, 1:1.

SYNTHESIS OF MORPHINE FROM NORMORPHINE AND STUDIES IN THE BIOSYNTHESIS OF MORPHINE

Melvin Look and Henry Rapoport

The degradation of morphine to normorphine and resynthesis to morphine without isolation went in 47% to 71% over-all yield. In order to prove that the N-methyl group is removed completely in our degradation scheme, we degraded some morphine-N-methyl-C^{14}, prepared in 1950[1], to normorphine and then it converted to morphine. The morphine isolated was still radioactive. Paper chromatography showed that at least 10% of the morphine-N-methyl-C^{14}, synthesized more than 5 years ago, had decomposed into one or possibly two radioactive unknowns. Methods are being developed to purify macroquantities of the radioactive morphine. Various extraction steps were developed to remove impurities and nondegraded morphine along the degradation runs. Dilute hydrochloric acid was found unsatisfactory for extracting morphine or diacetylmorphine from chloroform solution; the alkaloid hydrochlorides were soluble in the polar organic solvent. Dilute phosphoric acid, however, removes morphine and diacetylmorphine almost quantitatively from chloroform solutions.

The gas manifold and feeding systems are being constructed and tested for the growing of Papaver somniferum plants in the presence of radioactive carbon dioxide.

DARK FIXATION OF CARBON-14 DIOXIDE BY ALGAE

Duncan F. Shaw

Previous work in this laboratory has indicated that the distribution of radioactivity between various compounds during the dark fixation of $C^{14}O_2$ is not the same in the green alga, Scenedesmus obliquus, and the blue-green alga, Nostoc muscorum. The main difference found was a much more rapid introduction of activity into the sugar phosphates in Nostoc than in Scenedesmus. In order to confirm and explain this result the dark fixation of $C^{14}O_2$ by the two algae is being investigated.

A suspension of the algae in nutrient solution was treated with CO_2 in air in a blackened flask. After 2 hours, $C^{14}O_2$ (as $NaHCO_3^-$) was added. At known intervals samples were removed from the flask, by means of a blackened hypodermic syringe, and run into boiling ethanol. The total activity fixed was determined by drying some of the resulting suspension on an aluminum planchet, and measuring the activity present on the plate. The activity in the clear supernatant liquid was also determined in the same way.

---

[1] Rapoport, Lovell, and Tolbert, J. Am. Chem. Soc. 73, 5900 (1951).

In both algae the rate of fixation was almost the same, and for the first few minutes all the activity was present in the supernatant liquid. After about two hours the activity in the supernatant liquid remained constant, but the total activity continued to rise. The compounds responsible for the initial fixation of CO_2 must be present in the supernatant liquid only, and it was decided to try to find out if these compounds were the same for both algae.

In the experiment just described, the algae had been given inactive CO_2 for 2 hours before addition of the radioactive CO_2. It was possible, therefore, that during this time they had depleted the supply of some compound necessary for CO_2 fixation, and that the fixation rate was much lower after 2 hours than it was originally. Also, the constancy of activity in the supernatant liquid after 2 hours' fixation of $C^{14}O_2$ could be due to the complete exhaustion of the supply of this compound. To find if this was, or was not, the case, an experiment was carried out in which the algae were given inactive CO_2 for various times before being given $C^{14}O_2$. So far, this experiment has been done only with Scenedesmus, and it has shown that both the initial rate of appearance of activity and the final level of activity in the supernatant liquid fell by only a small amount for periods of pretreatment with inactive CO_2 varying from 4 minutes to 2 hours.

This result shows that the activity in the supernatant liquid levels off owing to the reaching of an equilibrium with the activity in the nutrient solution, and not as the result of the exhaustion of the supply of a compound necessary for CO_2 fixation. Also, it indicates that two hours in the dark does not seriously change the mechanism of dark fixation of CO_2. This experiment will be repeated using Nostoc muscorum.

The low rate of $C^{14}O_2$ fixation by these algae in the dark makes the detection of the active compounds present on a paper chromatogram of their extracts very difficult if the normal method of exposure to x-ray plates is used. Therefore, a more sensitive method of detecting C^{14}-labeled compounds on a paper chromatogram is required. Two ways of doing this, which are being investigated, are (a) the possibility of using a faster film than x-ray plates, and (b) the possibility of using Ilford Nuclear Emulsion G. 4, and scanning the developed plates with a microscope for β-particle tracks.

RELATIVE RADIOACTIVITY OF CHLOROPHYLL a AND b FROM PHOTOSYNTHETIC STUDIES

Jan Anderson

From photosynthetic tracer studies with Scenedesmus, it appeared that the chlorophylls were not as radioactive as the other lipid fractions.[1] The radioautograph from a 12-hour exposure to light and $C^{14}O_2$ suggested that chlorophyll a contained more radioactivity than chlorophyll b. An attempt has been made to see whether this apparent difference is real.

Scenedesmus was allowed to photosynthesize under standard conditions (lollipop, 1% suspension) in the presence of $C^{14}O_2$. The samples removed were heated in methanol and centrifuged, the supernatant was diluted to 90% methanol, and petroleum ether was added. After shaking and centrifuging, the petroleum ether extract contained the chlorophylls together with some lutein, but the bulk of the oxygen-containing carotenoids had been removed by the 90% methanol.

Chromatograms were made of the 1-, 4-, and 8-hour extracts, the zones of chlorophyll a and b counted, and radioautographs taken to check the distribution of radioactivity in comparison to the colored a and b zones. All chromatograms were on Whatman No. 3 filter paper. The only two useful solvent systems found for the descending method were toluene-acetone-isopropanol (100:4:2) and petroleum ether–n-butanol (100:0.5).

A variety of adsorbents were used in column chromatography to attempt the separation of chlorophyll a from b. Of these, only cellulose and polyethylene were successful. Rapid decomposition of the chlorophylls took place on alumina or celite, giving good separation of pheophytin a and b. Pure chlorophyll a was eluted from a cellulose column (petroleum ether) in a petroleum ether–n-butanol mixture (100:0.5).

Determination of Chlorophyll a and b

The most convenient way to measure the concentration of chlorophyll a and b, which are present together in the extracts, is by spectrophotometric analysis. The values obtained by Comar and Zscheile[2] and by Mackinney[3] were used as standards for the specific absorption coefficient a(liter/g/cm) (see Table XV).

---

1 Ulrich Blass, in UCRL-3595, Sept. 1956, p. 48.

2 C. L. Comar and F. P. Zscheile, Plant Physiol. 17, 198 (1942).

3 G. Mackinney, J. Biol. Chem. 140, 315 (1941).

Table XV

Specific absorption coefficients for chlorophyll a and b

max (mμ)	Chlorophyll a	Chlorophyll b
660	102	4.5
642	16.3	57.5
613	15.6	8.05
600	9.95	9.95

By substituting the optical densities found at these wave lengths into equations derived from the above constants, it is possible to calculate both the chlorophyll a and the chlorophyll b concentrations and to check these values against the total chlorophyll concentration, which can be calculated at the several wave lengths at which the chlorophyll a and b spectra intersect each other.

The ratio of chlorophyll a to b in Scenedesmus varies from 2.5 : 1 to 4 : 1. The specific activity therefore could be determined from the activity and concentration of each component.

Results

The figures obtained for the specific activity of chlorophyll a and b were very similar, indicating that the apparent large difference detected by looking at radioautographs is not real. It is uncertain, however, what significance may be attached to these figures, as the radioautographs indicate that some chlorophyll a or colorless material may be "tailing" into b. It is planned to separate a and b by column chromatography and determine the activity of spectrophotometrically pure samples, which would allow more absolute figures to be obtained.

DARK FIXATION OF CARBON-14 DIOXIDE BY NOSTOC MUSCORUM AND SCENEDESMUS OBLIQUUS

Osmund Holm-Hansen

During the study of citrulline formation by blue-green algae it was seen that not only was the rate of CO_2 incorporation in the dark by Nostoc muscorum very high compared with the fixation of CO_2 in the light, but also that much activity was incorporated into the sugar phosphates.[1] Other work in this laboratory has demonstrated that in Scenedesmus the dark fixation products of $C^{14}O_2$ are predominantly the dicarboxylic acids, with very little activity in the sugar phosphate compounds.[2] In contrast to this was the work showing that in Euglena the pattern of $C^{14}O_2$ incorporation was very similar to the pattern found during photosynthesis.[3] The mechanism whereby this occurs has not yet been determined. As the blue-green algae represent a very primitive group of the plant kingdom, it is of interest to elucidate the mechanism by which Nostoc can fix $C^{14}O_2$ in the sugar phosphate compounds, for it may shed some light on the evolution of the photosynthetic mechanism.

Many experiments have been performed with Nostoc and Scenedesmus to show the rate of fixation of CO_2 in the dark and to indicate which compounds contain the radioactive carbon. Data from two typical experiments are shown in Table XVI. Nostoc muscorum was grown on the shaker apparatus in a 2500-ml flask containing PG nutrient solution, with 4% CO_2-in-air being flushed through the flask. Immediately upon harvesting, the cells were centrifuged, rinsed once with distilled water, and resuspended (4 to 5% by volume) in distilled water and placed in a "lollipop" flask. The adaptation period consisted of flushing N_2 through the suspension in complete darkness or in high light intensity for 15 minutes. The labeled bicarbonate was then added, the "lollipop" stoppered and shaken by hand for the appropriate time. The killing with ethanol, the extraction and subsequent chromatographic separation, etc., were by the routine methods used in the laboratory. The Scenedesmus experiment was similar to the above except that the cells had been grown in Myer's medium in a continuous-culture tube, and during the adaptation period the suspension was flushed with 4% CO_2 in air. The cells adapted in strong light were, of course, kept in the light during the exposure to labeled bicarbonate, while those adapted in the dark were kept in complete darkness during the $C^{14}O_2$ fixation period. It is seen from Table XVI that the amount of $C^{14}O_2$ fixed after 10 minutes of darkness is about 36% of that fixed after 9 minutes of photosynthesis in Nostoc, while for Scenedesmus the total amount of $C^{14}O_2$ fixed after 15 minutes in the dark was only about 0.6% of that fixed during 6 minutes of photosynthesis. Equally striking is the difference between patterns of incorporation of the $C^{14}O_2$ in the two different organisms. In Scenedesmus, the activity is restricted mainly to the dicarboxylic acids, while in Nostoc the pattern strongly resembles that obtained in the photosynthetic experiment.

---

[1] Linko, Holm-Hansen, Bassham, and Calvin, J. Exp. Botany, in press.

[2] Calvin, Benson, Bassham, Goodman, Kawaguchi, Lynch, and Stepka, in UCRL-1365, June 1951, page 44.

[3] V. H. Lynch and M. Calvin, Ann. N. Y. Acad. Sci. 56, 890 (1953).

Table XVI

Comparison of light and dark fixation of $C^{14}O_2$ in Nostoc muscorum and Scenedesmus obliquus

	Nostoc muscorum				Scenedesmus obliquus			
	Light		Dark		Light		Dark	
Length of exposure to $HC^{14}O_3$	9 min.		10 min.		6 min.		15 min.	
Total cpm fixed per ml cells	7,470,000		2,720,000		52,000,000		291,000	
Total cpm in solubles per ml cells	4,450,000		1,570,000		51,000,000		218,000	
Percent soluble activity	60%		58%		98%		72%	
	cpm in compounds per ml cells	% of total soluble activity	cpm in compound per ml cells	% of total soluble activity	cpm in compound per ml cells	% of total soluble activity	cpm in compound per ml cells	% of total soluble activity
Aspartic acid	348,000	7.8	272,000	17.3	2,760,000	5.4	80,400	37.0
Glutamic acid	51,300	1.2	67,800	4.3	903,000	1.8	27,700	12.7
Citric acid	19,200	0.04	19,500	1.2	312,000	0.6	16,600	7.6
Malic acid	75,600	1.7	51,700	3.3	4,980,000	9.8	21,000	9.6
PGA	717,000	16.1	177,000	11.3	4,720,000	9.2	-	-
Hexose monophosphates	360,000	8.1	313,000	20.0	2,900,000	5.7	-	-
UDPG	165,000	3.7	129,000	8.2	1,630,000	3.2	-	-
Diphosphates	218,000	4.9	24,400	1.5	400,000	0.8	-	-
PEP	34,800	0.8	17,700	1.1	170,000	0.3	-	-
Spot A(unknown phosphate)	-	-	22,000	1.4	-	-	-	-
Serine	54,000	1.2	50,000	3.2	1,100,000	2.1	-	-
Sucrose	57,600	1.3	17,500	1.1	308,000	0.6	-	-
Glucose cyclic 1-2 P	-	-	-	-	470,000	0.9	-	-
Sum of aspartic, glutamic citric and malic	494,000	11.1	411,000	26.2	8,955,000	17.5	145,000	66.7
All phosphate - compounds	1494,000	33.6	683,000	43.5	10,290,000	20.2	28,500*	13.1

* The phosphate-compounds could not be adequately identified, and therefore the entire phosphate area was counted to yield this value of 28,000 cpm.

In regard to the possible mechanism whereby Nostoc is able to fix such large amounts of $C^{14}O_2$ in the dark, the data in Table XVI do not clearly differentiate between the two possibilities--of its being either a "pre-illumination" effect or a dark reaction independent of any immediately preceding light period. Scenedesmus has been shown to be able to fix considerable amounts of CO_2 owing to "pre-illumination" effects, which are dependent upon exposure of the algae to high light intensity in low concentrations of carbon dioxide and the addition of labeled bicarbonate at the moment the light is turned off.[2] Euglena has, on the other hand, been shown to be able to fix considerable amounts of CO_2 in the dark after dark-adaptation periods of up to 16 hours. With the same experimental conditions as described for Nostoc from Table XVI, the total amount of dark-fixation products has been studied at times of 2, 5, 10, 20 and 30 minutes (Table XVII A). It can be seen that the rate of dark fixation is considerably faster from 0 to 10 minutes than during the 10-to-30-minute interval. The striking similarity of the dark-fixation products during the first 10 minutes to the pattern of incorporation during photosynthesis can be seen by comparing Tables XVII A and XVII B. In both light and dark fixation in Nostoc the hexose monophosphates, aspartic acid, PGA, and UDPG all become heavily labeled, while the diphosphate compounds become labeled much more slowly in the dark than in the light. The reason for the pronounced drop in activity in many of the compounds between 10 and 30 minutes' dark fixation is not known, but the drop indicates that the high rate of dark CO_2 fixation may not be possible during long steady-state dark conditions.

Further progress on this problem is reported in another section of this quarterly.

Table XVII A

Time sequence of the incorporation of $C^{14}O_2$ by Nostoc muscorum in the dark (A) and in the light (B)

	Time				
	2 min	5 min	10 min	20 min	30 min
Total cpm fixed per ml cells	150,000	986,000	2,720,000	3,464,000	4,240,000
Total cpm in solubles per ml cells	98,600	700,000	1,570,000	1,640,000	1,760,000
Percent soluble activity	66%	71%	58%	47%	42%
Aspartic acid	31,200	117,000	272,400	119,200	25,900
Glutamic acid	1,704	8,160	67,800	98,400	37,380
Malic acid	8,340	28,800	51,700	78,600	54,200
Citric acid	1,750	5,320	19,500	9,480	7,620
PGA	15,800	81,900	177,000	30,600	9,600
Hexose mono-phosphate	33,400	130,000	313,000	a	278,000
UDPG	4,330	28,400	129,000	a	98,400
Diphosphates	-	10,600	24,400	105,000	95,400
PEP	4,200	6,400	17,700	3,700	-
Citrulline	2,680	16,980	47,400	53,400	52,380
Alanine	1,300	6,600	28,600	19,700	10,200
Serine	2,500	21,900	50,500	34,000	8,600
Sucrose	-	4,920	17,500	83,100	38,500

[a] These two spots were not resolved in the chromatogram. B-T together had 531,000 cpm.

Calvin

Table XVIIB

Time sequence of incorporation of $C^{14}O_2$ by Nostoc muscorum in the light

	Time						
	1 min	3 min	5 min	7 min	9 min	11 min	15 min
Total cpm fixed per ml cells	420,000	1,110,000	3,090,000	5,170,000	7,480,000	8,920,000	11,700,000
Total cpm in solubles per ml cells	308,000	1,040,000	2,330,000	3,450,000	4,450,000	5,440,000	7,580,000
Percent soluble activity	73%	93%	75%	67%	60%	61%	64%
Total cpm in CPD per ml cells:							
Aspartic acid	26,760	79,500	198,000	286,000	348,000	357,000	375,000
Glutamic acid	4,100	7,700	15,500	32,700	51,300	72,000	123,000
Malic acid	4,200	14,500	51,300	87,900	75,600	63,600	69,600
Citric acid	3,400	3,700	3,780	16,500	19,200	31,800	32,100
PGA	91,800	224,000	414,000	633,000	717,000	936,000	1,059,000
Hexose mono-phosphates	10,100	103,000	186,000	261,000	360,000	387,000	474,000
UDPG	1,600	43,900	76,500	120,000	165,000	231,000	312,000
Diphosphates	3,340	33,000	75,000	80,000	218,000	269,000	564,000
PEP	1,290	8,300	14,200	21,450	34,800	39,000	40,500
Citrulline	10,950	48,600	97,500	111,000	111,000	78,300	71,400
Alanine	3,000	3,800	9,800	17,600	19,900	21,800	18,600
Serine	--	--	22,200	43,800	54,000	53,100	57,000
Sucrose	--	6,990	19,200	45,600	57,600	70,500	93,300

EFFECT OF DIISOPROPYLFLUOROPHOSPHATE UPON CHOLINESTERASE AND BEHAVIOR

Edward L. Bennett and Hilda Karlsson

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

In previous reports we have shown that individual differences in the cholinesterase (ChE) activity of the cerebral cortex of the rat are correlated with individual differences in their adaptive behavior in a maze.[1,2] We have since attempted to alter the activity of the acetylcholine-cholinesterase system in order to study the effects of such alteration on behavior. Recently we have been using diisopropylfluorophosphate (DFP), a potent ChE inhibitor, for this purpose. Reports in the literature have suggested that ChE exists in considerable functional excess in the nervous system, since it must be almost completely inhibited before conduction is abolished in peripheral nerves. Studies on the intact animal have shown gross behavioral abnormalities if brain ChE activity was reduced by more than 50% to 60%.[3] We hoped that our more sensitive behavioral tests might detect effects of more moderate reductions of ChE, and this has proved to be the case.

Rats of our S_{13} strain were given intraperitoneal injections of 1.0 mg DFP per kilo body weight. While most of the brain ChE is initially inactivated, it continues to be reactivated during the pretraining and testing period. Pretraining was begun 4 days after the injection; maze testing was begun 14 days after the injection and lasted for 7 days. The animals were run in the Krech Hypothesis Apparatus under the progressively solvable schedule, with the lighted alleys becoming progressively more frequently correct.[1]

Littermate control injected rats were sacrificed for analysis of brain ChE at 2 and 13 days after the injection, so that we could measure the course of recovery of ChE. Fourteen injected rats and eight noninjected littermate controls were run in the behavioral test; these 22 animals were sacrificed for analysis of brain ChE at the end of the experiment. For each animal, ChE activity was measured in the visual and somesthetic areas of the cerebral cortex and the brain after all cortex had been stripped off. Lactic dehydrogenase was measured in each of these tissue samples for all animals. (The lactic dehydrogenase method and results will be reported at a later time.)

Recovery of ChE activity vs time after injection was compared for the different brain areas. The curve of [100-(percent of normal activity)] vs time was approximately linear on a semilog scale. However, more time intervals are needed to establish the recovery curves. Davison[4] has indicated

---

1 E. L. Bennett, in UCRL-3351, March 1956, p. 14.

2 E. L. Bennett and Ruth Deane, in UCRL-3415, June 1956, p. 21.

3 R. P. White, Proc. Soc. Exptl. Biol. Med. 93, 113 (1956).

4 A. N. Davison, Biochem. J. 60, 339 (1955).

in related experiments that the rate of recovery is relatively rapid to approximately 60% recovery, and then decreases markedly. On the basis of our limited data, the half life of inhibition appears to be about 10 to 11 days for brain and 14 days for the cortex. Thus, cortical ChE activity appears to be more sensitive to DFP. Visual and somesthetic cortex showed very similar curves, but the whole brain recovered at a slightly faster rate, as Table XVIII shows.

Table XVIII

Recovery of ChE activity in cortex and whole brain after DFP injection

Days after Injection[a]	2	13	20	21
Number of Animals	4	8	9	5
	% of normal ChE activity			
Visual cortex	23.2	57.4	63.0	71.4
Somesthetic cortex	24.4	58.6	64.1	72.0
Whole brain	28.4	72.0	74.0	78.8

[a] Rats were injected with 1 mg DFP/kg and sacrificed at the indicated time intervals after injection. Eight control animals were used.

During pretraining, while the cortical ChE activity was progressing from about one-third to about two-thirds of normal, the behavior of the injected animals differed clearly in two ways from that of their noninjected littermate controls. The injected rats ran down the apparatus to their food in about half the time required by the control animals, but they ate significantly less. During pretraining and testing, the animals consume their entire daily ration in 6 minutes in the apparatus. By the end of pretraining, the injected animals weighed a mean of 36 grams less than the controls, and this loss was maintained throughout the test period. It appeared that the injected animals could eat less in a restricted period; some of them had to be given supplementary feeding periods to keep their weight up.

During testing, when cortical ChE activity had already recovered to about two-thirds of normal, differences in speed of running were again clear. The injected animals usually required less than two-thirds the running time of the controls on the initial trials of each day. But toward the last of the twelve daily trials, the injected animals showed a sharp rise of running time, so that on some days they became slower than the control animals. This seems to be in keeping with the fact that they could eat less in a restricted period. Presumably the injected animals could not consume much on their last trials and therefore they became less motivated to run rapidly.

In the maze under the progressively solvable condition, the behavior is quantified in a Visual Preference Score. Each of the fourteen injected animals was the littermate of one of the eight noninjected controls. We could then measure the change in ChE compared with the control littermate and the change in behavior compared with the control littermate. Five injected animals had more than 67% of the cortical ChE activity of their littermate controls; their mean Visual Preference Score showed virtually no change from their controls. Nine injected animals had less than 67% of the cortical ChE activity of their littermate controls; their scores were considerably higher in visual preference than their controls. The rank correlation between percentage of remaining ChE and change in Visual Preference was -0.70. The ChE activity of all animals was at least 50% of normal during the maze testing. This appears to be the first study to demonstrate that changes of less than 50% in brain ChE activity can entail measurable changes in animal behavior. More animals are currently being run on this problem.

We have also begun to test the effects of DFP injection on behavior in a multiple-unit T maze. This apparatus is described in another section of the report.

BIOCHEMICAL FACTORS IN MAZE LEARNING: A PRELIMINARY REPORT

James L. McGaugh and Edward L. Bennett,

in collaboration with

Drs. Mark R. Rosenzweig and David Krech, Department of Psychology

In several previous papers we have presented evidence that in the rat the capacity for adjustive behavior is correlated with the level of cholinesterase (ChE) activity in the cerebral cortex.[1] In addition, we have shown that drugs that affect the acetylcholine system (pentobarbital sodium, DFP) also affect adaptive behavior.[2]

To date, all our findings have been based on studies of the preference behavior of rats trained in the Krech Hypothesis Apparatus.[1] Currently, however, we are conducting several studies in which a different apparatus is being used. In this paper, we present a description of this new apparatus and a brief summary of several current studies.

---

[1] Edward L. Bennett and Ruth Deane, in UCRL-3415, June 1956, p. 21; and Edward L. Bennett, in UCRL-3351, March 23, 1956, p. 14.

[2] Mark R. Rosenzweig, David Krech, and Edward L. Bennett, Science 123, 371 (1956).

Apparatus

The apparatus is a 14-unit automatic recording "alley maze." A top view of the maze is shown in Fig. 7. The starting box is at the upper right, and the goal box is at the upper left. The floor plan of the maze is patterned after the maze used by other investigators.[3] Each unit of the maze is 4 in. wide, 4 in. high, and 32 in. long. The walls and floor of the maze are painted a medium grey. In each unit on either side of the choice point there are doors, one leading to a blind alley and the other to the correct path. These doors are hinged at the top, and are painted black. If the rat (see first unit in Fig. 7) pushes on a door leading to a blind alley, a microswitch is activated, and the error is recorded on an electric counter on the control panel (not shown). There is a separate counter for each blind alley. If the rat pushes against the correct door, the door swings up and allows the rat into the next unit. The door then swings down behind the rat to prevent retracing. Thus, the rat must progress through the maze, and, as it does, its behavior is recorded automatically. A bell is sounded when the rat reaches the goal box, so there is no need for the experimenter to watch the rat. A cheesecloth stretched on a frame on the entrance-and-exit side of the maze serves as a sort of one-way screen to obscure the activities of the experimenter and other observers from the rats.

Training Procedure

All rats are first given 8 days of pretraining in a 7-ft straight runway (not shown). During this time they learn to operate the swinging doors, and to eat about 1/3 of their daily ration of food in less than 2 min. The other 2/3 of their ration (10 to 12 g wet mash) is given in the home cages immediately after the daily trials are finished. During the pretraining, all animals are fed sparingly so that they lose about 15% of their normal body weight.

The rats are then trained in the maze, one trial a day, for 14 days. At the end of each run the rats are allowed to eat in the goal box for 2 min. For each animal, daily records are kept of the number of errors made on each trial and of the number of seconds taken to run the maze. These records provide a measure of the individual differences in maze-learning ability. Thus in this apparatus we obtain a measure of adaptive capacity, which is somewhat different from measures we have used previously.

As a behavioral test, this maze has several features to recommend it. First, the scoring is completely objective. Because the behavior is recorded automatically, the probability of making errors in recording is almost eliminated. Secondly, this maze provides a very reliable measure of rate of learning. In this type of maze, reliability coefficients have been found to be about + 0.95. In other words, the individual differences in performance in this apparatus are very stable. Third, this maze provides a very convenient measure of behavior. Since the maze is easy to operate, large numbers of animals can be trained in a fairly short period of time.

---

[3] See section on Effect of Diisopropylfluorophosphate upon Cholinesterase and Behavior, p. 46 of this report.

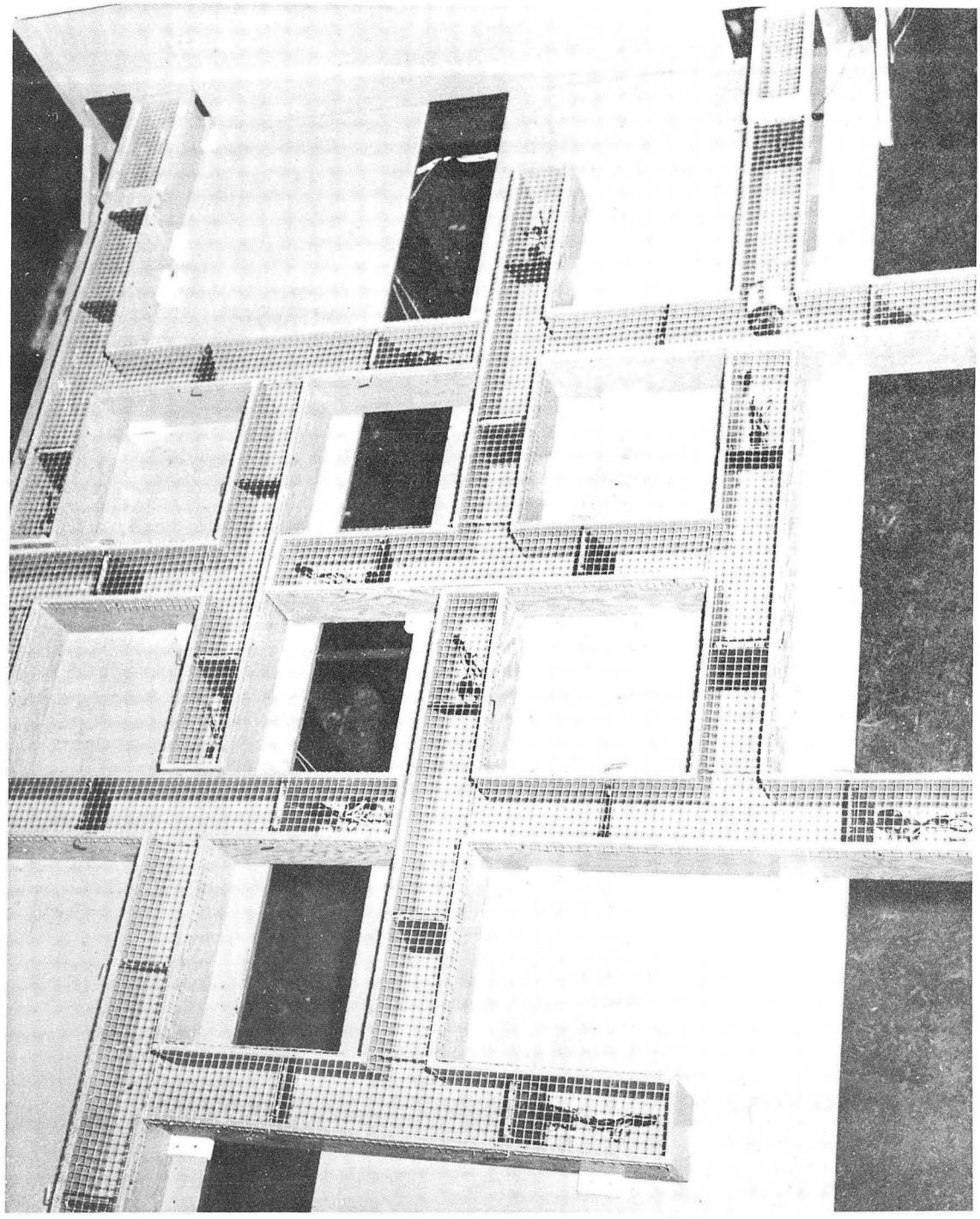


Fig. 7. Top view of 14-unit automatic recording maze. Rat in upper right has just left the starting box and is entering the first unit. Goal box is at upper left.

Experiments in Progress

Strain Differences in Maze-Learning Ability

Figure 8 shows the learning curves for two strains of rats. The upper curve is based on the performance of 16 rats from the S_1 strain of the Department of Psychology colony, and the lower curve is based on the performance of 16 rats from the S_3 strain. From the third day on, the S_3 strain shows a superior performance (fewer errors) compared with the S_1 strain. Although these results are only preliminary, the differences between the two strains are quite interesting, since these strains have been found to differ on other behavioral tests and also to differ in level of brain ChE activity. Since the animals on whose performance these curves are based are still being trained at the present time, we have no measure of their level of brain ChE activity. The speed-of-running curves (not shown) indicate that the two strains also differ in average running speeds. After 10 days of training, the S_1's have an average running speed of about 30 seconds, while the S_3's have an average of about 15 seconds.

Maze Learning and ChE Activity

We are presently analyzing the brains of 12 animals of the S_{13} strain for level of ChE activity. These animals were trained in the maze under the standard procedures described above. Since ChE activity was found to correlate positively with other measures of adaptive behavior, it is expected that animals with high rates of ChE activity will have made fewer errors than animals with low rates of activity.

The Effect of Drugs on Maze Learning

Diisopropylfluorophosphate (DFP) is known to inhibit ChE activity. Depending upon the dosage, the inhibition lasts for 2 to 4 weeks. In another article in this report, we show that DFP affects the behavior of rats in the Krech Hypothesis Apparatus. Platt reported that small doses of DFP facilitated discrimination learning in rats.[4] Since we have shown that ChE is correlated with behavior, we would expect that inhibition of ChE would also affect behavior. In a current experiment a number of rats from the S_1 and S_3 strains were injected with 1.0 mg DFP per kg, and are being trained in the maze. Their behavior will be compared with that of their noninjected littermates. In a preliminary observation, we found that 3 DFP-injected animals were at least as capable, if not somewhat better, learners than 2 littermate controls. These results were only suggested, however, since the number of animals used was so small.

In a recent study by McGaugh and Petrinovich it was found that the learning of a simple maze was facilitated by small doses (0.33 to 1.0 mg/kg) of strychnine sulphate.[5] We are presently repeating this study on the automatic maze, to test the generality of the effects of strychnine. In addition we plan to train rats under a number of different conditions in order to see if strychnine affects only maze learning, or only maze performance, or both learning and performance.

---

[4] C. E. Platt, unpublished doctoral dissertation, Ohio State University, 1951.

[5] J. L. McGaugh and L. Petrinovich, paper read at Western Psychological Association Meetings, 1956.

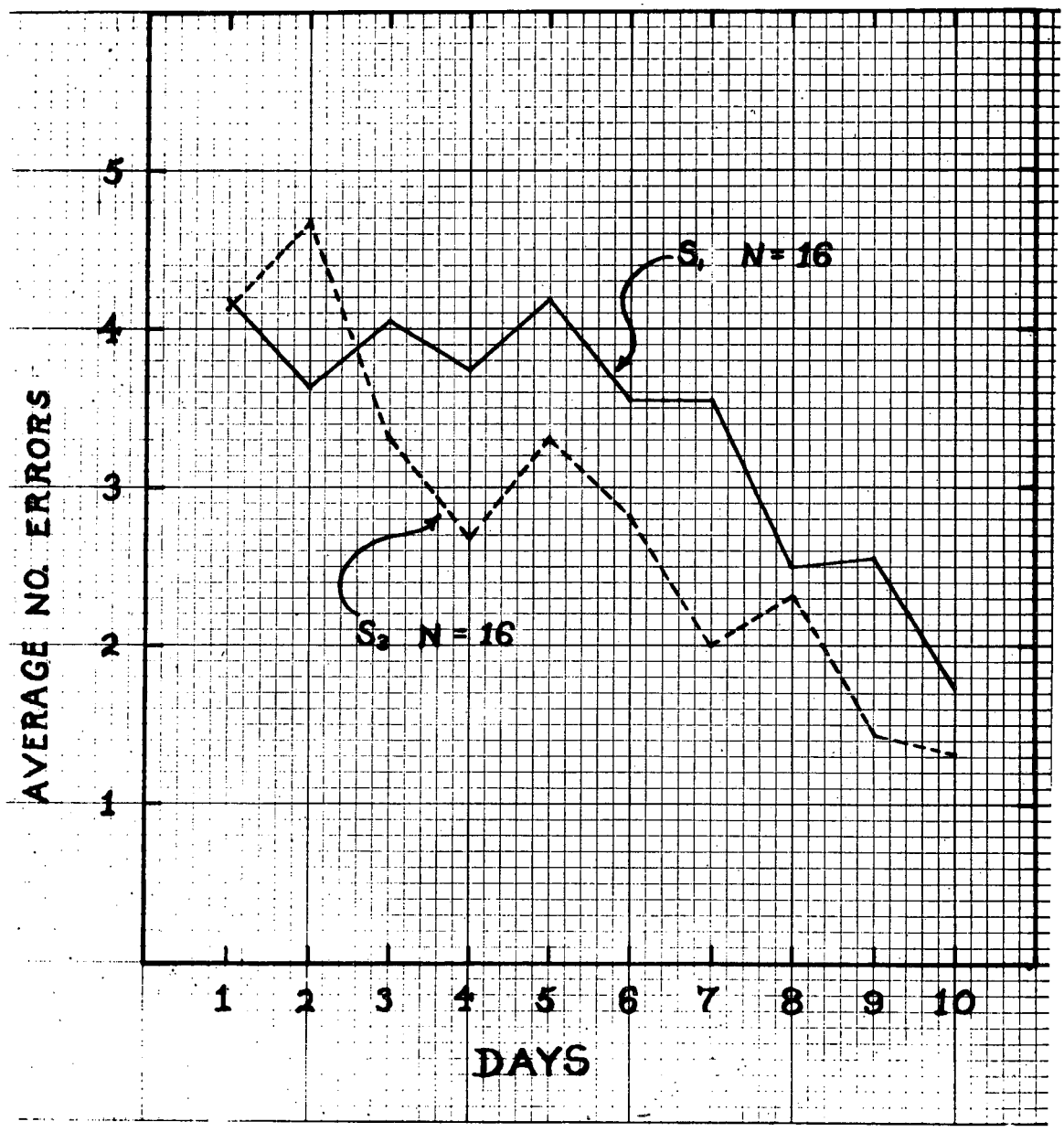


Fig. 8. Learning curves for two strains of rats (S_1 and S_3 of Phychology Department colony).

THE RADIO-WARBURG APPARATUS

B. M. Tolbert and Ann M. Hughes

The standard apparatus used for the study of the metabolism of specific tissue slices or homogenates is the Warburg apparatus. Basically, it consists of a small flask attached to one side of a manometer, the other side of which is open to the atmosphere. The flask is placed on a shaking mechanism and immersed in a water bath at 37°C. Slices of tissue, about 0.5 mm in thickness, are placed in the flask in a nutrient solution. In the course of an experiment, as the tissue respires it absorbs oxygen from the air and liberates carbon dioxide. If the latter is absorbed (usually by a piece of filter paper wet with potassium hydroxide and placed in a well in the center of the flask), then any changes in the pressure in the closed flask system will reflect the utilization of oxygen. The total carbon dioxide produced can also be determined. Thus, one measures rate of oxygen consumption and total carbon dioxide production for specific tissues.

For several years this laboratory has been studying the metabolism to $C^{14}O_2$ of carbon-14 labeled compounds in the intact animal, using an apparatus developed in this laboratory.[1] This instrument measures the rate of $C^{14}O_2$ production and gives data in terms of percent of injected dose excreted as CO_2 as a function of time. Following a single injection of a radioactive substrate, $C^{14}O_2$ measurements are made using a flow-type ionization chamber, together with a vibrating-reed electrometer and recorder. Measurements from this instrument give information that can be interpreted in terms of the metabolism of the specific substrates in vivo.

The two techniques described above have been combined into a new system which we are calling a Radio-Warburg apparatus. It consists of a flask which is held in a shaking mechanism and placed in a water bath. Gas from a reservoir is passed, in turn, through a water-vapor saturate, the flask, a sulfuric acid dryer, the ionization chamber of an ion chamber–electrometer–recorder combination, and into a CO_2 absorber. (See Fig. 9) Tissue slices are placed in the flask in a suitable medium, a labeled substrate is added, and a gas is passed through the system, carrying the $C^{14}O_2$ respired by the tissue through the ionization chamber for measurement and recording of the $C^{14}O_2$ concentration. The $C^{14}O_2$ curves obtained are analyzed in terms of rates of biochemical reactions.

A first major problem involved in developing a workable system based on this idea was to determine conditions which would be reasonably physiologic for the tissue, but which would allow a rapid diffusion of CO_2 from the solution into the gas. The usual media used in Warburg experiments are either Krebs-Ringer phosphate or Krebs-Ringer bicarbonate buffers. The optimum physiologic pH is 7.4, while an optimum for rapid CO_2 turnover would be as acid as possible. Canzanelli and Rapport[2] have studied the effects of pH changes on oxygen metabolism by tissue slices and found that the Q_{O_2} (oxygen quotient) did not drop off significantly within a pH range from 7.0 to 9.4.

---

[1] Tolbert, Hughes, Kirk, and Calvin, Arch. Biochem. and Biophys. 60, 301 (1956).

[2] A. Canzanelli and D. Rapport, Am. J. Physiol. 127, 290 (1939).

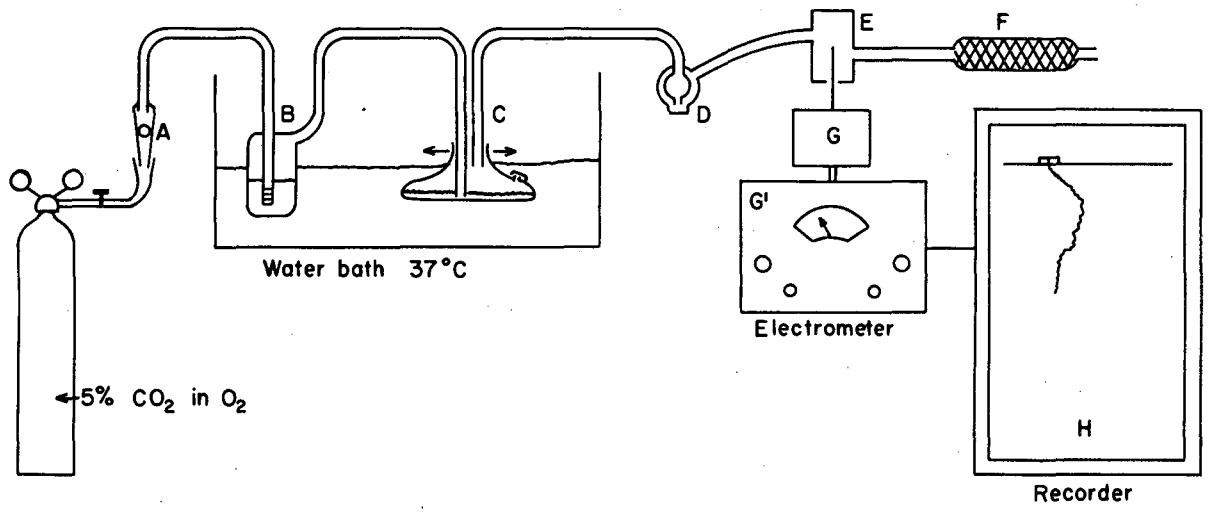


Fig. 9. Radio-Warburg apparatus.

Measurement of the rate at which $HC^{14}O_3^-$ is swept out of a Krebs-Ringer buffer solution as $C^{14}O_2$ was made at various combinations of gas mixtures and pH to determine conditions that would be optimum for a rapid CO_2 turnover, and would still be within physiologic limits for the tissue. The results of these experiments are shown in Tables XIX and XX.

The addition of a small amount of carbonic anhydrase, which catalizes the reaction,

$$HCO_3^- \rightarrow CO_2,$$

greatly shortened the half time for the removal of CO_2 from the solution studied (see Table XXI).

From the data of Table XIX it is evident that the most acid pH consistent with physiologic conditions for the tissue is the best choice from the standpoint of CO_2 turnover. Because a pH of 7.0 is still physiologic for tissue metabolism, that pH was selected for future experiments. The data indicate that a CO_2-O_2 mixture is more effective than pure oxygen as the gas phase, although within the range studied the actual percent CO_2 does not change the half time appreciably. Therefore 5% CO_2 in O_2, which is commercially available and is a standard gas mixture for Warburg work, was chosen as the gas phase. The addition of carbonic anhydrase greatly shortens the half time for CO_2 turnover. However, a small amount is as effective as larger amounts. Therefore 0.1 mg/ml was selected as the amount to be used in future work.

Table XIX

Effect of varying pH on the half time for CO_2 turnover with gas phase maintained at 5% CO_2 in O_2

pH	$T_{1/2}$ (min)
7.2	3.7
6.0	2.7
6.8	1.6

Table XX

Effect of varying CO_2 concentration on half time for $C^{14}O_2$ turnover, with pH maintained constant at 7.2

% CO_2 in O_2	$T_{1/2}$ (min)
0	6.6
0.09	3.6
0.48	2.8
2.98	3.0
5.00	3.7

Table XXI

Effect of carbonic anhydrase on half time for $C^{14}O_2$ turnover, with gas phase maintained constant at 5% CO_2 in O_2, varying the pH

pH	Carbonic anhydrase (mg/ml)	$T_{1/2}$ (min)
7.2	0	3.7
7.2	0.25	1.5
7.2	0.50	1.8
7.0	0	2.7
7.0	0.10	1.3
7.0	0.30	1.3
6.8	0	1.6
6.8	0.10	1.1
6.8	0.30	1.1

AN ATTEMPTED METHOD FOR INSULIN ASSAY USING CARBON-14 RESPIRATORY PATTERNS

B. M. Tolbert and Martha R. Kirk

Data given in a previous report show clearly that the oxidation of glucose to $C^{14}O_2$ in the intact rat can be used as an excellent and reliable criterion of the diabetic state of the animal.[1] Since this oxidation rate is dramatically and consistently changed by the administration of insulin, it was postulated that an index of the insulin activity of human serum might be obtained by injecting a small quantity of the serum into a diabetic animal along with a small amount of C^{14}-labeled glucose and making C^{14} respiration measurements. The amount of increase in glucose oxidation over that which would be found without the insulin-containing serum would thus be a measure of the insulin content of the serum.

Since amounts of insulin in serum are quite small, it is desirable to inject as large a quantity of serum as feasible, but, at the same time, keep the amount of blood which must be withdrawn from a patient as low as possible. For this reason, and also because smaller amounts of C^{14} are sufficient for a good respiration pattern, mice were chosen for this study.

Experimental

It was first necessary to determine what effect, if any, repeated injections of rather large quantities of human serum might have on mice. Therefore, three Swiss mice were each injected intraperitoneally with 1 ml human serum every third or fourth day for six injections. At first the animals lost hair, especially around the abdomen, and the skin of one showed an allergic-type reaction. Otherwise, they seemed healthy and normal and most of the bare patches were again covered with hair before the injections were stopped. Such a marked reaction to the protein does not augur well for a test requiring such injections.

In order to obtain diabetic mice, twelve Swiss male mice, 4 to 6 weeks of age and weighing 12 to 20 grams, were injected intravenously by tail vein with a fresh saline solution of alloxan monohydrate (4 mg/ml) at the rate of 100 mg/kg body weight.[2] Twenty others received 150 mg/kg. There was no consistent difference between the two groups of mice in the experiment described below which attempted to evaluate the degree of diabetic abnormality produced by the alloxan.

Carbon-14 respiration was determined by the ion-chamber continuous-flow-system method described previously.[3] A 250-cc ion chamber and a flow rate of 100 cc/min were used.

---

[1] B. M. Tolbert and Martha R. Kirk, Experiments with Normal and Diabetic Rats Using Carbon-14 Respiration Patterns, UCRL-3503, Sept. 1956.

[2] B. A. Wasbren, Proc. Soc. Exptl. Biol. Med. 67, 154 (1948).

[3] Tolbert, Kirk, and Baker, Am. J. Physiol. 185, 269 (1956).

Calvin

Results

Normal Swiss male mice were shown to oxidize about 50% of an injected glucose-C^{14}_6 dose (0.1 mg, 2 μC C^{14}, injected intraperitoneally) to $C^{14}O_2$ in 2 hours. Injection of 0.5 unit of insulin per kg body weight 15 or 20 minutes before the glucose injection decreases the amount of a tracer dose of glucose oxidized by normal mice in 2 hours to about 30% of the injected dose.

Testing of the diabetic mice was begun about 1 week after alloxan injection. At this time a qualitative test of the urine showed that most were spilling some sugar. The majority of the mice had as much as 2% sugar in the urine, but some were as low as 0.5% and a few were negative. Of the 32 mice alloxanized, two died within 5 days. Two-hour respirations on the rest gave the results shown in Table XXII. Of the 30 animals tested, only two were severely diabetic and six others were potentially severe diabetics. Thus, only those in the first two categories could be used for further testing, and even some of those in the 12–18% group were doubtful severe diabetics. Further tests were made that showed which mice gave consistently quite diabetic C^{14} respiration patterns. In Table XXIII the results of a series of tests with these mice are given. These measurements show that in diabetic mice insulin does increase glucose oxidation to CO_2 by a factor of three to four. However, the increase is not very consistent with insulin dosage, so that the possibilities of a consistent insulin assay based on these measurements are not very good.

In another experiment a diabetic mouse was injected with glucose-C^{14}_6 and a 2-hour respiration pattern made. At the end of this time he was given 40 units insulin per kg and another injection of glucose. Glucose injections were continued at 2-hour intervals for a total of four injections. Each 2-hour section was analyzed separately; the background from the previous injections was subtracted. Total glucose oxidation for each 2-hour period was as follows:

first (no insulin) 15.18%;
second (0 to 2 hours after insulin) 16.17%;
third (2 to 4 hours after insulin) 52.70%;
fourth (4 to 6 hours after insulin) 33.05%.

It thus appears that the maximum increase in glucose oxidation occurs 2 to 4 hours after an injection of insulin.

The above results indicate that mice are rather difficult to make alloxan-diabetic. Mice that do become diabetic, as determined by sugar in their urine, show a decrease in oxidation of glucose to CO_2, although the results are rather variable. Insulin is capable of enhancing the glucose-to-CO_2 oxidation in diabetic mice. Sixty units of insulin per kg cause a diabetic mouse to oxidize 50% of a given dose of glucose-C^{14} to $C^{14}O_2$ in 2 hours, whereas without the insulin only 10% would be so oxidized to CO_2. These results are consistent with our previously reported studies on diabetic rats.

The insulin assay as we have tried it does not seem feasible because of (a) the variability of the response of diabetic mice to insulin, (b) the reaction of mice to serum, (c) the lack of a measurable response to the insulin in 1 ml of normal serum, and (d) the difficulty in preparing suitably diabetic mice.

Table XXII

Glucose oxidation by alloxanized mice

Glucose oxidized to CO_2 in 2 hours (%)	Number of mice	Urine sugar (%)
Less than 10	2	2 or more
12 to 18	6	2
20 to 30	3	0.5 to 0.1
30 to 45	9	0.5 to 0.1
More than 45	10	0.5 to negative

Table XXIII

Glucose oxidation by diabetic mice given insulin and serum

Drugs	Time between drug and glucose	% Glucose oxidized to CO_2 in 2 hours (%)
0 units		15
12 units insulin/kg	15 min	33.27
25 units insulin/kg	15 min	22.78
40 units insulin/kg	15 min	33.54
52 units insulin/kg	15 min	28.55
40 units insulin/kg	1 hour	32.11
60 units insulin/kg	2 hours	49.64
1 ml serum	2 hours	11.57
1 ml serum and 40 units insulin/kg	2 hours	45.66

RESPIRATORY CARBON-14 PATTERNS FOR D- AND DL-LEUCINE AND NORLEUCINE

Martha Kirk and B. M. Tolbert

In a previous report a method for the small scale resolution of the optical isomers of DL-valine are described.[1] In subsequent quarterly reports[2,3] results of the resolution of several C^{14}-labeled amino acids are given, together with respiratory patterns for D-, L-, and DL-valine-4,4'-C^{14}. This report includes results of respiration measurements using leucine-3-C^{14} and norleucine-3-C^{14}.

Experimental

Male rats of the Long-Evans strain and weighing approximately 250 grams were used in these experiments. Animals were fasted for about 20 hours before the beginning of each experiment. Each was then injected intraperitoneally with 2 mg of the amino acid to be tested, containing approximately 7 μC carbon-14. Respiration patterns were determined by use of an ionization chamber in a continuous-flow system.[4]

Results and Discussion

Figures 10 and 11 show a plot of time vs. the total percent injected carbon-14 which is converted to $C^{14}O_2$ in 7 hours after the injection of D-leucine-3-C^{14}, L-leucine-3-C^{14}, and DL-leucine-3-C^{14}. Each curve is the average from several animals. As with valine-4,4'-C^{14},[2] much more of the D-isomer is oxidized to $C^{14}O_2$ in the 7-hour period than of the natural L-isomer. Interestingly enough, the racemic compound is not an average of the two isomers, but shows an entirely different pattern, both in rate and in total excretion. This suggests some antagonism between the isomers when both are present, but further studies are needed to confirm this.

In Fig. 12 are shown the comparative respiratory C^{14} patterns for D-norleucine-3-C^{14} and L-norleucine-3-C^{14}. The difference between these two isomers is hardly significant, and certainly does not compare in magnitude with the differences between the isomers of the other amino acids

---

[1] Patricia T. Adams, in UCRL-3240, Dec. 1955, p. 24.

[2] Martha Kirk, Patricia T. Adams, and B. M. Tolbert, in UCRL-3351, March 1956, p. 21.

[3] Patricia T. Adams, in UCRL-3415, June 1956, p. 50.

[4] B. M. Tolbert, Martha Kirk and E. M. Baker, Am. J. Physiol. 185, 269 (1956).

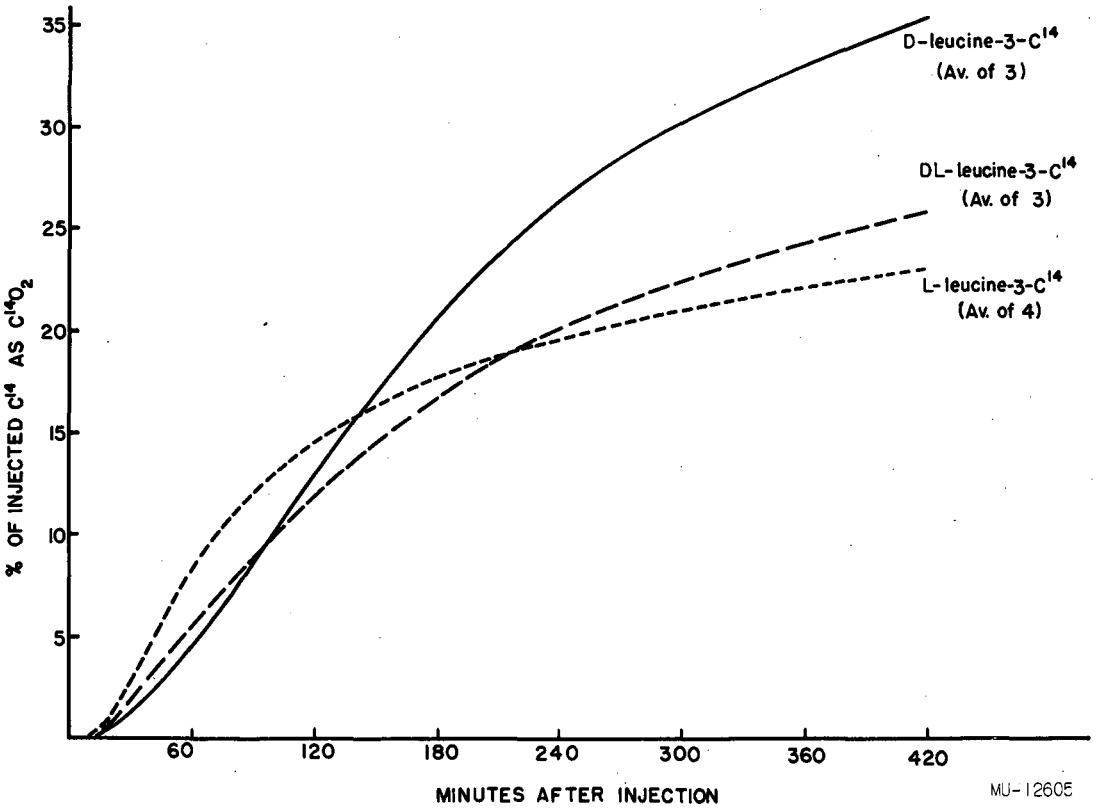


Fig. 10. Respiratory C^{14} patterns: leucine-3-C^{14} isomers.

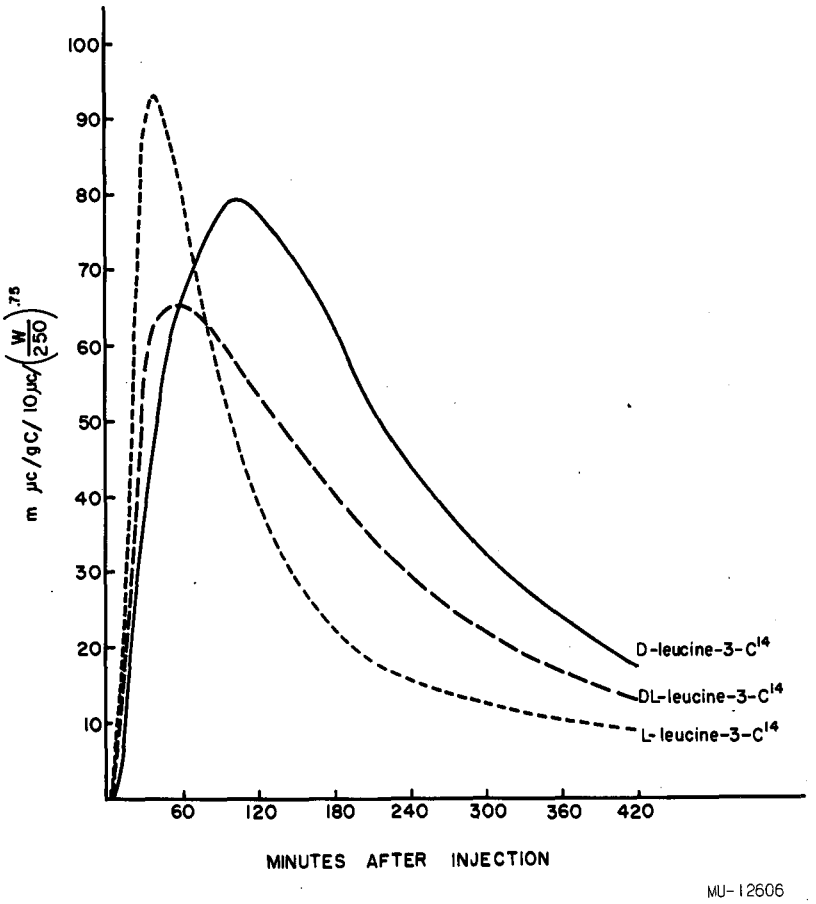


Fig. 11. Specific activity of $C^{14}O_2$: leucine-3-C^{14} isomers.

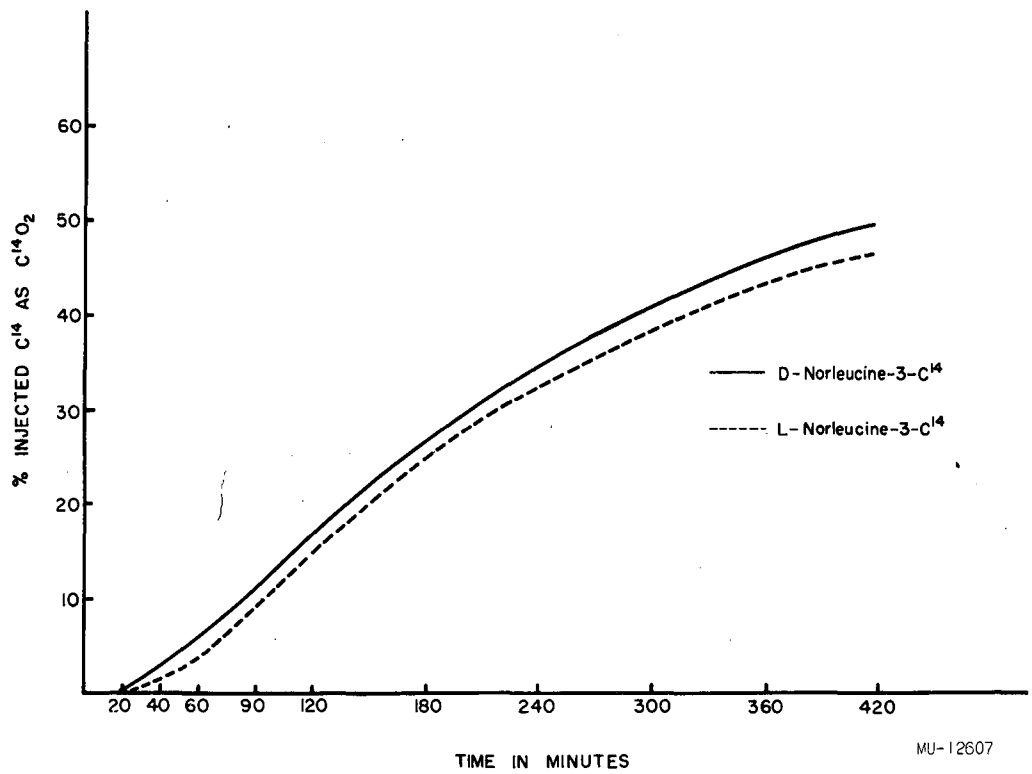


Fig. 12. Cumulative excretion of $C^{14}O_2$ after injection of norleucine-3-C^{14} in normal fasted rats: Comparison of D- and L-isomers (average of 4 rats each).

studied, including methionine.[5] Within the range of the amino acids studied thus far, large differences between isomers occur in valine, leucine, and methionine, which are not synthesized by the body; whereas in norleucine, which is a nonessential acid, there is virtually no difference.

Fasted animals were used in all these experiments because, while oxidation to CO_2 is somewhat slower, it is more consistent than in those animals allowed food "ad libitum," this makes it possible to obtain a more uniform level of metabolic activity among animals.

---

[5] Patricia T. Adams, Martha Kirk, Ann C. Kleerup, and B. M. Tolbert, in UCRL-3351, March 1956, p. 22.

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

DECAY OF EUROPIUM-154

Jose O. Juliano

Numerous investigators have reported on the decay of the 16-year nuclide Eu^{154} to the excited states of Gd^{154}, and there seems to be no agreement so far.[1-5] This fact may be ascribed to the difficulties encountered in having a relatively pure sample, i.e., free from other europium isotopes whose excited states are quite similar. Recently, intensive gamma-gamma coincidence measurements were done by Stephens,[5] and he presented a decay scheme.

The conversion electrons of Gd^{154} from the beta decay of Eu^{154} were studied with the use of permanent magnet spectrographs[6] with resolutions of about 0.1%. The sample's purity was verified by mass analysis to be 154 and 155.[7] With this determination the energies of the different transitions were known accurately and were assigned without any doubt to the gadolinium daughter nuclide. The following transitions were from Gd^{154}.

Energy of transition (kev)	Conversion electrons observed	Energy of gamma rays (kev)	Multipolarity of transition
122.9	K, L_I, II, III, M,N	120	E2
247.9	K, L_I	245	E2
593.3	K, L_I	590	
724.9	K	730	
758.9	K	750	
875.3	K	880	
998.2	K	1010	
1006.9	K	1010	
1276.7	K	1270	
--	--	1600	

1 Hollander, Perlman, and Seaborg, Revs. Modern Physics 25, 469 (1953).

2 Slattery, Lu, and Wiedenbeck, Phys. Rev. 96, 465 (1954).

3 Church, Phys. Rev. 95, 626A (1954).

4 Cork, Keller, Rutledge, and Stoddard, Phys. Rev. 77, 848 (1950).

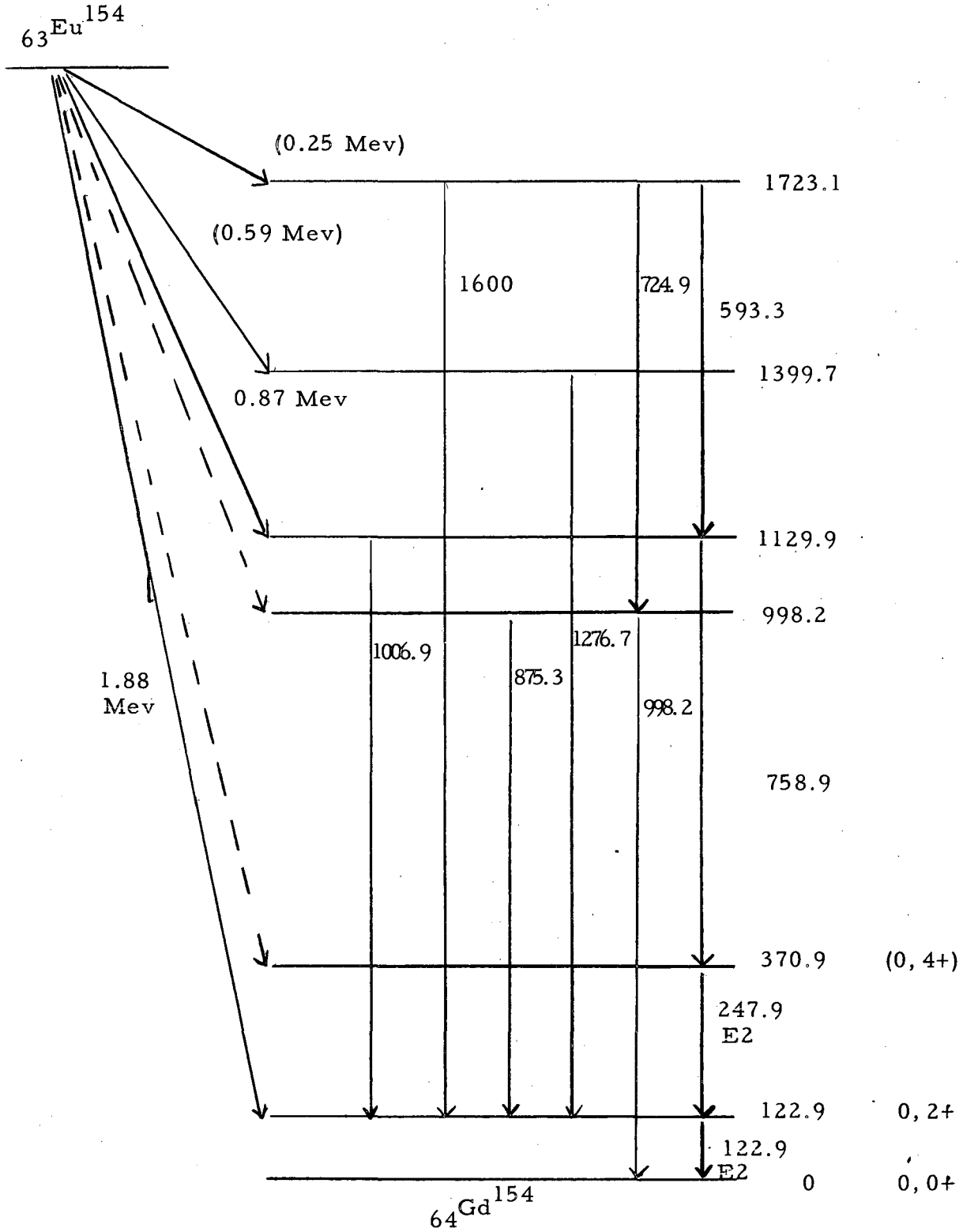
5 Frank S. Stephens, Jr., Decay Schemes and Nuclear Spectroscopic States in the Heavy-Element Region (Thesis), UCRL-2970, June 1955.

6 Smith and Hollander, Phys. Rev. 101, 746 (1956).

7 The mass analysis was performed by Dr. Maynard Michel.

The results confirm the gamma rays observed by Stephens.[5] His decay scheme had two transitions of 1.01 Mev and this is well verified by the 998.2- and the 1006.9-kev transitions observed from the conversion electron spectrum. The multipolarities of the two lowest transitions were assigned from the relative intensities of the L-subshell conversion electrons using Rose's[8] conversion coefficients. This is in agreement with previous reports. In this sample the two highest beta transitions were identified from a magnetic spectrometer measurement as 1.88 and 0.867 Mev. A tentative decay scheme is given on the following page.

[8] M. E. Rose, (privately circulated tables).



Seaborg, Perlman

DECAY OF EUROPIUM-155

Jose O. Juliano

The conversion electrons of Eu^{155}, which beta-decays to Gd^{155} with a reported half life of 2.7 years,[1] was studied by the use of permanent-magnet electron spectrographs of 0.1% resolution.[2] The following transitions were assigned to the Gd^{155} nuclide:

Energy of transition (kev)	Conversion electrons observed	Energy of gamma rays (kev)	Multipolarity of transition
18.8	$L_{II, III}$(?)	18	--
59.9	K, $L_{I, II, III}$, M	--	M1(+E2)
86.5	K, $L_{I, II, III}$, M, N	84	E1
105.2	K, $L_{I, II, III}$, M	102	E1

From Coulomb excitation the following levels were observed by Bjerregaard and Meyer-Berkhaut:[3] 60.2 ± 1.0 and 146 ± 2 kev. The spin of Gd^{155} was assigned by Speck and Jenkins[4] as 3/2. This agrees well with the rotational states with spins 3/2, 5/2, and 7/2 with energies 0, 59.9, and 146 kev as predicted by Bohr and Mottelson.[5] It was interesting to compare the relative intensities of the beta groups decaying to the members of the rotational band 3/2-, 5/2- and 7/2-. Using Alaga's[6] intensity rules and basing on 77% decaying to the 105.2 kev level, one can calculate that only 5% should decay to the 59.9-kev and only 0.6% to the 146-kev level. Therefore, transitions from the 146-kev level, and also the beta group decaying to this level, would be difficult to detect in the decay of Eu^{155}. This is consistent with the conclusion that the 86.5-kev γ ray is not the transition between the levels at 146 kev and 59.9 kev. The tentative decay scheme is given in the diagram. As can be seen, the 18.8- and 86.5-kev transitions can be placed in either of two ways. The multipolarities were assigned from the relative L-subshell conversion electron intensities.

---

1 Hollander, Perlman and Seaborg, Revs. Modern Phys. 25, 469 (1953).

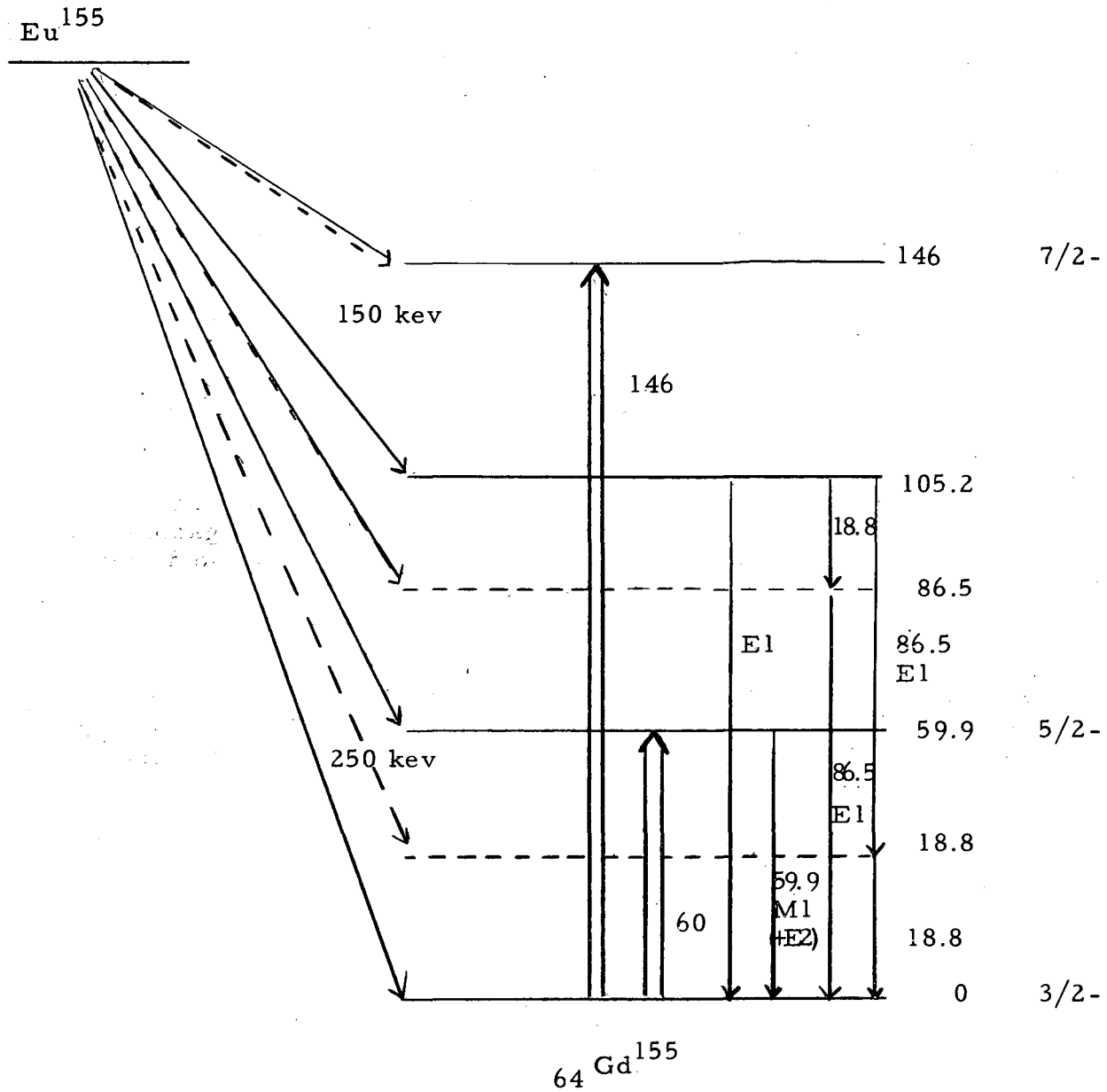
2 Smith and Hollander, Phys. Rev. 101, 746 (1956).

3 Bjerregaard and Meyer-Berkhaut, Z. Naturforsch. 11a, 273 (1956).

4 Jenkins and Speck, Phys. Rev. 100, 973A (1955).

5 Bohr and Mottelson, Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd. 27, No. 16 (1953).

6 Alaga, Alder, Bohr, and Mottelson, Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd. 29, No. 9 (1955).



Seaborg, Perlman

ELECTRON-CAPTURE DECAY OF GADOLINIUM-151

Virginia S. Shirley and John O. Rasmussen

In the study of the decay schemes of Gd^{147} and Gd^{149}, transitions in Eu^{151} resulting from the electron capture decay of Gd^{151} were found. Conversion electrons were seen for strong transitions of 21.59, 153.73, and 175.00 kev and for weak transitions of 63.1, 243.85, and 308.4 kev. Photons were seen for the 153.73-, 175.00-, 243.85-, and 308.4-kev transitions.

Other workers have proposed levels of 20,[1] 195,[2] and 304[2] kev and reported gamma transitions of 20,[1] 110,[2] 195[2], 304,[2] 310 ± 3,[3] 300 ± 15,[4] and 265 kev.[5]

ALPHA-GAMMA COINCIDENCE SPECTRA OF RADIUM-223

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

An electromagnetic alpha-particle spectrograph was used in conjunction with a scintillation pulse-height analyzer to obtain the gamma-ray spectra coincident with each of the prominent alpha groups in the decay of Ra^{223}(AcX).

The spectrograph is a double-focusing 180° machine with a trajectory of 35 cm normal radius; the source and receiver are located outside the magnetic field. The alpha receiver used in this work was a zinc sulfide screen masked with a slit, 1 mm by 1 in., coupled through a light pipe to an RCA-5819 photomultiplier. An alpha transmission of approximately 5×10^{-4} was obtained.

The source chamber is walled with beryllium, for which the gamma-ray absorption is negligible, hence it was possible to use as the gamma detector the usual 1.5-by-1-in. sodium iodide (thallium activated) crystal and RCA-5819 photomultiplier, with a geometry of 0.13.

---

1 H. M. Wilson and G. M. Tervis, Proc. Phys. Soc. (London) 65A, 656 (1952).

2 N. P. Heydenburg and G. M. Temmer, Bull. Am. Phys. Soc. 1, No. 4, 164c3 (1956), (oral report).

3 N. P. Heydenburg and G. M Temmer, Phys. Rev. 100, 150 (1955).

4 H. Mark and G. T. Paullissen, Phys. Rev. 100, 813 (1955).

5 R. E. Hein and A. F. Voight, Phys. Rev. 79, 783 (1950).

The pulses from the alpha and gamma detectors, after suitable amplification, were used as gate and signal respectively, for a 50-channel pulse-height analyzer. A coincidence resolving time of approximately 8 to 10 μsec was employed. In previous experiments of this sort, the very high gamma-ray flux has caused overloading effects in the amplifier to become serious; the use of a double-differentiating amplifier in this work was found to eliminate these difficulties.

The alpha spectrum reported by Rosenblum (HPS, Table of Isotopes) is:

	Mev	Intensity
α_0	5.860	weak
α_{122}	5.730	9%
α_{155}	5.704	53
α_{270}	5.596	24
α_{378}	5.528	9
α_{380}	5.487	2
α_{450}	5.419	3

Asaro and Perlman did not observe the groups at 5.860 and 5.487, setting limits of $\leq$ 1%. A current reinvestigation of the spectrum in this laboratory indicates that both are present in somewhat less than 1%.

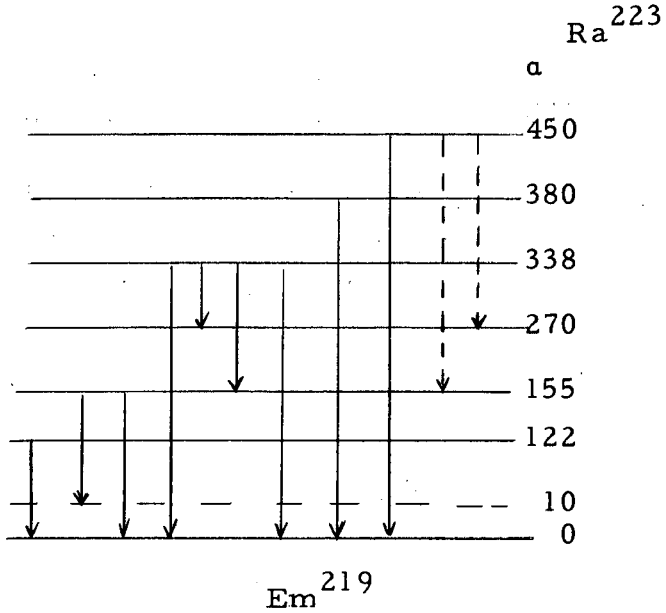
The data obtained in this work are summarized in Table I.

Table I

Characteristics of coincident alpha-gamma radiation from Ra^{223}

Alpha Group	Coincident gamma radiation	
	Energy (kev)	Abundance (photons/alpha particle)
α_{122}	122	0.19
	K x-ray	0.65
α_{155}	145	0.08
	155	0.11
	K x-ray	0.51
α_{270}	270	0.50
	K x-ray	0.39
α_{338}	145 + 155	0.05
	180	0.05
	270	0.02
	338	0.49
	K x-ray	0.24
α_{380}	380	0.60
	K x-ray	0.41
	possible small stopover contributions	
α_{450}	450	0.34
	K x-ray	0.82
	290 + 290	$\leq$ 0.1
	180	$\leq$ 0.03
	155 - 145	$\leq$ 0.03

On the above basis, the following partial decay scheme may be proposed:



The alpha-particle spectrum itself reveals a state lying close to the ground state. The energy separation, although of the order of 10 kev, is not certain.

The angular distribution of the 270-kev photons with respect to the alpha particles populating the state was found to be

$$W(\theta) = 1 + (0.24 \pm 0.10)\, P_2(\cos\theta).$$

Further determination of the alpha spectrum and of the decay scheme is in progress.

NEW NEUTRON-DEFICIENT DYSPROSIUM ISOTOPES

Kenneth S. Toth and John O. Rasmussen

Work has continued on the neutron-deficient dysprosium isotopes.

A stacked-foil bombardment was carried out on gadolinium enriched in Gd^{152} to 15%. The gadolinium was painted on four aluminum foils and bombarded with alpha particles in the 60-inch cyclotron. Suitable aluminum absorber foils were placed between the target foils to give the following approximate energies on the four targets:

Target 1, 48 Mev;
Target 2, 41 Mev;
Target 3, 33 Mev;
Target 4, 23 Mev.

No chemistry was done and only alpha radiation was counted. Alpha-counter curves yielded these activities:

Foil 1, 2.5 hr, with a 17-hr tail;
Foil 2, 5 hr, with a 15-hr tail;
Foil 3, scattered counts;
Foil 4, no alpha activity.

Peaks were not seen in the 48-channel alpha-pulse analyzer. However, when the counts on various channels were plotted on semilog paper vs time, the same activities were seen on Foils 1 and 2 as above. Foils 3 and 4 were not counted in the pulse analyzer.

We believe this experiment mass-assigns the 2.5-hr alpha emitter to be Dy^{152}, and the new 5-hr activity, with an alpha energy of 3.48 Mev (reported in the preceding Quarterly Report) to be from Dy^{153}.

A full-energy (approximately 48-Mev) alpha-particle bombardment with the 60-inch cyclotron was carried out on gadolinium enriched in Gd^{154} to 33%.

Chemistry was performed on the target, including a resin-column separation.

Alpha activity was low. Geiger-counter curves did show a 5-day activity, which accounted for 10% of the total activity. Because of this abundance, it was thought to be Dy^{155}, since the isotope could have been produced by two reactions:

1. $(\alpha, 4n)$ on Gd^{155}, 37% of material bombarded;

and 2. $(\alpha, 3n)$ on Gd^{154}, 33% of material bombarded.

The activity also appeared in a large quantity in a full-energy bombardment on natural gadolinium. This experiment indicates that the activity could not be Dy^{154}, because this latter isotope can be produced only by an (a, 4n) reaction on Gd^{154}, 2% abundance in natural gadolinium. Dy^{155}, on the other hand, can be made in two reactions:

1. (a, 4n) on Gd^{155}, 15%;
and 2. (a, 3n) on Gd^{154}, 2%.

Finally, a low-energy bombardment was carried out on gadolinium enriched in Gd^{154}. This energy, 27 Mev, is below the (a, 3n) threshold. No 5-day activity was seen.

We thus feel that we have mass-assigned the 5-day activity as Dy^{155}, whose principal mode of decay is electron capture.

Stable praseodymium was bombarded with nitrogen ions in the 60-inch cyclotron to form dysprosium isotopes.

The target material was counted directly in the 48-channel alpha-pulse analyzer. No chemistry was done, and with the thick sample no peaks were evident. Half lives noted were 7 min and 19 min, probably corresponding to the alpha emitters previously reported. [1]

Tb^{149}, a 4.1-hr alpha emitter with an alpha energy of 4.0 Mev, also appeared in the alpha-pulse analyzer counts. However, this particular activity grew in with a half life between 6 and 10 min.

It is therefore felt that Dy^{149}, the alpha branching of which is expected to be low, was made in the bombardment, and then electron-captured to Tb^{149} with a half life of 8 ± 2 min.

---

[1] Rasmussen, Thompson, and Ghiorso, Phys. Rev. 89, 33 (1953).

SEARCH FOR SULFUR-38

David R. Nethaway and Albert A. Caretto, Jr.

An attempt has been made to produce S^{38}, which is of interest as it is the lightest mass of the isodiapheric series with N-Z = 6. Since the next higher-mass members of this series are 4-year A^{42} and stable Ca^{46}, one might expect S^{38} to have a measurable half life. This would also be the first example of an unstable even-even sulfur isotope.

A search for S^{38} has also been reported by J. W. Jones,[1] who produced the S^{38} by bombarding Sc_2O_3 with 350-Mev protons, and looked for the well-known Cl^{38} (37.3 min) daughter. He was unable to find direct evidence for its existence, but was able to set exclusion limits for the half life of < 2 hours and >50 years.

In this experiment S^{38} was produced by bombarding NaCl(99.9+%) with 47-Mev alpha particles by the reaction: $Cl^{37}(\alpha, 3p)S^{38}$. This method has the advantage that the number of interfering radioisotopes produced is smaller. The $(\alpha, 3p)$ cross section at 47 Mev for elements in this mass region was measured by isolating the Mg^{28} produced by the $Al^{27}(\alpha, 3p)Mg^{28}$ reaction in the 1-mil aluminum foil used to cover the NaCl during bombardment. The average of three determinations gave σ = 0.13 mb for the latter reaction.

After bombardment the NaCl was dissolved and H_2SO_4 carrier added. The naturally formed Cl^{38} was then removed from the sulfate fraction by repeated boiling with HCl and HNO_3, AgCl scavenges, and $BaSO_4$ precipitation. The sulfate fraction was then allowed to stand so that the Cl^{38} daughter activity could be formed. NaCl carrier was added and a chloride fraction isolated. AgCl was finally precipitated, mounted, and counted in a beta proportional counter. Pure Cl^{38} activity was found on three bombardments.

Calculations made on the best two runs by assuming a 0.13-mb cross section for S^{38} formation gave values for the S^{38} half life of 3.8 and 5.0 min, with an average of 4.4 min. Direct counting of the S^{38} is made difficult by the presence of 5.0-min S^{37}. However, attempts were made to look at both the gamma-ray spectrum and the beta activity. Nothing definite corresponding to an unknown activity was found. It is hoped that future experiments, planned with a quicker chemical separation, repeated milkings of the daughter activity, and gamma-ray spectrum measurements, will produce more conclusive results.

T. P. Kohman and Jean-Claude Roy[2] at the Carnegie Institute of Technology have also found evidence for the existence of S^{38}. Their experiments indicate that the half life is approximately 6 min.

---

[1] J. W. Jones, Spallation Yields from Chlorine with 45-430 Mev Protons (Thesis), Carnegie Institute of Technology, May 1956.

[2] T. P. Kohman and Jean-Claude Roy, private communications.

SELECTION RULES FOR GAMMA TRANSITIONS IN HIGHLY DEFORMED SPHEROIDAL NUCLEI

Richard R. Chasman and John O. Rasmussen

Because of the success of Alaga[1] in correlating beta-decay ft values in the region of large nuclear deformation, with selection rules applicable to single-particle harmonic-oscillator wave functions in cylindrical coordinates, it was decided to derive gamma-transition selection rules in the same representation. In this representation, the quantum numbers of interest are N, Λ, n_z, and Σ. N denotes the total number of nodes in the wave function describing a particular state; Λ, the number of nodes along planes through the cylindrical symmetry axis; n_z, the number of nodes along planes of constant z. Σ denotes the projection of the intrinsic spin along the symmetry axis.

The wave function ψ is of the form[2]

$$\psi = F_{n_\rho}^{|\Lambda|}(\rho)\, e^{i\Lambda\theta}\, e^{-aZ^2/2}\, H_{n_z}(\sqrt{a}\, Z).$$

A transition is allowed if the integral of the transition operator between the initial and final states of the system is nonzero.

The selection rules are:

E1				M1			
ΔN	Δn_z	$\Delta\Lambda$	$\Delta\Sigma$	ΔN	Δn_z	$\Delta\Lambda$	$\Delta\Sigma$
+1	+1	0	0	0	0	0	±1
-1	-1	0	0	0, ±2	±1	±1	0
±1	0	±1	0	0, ±2	0	0	0
E2				**M2**			
+2	+2	0	0	±1, ±3	±1	0, ±2	0
-2	-2	0	0	±1, ±3	0	±1	0
0, ±2	0	0	0	±1, ±3	±2	±1	0
0, ±2	±1	±1	0	±1	0	±1	±1
±2	0	±2	0	±1	±1	0	±1

[1] G. Alaga, Phys. Rev. 100, 432 (1955).

[2] Pauling and Wilson, Introduction to Quantum Mechanics (McGraw-Hill, 1935), p. 105 ff.

PARAMAGNETIC RESONANCE OF CURIUM

M. Abraham, Burris B. Cunningham, Carson D. Jeffries, Robert W. Kedzie, and James C. Wallmann

(Department of Physics, Department of Chemistry, and Radiation Laboratory)

The microwave paramagnetic resonance of 185 μg of Cm^{244} as Cm^{+3} ions incorporated in a 183-mg single crystal of anhydrous $LaCl_3$ has been observed at 77°K. The spectrum consists of seven lines, and--to first order--may be fitted by the spin Hamiltonian

$$H = g\beta \vec{H} \cdot \vec{S} + D[S_z^2 - (1/3)S(S+1)],$$

where $g \simeq 2.00$, $S = 7/2$, and $D \simeq 0.0010\ cm^{-1}$. The spectrum is similar but not identical to that of Gd^{+3} in the same crystal structure. These results indicate that Cm^{+3}, like Gd^{+3}, has a ground state $^8S_{7/2}$. Qualitatively similar results have been obtained by B. Smaller of Argonne National Laboratory (private communication).

FLUORESCENCE AND ABSORPTION AND ZEEMAN SPECTRA OF THE ACTINIDES

John G. Conway, Ralph D. McLaughlin, George V. Shalimoff, Burris B. Cunningham, and James C. Wallman

Fluorenscence and Absorption of Cm^{+3}

A single crystal of $LaCl_3$ containing 0.1% $Cm^{244}Cl_3$ has been prepared. Seven fluorescence lines and four absorption lines have been photographed. A second crystal is being prepared so that these original observations may be verified.

Zeeman effect of Am^{+3}

Preliminary observations of the Zeeman effect on Am^{+3} have been made. Six lines show clearly resolved patterns. The line at 4626 Å which appears in fluorescence and absorption spectra is one of these lines. The interpretation of this line should greatly aid in the analysis of the fluorescence spectrum.

RADIATION CHEMISTRY

Amos S. Newton, Aldo Sciamanna, and Jean H. Futrell

Experiments have been done to check the effect of temperature on the radiolysis of some organic compounds, with special reference to unsaturated compounds that do not polymerize under irradiation. The results of increasing the temperature from 25°C to 80°C have not been as pronounced as expected. The principal result is a general increase in over-all yields of all products, suggesting much less back reaction at the higher temperature. The high-boiling products from trichlorethylene, 1,2 dichloroethylene, and isopropenyl acetate has increased by a factor of about 2 to 3, and this represents the largest effect that has been observed. Details will be published elsewhere. Attempts to determine the composition and structure of the high-boiling products from these compounds by infrared and high-temperature mass spectrometry have given no positive clue as to the compounds present other than indications from mass spectrometry that a dimer of the original molecule is present. Work is continuing on the separation of these high-boiling products into pure compounds.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

NOTES ON WORK IN PROGRESS

CORRELATION OF LIMITING CURRENT DENSITY AT HORIZONTAL ELECTRODES UNDER FREE CONVECTION CONDITIONS

Eugene J. Fenech and Charles W. Tobias

This work has been completed and will be reported separately in UCRL-3586.

STABILITY OF PERFORATED-PLATE TRAYS

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

Stability data have been taken, using the air-water system, on two plates each with 0.25-inch diameter perforations but with varying areas (4.2% and 15.7% of the column area). Equivalent clear liquid heads on the plate have been varied from 0.5 in. H_2O to 3.5 in. H_2O. The reproducibility is good.

No satisfactory generalized correlation of the results has been found to date.

GAS-LIQUID PARTITION CHROMATOGRAPHY (GLPC)

Robert H. Houston and C. R. Wilke

Factors for correlating experimental data from a GLPC column with theoretical solution of a model column have been established. Experimental data are being collected. A theoretical equation has been programmed for the IBM 650 computer, and solved for various values of column parameters.

MULTISTAGE SEPARATION PROCESS STUDIES WITH AN IBM 650 DIGITAL COMPUTER

J. H. Duffin, Donald N. Hanson, and C. R. Wilke

A distillation column has been simulated by the above computer, and the program has evaluated an infinite-petroleum-mixture problem for the normal fractionation case. Presently being attempted on the computer is the solution of a similar infinite-petroleum-mixture problem for the more complex case of a steam-stripped fractionator with steam-stripped side columns.

LIQUID-LIQUID EXTRACTION AND AGITATION: COALESCENCE IN TWO-PHASE AGITATED SYSTEMS

J. H. Vanderveen and T. Vermeulen

Numerous data have been obtained on the variation of specific interfacial area with height in the mixing vessel. These show that the rate of coalescence increases with decreasing interfacial tension and with increasing volume fraction of dispersed phase. Viscosity does not appear to affect the rate, but does influence the distribution of specific area at high coalescence rates. A quantitative correlation of these results is now being developed.

PERFORMANCE OF SPHERE-PACKED EXTRACTION COLUMNS

G. Jacques and T. Vermeulen

Three 6-inch-diameter columns have been assembled, and tested hydraulically. Calibration of the built-in conductivity cells is in progress. The first experimental stage, the determination of longitudinal-diffusion rates in single-phase flow, will follow immediately.

EXTRACTION RATES INTO SINGLE DROPS

Terukutsu Miyauchi and Theodore Vermeulen

The system uranyl nitrates–nitric acid–water–pentaether has been studied previously in this laboratory. Apparatus is now being assembled for a study of the mechanism and rate of extraction, for both uranyl nitrate and nitric acid, in this system.

ELECTROCHEMICAL STUDIES IN NONAQUEOUS SOLVENTS

W. S. Harris and Charles W. Tobias

Encouraging results given by a qualitative study of the conductivity and solubility of inorganic salts in propylene carbonate have led to a quantitative study. Special solubility and conductivity cells have been built to exclude moisture. A vacuum line has been constructed for the drying of salts. For the solvent, a method of drying to < 20 ppm has been devised.

GENERAL CHEMISTRY

Robert E. Connick and Leo Brewer in charge

METALS AND HIGH-TEMPERATURE THERMODYNAMICS

Absolute Lifetime Apparatus

C. Geoffrey James, Fred Stafford, and Earl Worden

The electronic equipment is expected to be sufficiently completed to install in the laboratory by February. Work is progressing on arc light sources. An electronic bombardment furnace for the TiO, NO, and C_2 sources is being designed.

Ground State of C_2

William T. Hicks

Calculations of C_2 data are almost complete and are being written up.

Study of MgOH Spectrum

Frank T. Greene and C. Geoffrey James

Deuterium gave no isotope shift of ultraviolet bands of MgO, thus excluding MgOH as the source of the bands.

Stability of SiO Solid

Frank T. Greene

A paper describing the final results of this work is almost complete.

Ruthenium Chemistry

Howard Cady

During the last quarter work has continued on the separation and identification of the species present in a perchloric acid solution of three-valent ruthenium. Both separation and identification are performed using ion-exchange resin. Since the ruthenium may hydrolyze or polymerize it is necessary to determine the charge per ruthenium atom as well as the charge per ruthenium species in solution. It is hoped that perchlorate will not complex the ruthenium.

One experiment separates the ruthenium species and yields information that can be used to determine the charge per ruthenium atom. This experiment requires an ion which is held more strongly by the resin than any of the ruthenium species of interest. The strongly held ion is used to push the ruthenium species out of a column ahead of it. The species separate from one another, with the most loosely held ions coming out of the column

first. The charge per ruthenium atom is determined by equating the charge per ml in the eluting solution to that in the solution containing ruthenium. After appropriate corrections for the other cations in the ruthenium solution the charge per ruthenium atom is calculated.

The charge per ruthenium species is determined by equilibrating a solution containing the ruthenium and another ion of known charge (H^+) with a small quantity of resin. The solution is diluted and reequilibrated. From the change in the aqueous ruthenium concentration with dilution the charge on the ruthenium species can be calculated.

Preliminary experiments indicate that the previously described colorless Ru(III) in perchloric acid is Ru^{+3}. There is also some other Ru(III) species, which as yet has not been identified, but it is held more strongly to the resin than Ru^{+3} and has a lower charge per ruthenium atom than +3.

Determination of the Molecular Structure of Aluminum Hydride

R. F. Nickerson

During the last quarter effort was made to eliminate difficulties encountered with the nuclear induction probe described in previous reports. The major difficulty was in obtaining a sufficiently stable balance of the radiofrequency leakage to the receiver coil from the transmitter coil. The method used was to tap radiofrequency current from the transmitter circuit, attenuate it with a ladder-type attenuator, and introduce the necessary phase shift with a continuously variable delay line. The resultant radiofrequency voltage was then coupled into the receiver circuit through a small capacitor. Hence the leakage to the receiver coil that cannot be eliminated geometrically is balanced by additional external leakage of the proper phase and amplitude.

The Heat of Formation of the Ferrate Ion

Robert Wood

The heat of reaction of 50 ml of from 0.005 to 0.02 M K_2FeO_4 in 10^{-4}M NaOH with one liter of 0.500 M $HClO_4$ has been determined calorimetrically, using two different samples of K_2FeO_4. The calculated value for the ΔH_f° of $FeO_4^=$ was -116 ± 1 kcal/mole, using -107.3 kcal/mole as the estimated heat of formation of $Fe(ClO_4)_3$ in 0.500 M $HClO_4$. Using 23 and 9 eu respectively for the entropies of $Fe(OH)_3$ and $FeO_4^=$ gives a value of -0.91 ± .02 volt for the standard potential of the ferrate ion going to ferric hydroxide.

Attempts to reach an equilibrium between the $FeO_4^=$–$Fe(OH)_3$ couple and either the ClO^-–Cl^- or BrO^-–Br^- couple have failed.

Chemical Shifts in the Fluorine-19 Nuclear Magnetic Resonance in Aqueous Solutions of Inorganic Fluorides

Richard E. Poulson

Some nuclear magnetic resonance studies are covered in a separate report, recently issued.[1]

Kinetics of Rapid Reactions

Claude Coppel

At the time of the last report the rapid mixer was being used to check the heats of activation of the ferric thiocyanate complexing reaction. During these experiments it was found that a more accurate method for measuring the temperature in the mixer was needed. Previously samples had been removed carefully from the mixer and their temperature measured. This was found to give errors up to 1°, and even at room temperature showed deviations up to several tenths of a degree.

The method now used to measure temperature employs a calibrated thermistor inside the mixer. The resistance of this thermistor is measured with a simple instrument of the Wheatstone bridge type to an accuracy of about ± 2 ohms, which gives the temperature to about ± 0.05°. Because of fluctuations of temperature inside the mixer, it is felt that the actual temperature is known to about ± 0.10°.

The entropies of activation previously obtained by Dr. Below did not agree well with values in similar reactions, and were strongly dependent on a single low-temperature measurement. Also the temperatures themselves were questionable in view of the preceding discussion. The repeated experiments gave results quite different from those of Below. They were compared with entropies of activation for chromic thiocyanate complex formation; with entropies of activation of $Cr(NH_3)_5 H_2O^{+++}$ and $Co(NH_3)_5H_2O^{+++}$ reacting with thiocyanate to form complexes; and also with equilibrium entropies of formation of fluoride complexes of iron. All agreed within the probable errors. At the present time a final check is being made on the values, which was necessitated by a suspected difficulty in the response of the oscilloscope.

One further experiment was done in connection with this reaction. The equilibrium constants for the ferric thiocyanate complexing and the heat of this reaction had been taken from the leterature. These values had been measured at an ionic strength of 1.28 and our experiments were at an ionic strength of 0.40. The work was now repeated at our experimental conditions. This was done by measuring the concentration of ferric thiocyanate complex in known mixtures at various temperatures. The measurements were made in a Beckman DU spectrophotometer, and temperature measurements were made by placing a thermistor directly in

[1] Richard E. Poulson, Nuclear Magnetic Resonance Studies of Some Inorganic Molecules and Ions (M.S. Thesis), UCRL-3567, Oct. 1956.

the absorption cell. This gives a very easy method for measuring both optical density and temperature in the cell. These measurements gave results that supported both the K and OH values used in our calculations.

Oxygen-17 Relaxation by Paramagnetic Ions

Richard Poulson

From nuclear magnetic resonance line widths, the order of effectiveness in relaxing of the O^{17} nucleus in H_2O has been determined for the following paramagnetic ions.

Ion	Mn^{++}	Cu^{++}	Fe^{+++}	Ni^{++}	Co^{++}	Cr^{+++}
Concentration for equal broadening	10^{-4}M	10^{-3}	2×10^{-3}	10^{-2}	10^{-1}	1

Oxygen-17 Chemical Shifts

Richard Poulson

The O^{17} nuclear magnetic resonance of UO_2^{++} in 5 M UO_2Cl_2 has been observed to be 11.3 ± 0.1 gauss below the H_2O resonance at 10^4 gauss.

P_4S_3: Chemical Shifts and Spin-Spin Interactions

Richard Poulson

The P^{31} resonance in solutions of P_4S_3 in CS_2 consists of two chemically shifted groups in the ratio of 3 to 1. The resonance of relative abundance 3 is split into a 1:1 doublet. The single phosphorus resonance is a 1:2:1 triplet. The chemical shifts are: 3 P at + 119 ppm and 1 P at -68 ppm, referred to aqueous H_3PO_4.

Aluminum Fluoride Complexes in Concentrated Aqueous Solutions

Richard Poulson

By a nuclear magnetic resonance method, equilibrium quotients have been determined for the following reactions in concentrations up to 2 M aluminum nitrate and sodium fluoride:

$$AlF^{+} \rightleftharpoons Al^{+++} + F^{-},$$

$$AlF_2^{+} \rightleftharpoons AlF^{+} + F^{-}.$$

At low concentrations the quotients agree with those of Brosset and Orring for an ionic strength of 0.5. The value $(AlF^{+})/(AlF_2^{+})$ was obtained from the F^{19} resonances of these two ions.[1] The aluminum ion concentration was determined from the Al^{27} resonance in these solutions.

Connick, Brewer

Information Division
1-18-57 sa