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Radiation Laboratory

BIO-ORGANIC CHEMISTRY QUARTERLY REPORT
September, October, November 1958

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Lawrence Radiation Laboratory and Department of Chemistry
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EXTERNAL RADIATION AS AN AID IN EXCHANGE TRITIUM LABELING

Richard M. Lemmon, Bert M. Tolbert,
Walter Strohmeier, and Irville M. Whittemore

The method described by Wilzbach[1] for the labeling of organic compounds by exposure to tritium gas has come into wide use. The compound to be labeled is exposed to subatmospheric pressures of tritium gas at room temperature for a few days. The radioactive gas is then removed and the exposed compound is rigorously purified by recrystallization, distillation, or chromatography. By means of this technique, labeled organic compounds with specific activities of the order of 10 mC/g may be obtained.

It has been shown that reactions with recoiling tritons from the labeling gas are not the principal process by which the organic substrate becomes labeled.[2] The most likely process appears to be reactions between ionized or excited organic molecules and the tritium gas, a supposition furthered by the work of Ahrens et al.[3], in which was measured the product distribution obtained by exposing hydrocarbons to tritium-labeled hydrogen and to γ radiation. It therefore seemed to us that the exchange labeling could be greatly speeded up by providing the system with an external source of radiation. In order to test this conclusion we have applied, separately, γ rays from a Co^{60} source and a silent electrical discharge to a benzene-HT system.

Experimental Procedure

Storage and Dilution of Tritium

About 20 curies of tritium gas was converted to uranium tritide, UT_3. The apparatus used for this purpose, and for subsequent transfers of T_2 and H_2 to the benzene, is shown in Fig. 1. The volume of the entire vacuum-line assembly was known. The volumes were also known of the three individual segments, i.e., the part lying to the left of stopcock No. 1, the part lying between stopcocks No. 1 and No. 2, and the part lying to the right of stopcock No. 2. Uranium hydride was placed in the small stainless steel container and was heated at 450° under high vacuum. When all the hydrogen was removed (as shown by the manometer) 20 curies of isotopically

[1] K. E. Wilzbach, J. Am. Chem. Soc. 79, 1013 (1957).

[2] P. Riesz and K. E. Wilzbach, J. Phys. Chem. 62, 6 (1958).

[3] R. W. Ahrens, M. C. Sauer, and J. W. Williard, J. Am. Chem. Soc. 79, 3285 (1957).

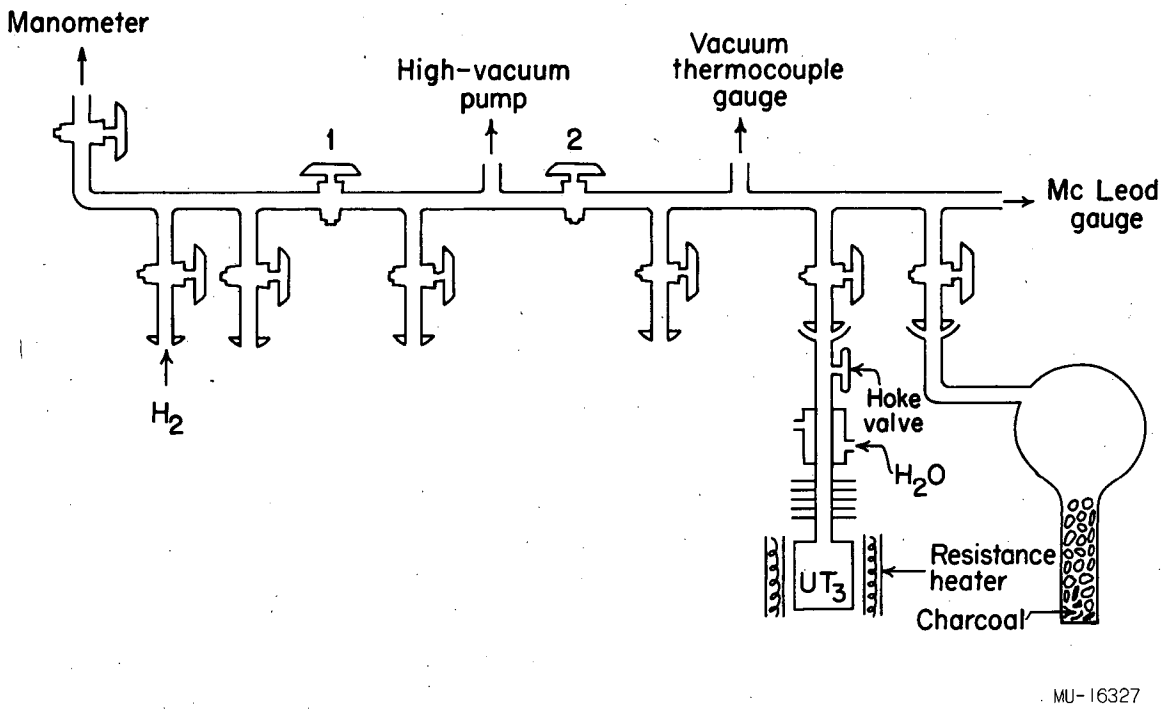

Fig. 1. Vacuum line for handling tritium.

pure T_2 was admitted to the system and absorbed on the uranium at room temperature.

For the irradiation experiments the tritium was diluted as follows: The UT_3 was heated (450°) until the manometer indicated that a desired amount of T_2 was present in the system. The stopcock leading to the furnace was then closed and the T_2 was absorbed on the charcoal at -196°. The stopcock leading to the charcoal was closed and H_2 was admitted into the system until a desired pressure was reached. The H_2 was then also absorbed at -196° onto the charcoal which already contained the T_2. In this way, any desired dilution of the tritium could be obtained. This mixture was removed from the charcoal by allowing it to come up to room temperature.

Irradiation Experiments

To determine the effect of γ radiation on the incorporation of tritium into benzene, the following experiment was performed: In each of two similar glass vessels (40 ml volume) were placed 600 λ of benzene and a mixture of H_2 and T_2 (0.08% T_2; sp. act. 2.014 mC/cc at STP; total activity 25 mC). The total pressure in each vessel was 330 mm (benzene = 80 mm, H_2-T_2 = 250 mm). The vessels were then glass-sealed. One vessel was allowed to stand for 24 hours at room temperature; the total energy expended in this vessel by the tritium was 4.7×10^{17} ev. Of this total energy, a little more than half, or about 2.5×10^{17} ev. was absorbed by the gas mixture. The other vessel was irradiated for 24 hours at room temperature with γ rays from a Co^{60} source. Its total dose was 2.7×10^{19} ev. The vessels were then opened, through break-off seals, on a vacuum line. The hydrogen-tritium gas was removed and the irradiated benzene samples were purified to constant specific activity by vapor-phase chromatography. The specific activity of the benzene was determined by scintillation counting in diphenyloxazole-toluene solutions. Two passes through a 5-ft paraffin column were enough to accomplish this; further passes through paraffin, silicone, "carbowax," or "Ucon" columns led to no changes in the specific activity. In the "control" benzene a total of 0.565 μC was incorporated; in the γ-irradiated sample a total of 3.85 μC was incorporated. The tritium labeling in the irradiated sample was therefore greater by a factor of 6.8.

A second experiment was performed in exactly the same way as the first except that the total pressure in the vessels was 150 mm (benzene = 80 mm, H_2-T_2 = 70 mm). The total energy expended in the control vessel was 1.3×10^{17} ev; the amount absorbed was about 7×10^{16} ev. The γ-irradiated vessel received an additional 2.7×10^{19} ev. The total incorporations obtained were 0.16 μC for the control and 1.11 μC for the γ-irradiated sample. The incorporation was therefore increased by a factor of 6.9.

Not all laboratories have a good source of γ radiation. We therefore decided to test the effect of a silent electrical discharge on the Wilzbach technique. Benzene (600 λ) was exposed to 40 mC of tritium (partial pressure of H_2 and T_2 = 210 mm) in the apparatus shown in Fig. 2. The volume of the space occupied by the benzene was about 60 ml; therefore

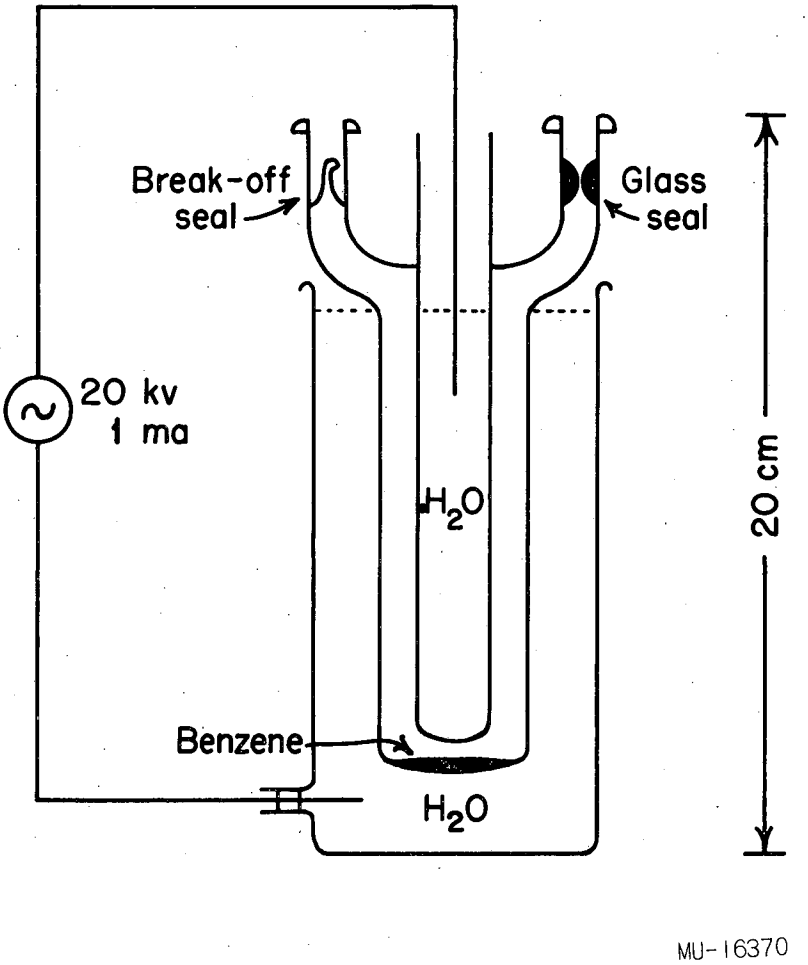

Fig. 2. Apparatus for tritium incorporation under silent electrical discharge.

only 4% of the benzene was in vapor form and the remainder was as a liquid in the bottom of the tube. When a silent discharge (ac, 20 kv, 1 ma) was passed through for 1 hour, 0.67 μC was found to have been incorporated into 500 λ of the purified benzene. In a second experiment (with the entire assembly in a horizontal position to spread out the liquid benzene) passage of the current for 5 hours (46.5 mC, 256 mm partial pressure of the H_2-T_2 gas) resulted in the incorporation of 5.56 μC into a 200-λ aliquot portion of the purified benzene. Without the discharge the Wilzbach technique would have given benzene with a total activity of only about 0.04 μC (1 hour) or 0.2 μC (5 hours). Consequently, there is a real advantage to the use of the electrical discharge.

Some preliminary experiments have been carried out in this laboratory to label sucrose by exposure to tritium gas and γ radiation. These experiments indicate that for increasing the exchange of tritium into the molecule, γ radiation is as successful with sucrose as it is with benzene.

CARBON-14 BOMBARDMENT OF ORGANIC COMPOUNDS

Robert Mullen, Richard M. Lemmon, and Frederick L. Reynolds

Recent studies have shown that C^{14} can be introduced into organic compounds by bombardment with C^{14} ions. It has been noted that by bombardment of various organic compounds with C^{14} ions, both labeled target compound and labeled methyl homologs of the target compound are often formed.[1-4]

Three methods have been used to generate the C^{14} ions:

1. Nuclear recoil, in which nitrogen-containing compounds are subjected to a neutron flux forming recoil C^{14} by the reaction $N^{14}(n, p)C^{14}$.

   If it is desired to investigate a compound that does not contain nitrogen, an inert nitrogen source must be homogeneously dispersed with the compound studied.

2. Radiation-induced gas-phase reactions,[5] in which some simple C^{14} source (such as CO_2, or C_2H_2), the compound to be irradiated, and a source of β-radiation (such as Kr^{85}) are mixed in the gas phase.

3. Ion acceleration, in which a mass spectrometer is used to isolate and accelerate the desired ions.

The ion-acceleration method possesses several distinct advantages over the first two methods. One is certain of the identity of the bombarding ion, which is not the case in the gas phase reaction, where $C^{14}O_2$, $C^{14}O$, and C^{14} ions are all formed as well as the respective C^{12} ions. No extraneous radiation is present, so that one avoids the radiation damage that is invariably associated with the nuclear-recoil method and, to a lesser extent, the gas-phase method. Finally, one has some control over the energy of the impinging ion, whereas in the recoil method, the C^{14} formed has a very high energy (around 45,000 ev) compared with that of chemical bonds, and is in itself a source of radiation damage.

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[1] Richard M. Lemmon, Frederick L. Reynolds, and Franco Mazzetti, in Chemistry Division Quarterly Report, UCRL-3595, Sept. 1956, p. 5.

[2] Richard M. Lemmon, Frederick L. Reynolds, Ann Coombs, and Melvin Look, in Chemistry Division Quarterly Report, UCRL-3629, Jan. 1957, p. 25.

[3] A. P. Wolf, B. Gordon, and C. S. Redvanly, Abstracts of Papers, 131st Meeting of the American Chemical Society, Miami, Florida, April 1957, p. 12-0.

[4] For a complete literature citation see A. P. Wolf, C. S. Redvanly, and R. C. Anderson, J. Am. Chem. Soc. 79, 3717 (1957); also Reference 5 below.

[5] C. N. Turton, Second United Nations International Conference on the Peaceful Uses of Atomic Energy, A/Conf. 15/P/284, United Kingdom, May 19, 1958.

In order to study the bombardment of organic compounds with C^{14} ions under optimum conditions, an isotope separator has been designed and the experimental model completed, with satisfactory results.

The isotope separator employs a capillary arc ion source and a 90° magnet. It is designed to yield a C^{14} beam of around 1 μamp intensity which can be delivered to the target with an energy variable down to essentially zero. The energy is controlled by a variable positive potential on a grid connected to the target container at the end of the magnet chamber. At present the voltage of the ion source, hence the maximum energy of the ions, is 6 kv. The ion source, magnet chamber, and target container can be closed off from one another and pumped out separately.

Carbon-14-enriched CO_2 gas is bled into the ion source through the hollow anode, and the positive ions formed are drawn out into the focusing electrode system, which focuses the C^{14} ions on a slit at the front of the magnet chamber. Liquid targets, or gaseous targets if their freezing point is not too low, may be bled into the target chamber and frozen on its surface; solids may simply be painted on the surface. Excessively low temperatures must be avoided to prevent condensation of CO_2 on the target.

Several problems encountered with the experimental model will be eliminated or at least lessened in the final isotope separator. The greatest difficulty found in the experimental model was that the glass-enclosed metal-ring focusing electrodes could not be moved while the beam was on, which made efficient focusing of the beam on the slit very difficult. This will be eliminated in the final model by using a movable, all-metal electrode system with a detachable tip, and an externally movable slit. The new ion source will have an easily changed filament, allowing a fairly rapid rate of filament burnout to be tolerated, if necessary. Diluting the CO_2 gas with argon has also been found to help the burnout problem.

It has been shown that the toluene formed on bombarding benzene with recoil C^{14} has about 86% of its activity in the methyl group, the remaining 14% in the ring.[3] In order to get some idea of the mechanism, or mechanisms, of substitution and addition of the impinging C^{14} ions, it is necessary to determine the position of the C^{14} in the ring relative to the methyl group. Methods are presently being tested for the complete degradation of toluene, a product of the C^{14} irradiation of benzene in previous experiments.

The general analytical procedure used involves the purification of the bombardment mixture, diluted with cold carrier of any suspected product, via gas-liquid partition chromatography to constant specific activity. This procedure generally requires the use of several different columns. Front and back portions of a given peak may be counted to insure homogeneity of product.[1, 2] Counting is done with a liquid scintillation counter. When appropriate and feasible, degradation of a product may be carried out. It is necessary, of course, that there be a sufficient amount of activity in the product that dilution for multistep degradative reactions will not reduce the specific activity to an unworkably low level.

In the case of toluene, around 1% of the C^{14} ions impinging on benzene can be expected, on the basis of previous reports of similar experiments,[1, 2] to result in toluene. Since the new ion separator has delivered an argon beam of around 1 μamp, the improved final version can be expected to provide at least as intense a beam of C^{14} ions. If this is so, a 24-hour run would allow the toluene formed to be diluted to a total volume of 10 ml, with a resultant specific activity of around 120 dpm per μl. If only 10% of the activity were in the ring, the specific activity of the ring would still be 12 dpm per μl, a sufficiently large value.

Two independent routes are available for the degradation of toluene, both depending on the initial conversion of toluene to phenol. One path, due to Reio and Ehrensvärd,[6] will be relied on for the complete degradation of the ring; the other could be so used, but it is designed primarily to give the phenyl carbon adjacent to the methyl group by itself. Most of the reactions involved have been satisfactorily carried out, but it is necessary to improve the yield in a few cases. A complete check on the reliability of the methods has not yet been completed.

The chromatograms of previous experiments,[1, 2] showed, in addition to toluene and benzene, many unidentified peaks, presumably due in part to fragmentation and recombination reactions. Attempts will be made to identify as many of these compounds as possible, and in particular, seven-membered compounds such as cycloheptatriene will be sought. The energy dependence of product formation, yield, and site labeling will also be investigated.

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[6] L. Reio and G. Ehrensvärd, Arkiv Kemi 5, 301 (1953).

KINETIC STUDIES OF RADIATION DAMAGE IN CHOLINE CHLORIDE

Robert O. Lindbolm and Richard M. Lemmon

In previous Quarterly Reports[1] we have discussed in some detail the shape of the electron-spin resonance (ESR) signal of a radical associated with radiation damage of choline chloride and its deuterated analogs.

It has been inferred that this radical is the species responsible for the chain decomposition of irradiated choline chloride, and in the past it has been assumed that the postirradiation decomposition of choline chloride was complete in about 24 hours at room temperature. These assumptions are now being investigated by kinetic studies. Table I shows the first set of kinetic data, giving the radiation damage after 88 days for samples receiving radiation doses ranging from 0.0071 to 23 megarads of 3-Mev electrons. Figure 3 is a graph of the G-value–dose relationships in Table I. Although measurements of radical concentration by ESR throughout the 88-day period would be very valuable, unfortunately the signal level decays below observable values during the first 48 hours. Therefore, correlation of the radical concentration and decay rate will have to be done during the first 48 hours of decay, when the radical concentrations can be observed directly by the ESR spectrometer.

The data of Table I show several interesting features that any model for choline chloride irradiation damage must explain: (a) the high G values, (b) the saturation of radiation damage, and (c) lack of saturation in the initial ESR signal levels.[2] These data are best explained by assuming that the ionic radicals are stabilized by two types of electron traps: (a) long-lived traps, which are naturally occurring imperfections and impurities in the crystal, and (b) short-lived traps, which are radiation-induced anionic vacancies in the crystal lattice and which result from atomic interactions with high-energy electrons. These traps are short-lived because thermal annealing allows the interstitual atoms to return to the vacancies.

These assumptions lead to the following hypothesis with respect to the effects of radiation on choline chloride. The radiation ionizes--in high yield--many molecules, and produces--in low yield--short-lived traps. The low yield of trap production is the consequence of the poor transfer of kinetic energy from a light electron to a heavy atom. Also, for every observable ionic radical there must be an electron stabilized in a trap. Radiation, then, slowly produces induced traps and saturates these as well as the natural traps with electrons from ionized molecules.

In the dose region below 0.007 megarad the natural traps are not yet saturated. The G value should be constant and at a maximum since the long-lived traps are being populated. From 0.023 to 0.71 megarads the natural

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[1] Robert O. Lindblom and Richard M. Lemmon, in Chemistry Division Quarterly Reports: UCRL-3629, Feb. 1957, p. 24; UCRL-3950, Sept. 1957, p. 73; UCRL-8141, Dec. 1957, p. 69; UCRL-8204, March 1958, p. 5; UCRL-8421, June 1958, p. 36.

[2] Robert O. Lindblom and Richard M. Lemmon, in Chemistry Division Quarterly Report, UCRL-8421, June 1958, p. 36.

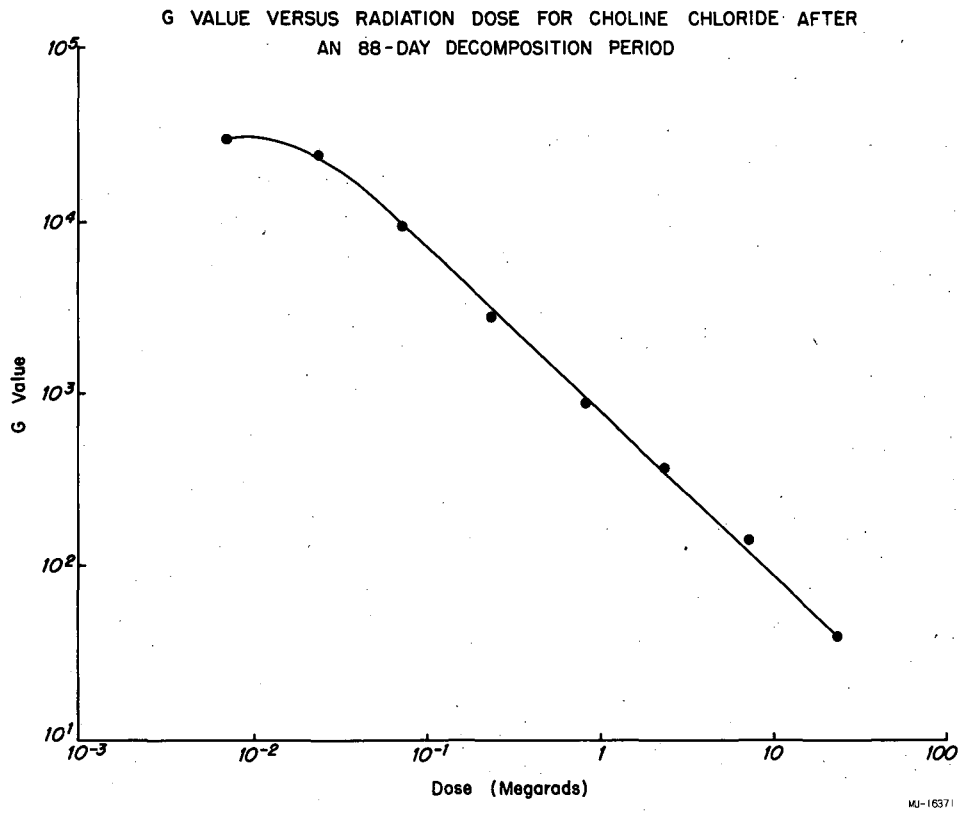


Fig. 3. G value vs radiation dose for choline chloride after 88-day decomposition period.

Table I

Radiation damage of choline chloride 88 days after irradiation with 3-Mev electrons

Dose (megarads)	% Decomposition (volatile amine analysis[a])	G value	ESR signal (initial)
23.7	13.6	39.6	61
7.10	14.6	142.0	37
2.37	12.6	367.0	20
0.710	9.2	895	5.6
0.237	9.5	2770	2.4
0.071	9.3	9050	1.08
0.023	8.4	24500	
0.0071	3.1	30100	
0.0000	0.40[b]	-	-

[a]The decompositions from 3.1 to 9.5% have also been checked by the reineckate method.

[b]All the other values have been corrected by the amount of this blank.

traps are all populated and the total number of traps is increasing, but the induced traps have too short a lifetime and are two few in number to contribute to the radiation damage. Since the decomposition is nearly constant, the G value falls rapidly with increasing dose. Above doses of 0.71 megarad the radiation-induced traps are becoming numerous enough to contribute to the radiation damage. Over the dose region shown, the long-lived traps are dominating the decomposition, although their concentration is too small to be seen with the ESR spectrometer. Hence it is not surprising that the initial ESR signal, which is a measure of the radiation-induced traps, does not correlate with the radiation damage, which is controlled by the natural traps.

The model also provides an explanation for the different G values for several radiation sources reported earlier:[3]

Source	G
C^{14} (0.145-Mev β)	1250
3-Mev electrons	98
Co^{60} (1.23-Mev γ)	175

[3]Lemmon, Gordon, Parsons, and Mazzetti, J. Am. Chem. Soc. 80, 2731 (1958).

A denser ionization path, with greater opportunity for radical-electron recombinations, could explain different G values for Co^{60} and for 3-Mev electrons, but for C^{14} β radiations and for 3-Mev electrons the path density should be the same and the G values should be comparable, not thirteenfold different. The explanation is that only a relatively low dose rate is possible with C^{14} self-irradiation, and the decomposition occurred over a period of 46 days. Under these conditions the long-lived natural traps had an opportunity to dominate the radiation damage. This results in long lengths for the chain decomposition and high G values.

Experimental Procedure

Samples of approximately 5 mg of choline chloride were sealed in 3-mm Pyrex tubes after evacuation to 100 microns pressure and baking out at 100° for 1 hour. The samples were irradiated at liquid nitrogen temperatures by electrons from a 3-Mev linear accelerator. Only one end of the tube was irradiated, so that the other end was available for ESR observation of the sample. An amine-diffusion method was used to analyze for the sample's decomposition. In previous work, the analysis for radiation damage has been done with either the reineckate colorimetric method[4] or the tetraphenyl boron gravimetric method.[5] In either of these latter methods the amount of radiation damage is indicated by the difference between the amount of choline chloride initially taken and that which remains after the decomposition. For small values of radiation damage, the value sought is a small difference between the two large measured values, and consequently, the accuracy falls off rapidly as the amount of damage decreases. Since acetaldehyde and trimethylamine have been established as the major products of the radiation-induced decomposition,[6] it was decided to use an amine-diffusion analysis method.

The vacuum Tompkin-Kirk diffusion cell used is shown in Fig. 4. A large excess of saturated K_2CO_3 is put in position A, position B contains the amine salt, and cup C has an aliquot of sulfuric acid solution, containing acid equivalent to 2 to 5 times the expected amount of volatile amine. After the solutions are all in place, the cell is evacuated to 30 mm pressure (just above the boiling point). At this point, tube D, which serves as an evacuation point as well as a valve, is pulled out until its valve opening is just past the stopper. The solutions in A and B are mixed carefully to avoid spilling solution in cup C or spattering of the other solutions into C. After at least 3 hours the vacuum is carefully released and the acid in cup C is back-titrated to determine the volatile amine. The ultimate sensitivity of this system is estimated to be 0.02% decomposition. The previously used methods have an ultimate sensitivity of the order of 1% decomposition.

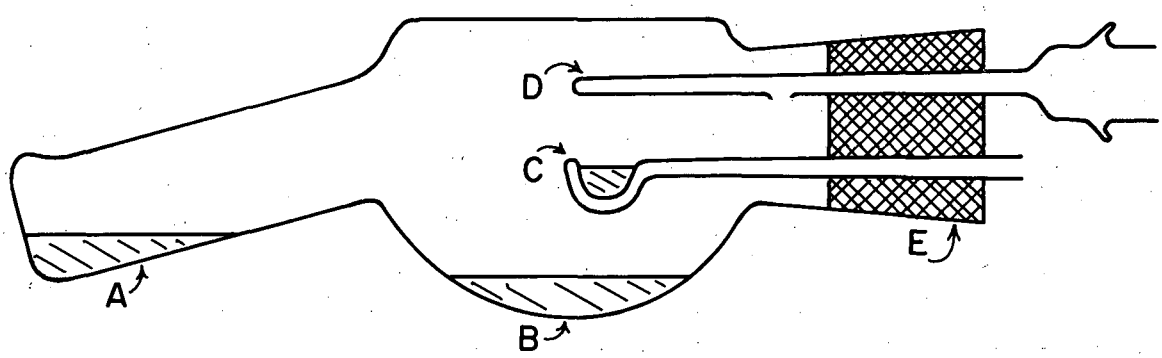
The dose and G values have been calculated from the following formulas:

$$\text{G value} = \frac{\text{molecules decomposed}}{\text{100 electron volts dissipated}} = \frac{69.0 \times \text{\% decomposition}}{\text{Dose (in megarads)}} ,$$

[4] D. Glick, J. Biol. Chem., Hoppe-Seyler's 156, 643 (1944).

[5] P. Marquardt and G. Vogg, Z. physiol. Chem. 291, 143 (1952).

[6] R. M. Lemmon, Nucleonics 11, No. 10, 44 (1953).



Tompkin-Kirk Diffusion Cell

MU-16369

Fig. 4. Tompkin-Kirk diffusion cell.

where dose (in megarads) = 0.186σ (a megarad is 10^8 ergs/g),
σ = microcoulombs/cm^2.

The dose relationship is derived from the Feather relationship:

$$\Delta E \text{ (in Mev/electron)} = \frac{\Delta R \text{ (in g/cm}^2)}{0.54} .$$

ELECTRON-SPIN RESONANCE OF ORGANIC ELECTRON DONOR-ACCEPTOR PAIRS

John Eastman and Melvin Calvin

Some organic electron donor-acceptor pairs in the solid state have been characterized by their paramagnetism[1] and semiconductivity.[2] Equilibrium constants for the formation of charge-transfer molecular complexes have been assigned to other pairs in solution. These complexes are characterized by charge-transfer spectra,[3] and dipole moments or dielectric constants.[4] It is the purpose of this work to determine the origin of paramagnetism in the solids, and to determine if, and to what degree, free radicals may be produced from donor-acceptor pairs in general. The results reported here are of a preliminary nature, and experiments are described in order to attempt quantitative interpretations in the future.

Aromatic Hydrocarbons

When perylene or coronene was combined with the acceptors, tetrachloro-o-benzoquinone (o-chloranil), picric acid, or bromine, electron-spin resonance (ESR) was observed which was not reproduced in solution when an attempt was made to dissolve the species containing unpaired spin. A low-intensity absorption occurred in solution, and careful experiments are planned to determine the number of unpaired spins in the solid and solution as a function of composition, for the combination o-chloranil-perylene.

Arylamines

ESR was observed when N, N, N', N'-tetramethyl-p-phenylenediamine (TMϕD) was combined in the solid state with o-chloranil. Solutions of this combination in low-dielectric solvents exhibited only a minimum detectable electron-spin resonance. Kainer reported the paramagnetism of TMϕD-chloranil solid and also the spectra and conductivity of solutions in acetonitrile.[1] His qualified interpretation was that the solid contained unpaired spins by virtue of triplet-state population of a charge-transfer complex, and that the solution contained radical ions, being the dissociated complex. In order to characterize these two systems more fully, the following determinations will be made: number of unpaired spins, spectra, and solution conductivity, all as functions of composition and temperature.

When chloranil was dissolved in N, N-dimethylaniline and N, N-diethylaniline, a deep blue solution resulted, and high ESR absorption was observed in each case. When an equal volume of CCl_4 was added to this

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[1] H. Kainer and A. Uberle, Chem. Ber. 88, 1147 (1955).

[2] H. Akamatu, H. Inokuchi, and Y. Matsunaga, Bull. Chem. Soc. Japan 29, 213 (1956).

[3] L. E. Orgel, Quart. Revs. (London) 8, 422 (1954).

[4] G. Kortum and H. Z. Walz, Z. Elektrochem. 57, 73 (1953).

sample, no ESR was observed. The number of chloranil and aniline molecules in the microwave cavity was constant. When high-dielectric solvents were added, again keeping constant the number of molecules in the cavity, a moderate ESR absorption was observed. A CCl_4-diluted donor-acceptor combination gave the known charge-transfer spectra[5] of the one-to-one molecular complex.

In another experiment, the conductivity of the original sample was barely detectable at 80° with the apparatus used. When the sample was diluted with chlorobutane, the conductivity increased, and it increased with temperature. These results are summarized in Table II.

Table II

ESR absorption and specific conductance of chloranil + N, N-dimethylaniline solutions

Original sample	ESR	Temp(°C)	Specific Conductance (Ω^{-1} $cm^{-1} \times 10^7$)
	+	22	< 4
		40	< 4
		80	6
+ 1 drop CCl_4	-		
+ 1 drop CCl_4 + 1 drop $CH_3(CH_2)_3Cl$	+		
diluted with CH_3 $(CH_2)_3$ Cl		0	7
		22	10
		40	13
diluted with CCl_4		charge-transfer spectra	

+ ESR absorption detected

- ESR absorption not detected

Quantitative experiments are now in progress to determine the number of unpaired spins, the spectra, and the conductivity of these donor-acceptor combinations as functions of composition, temperature, and solvent dielectric constant.

[5] J. M. Corkill, R. Foster, and D. Hammick, J. Chem. Soc. (1955), 1202.

It is speculated that the ESR absorption in the solids and liquids is due to unpaired electrons resulting from charge transfer in a molecular complex followed by a second electronic transfer giving two radical ions separated by a neutral molecule. The necessary condition for observed ESR is that there be proximity of at least three molecules capable of electron transfer. On the other hand, solutions exhibit ESR and conductivity only when the solvent is polar enough to split the stable charge-transfer complex into radical ions.

USE OF AMMONIUM CHLORIDE-COPPER-COPPER OXIDE MIXTURES THE SEALED-TUBE OXIDATION OF ORGANIC COMPOUNDS

Irville M. Whittemore and Sibyl Kocher

The sealed-tube combustion of organic compounds with a copper-copper oxide mixture described by Wilzbach and Sykes[1] has been used extensively in this laboratory for the precision assay of carbon-14, using ionization chambers. The method, however, is not generally useful in the combustion of compounds containing metals, as the metals tend to form stable carbonates which may contain nonreproducible amounts of the total activity present. We have tried to overcome this defect by various methods.

Our earliest attempt was to use $CuSO_4$ as an oxidizing acid.[2] Although the CO_2 yield here was quantitative, the SO_2 formed in the combustion accompanied the CO_2 into the ionization chamber. This electronegative impurity decreases the efficiency of a CO_2-filled chamber. When standard benzoic acid is oxidized with $CuSO_4$, the results are lower than when the same compound is oxidized with Cu-CuO.

Another approach to the problem of oxidation of organic compounds containing metals was to use vanadium pentoxide as an oxidizing agent. Here, too, quantitative release of the CO_2 was obtained and without troublesome electronegative impurities. A different difficulty appeared, however. V_2O_5 may contain a large quantity of radioactive impurities which may accompany the CO_2 into the ionization chamber.[3] This results in a high background reading which interferes with determinations at low levels of activity. While one may make corrections for this increased background, the desirability of having a single method of calculation for all types of samples led us to search for another, correction-free, combustion technique.

In general, solid-solid reactions proved unreliable. The V_2O_5 method mentioned above seems to be an exception to this rule.

A very satisfactory method has been developed using NH_4Cl in combination with Cu-CuO. The sample (approx. 10 mg) containing the metal is sealed with a gram of 1:3 Cu-CuO mixture and about 10 mg of NH_4Cl, mixed, and burned in the usual way.[1] Results obtained with a sample of sodium acetate-C^{14} are listed in Table III.

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[1] K. E. Wilzbach and W. Y. Sykes, Science, 120, 494 (1954).

[2] I. M. Whittemore and B. M. Tolbert, in Chemistry Division Quarterly Report, UCRL-3415, June 1956, p. 45.

[3] I. M. Whittemore and Sibyl Kocher, in Chemistry Division Quarterly Report, UCRL-8421, June 27, 1958. p. 42.

Table III

Results of NH_4Cl-Cu-CuO method for sealed-tube oxidations

Determination	μC/mg NaOAc-C^{14}
1.	0.0228
2.	0.0224
3.	0.0227
4.	0.0221
5.	0.0222
6.	0.0225
7.	0.0222
8.	0.0222
Average	0.0224
standard deviation	0.0002

The reaction between sodium acetate, NH_4Cl, and CuO may be written as follows:

$$2\ H_3C_2O_2Na + 2\ NH_4Cl + 11\ CuO \qquad 2\ NaCl + 4\ CO_2 + 7\ H_2O + N_2 + 11\ Cu$$

The presence of NH_4Cl does not interfere with the determination of carbon-14 in compounds containing no metals. Because of this, it may be included in the standard oxidizing mixture for all compounds, especially those whose exact composition is not known, such as biological specimans.

PREPARATION OF HAMAMELONIC ACID

Jan Anderson

In the research on the 2-C-hydroxymethyl pentonic acids, whose synthesis by the action of cyanide on the pentoses has been previously described,[1] some difficulties have been encountered in the separation and subsequent identification of these epimeric acids. It would therefore seem desirable to have an authentic sample of one of these acids in the pure form, in order to study their chemistry. It is the purpose of this report to describe the preparation of hamamelonic acid (2-C-(hydroxymethyl)-D-ribonic acid).

Discussion and Method

The bark of the North American shrub Hamamelis virginica (commonly referred to as witch hazel) contains, in addition to other tannins, about 1 to 1.5% of hamameli-tannin.[2] This tannin was shown to have the composition of a di-O-galloylhexose (I).[3,4] Acid or enzymic hydrolysis of hamameli-tannin yields two moles of gallic acid, and one mole of a strongly reducing hexose, hamamelose (II), whose structure has been established as 2-C-(hydroxymethyl)-D-ribose (II).[5,6]

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[1] J. M. Anderson, in Bio-Organic Chemistry Division Quarterly Report, UCRL-8421, June 1958, p. 21.

[2] F. Grüttner, Arch. Pharm., 236, 278 (1898).

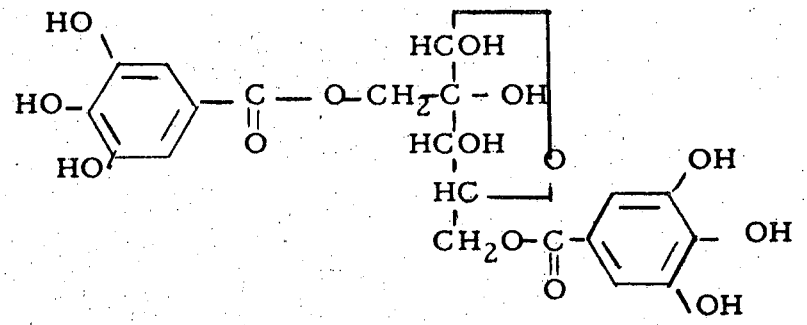
[3] K. Freudenberg and D. Peters, Ber. deut. chem. Ges. 53, 953 (1920).

[4] K. Freudenberg and F. Blümmel, Ann. chem. Liebigs 440, 45 (1924).

[5] O. Th. Schmidt, ibid., 476, 250 (1929).

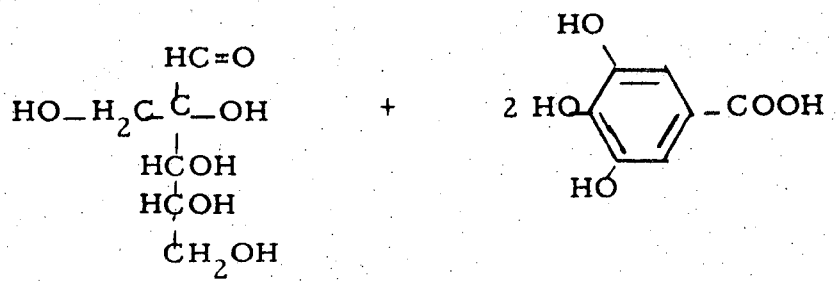
[6] O. Th. Schmidt and K. Heintz, ibid., 515, 77 (1934).

(I)



hydrolysis

(II)



Several procedures for the extraction of the tannin from the dried bark have been described;[4,7] these are summarized in the following flow-sheet diagrams. It was found that Method III gave the best yield of purified hamameli tannin, corresponding to 1.4% of the dried bark; however, this is a long and tedious extraction procedure. The complete removal of a red gelatinous material, upon which the white crystals of the tannin are formed, is extremely difficult. This material is present in large amounts only if acetone has been used in the initial extraction of the bark. Method III gave the lowest yield, 0.75%, and had no advantageous features. Although Method I gave a slightly lower yield (1.25%) than Method III, it is definitely the method of choice. It is relatively quick and easy to perform and the crystallized tannin is relatively free from the red gelatinous material of Method III.

[7] K. Freudenberg, Ber. deut. chem. Ges. 52, 117 (1919).

Extraction of Hamameli-tannin

Method I

Dried Bark (500 g)

↓ ether-acetone (5:1) in the cold (5×700 ml)

Extract

↓ evaporated under reduced pressure

Residue

↓ dissolved in acetone (~50 ml) and ether added until precipitation complete (~200 ml)

Residue | Filtrate

↓ evaporated under reduced pressure

Extract

↓ dissolved in water and extracted with petroleum ether

Organic layer | Aqueous layer

↓ evaporated under pressure

Crude hamameli-tannin (8 g)

Method II

Dried Bark (500 g)

↓ chloroform-water (9:1) in the cold (5×500 ml)

Extract

↓ evaporated under reduced pressure

Residue

↓ dissolved in acetone (50 ml) and ether added until precipitation complete (150 ml)

Residue | Filtrate

↓ evaporated under reduced pressure

Residue

↓ dissolved in water (50 ml)

Crude hamameli-tanin (5 g)

Method III

Dried Bark (500 g)

↓ acetone (5×600 ml) in the cold

Extract

↓ evaporated under reduced pressure

Extract (200 ml)

↓ extracted with benzene (liquid-liquid extraction)

Benzene layer | Acetone-water layer

↓ evaporated under reduced pressure

Residue

↓ dissolved in water and extracted with ethyl acetate (liquid-liquid extraction)

Organic layer | Aqueous layer

↓ evaporated under reduced pressure

Residue

↓ dissolved in water (500 ml)

Extract

↓ shaken with talc, filtered, and 10% Pb $(C_2H_3O_2)_2$ added dropwise until precipitation occurs

Filtrate

↓ H_2SO_4 and filtered by centrifugation

$PbSO_4$ + Filtrate

↓ stored at 5°

Crude hamameli-tannin + red gelatinous material (30 g)

↓ dissolved in water (60 ml) and dried very slowly (this step omitted as no purpose for it seen)

Residue

↓ *dissolved in acetone and benzene added until precipitation complete

Residue | Filtrate

↓ *evaporated under reduced pressure

Crude hamameli-tannin

*Two steps repeated until the red gelatinous material is removed to give pure hamameli-tannin (6.7 g).

The crystalline hamameli-tannin may be hydrolyzed, enzymatically by tanase,[4] or by acid.[7] Freudenberg used 1 M H_2SO_4 for the hydrolysis, but a slightly improved yield of hamamelose and an easier work-up procedure was obtained using 2 M HCl. The separation of gallic acid from the sugar hamamelose is difficult. The original method used involves precipitation of the gallic acid by the alternate addition of small quantities of $Pb(CO_3)_2$ and $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ and subsequent removal of the excess cation.[8] It was hoped that the use of ion-exchange resins might facilitate the removal of gallic acid, but this proved unsatisfactory.

The hamamelose was oxidized with mercuric oxide in the presence of calcium carbonate to give a noncrystalline calcium salt from which the free acid was generated.[4] An improved yield was obtained by using bromine as the oxidizing agent.

Further research is now in progress, and it is hoped that following studies of the chemical properties of hamamelonic acid, the epimeric 2-C-(hydroxylmethyl)-D-pentonic acids previously synthesized may be separated.

Experimental Procedure

Extraction of hamameli-tannin

Methods I, II, and III were investigated as shown in the flow-sheet diagrams; Method I was used in all subsequent extractions. Pure hamameli-tannin crystallizes in fluffly white clusters from water, m. p. 154°. (Found: C, 49.6; H, 4.12. Calc. for $C_{20}H_{20}O_{14}$: C, 49.5; H, 4.15%).

Hydrolysis of hamameli-tannin

Hamameli-tannin (5 g) was hydrolyzed with 2 M HCl (50 ml) for 50 hours at 60°. The reaction mixture was partially concentrated under reduced pressure and left at 0° for some hours; the precipitated gallic acid was removed by centrifugation. The filtrate was concentrated under reduced pressure to dryness several times to remove the hydrochloric acid, and then dissolved in water (40 ml). The solution was then extracted with ether in a continuous liquid extractor for 48 hours and the aqueous layer evaporated to give a colorless syrup of hamamelose (1.9 g).

Oxidation of hamamelose

Hamamelose (1.8 g) was dissolved in water (20 ml) containing suspended $CaCO_3$ (12 g), the solution cooled to 5°, and the bromine (1 ml) slowly added. After the mixture had been shaken for 24 hours at 25°, air was bubbled through the solution to liberate any gaseous bromine, the mixture was filtered, and the filtrate concentrated under reduced pressure. The syrup was diluted and filtered through a column of Amberlite IR-120, H^+ form (100 ml resin), and again concentrated under reduced pressure several times to remove the hydrogen bromide. A solution of brucine (12.5 g) in EtOH (40 ml) was added to the syrup and the solution kept at 5° for several days. The crude salt (7.5 g) was dissolved in a minimum amount of water and the excess brucine removed by extraction with chloroform. After the addition of ethanol, white needles of the brucine salt were obtained (4.0 g), m. p. 174°. (Found: C, 61.51; H, 6.39; N, 4.98, Calc. for $C_{29}H_{36}N_2O_{10}$: C, 60.83; H, 6.34; N, 4.89%.)

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[8] E. Fischer and K. Freudenberg, ibid., 45, 915 (1912).

THE HILL-REACTION ACTIVITY OF CHLOROPLAST FRAGMENTS AS COMPARED WITH INTACT CHLOROPLASTS

Roderic B. Park and Ning G. Pon

Evolution of O_2 by photosynthetic tissues in the presence of light and an oxidant such as ferricyanide ion or quinone was first described by Robert Hill[1] and is called the Hill reaction. Since this reaction involves photolysis of water with concomitant O_2 production, it is a convenient system with which to study the first steps in the light reaction of photosynthesis.

We are presently isolating from spinach chloroplasts various chloroplast fragments which carry out the Hill reaction at a rate approximating that of intact chloroplasts. Our method involves preparation of chloroplasts[2] followed by sonication at 9 kc. Four minutes is the optimal sonication time for our purposes. Sonication for a shorter time gives too small a yield of chloroplast fragments, while sonication for a longer time gives decreasing Hill-reaction rates on a unit chlorophyll basis. The sonicated chloroplast preparation is then separated centrifugally into various fractions which are assayed for Hill-reaction activity. Using $K_3Fe(CN)_6$ as the oxidant, we have noted that the Hill-reaction rate of chloroplast fragments is as much as 2.5 times the rate observed for intact chloroplasts on a unit chlorophyll basis. To confirm this observation we performed the following experiment. A chloroplast suspension was divided into two portions. One portion was assayed for Hill-reaction activity without further treatment. The other portion was sonicated at 9kc in a Raytheon 100-watt magnetostriction oscillator. Equal volumes of the two preparations contained equal amounts of chlorophyll and differed only in their state of organization. Each preparation was assayed manometrically for O_2 production in the presence of light and ferricyanide ion. The results of such an experiment are plotted in Fig. 5.

The data in Fig. 5 show that fragmentation of chloroplast material increases the Hill-reaction rate 2.5-fold. This observation can be explained by assuming that the Hill-reaction activity of intact chloroplasts under the conditions of our experiment is limited by ferricyanide availability. Sonication apparently removes the barrier to ferricyanide penetration. If ferricyanide availability also limits the Hill-reaction rate *in vivo*, care must be taken in the interpretation of Hill-reaction rates observed for intact tissues.

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[1] R. Hill, and R. Scarisbrick, Proc. Roy. Soc. (London) B., 129, 238 (1940).

[2] Sogo, Pon and Calvin, Proc. Natl. Acad. Sci. U.S. 43, 387 (1957).

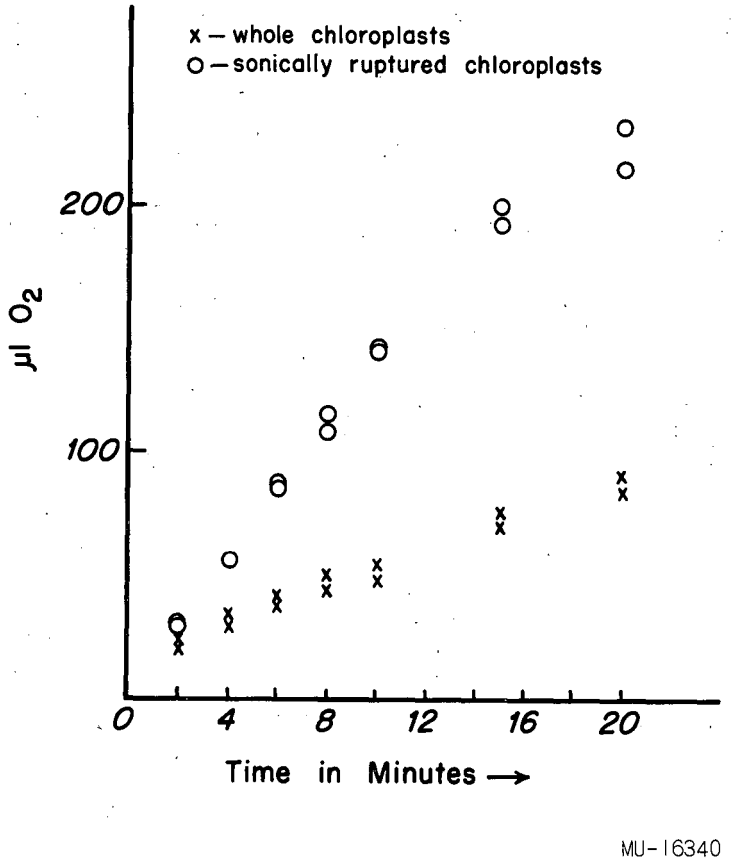

Fig. 5. Each flask contained 1.45 mg chlorophyll as intact chloroplasts or chloroplast fragments, and 60 μM $K_3Fe(CN)_6$ in 2.9 ml of 0.5 M sucrose at pH 7.2. Bath temperature was 19°C. Four photospots were used for illumination.

A GLUTAMIC-ACID-CONTAINING COMPOUND FORMED DURING PHOTOSYNTHESIS

Karel P. Louwrier and James A. Bassham

Chlorella pyrenoidosa, suspended in a nutrient solution, grown in a bright light, and aerated with air containing $C^{14}O_2$, produces many substances containing C^{14}. These compounds have been analyzed by paper chromatography and radioautography, as described in many previous reports from this laboratory. Some of these compounds were not observed in earlier experiments, in which the chlorella were exposed to $HC^{14}O_3^-$ in distilled water. After a period of photosynthesis of some minutes in steady-state apparatus[1] in a nutrient solution containing

$(NH_4)_2HPO_4$,	0.3 g/l
KNO_3	0.1 g/l
Hoaglund's solution of micro elements	0.5 ml/l
$MgSO_4 \cdot 7H_2O$	0.1 g/l
NH_4VO_3	11.5 μg/l
Fe-EDTA solution[2]	0.5 ml/l

a new spot appears on the radioautogram. This compound, which will be called No. 13 in this report, has an Rf in phenol of 0.16 and in butanol-propionic acid of 0.22.

Treatment of spot No. 13 with a preparation containing human prostate phosphatase yields many new compounds. Most of the radioactivity is found in three spots, the most radioactive of which has been identified as glutamic acid. It has not yet been possible to identify the other two spots. Treatment with Polydase yields two spots not yet identified. Several attempts have been made to show the presence of phosphorus in spot No. 13 by the use of P^{32}-phosphate. The algae were suspended in a Myers' nutrient solution and exposed to brilliant light for 6 hours with each hour of exposure to light followed by a 10-min period of darkness. During photosynthesis air containing 2% CO_2 was bubbled through the suspension. The algae were then filtered and killed in 20% ethanol. Upon subsequent chromatography and radioautography it was not possible to find any P^{32} activity in spot No. 13.

There is no hydrolysis of spot No. 13 after treatment in 1 M HCl for 30 min at 100°C, but a clearly defined circular spot appeared in the radioautogram.

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[1] Bassham, Shibata, Steenberg, Bourdon, and Calvin, J. Am. Chem. Soc. 78, 4120 (1956).

[2] Containing 59 g/l EDTA and 24 g/l $FeSO_4 \cdot 7H_2O$; pH 9.7.

After treatment with 1 M NaOH for 30 min at 100°C there is still no hydrolysis and the spot on the radioautogram has become elongated. However, after an attempt to hydrolyze spot No. 13 in 0.1 M HCl at 120°C under pressure, we found several products that were different from those formed by phosphatase treatment.

Reduction with BKH_4 in neutral solution or with sodium amalgam in acid medium gave a new compound with Rf values of 0.18 and 0.03. Reaction of spot 13 with 2,4-dinitrofluorbenzene in 0.1 M $NaHCO_3$ produced a new compound which is extractable from an acid solution by ether. This compound is not identical with that which is formed by treatment of glutamic acid with 2, 4-dinitrofluorbenzene. This reaction indicates the possibility of a free NH_2 group in spot No. 13. Reaction with dinitrophenylhydrazine in acid solution does not form a new compound. This indicates that there is no carbonyl group in the compound.

After elution and rechromatography, spot No. 13 was found to streak in a phenol direction with three points of high activity. The same thing occurred in the butanol-propionic acid direction. Only one of these spots is the original one. The others were products which were also formed after reaction with phosphatase. There is most likely an oxidation or a decomposition of spot 13 after its exposure to air.

In another experiment, the algae were allowed to photosynthesize for 5 hours in medium with C^{14}. After extraction and phosphatasing of spot No. 13 with prostate phosphatase the activity of the glutamic acid was found to be about 7 times the activity in the other two spots. An attempt to observe the uv absorption spectrum of spot No. 13 failed because of impurities in the filter paper.

(Since the above report was first written spot No. 13 has been tentatively identified by cochromatography as glutathione.)

BIOSYNTHESIS OF OPIUM ALKALOIDS

Henry Rapoport and Frank Stermitz

In continuing work on the biosynthesis of opium alkaloids,[1, 2] morphine, codeine, and thebaine were isolated from a poppy plant (Papaver somniferum) that had absorbed 20.4 millicuries of $C^{14}O_2$ in 2 hours. The specific activity of the morphine was 104,000 dpm/μmole. The codeine had a specific activity of 865,000 dpm/μmole. The codeine-morphine specific activity ratio is 8.3, which compares to a ratio of 9.6 found by Baker. Sufficient thebaine was not obtained, so that no check of its specific activity could be made.

Degradation indicated that 37% of the radioactivity in the morphine was located in the N-methyl group (as compared to 41% found by Baker) and that 50% of the radioactivity of the codeine was located in the N-methyl group (as compared to 47% found by Baker). Further degradation work is in progress.

An additional poppy plant absorbed 23.7 millicuries of $C^{14}O_2$ during 5 hours. The plant was harvested immediately. Alkaloid fractions were separated as indicated previously[1] and will be used for further degradation.

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[1] Don R. Baker, Biosynthesis of Morphine (Thesis), University of California, Berkeley, California, 1958.

[2] Henry Rapoport, Don R. Baker, and Melvin Look, in Chemistry Division Quarterly Report, UCRL-8204, March 1958, p. 21.

SOME EFFECTS OF D_2O ON *DROSOPHILA MELANOGASTER*

Ann M. Hughes

The possibility of producing a recessive lethal mutation in *Drosophila melanogaster* by treatment with D_2O has been investigated, by using a modification of the Muller test. In this method, males of one strain are crossed with females of a different strain (P generation). Their offspring (the F_1 generation) are inbred (brothers × sisters.) When their offspring (the F_2 generation) are examined, the absence of a male fly like the P male indicates that a lethal mutation has been produced in the P males.

In our experiments, carnation-vermilion strain (*carv*) males were grown on either 10% or 20% D_2O media.[1] They were then mated with yellow-bar-apricot (*bascy*) females grown on normal media. This was the P generation. Individuals of the F_1 generation from these matings were placed by pairs (one female and one male) in creamers (the 1-oz bottles frequently used in restaurants for individual servings of coffee cream) also containing normal media. The F_2 generation was examined for *carv* males (i.e., similar to P males.) At the same time the F_2 was examined for unusual eye colors and shapes, which would indicate crossing-over in the chromosomes.

A control experiment was carried out in similar fashion, except that the *carv* males were grown on ordinary media.

Results are summarized in Tables IV and V.

Table IV

Tabulation of creamers examined (F_2)

	Numbers of creamers		
	Controls	10% D_2O	20% D_2O
Nonlethal	423	1385 (1415)[a]	1302
Lethal	1	4	3
Doubtful	5	8 (9)	1
Sterile	32	146 (188)	193
Crossover	0	9	8

[a]Numbers in parentheses include data from one group of F_1 flies in which the sterility was extraordinarily high.

[1]Ann M. Hughes, in Chemistry Division Quarterly Report, UCRL-8457, September 1958, p. 52.

Table V

Recessive lethals, sterility, and crossing-over produced in *Drosophila* by D_2O

	Controls	10% D_2O	20% D_2O
Total sperm tested	461	1543 (1616)[a]	1499
% Sterility	6.9	9.5 (11.6)	12.9
% Lethals	0.22	0.26 (0.24)	0.20
% Crossovers	0	0.65 (0.64)	0.61

[a]Numbers in parentheses include data from one group of F_1 flies in which the sterility was extraordinarily high.

The designation of a creamer as nonlethal (or lethal) indicates that at least one carv male was (or was not) seen respectively. The designation "doubtful" indicates that no carv male was seen, but that there were less than 50 flies in the creamer. The designation "crossover" indicates that at least one crossover type was seen.

The number of creamers examined indicates the number of sperm grown in a deuterated media. The number of sterile creamers indicates the number of sterile matings of the F_1 generation.

Our data indicate that D_2O, in the concentrations used, does not increase the rate of lethal mutation in Drosophila. However, the percentage of sterility in the F_1 generation is definitely increased, and is probably an indication of an effect of D_2O on the P males. In addition, there is an apparent enhanced crossing-over in the F_2 generation.

Another series of controls has been started to increase the validity of our data. Since this experiment was not set up specifically for the testing of crossing-over, another type of experiment, designed especially to test crossing-over, has been started.

BRAIN CHEMISTRY AND BEHAVIOR

Edward L. Bennett, Hilda Roderick, Ann Ohlander, Thomas H. Roderick, Hiromi Morimoto, and Charles Longueil

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

In numerous experiments and with several maze-test procedures we have shown that descendants from two strains of rats, S_1 and S_3, selected 30 years ago on the basis of another maze-testing procedure, differ behaviorally. The S_1 animals are descendants from Tryon's "Maze Bright" animals, and the S_3 animals are descendants from Tryon's "Maze Dull" animals. It was also found that the cortical cholinesterase (ChE) activity of these rats differed significantly, and it was postulated that the level of the enzyme ChE may be associated with maze performance or adaptability.[1]

As has been previously discussed, the differences between the S_1 and S_3 strain in ChE activity and behavior may be only fortuitous. A very necessary part of the investigation is an independent genetic experiment in which animals from a heterogeneous stock colony are selectively bred for differences in cortical ChE activity, and then after such differences are obtained, these animals, or descendents from these animals, are tested in various procedures as well as analyzed for ChE activity. This selective breeding program has been successful and has been summarized.[2,3]

From the fifth selected generation, additional animals have been raised and a stock colony has been established. Four lines have been established, RCL, RCH, RDL, and RDH. In this report we describe some behavioral tests used recently and some preliminary results obtained with these different strains of rats and another strain derived by crossing the S_1 and S_3 rats.

Maze Tests

1. The Hebb-Williams maze is a multiple-problem maze, first developed by D. O. Hebb at Montreal University for his studies of learning in the rat. He refers to it as an "intelligence test" for rats, since it is modeled somewhat after the design of the usual "intelligence tests." That is, the subject is presented with a number of problems to solve, and his total score is determined by his performance on all the tests. The maze (Fig. 6)

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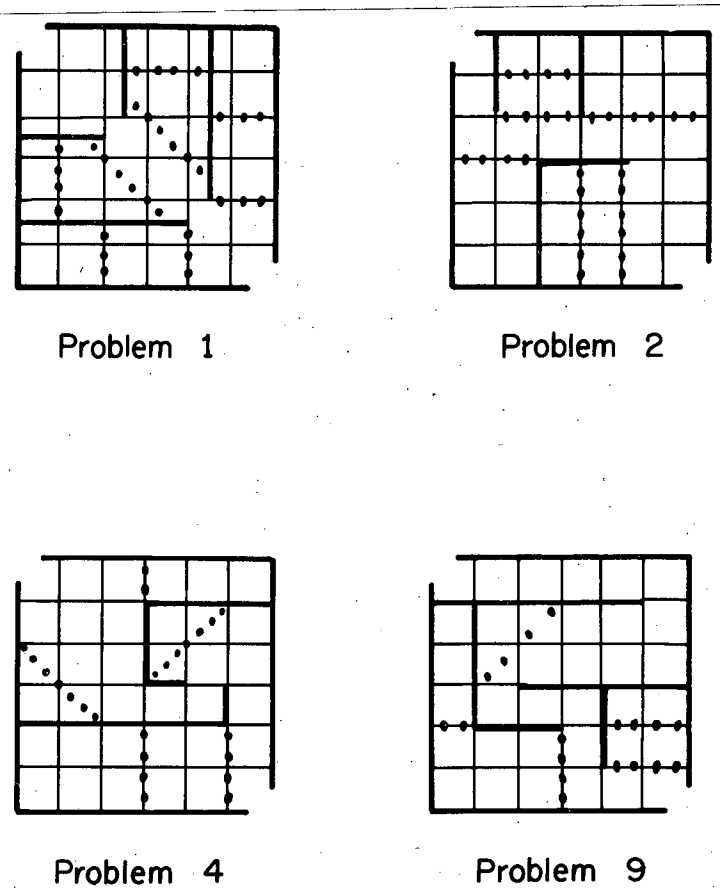
[1] Edward L. Bennett, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 14.

[2] Thomas H. Roderick, Edward L. Bennett, and Hilda Karlsson, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 86.

[3] Edward L. Bennett, Hilda Karlsson, Nancy Dye, Ann Ohlander, and Thomas H. Roderick, in Chemistry Division Quarterly Report, UCLR-8421, June 1958, p. 44.



HEBB–WILLIAMS MAZE

(4 of 12 problems used)

MU-15,827

Fig. 6. Hebb-Williams maze.

consists of a field in which different problems can be set up by means of movable barriers (indicated by heavy lines in Fig. 6). Four of the twelve problems are shown in Fig. 6. The rats are given two different problems a day for six successive days. The starting point is in the lower right-hand corner, the goal or food box is in the upper left-hand corner. An error is scored when the rat crosses the dotted lines when going from starting box to food box. Only initial errors are counted.

2. The Dashiell maze (Fig. 7) has a checkerboard pattern of blocks. There are 20 different errorless paths from starting box to goal box; an error is scored whenever a rat crosses a boundary leading away from the goal. In addition, the ability of the animal to select the more direct central route can be measured. Typically, when an animal is first presented with this problem, he adopts the more peripheral route, but as he gains experience with the maze, he begins to take the more central path. Dr. Krech demonstrated a number of years ago that rats with brain lesions were less capable of adopting the central path than were intact animals.

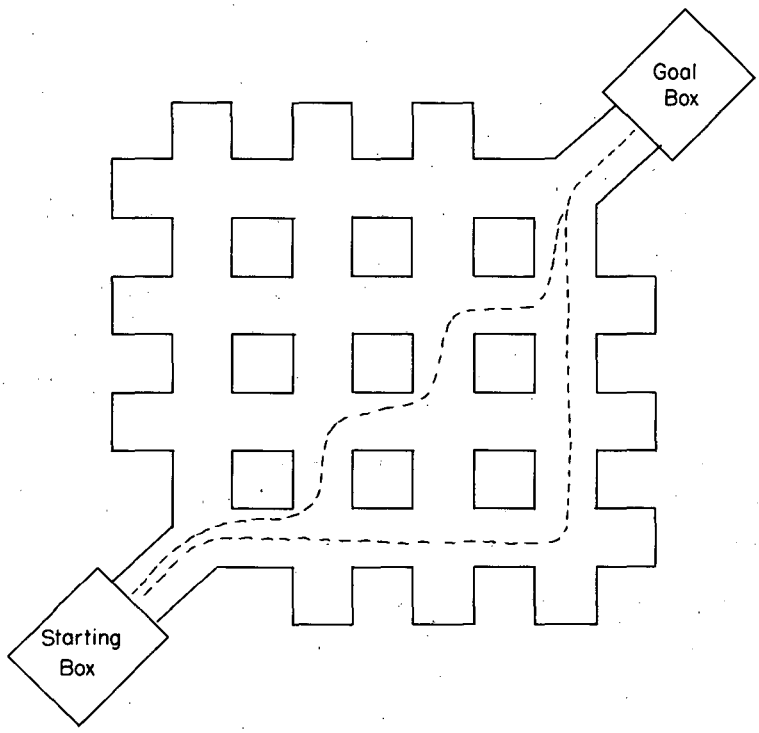
3. The Lashley maze (Fig. 8) is a simple alley maze with eight blinds. An animal is given five trials a day for five days (25 trials total) and the numbers of initial errors (entry into a blind alley) are recorded. Lashley found that brain-injured animals made more errors in this maze than their normal controls.

Results of Maze Tests

A relatively large number of S_1 and S_3 rats have been tested behaviorally by the three maze procedures described. As summarized in Table VI, the S_1 animals made significantly fewer errors in each maze and took fewer peripheral units in the Dashiell maze than did the S_3 rats. The difference in errors made by the two strains is significant at better than the 1% level in each test. The distribution of ChE activity of a representative group of these animals is summarized in Fig. 9 for 262 animals 90 to 119 days old. Thus, the strain with the higher ChE activity made fewer errors in these mazes, in agreement with our original hypothesis that ChE activity is positively associated with maze ability.

At present, relatively few of the Roderick animals (RCH, RCL, RDL, RDH,) have been tested in the maze procedures. The behavioral results for those tested are summarized in Table VI. In five out of six comparisons, the low-cortical-cholinesterase lines (L) made fewer errors than did the high-cholinesterase lines (H). (The separation of the cortical ChE activity for the RDL-RDH lines is shown in Fig. 10). ChE activity does seem to be related to learning capacity in the Roderick animals, but in just the opposite way from what we originally expected. To date, we have found no difference between strains for either cortical protein content or cortical lactic dehydrogenase activity, nor any correlation between these measures and behavior.

A separate genetic experiment is in progress using descendants from a cross of the S_1 and S_3 strains. In this experiment, third- and fourth-generation animals from this cross, designated as the K strain, are being used for maze and chemical testing. Individual learning scores obtained in the maze procedures are being compared with individual ChE activity levels.

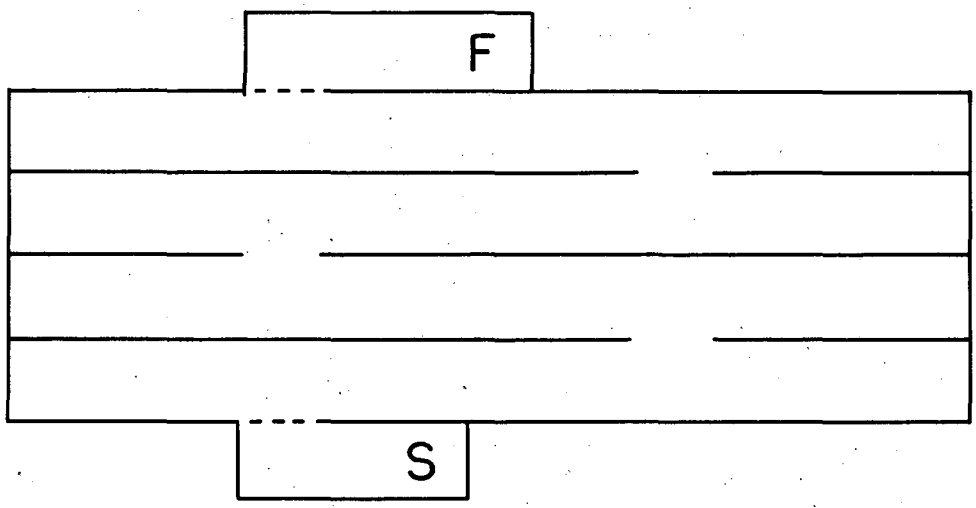


DASHIELL MAZE

(Two of 20 possible paths indicated)

MU-15,829

Fig. 7. Dashiell maze.



LASHLEY TYPE 3 MAZE

MU-15,828

Fig. 8. Lashley maze.

Table VI

Strain differences in behavioral tests (and probabilities of significance)

Maze	Behavioral scores (averages for groups)					
	S_1	S_3	RCH	RCL	RDH	RDL
Hebb-Williams	66	78	89	86	90	83
	($p > 0.01$)				($p > 0.10$)	
Dashiell						
Errors	21	34	33	26	19	21
	($p < 0.01$)					
Peripheral units	3.2	4.2	4.0	4.2	3.4	3.6
	($p < 0.05$)					
Lashley	19	42	40	29	33	19
	($p < 0.01$)		($p > 0.10$)		($p > 0.05$)	

On the basis of our original hypothesis -- i.e., that ChE activity and maze behavior are related--we would not have expected that the genetic recombinations occurring in this cross would affect our correlations between maze behavior and ChE activity. On the other hand, if our original hypothesis is not valid, the random genetic recombination of the factors that determine learning ability and those which determine ChE activity should lead to zero correlation between learning ability and ChE activity in the K strain. Neither of these expectations has been realized.

As with the Roderick animals, the preliminary results indicate a positive correlation instead of the negative or zero correlation predicted between errors and ChE activity (Table VII). The higher the ChE level of the rate, the worse is his adaptive behavior. ChE activity is related to learning in the K strain, but in just the opposite way from that expected on the basis of data from the S_1 and S_3 rats.

In view of the above findings, we are tentatively revising our original hypothesis that ChE activity is necessarily positively associated with maze ability. In part, this hypothesis rested on the simplifying assumption that there was a high positive correlation between the enzyme ChE and its substrate acetyl choline (ACh). It is possible that the contents of ACh and ChE activity are relatively independent, and the correlation between ACh and ChE is low. We now suggest that the efficiency of cortical conduction and transmission (and thus the efficiency of learning) is a function of cortical ACh-ChE balance. Most of our data can be accommodated by this new hypothesis. The Tryon maze-bright strain (S_1) may have been bred for both higher ACh and ChE activity than the S_3 strain, since maze-learning ability

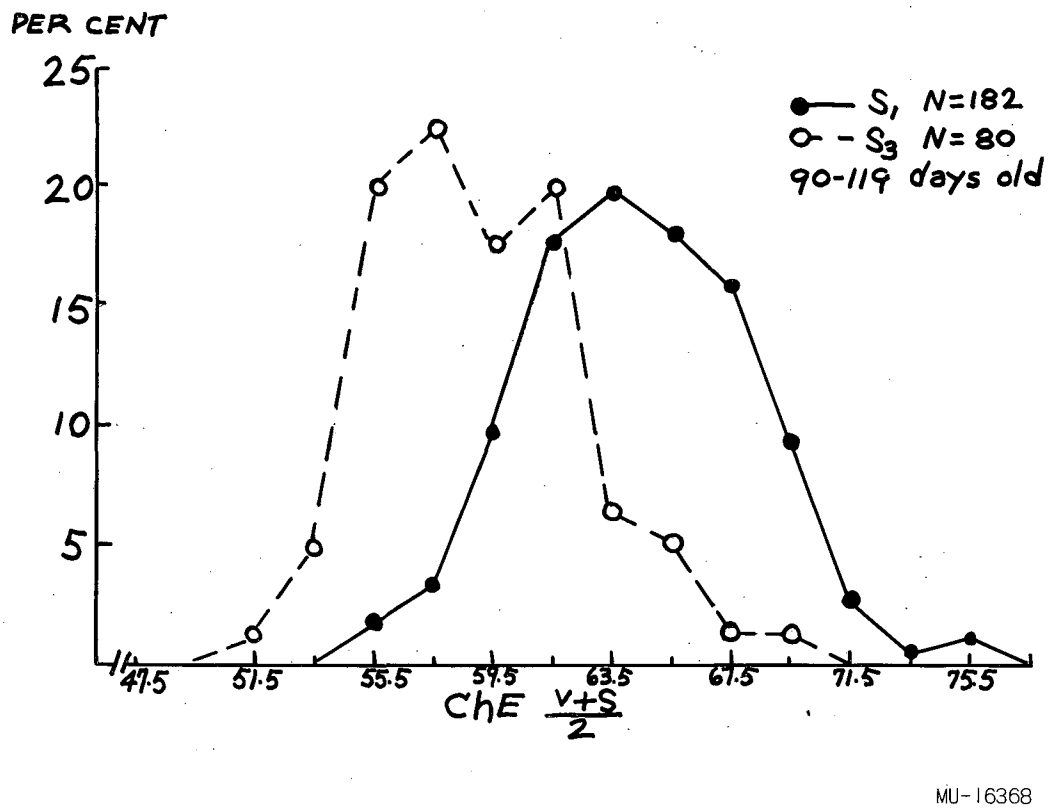


Fig. 9. Cortical ChE frequency distribution of S_1 and S_3 male rats 90 to 119 days old.

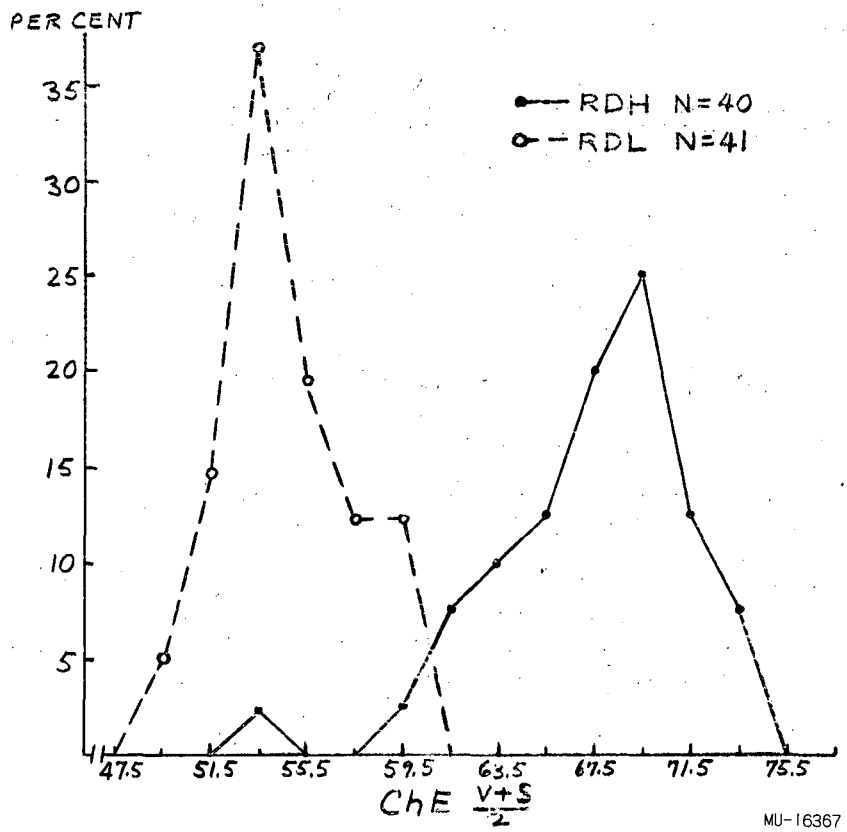


Fig. 10. Cortical ChE frequency distribution of RD male rats 90 to 119 days old.

was used as the breeding criterion. The Roderick strains were bred only for differences in ChE activity, perhaps leaving the ACh levels unaltered. One might expect the Roderick high-ChE rats to be inferior in adaptive behavior to the low-ChE rats, since raising the ChE activity level and leaving the ACh unaltered would lower the ChE-ACh ratio and result in too rapid a breakdown of ACh for efficient synaptic transmission.

Table VII

Correlations between behavioral test scores and ChE in K strain

Maze	r	P
Hebb-Williams	0.06	
Dashiell		
Errors	+0.53	0.01
Peripheral units	+0.44	0.05
Lashley	0.28	

As a result of this revised hypothesis, we predict relatively large differences between the ACh level of the S_1 and S_3 strains, but smaller differences between the Roderick high-ChE and the Roderick low-ChE lines. We hope to test these predictions in the near future.

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