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CHEMISTRY DIVISION QUARTERLY REPORT

March, April, and May 1957

BERKELEY, CALIFORNIA

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CHEMISTRY DIVISION QUARTERLY REPORT

March, April, and May 1957

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

June 28, 1957

BIO-ORGANIC CHEMISTRY

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

A TRACER STUDY WITH OXYGEN-18 IN PHOTOSYNTHESIS BY ACTIVATION ANALYSIS

Ingrid Fogelstrøm-Finemann, Osmund Holm-Hansen, and Bert M. Tolbert

Oxygen is an important element in biological processes and in organic chemistry, hence it is of considerable interest to develop additional methods whereby tracer studies with oxygen can be carried out. Because the half lives of the radioisotopes of oxygen are all short, it is necessary for tracer studies to use one of the stable isotopes that occur naturally, either oxygen-17 or oxygen-18. In previous reports of this group, we have discussed the possibilities of nuclear magnetic resonance spectroscopy as a tracer technique, making use of the O^{17} isotope. In this report, we are describing techniques for the activation analysis of O^{18} and the application of this procedure to a study of the oxygen-evolving reactions in photosynthesis.

Oxygen-18 has a high capture cross section for protons (2×10^{-25} cm^2 for 4-Mev protons) yielding F^{18} by the reaction $O^{18}(p,n)F^{18}$. Flourine-18 has a half life of 1.8 hr and emits 0.64-Mev positrons. Calculations show that 0.1 to 1.0 μg of O^{18} could easily be detected from the F^{18} radiation if a 4-Mev proton beam of 1 to 10 μa is allowed to hit an oxygen target for a few minutes.

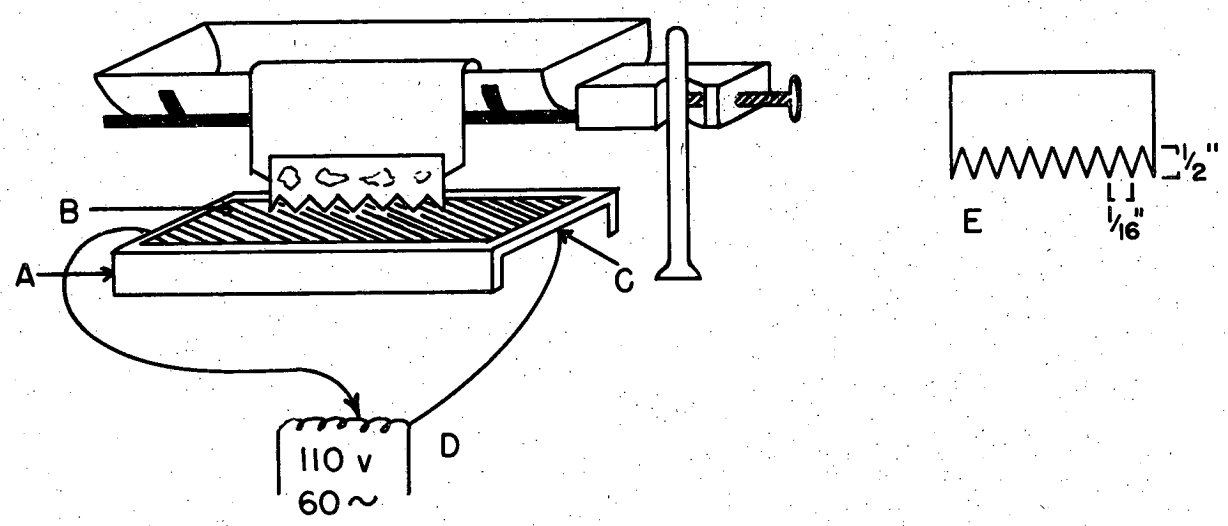
The application of a tracer technique to a chemical or biochemical problem involves detecting the isotope qualitatively and then determining its concentration before or after chemical separations. Because the analytical method for O^{18} that we are to describe involves the destruction of the sample, it is necessary that chemical separations, if they are to be made, be done prior to any isotope assays. This separation has been accomplished by paper chromatography. Apparently, it is not possible to perform an activation analysis of O^{18} directly on paper such as is used in paper chromatography. Therefore it has been necessary to devise a method to transfer the water-soluble compounds from the paper chromatogram to a metal sheet and thereafter to perform the activation and detection of the radioisotope directly on the metal sheet. The experimental development of the technique outlined above is described below.

A one-dimensional paper chromatogram containing several C^{14}-labeled sugar phosphates was serrated on one edge and the water-soluble compounds were transferred to a tantalum metal sheet. Autoradiograms of the original paper chromatogram and of the metal sheet containing the transferred compounds are shown in Fig. 1. The similarity of these two autoradiograms indicates that it is possible to transfer compounds from a one-dimensional chromatogram to a metal sheet by this procedure and yet retain the original distribution of compounds along the chromatogram.

For the first biological experiment utilizing O^{18} Chlorella pyrenoidosa were grown in O^{18}-enriched water for 2-1/2 days. Samples of these algae were then put on a tantalum target sheet and irradiated on a rotating target for two hr with 4.5 ± 0.2-Mev protons at an average current of 2.75 μa. This amounted to an average exposure of 3 min. in the beam for each spot, as calculated from the beam width and the size of the target. The autoradiograms from the bombarded spots showed that samples containing as little as 0.1 μg of O^{18} could be detected, and 1 μg of O^{18} produced a very easily detectable spot.

In an application of this technique to a specific problem, 250 λ of packed cells of Chlorella pyrenoidosa was suspended in 4 ml of 20% O^{18}-enriched water, killed, and extracted with hot ethanol. An aliquot of the extract was chromatographed in butanol–propionic acid–water. The chromatogram was then transferred to a tantalum target strip, which was bombarded with protons and assayed by means of autoradiograms. Figure 2 shows the result of this experiment, and reveals three peaks of radioactivity (corresponding to concentrations of O^{18} on the original chromatogram) that were not identified but correspond to the mono- and diphosphate and phosphoglyceric acid areas on the paper chromatogram as they usually appear in $C^{14}O_2$-fixation experiments.

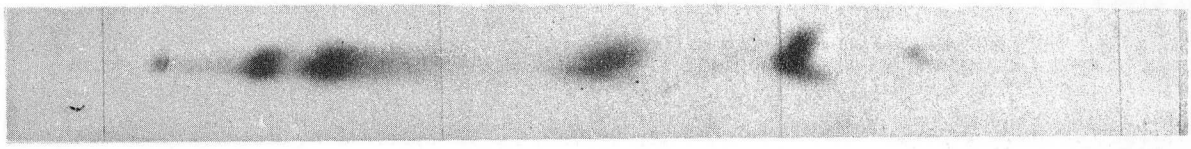
In summary, a method has been devised for tracer studies with oxygen, using paper chromatography and proton activation of O^{18} to F^{18}. This technique is relatively simple and can detect a fraction of a microgram of O^{18} as well as giving some information about the chemical nature of the compound into which it is incorporated. The procedure is being applied to a preliminary study of the path of oxygen in green algae.



MU-13170-A

Fig. 1. Apparatus for transferring a developed one-dimensional paper chromatogram to a metal sheet.
A, metal supporting plate;
B, tantalum sheet;
C, heating tape under sheet;
D, variable voltage control;
E, sheet of brass used to serrate edges of chromatogram.

Direction of chromatogram development ⟶



a: Autoradiogram of a paper chromatogram of C^{14}-labeled sugar phosphate developed in butanol–propionic acid. Exposure time 12 hr.

b: Autoradiogram of the metal sheet to which the paper chromatogram in "a" had been eluted. Exposure time 12 hr.

ZN-1746

Fig. 2. Films showing transfer of paper-chromatogram pattern to metal sheet.

HUMAN CARBON-14 RESPIRATION-PATTERN ANALYZER

B. M. Tolbert and Martha R. Kirk

The use of large ionization chambers to detect low levels of $C^{14}O_2$ from patients injected with carbon-14-labeled metabolites has been described previously.[1,2] During the past few months we have constructed a new machine for this purpose that uses the basic principles of the original but has many refinements in design. Changes were made to afford more comfort for the patient, provide greater facility of operation for the technician, improve CO_2-analyzer and ratio-recorder stability, and redesign certain engineering aspects.

The component parts of the new apparatus are mounted in two Budd cabinets. In addition to these cabinets, there are a lounge chair, the necessary compressed air, standard CO_2 tanks, and the exhaust pump. These are shown in Figs. 3 and 4.

One of the Budd cabinets contains the electrical circuitry and control panels. In it are, from top to bottom, the modified Leeds and Northrup multichannel recorder,[1] the amplifying circuit of the infrared CO_2 analyzer, the vibrating-reed electrometer, a control panel with helipots for regulating the various signals to the recorder, and a Sorenson voltage regulator.

In the other Budd cabinet we have placed, from top to bottom, the wet-test meter and calcium chloride moisture absorber; the infrared CO_2 analyzer-detector unit; the head of the vibrating-reed electrometer with its turret switch containing 10^{11}-, 10^{12}-, and 10^{13}-ohm resistors; and the 20-l stainless steel ion chamber. A schematic diagram of the airflow system that connects the various components of this section with one another and also with the air tanks, patient's helmet, and the exhaust pump is shown in Fig. 5. A series of solenoid valves has been used to control the "bypass flow" around the CO_2 analyzer so that this instrument may be standardized without interrupting the operation of the rest of the unit. Solenoid valves have also been used to bypass the helmet when the system is being flushed and to open the outlets of the wet-test meter to air pressure in order that the water level may be checked. The control panel on this Budd cabinet has the two flowmeters and needle valves necessary for regulating the airflow into the helmet and the exhaust from the helmet through the system. It also has one small flowmeter for indicating flow rate from the standard CO_2-air tanks and the necessary switches for controlling the exhaust pump and the various solenoid valves.

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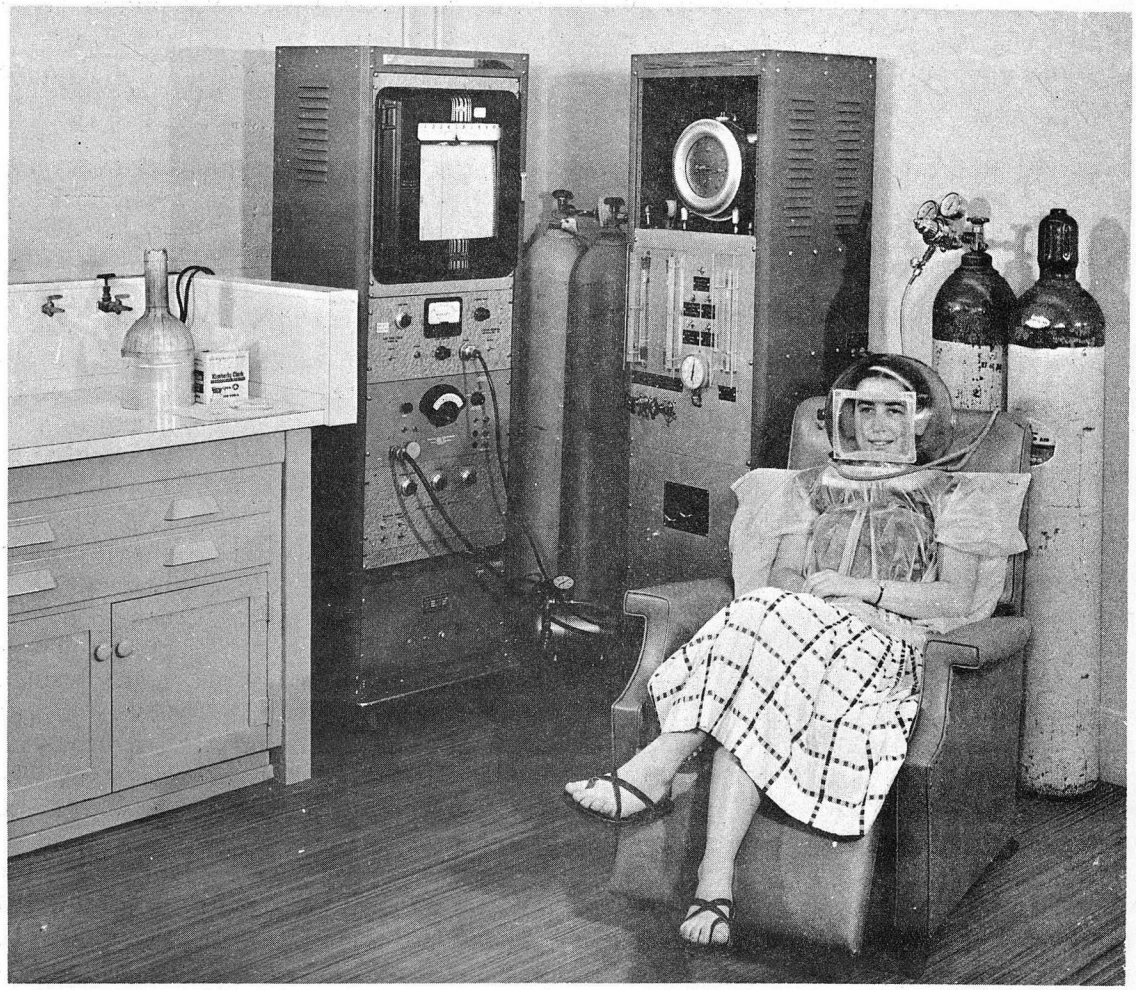
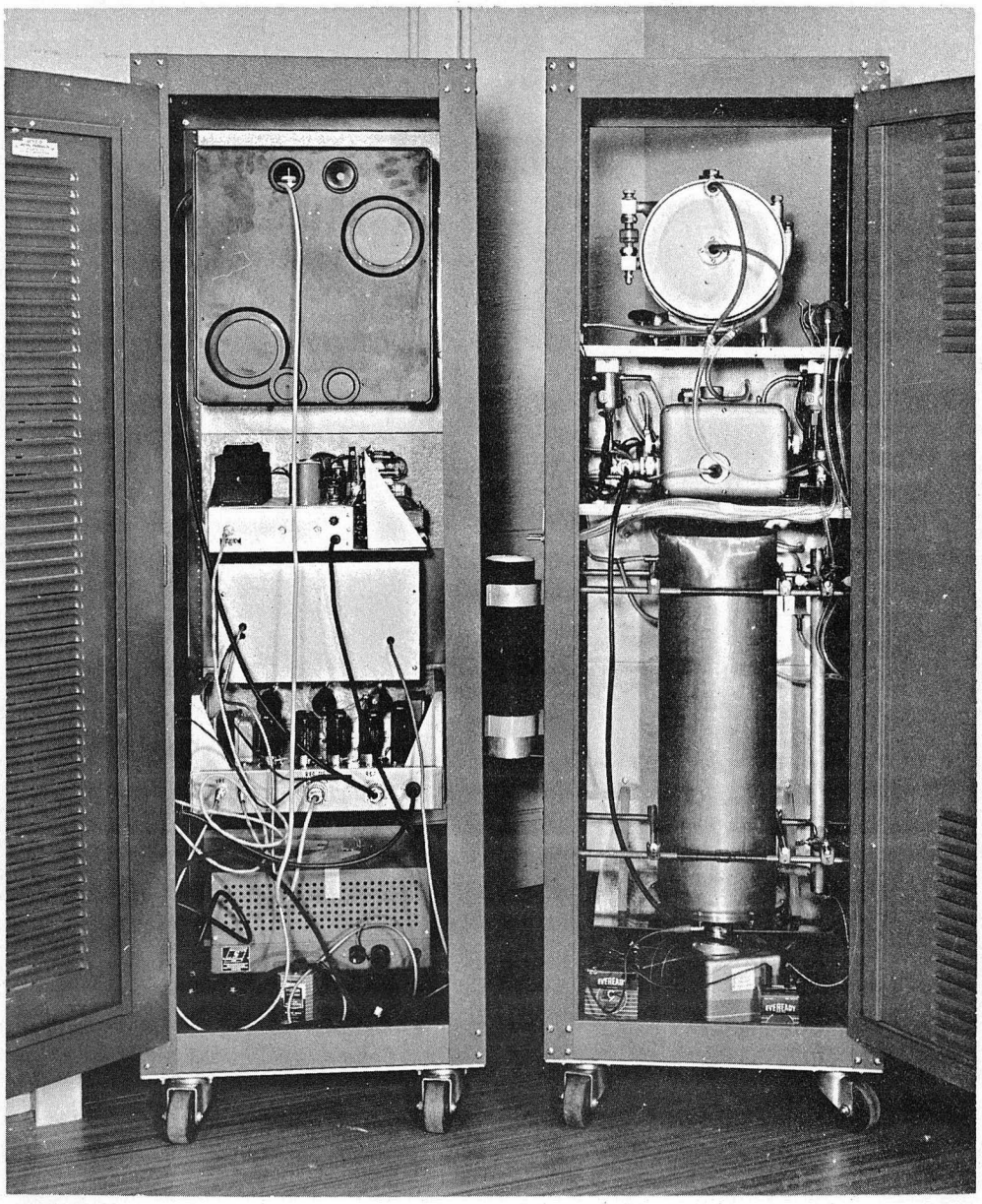
[1] Tolbert, Kirk, and Baker, Am. J. Physiol. 185, 269 (1956).

[2] Tolbert, Lawrence, and Calvin. Proceedings of the International Conference on Peaceful Uses of Atomic Energy 12, 281 (1956).



ZN-1744

Fig. 3. Carbon-14 respiration-pattern analyzer for humans.



ZN-1745

Fig. 4. Back view of electrical circuitry and flow system for the human carbon-14 respiration-pattern analyzer.

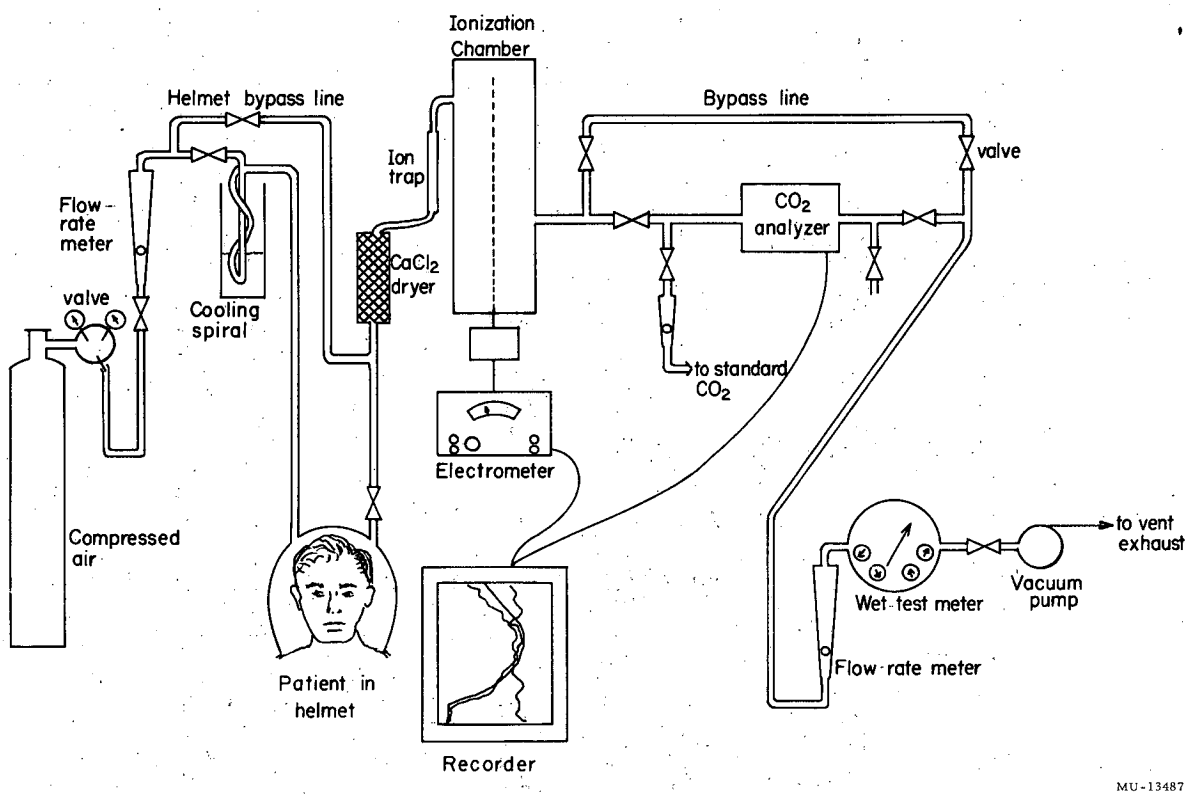


Fig. 5. Schematic diagram of airflow system, human carbon-14 respiration-pattern analyzer.

A flat face plate covered with a thin mylar window has been attached to the helmet in place of the rounded one formerly used. This greatly improves visibility for the patient, thus making him better able to read or observe what is going on around him. A thin pliable polyethylene sheet is attached to the helmet and forms a shawl for the patient's shoulders. This allows greater freedom of movement and is cooler than the sponge rubber sheet formerly used.

IONIZATION-CHAMBER ASSAY OF LOW-LEVEL $C^{14}O_2$

Irville M. Whittemore, B. M. Tolbert, and E. Ann Ludwigsen

In previous reports[1] the authors have described an apparatus for the precision assay of carbon-14 in organic compounds. A combination of Pregl-combustion and ionization-chamber assay of the $C^{14}O_2$ was used. In the earlier work, the assay was done on samples containing 0.05 μC or more of C^{14}. The useful range of the analysis has been extended down to 500 dis/min or about 2×10^{-4} μC, but with a lowering of the precision of the analysis.

Instead of using the high-resistance-leak measurement technique as previously described, we used the rate-of-drift method.[2,3] Benzoic-carboxyl-C^{14} acid of specific activity of about 4.5×10^{-5} μC/mg (100 dis/min/mg) was prepared for this test. Forty samples ranging in weight from 4.580 mg to 7.814 mg were burned and measured. The results are shown in Table I.

Samples of this activity gave rates of drift, which were about five to eight times the natural backgrounds of the 250-cc chambers used. The C^{14}-radioactivity variation should be about ± 0.6%, thus allowing about 5% variation in the analytical precision of this method to arise from background radiation and instrumental variations. The two chambers used showed a background standard drift-rate deviation of ± 13% and ± 6%. These variations correspond to about ± 2% of the sample drift rate. Thus most of the analytical variation seems to be present in the sample drift-rate determination.

Our previous reports show that the weighing, combustion, and chamber-filling technique should have an error of not more than 0.5%. We must therefore infer that most of our sample variation came from electrical variations in the ionization chamber and electrometer. It is probable that mechanical- and voltage-stress currents in the insulators were the critical item, because aging times and an assay schedule were not rigidly adhered to.

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[1] Whittemore, Ludwigsen and Tolbert in Chemistry Division Quarterly Report, UCRL-3595, Sept. 1956, p. 12; and Chemistry Division Quarterly Report, UCRL-3629, Jan. 1957, p. 29.

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

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

A second series of samples of this same benzoic acid ranging in weight from 1 to 2 mg were burned and measured. The mv/min/mg drift rate varied from 0.84 to 2.54, indicating that at the present time activities of this order of magnitude cannot be measured with any degree of precision.

If the background drift rate of the ionization chambers can be made less subject to random errors, the precision of the first type of analysis might be made even higher, and the very-low-level analysis could become useful.

Table I

Drift rate (in mv/min/mg) for 4 to 8 mg benzoic-C^{14} acid samples combusted to $C^{14}O_2$. Values corrected for background.

1.14	1.00	1.14	1.25	1.13	1.19
1.12	1.13	1.14	1.24	1.23	1.27
1.11	1.13	1.05	1.16	1.12	1.18
1.13	1.24	1.15	1.11	1.11	1.23
1.31	1.08	1.14	1.06	1.11	1.17
1.00	1.24	1.18	1.27	1.19	1.14
1.20		1.20	1.25	1.10	

average = 1.17

average deviation = 0.06

standard deviation = 0.07

% standard deviation = 6%

LUMINESCENCE OF PLANT MATERIALS

Gordon Tollin

The work described here was undertaken to learn more about the physical and chemical nature of chlorophyll in living plants, particularly in the period just after the chlorophyll is exposed to light. For this purpose, we have studied the luminescence spectrum of chloroplasts immediately after they were exposed to a flash of light.

Experimental

An apparatus was constructed that consisted of a lighttight housing having two openings at right angles to each other. These openings were fitted with camera shutters. One of the openings led into a cooled K1292 Dumont photomultiplier tube, the anode current of which was fed into a dc electrometer through a resistance of 10^9 ohms. The electrometer signal was fed into a Sanborn recorder. The samples were painted upon an aluminum rod fitted with a heater and a thermocouple. The bottom of the rod projected out of the housing and could be immersed in a cooling bath. An impulse from a storage battery triggered the first shutter, and the flash contacts on this shutter fired a General Electric FT-503 flash tube. The light from the flash was focused on the sample. The same battery impulse was sent through a delaying circuit and triggered the second shutter immediately after the first shutter had closed. The light emitted from the sample as a result of excitation by the flash was directed through the second shutter into the photomultiplier tube. In this manner the emitted light could be observed in a minimum of 0.05 sec after the flash was completed.

Luminescence of Whole Spinach Chloroplasts

The chloroplasts were prepared by the method of Sogo, Pon, and Calvin.[1,2] At room temperature these show a luminescence-decay curve consisting of two components, with half lives on the order of 0.1 sec and 2 sec. The former appears to decay exponentially, whereas the latter appears to follow a bimolecular rate law. Further studies are necessary, however, in order to establish this firmly. Upon cooling of the chloroplasts, the 2-sec component has essentially disappeared at about 0°C, whereas the 0.1-sec component slowly diminishes in intensity and is essentially gone at about -100°C. At about -90°C, a third emission commences to grow in; it reaches a maximum intensity at about -160°C, and remains constant down to -196°C. This latter emission has a half life of about 0.3 sec and appears to decay exponentially. These cooling effects are completely reversible.

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[1] Sogo, Pon, and Calvin. Photo Spin Resonance in Chlorophyll-Containing Plant Material, UCRL-3713, March 1957.

[2] Ibid., Proc. Nat. Acad. Sci. 43, 387 (1957).

The 0.1-sec and 2-sec half-life emissions are excited by light between 6500 A and 7000 A, indicating absorption by chlorophyll, although light between 3500 A and 4000 A is somewhat effective in exciting them. They both appear to have the same spectral distributions, lying between 7000 A and 9000 A. The 0.3-sec low-temperature emission is excited by light between 3500 A and 4000 A. Light between 6500 A and 7000 A is completely ineffective. This emission consists of light of longer wave lengths than 10,000 A and probably has a maximum around 12,000 A. When the chloroplasts are cooled to -196°C, their absorption spectrum appears to undergo no changes that could account for the appearance of the 0.3-sec emission. Both at room temperature and at -196°C, the chloroplasts are absorbing strongly between 3500 A and 4000 A, however, presumably in the second excited singlet of chlorophyll. It is striking that the energy difference between 0, 0 bands (transition from the lowest vibrational ground state to the lowest vibrational excited state) of the ultraviolet absorptions and the visible absorption corresponds to a wave length of emission of 12,200 A. Also, the lowest triplet states of either chlorophyll a or b would not be expected to lie out beyond 10,000 A.

From an analysis of the above data and a comparison of these data with those of Sogo, Pon, and Calvin,[1, 2] it seems likely that the room-temperature luminescences are the result of the recombinations of electrons and holes trapped in a quasi-crystalline lattice. The two time constants would thus correspond to two different trap depths. Tentatively, it is possible to assign the low-temperature infra-red emission to the radiative transition from the second excited triplet of chlorophyll to the lowest excited triplet.

Luminescence of Large and Small Spinach-Chlorophyll Fragments

These materials are prepared according to the method of Sogo, Pon, and Calvin.[1] They give room-temperature luminescence decay curves quite similar to those given by the whole chloroplasts.

Luminescence of Whole Chlorella

This organism gives a decay curve at room temperature apparently consisting of only one component, with a half time of about 1 sec. When the sample cools, the light emission is entirely gone at about -20°C. It has not been learned whether a new emission comes in upon further cooling. If dry nitrogen is allowed to blow on the Chlorella for about 15 min, the light emission disappears entirely. If, then, the algae are rewet, the light emission comes back immediately. The decay curve of this emission is, however, quite different from that of the undried material. The half time is approximately 0.2 sec, and the decay appears to follow bimolecular kinetics. It is not possible to say at this time whether the undried material decays exponentially or bimolecularly.

PREPARATION OF HEXATRIENE AND CYCLOHEPTATRIENE

Ann Coombs and Richard M. Lemmon

A previous report showed that both benzene-C^{14} and toluene-C^{14} are formed when ordinary benzene is irradiated with C^{14} ions in a mass spectrometer.[1] In the chemical processes that take place during such an irradiation, labeled hexatriene and cycloheptatriene may also be expected to be formed. The hexatriene-C^{14} would be formed by a rupture of the benzene ring accompanying the replacement of a C^{12} by an impinging C^{14} particle. Labeled cycloheptatriene is expected because of the work of Wolf and co-workers.[2] They have shown that when benzene is irradiated by neutrons in the presence of 2-methylpyrazine (as a source of nitrogen atoms), toluene-C^{14} is obtained. About 86% of the toluene's radioactivity is in the methyl group and the other 14% is in the ring. To explain the presence of activity in the ring, Wolf postulates an intermediate of either cycloheptatriene or tropylium ion. In either case, one should expect to find labeled cycloheptatriene as a product of the ion irradiation of benzene.

In order to have carrier amounts of the two compounds available for future attempts to isolate the labeled compounds, we have synthesized some by the methods described below.

1, 3, 5-Hexatriene

The hexatriene was prepared by the reaction[3]

$$NaNH_2 + CH_2 = CHCH_2C\ell \xrightarrow{\text{liq. } NH_3} CH_2 = CH\text{-}CH{=}CH\text{-}CH{=}CH_2.$$

About 11 g of sodamide was prepared by the addition of metallic sodium to liquid ammonia in the presence of a small amount of ferric nitrate. The excess ammonia was evaporated and the sodamide removed in a dry N_2 atmosphere. Twenty-three milliliters of freshly distilled allyl chloride was dissolved in 30 ml of liquid ammonia, and the sodamide was then added slowly with stirring and with a slow stream of N_2 moving through the system. After all the sodamide had reacted, the ammonia was evaporated, the salt dissolved in water, and the product extracted with peroxide-free ether. At this point, both vaporphase chromatography and the ultraviolet spectrum indicated a yield of about 35% of hexatriene. Then most of the ether was removed by room-temperature distillation, and the hexatriene was separated from higher-boiling material by vacuum distillation. The final purification was made by vapor-phase chromatography (Aerograph Model A90, 5-ft paraffin column at 70°, helium-flow rate of 50 cc/min). A compound ("peak") emerging from the column 6-min after injection was trapped and identified as 1, 3, 5-hexatriene. About 0.7 ml of

[1] Lemmon, Reynolds, and Mazzetti, in Chemistry Division Quarterly Report, UCRL-3595, October 1956, p. 5.

[2] Wolf, Gordon, and Redvanly, Abstracts of the 131st Meeting of the American Chemical Society, Miami, Florida, April 1957, p. 12-O.

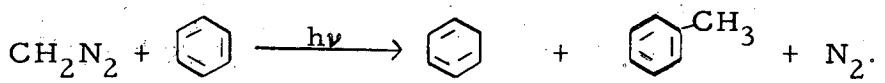
[3] M. S. Kharasch and E. Sternfeld, J. Am. Chem. Soc. 61, 2318 (1939).

this peak was isolated and then rechromatographed on paraffin, silicone, and carbowax columns. Its identity was established by its boiling point (80°), its microanalysis, its ultraviolet spectrum, and its mass spectrum (taken by Amos S. Newton of the Nuclear Chemistry Group).

A second peak, which emerged from the column 3 min after injection, was probably the other geometrical (cis-trans) isomer of hexatriene, as its ultraviolet spectrum appeared to be the same. However, after this compound was stored in a vial at -20° for 7 days, its spectrum was radically altered.

1,3,5-Cycloheptatriene

The cycloheptatriene was prepared by the method of Doering and Knox:[4]



A solution of approximately 0.15 mole of diazomethane in 500 ml of dry benzene was placed in a 1-liter round-bottom flask which was cooled by running water. The flask was irradiated with two General Electric reflector sunlamps at a distance of 6 in. from the flask. The reaction was complete when the evolution of nitrogen ceased (10 to 20 hr). About 350 ml of benzene was distilled from the flask at atmospheric pressure. The residue was distilled under nitrogen at 135 mm pressure, and the bulk of the product appeared in the material boiling at 45° to 90° C. Purification was accomplished by vapor-phase chromatography (Aerograph Model A90, 5-ft paraffin column, 74°, He flow 76 cc/min.) The toluene peak appeared 6.5 min after injection and the cycloheptatriene peak appeared 1.5 min later. About 30% of the cycloheptadiene peak was discarded in order to get rid of all the toluene. The completeness of the separation was shown by adding toluene-C^{14} to the mixture. After the chromatography, no radiactivity was found in the purified cycloheptatriene. The product was rechromatographed on paraffin, and the final yield of purified cycloheptatriene was 0.7 ml (5%). The product was identified by its boiling point (116°C), its ultraviolet spectrum, its microanalysis, and by the preparation of its maleic anhydride addition product. This product's melting point (103°-4°C) was in agreement with literature values, and its microanalysis was in agreement with the calculated values.

[4] W. von E Doering and L. H. Knox, J. Am. Chem. Soc. 75, 297 (1953).

PREPARATION OF DRY $KC^{14}N$ FROM $BaC^{14}O_3$

Richard M. Lemmon, Bernice Fingerman, and Helmut Simon

The procedure of Sixma et al.[1,2] has been used to prepare aqueous solutions of labeled sodium or potassium cyanide. Recently it became necessary to prepare a sample of dry $KC^{14}N$ with a specific activity of 360μC/mg. The following procedure was used for this purpose:

Fifty milligrams (0.25 mmole) of labeled barium carbonate and 100 mg of ammonium chloride were placed in a tube (25 cm by 17 mm o.d.) of Pyrex 1720 (high-temperature) glass. Air was displaced from the tube by a slow stream of nitrogen, and 0.3 to 0.5 g of metallic potassium (freshly cut under ether) was added. The tube was alternately evacuated and filled with nitrogen several times; simultaneously it was heated and shaken on a steam bath in order to melt the potassium and mix it thoroughly with the salts. The tube was then sealed under vacuum and placed in an oven at 640° for 70 min. After the tube was cooled, the capillary tip was broken and a partial vacuum applied to remove any hydrogen. The tube was broken into short sections, which were placed in a generating flask (a 125 ml Erlenmeyer flask equipped with a standard-taper joint). About 30 ml of n-butanol was then added to the flask to decompose the potassium.

The $HC^{14}N$ was generated by the slow addition from a dropping funnel of about 40 ml of 40% aqueous perchloric acid to the generating flask while a slow stream of nitrogen was kept moving through the all-glass system. The evolved $HC^{14}N$ was led through a small tube (12 mm by 5 in) filled with glass wool that had been dipped into P_2O_5 powder. The gas was then bubbled through two small traps that contained a total of 105% of the theoretical amount of potassium methoxide dissolved in about 1 to 2 ml of dry methanol. The traps were maintained at -78°C during an overnight generation of the $HC^{14}N$; during this time the generating flask was kept at 75°C. The traps were then allowed to come to room temperature while the passage of nitrogen through the system was maintained.

After the traps were removed from the system, the inlet tubes were raised above the surface of the liquid (by inserting a short piece of tubing containing the appropriate standard-taper joints). The inlet tubes were washed down with a small amount of methanol, and the methanol was evaporated at room temperature by a stream of dry nitrogen. The first trap, in which had been placed about 75% of the theoretical amount of methoxide, contained pure, dry $KC^{14}N$. The second trap had a mixture of methoxide and cyanide.

The yields by this procedure, determined by argentimetric titration ($AgNO_3$ after addition of excess NH_4OH plus a KI crystal) or by weighing the dry product, were 70 to 85%. Yields of 97 to 102% have been obtained on a 1-mM scale.[3]

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[1] Sixma, Hendriks, Helle, Hollstein, and van Ling, Rec. trav. chim. 73, 161 (1954).

[2] P. Kwong and R. M. Lemmon in Chemistry Div. Quarterly Report, UCRL-3415, June 1956, p. 47.

[3] Helmut Simon, Technical University, Berlin, Germany, unpublished work. Calvin

SYNTHETIC AND BIOCHEMICAL STUDIES OF MORPHINE

Melvin Look and Henry Rapoport

For reasons reported previously,[1] a scheme must be devised which completely removes the N-methyl group of morphine and then replaces the group. As reported previously, morphine-N-methyl-C^{14} was degraded to normorphine and then converted to morphine without the isolation of the intermediates.[2] The morphine isolated was still highly radioactive. It has now been shown that the morphine-N-methyl-C^{14} was incompletely degraded. The primary reason for the incomplete degradation was the formation of heroin acetate (which cannot be degraded) from the acetylation of morphine. Alkaline treatment of the products from the acetylation of morphine prior to the von Braun degradation increased the over-all yield of normorphine (from morphine) to 70 to 75%. More extraction steps were added to remove impurities arising from the degradation of morphine to normorphine and from the resynthesis to morphine. Using the new procedure, we have obtained over-all yields of 36% to 65%. The final testing of the scheme awaits the synthesis of more morphine-N-methyl-C^{14}. If it proves adequate, the new procedure will be described in a later report.

Adsorption and ion-exchange chromatography of morphine and morphine derivatives were studied as a method of purifying morphine-N-methyl-C^{14}. Adsorption chromatography was conducted with heroin (diacetylmorphine) because it has more favorable solubility properties than morphine. Morphine can be converted to heroin quantitatively, and the latter can be hydrolyzed back to morphine in good yields.[3] "Neutral" alumina adsorbed and held the heroin quite effectively. Elution with solvents having a polarity up to and including that of chloroform was ineffective in removing the compound from the alumina. A mixture of isopropyl alcohol and benzene removed the compound as an uncharacterized, hydrolyzed product. Triacetyl-10-hydroxymorphine can be successfully chromatographed on acid-washed alumina with chloroform and benzene.[4] The method was not successful in the chromatography of heroin; small amounts of heroin were removed by unreasonably large amounts of solvents. Ion-exchange chromatography of morphrine was much more successful.

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[1] M. Look and H. Rapoport, in Chemistry Division Quarterly Report, UCRL-3415, June, 1956, p. 4.

[2] M. Look and H. Rapoport, in Chemistry Division Quarterly Report, UCRL-3629, Jan. 1957, p. 37.

[3] C. R. A. Wright, J. Chem. Soc. (London) 27, 1031 (1874).

[4] H. Rapoport and S. Masumune, J. Am. Chem. Soc. 77, 6359 (1955).

Van Etten's method[5] with Dowex 50 cation exchanger was employed. An eluting solution of ammonium hydroxide more diluted than the one used by van Etten achieved a reasonable purification of morphine. A sample of morphine-N-methyl-C^{14} previously shown to be impure[2] was separated into two parts by this method. A radioactive impurity of about 1% of the total activity was removed from the morphine-N-methyl-C^{14}.

Morphine-N-methyl-C^{14} is being synthesized for the study of metabolism of morphine in humans and for use as a tracer for the degradation scheme described above. Modifications of the methods of Rapoport, Lovell, and Tolbert[6] were devised, and the over-all yields for the synthesis of morphine-N-methyl-C^{14} from codeine-N-methyl-C^{14} were increased significantly. Alkylation of norcodeine with methyl iodide to form codeine was carried out under a cold-finger condenser cooled with a mixture of n-butyl carbitol and dry ice instead of the ice water previously used. The evaporation of methyl iodide was lowered by this technique so that the yield of codeine was from 57% to 66% (based on methyl iodide), as compared with 35% by the old method.

The cleavage method of converting codeine to morphine was modified considerably; a complete description follows. A mixture of 0.8 g codeine and 2.4 g pyridine hydrochloride in a 25-cc flask fitted with a reflux condenser and a nitrogen bubbler (for dry nitrogen) was heated 6.0 min at 220-230°C in a preheated bath. The dark solution was allowed to cool to room temperature and 20 cc of water was added to dissolve the solidified material. The solution was made basic with 8 cc of 4 N sodium hydroxide containing a few milligrams of sodium hydrosulfite. The solution was extracted with four 15-cc portions of chloroform. The combined chloroform extract was washed with 10 cc of 0.5 N sodium hydroxide containing a few milligrams of sodium hydrosulfite, and then with 10 cc of water. The combined aqueous extract was acidified with concentrated hydrochloric acid and was adjusted to pH 9 with concentrated ammonium hydroxide. The dark solution was extracted under nitrogen with methylene chloride for 20 hr.

The methylene chloride solution was evaporated to leave a gummy solid. The solid was dissolved in 40 cc of methanol, and the dark solution was poured on a 1-by-16 cm column of Dowex 50. The column was washed with 10 cc of methanol and 10 cc of water and eluted with 150 cc of 0.5 N ammonium hydroxide. The eluate was adjusted to pH 9 with hydrochloric acid. Morphine crystallized (0.24 g; 29%); the solid was filtered and was washed with water. A further 18 mg of morphine was in the mother liquor, giving a total yield of 31%. The previous yield of morphine from codeine was 22%.[6] Low-specific-activity codeine-N-methyl-C^{14} (0.019 and 0.505 mC/mmole) and morphine-N-methyl-C^{14} (0.051 mC/mmole) were synthesized by these methods.

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[5] C. H. van Etten, Anal. Chem. 29, 954 (1956).

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

CARBON-14 RESPIRATION PATTERN ABNORMALITIES IN DIABETIC RATS

Hector Carminatti, Martha Kirk, Edward L. Bennett, and Bert Tolbert

Introduction

The changes in the metabolism of glucose to CO_2 in rats that have been made diabetic either by injection of alloxan or by pancreatectomy, and the influence of insulin and two of the sulfanylurea drugs on these changes have been discussed previously.[1,2] In order to gain further knowledge of the site of impairment of metabolism along the pathway from glucose to CO_2, several intermediates have been studied. This report deals particularly with the C^{14} respiration patterns of fructose-C_6^{14} and glucose-C_6^{14}-1-phosphate.

Experimental

Fructose-C_6^{14} for these experiments was obtained from canna leaves by biosynthesis[3] and purified by Dr. Richard M. Lemmon by paper chromatography. The glucose-C_6^{14}-1-phosphate was synthesized from sucrose-C_6^{14} by enzymatic phosphorylation with sucrose phosphorylase[4] and purified by paper chromatography. Both compounds were administered intraperitoneally in quantities of 1 mg. The fructose-C_6^{14} was dissolved in 0.1 ml H_2O and contained about 5 μC of C^{14}. The glucose-1-phosphate was dissolved in 0.25 ml H_2O and contained from 1.25 to 1.50 μC of C^{14}.

Long-Evans males were made diabetic by a single intravenous injection of alloxan (50 mg/kg) dissolved in physiological saline. The depancreatized rat was a Sprague-Dawley female obtained from Dr. Clarissa Beatty of the Oregon Medical School. Unless otherwise specified, the insulin used was crystalline insulin (Eli Lilly), and was injected intraperitoneally one hour before injection of the labeled substrate. Alloxan-diabetic rats received 40 units/kg per injection, and the depancreatized rat received 3 units. Carbon-14 respiration-pattern analyses were made using the ion-chamber infrared CO_2-analyzer ratio-recorder system described previously.[5]

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

[2] B. M. Tolbert and Martha Kirk, in Chem. Div. Quarterly Report, UCRL-3710, March, 1957, p. 52.

[3] E. W. Putman and W. Z. Hassid, J. Biol. Chem. 196, 749-52 (1952).

[4] R. Weinberg and M. Doudoroff, J. Bacteriol. 68, 381 (1954).

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

Results

The oxidation patterns for fructose-C_6^{14} show the same trends as glucose-C_6^{14}, but results are not nearly so consistent.[2] These results are summarized in Table II. Figures 6 and 7 show typical day-to-day variations in the same animal of the specific activity (C^{14}/CO_2) curves after injection of fructose-C_6^{14}.

The summarized data for glucose-C_6^{14}-1-phosphate are presented in Table III. The results again show changes in the same direction as those shown by glucose, but also exhibit considerable variation from animal to animal, or in the day-to-day tests on the same animal. Response to regular insulin seems to be quite consistent, but the anomalous results with Rat D2 on days 61 and 82 led to an investigation of the duration of action of protamine zinc insulin. This is a longer-acting insulin manufactured by Eli Lilly and Company, and is given to the animal subcutaneously when it is necessary to sustain him for several days without any further treatment. By numerous early tests, we have shown the action of crystalline insulin to be exhausted within 14 to 16 hours.[1] To test the protamine zinc insulin, Rat D2 was given a subcutaneous injection of insulin (40 μ/kg), and a glucose-C_6^{14} respiration pattern was made at least daily thereafter for four days. Figure 8 shows the total glucose-C_6^{14}-to-$C^{14}O_2$ oxidation for a 2-hr period for various intervals after the protamine zinc insulin injection. During the first two days the action of the insulin is sustained but it breaks sharply after 48 hr, so that by the end of 66 hr any effect is dubious, and at 72 hr there is none at all.

Discussion

Although the duration of the action of protamine zinc insulin may explain the ability of Rat D2 to oxidize glucose-C_6^{14}-1-phosphate at a rate even somewhat greater than normal, there is no such obvious explanation of the ability of a diabetic rat to oxidize fructose at a normal rate at some times and not at others. Also, there seem to be conditions under which fructose metabolism does not respond to insulin in the normal way.

Table II

Fructose-C_6^{14} oxidation by diabetic rats

Rat	Days after alloxan	Drug	% of injected fructose-C_6^{14} as $C^{14}O_2$				Previous treatment that might alter $C^{14}O_2$ pattern
			0 to 20 min	0 to 40 min	0 to 60 min	0 to 120 min	
Av. of 17 runs using Rats B1, B3, C1, C3, D1, D2 and E1	10 to 53	-	1.08	3.73	6.25	11.83	
C1	18	-	2.24	9.25	15.54	29.63	8u PZI* - 14 days
C3	33	-	4.95	14.99	23.05	36.42	8u PZI* - 14 days
B3	23	-	6.82	17.00	23.05	29.91	No PZI* after 7 days
Av. of 9 runs using Rats C1, C3, B3, D1, D2, E1	11 to 59	insulin 40u/kg	3.58	13.09	20.83	32.07	
B3	46	insulin	1.63	6.05	10.04	17.22	
D1	20	insulin	1.15	3.93	6.60	13.37	
Av. of 4 runs	†	-	0.98	3.56	5.79	10.79	
Av. of 2 runs	†	3u insulin	2.47	9.33	15.03	25.00	

† Depancreatized rat

* Protamine zinc insulin

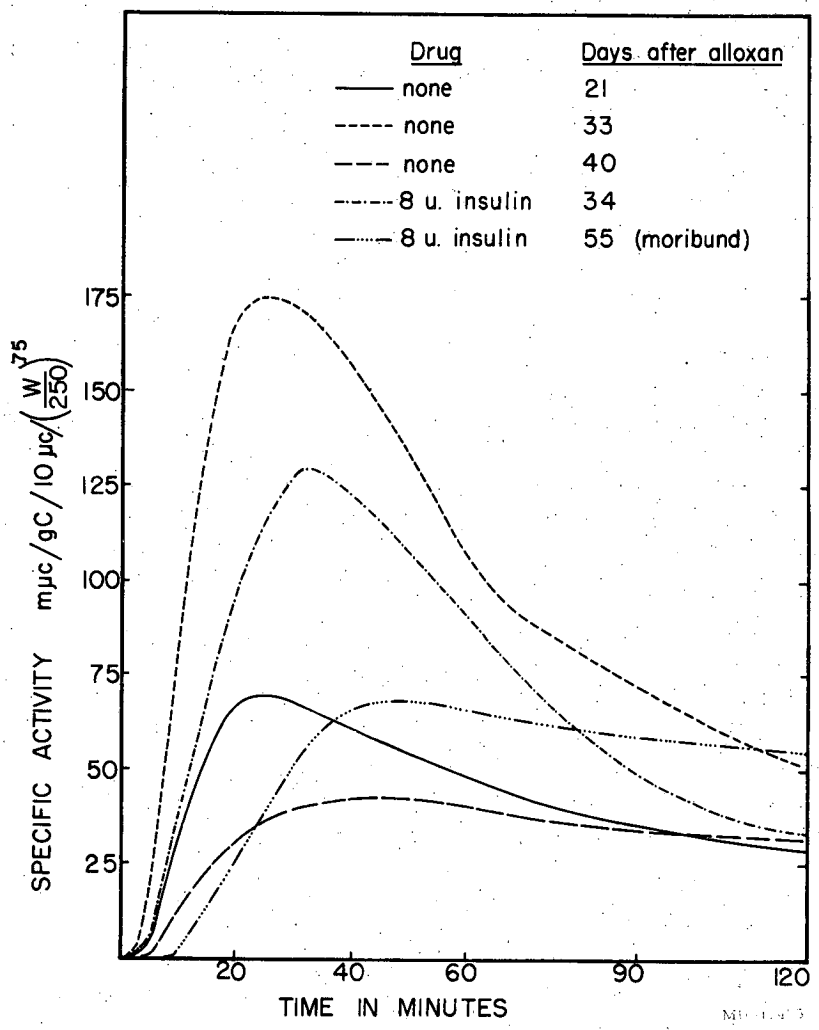

Fig. 6. $C^{14}O_2$ Respiratory patterns: variations in fructose-C_6^{14} oxidation in Rat C3.

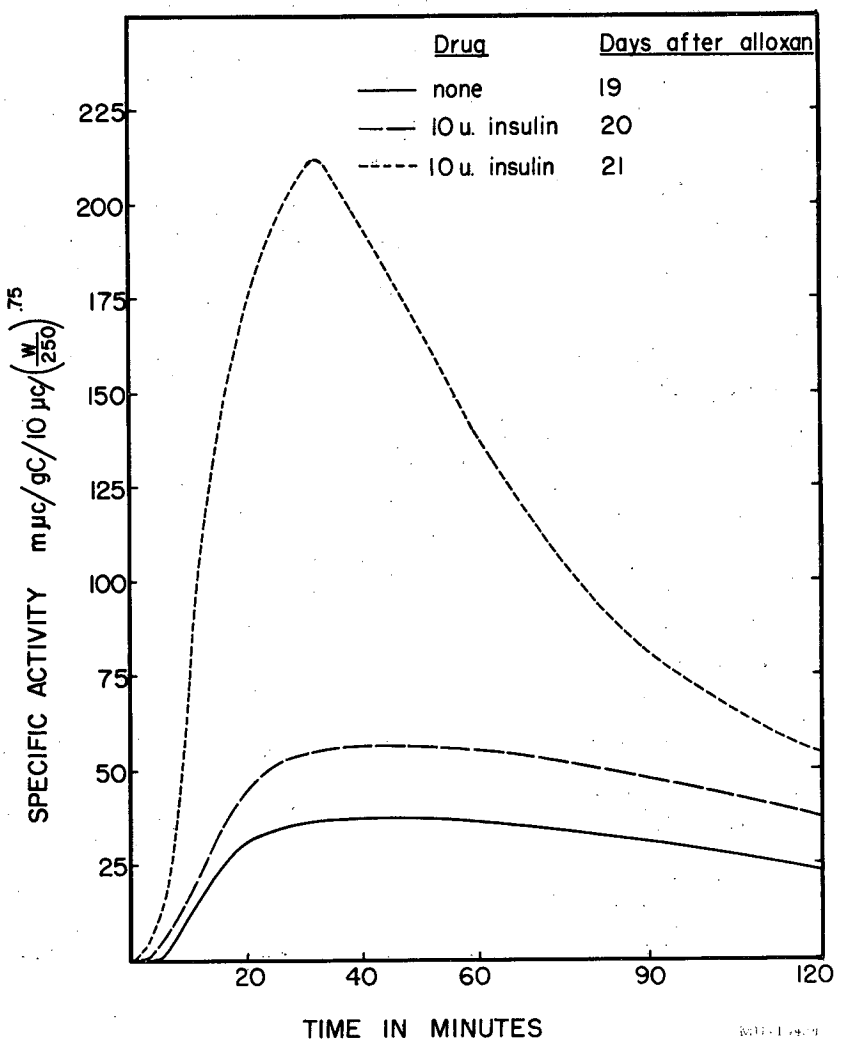

Fig. 7. $C^{14}O_2$ Respiratory patterns: variations in fructose-C_6^{14} oxidation for Rat D1.

Table III

Glucose-C_6^{14}-1-phosphate oxidation by alloxan-diabetic rats

Rat	Day after alloxan	Drug	% of injected glucose-C_6^{14} as $C^{14}O_2$				Previous treatment that might affect $C^{14}O_2$ pattern
			0 to 20 min	0 to 40 min	0 to 60 min	0 to 120 min	
C1	77	-	1.29	3.81	7.00	17.28	
C1	84	-	0.47	1.77	3.51	8.38	
D2	63	-	0.45	2.55	4.80	10.85	
D2	66	-	0.85	3.98	7.46	17.35	
E1	32	-	1.23	3.18	5.47	12.63	
E1	37	-	0.88	2.77	5.61	15.29	
D2	61	-	5.29	16.08	27.13	45.44	10u PZI 59 days
D2	82	-	8.30	26.35	36.75	48.10	12u PZI 80 days
C1	87	insulin 40u/kg	3.59	14.48	25.02	39.93	
D2	67	"	4.06	14.98	24.68	35.71	
E1	39	"	2.84	13.76	23.06	35.44	
E2	av. of (2 runs)	not-diabetic	2.73	12.38	21.35	41.42	

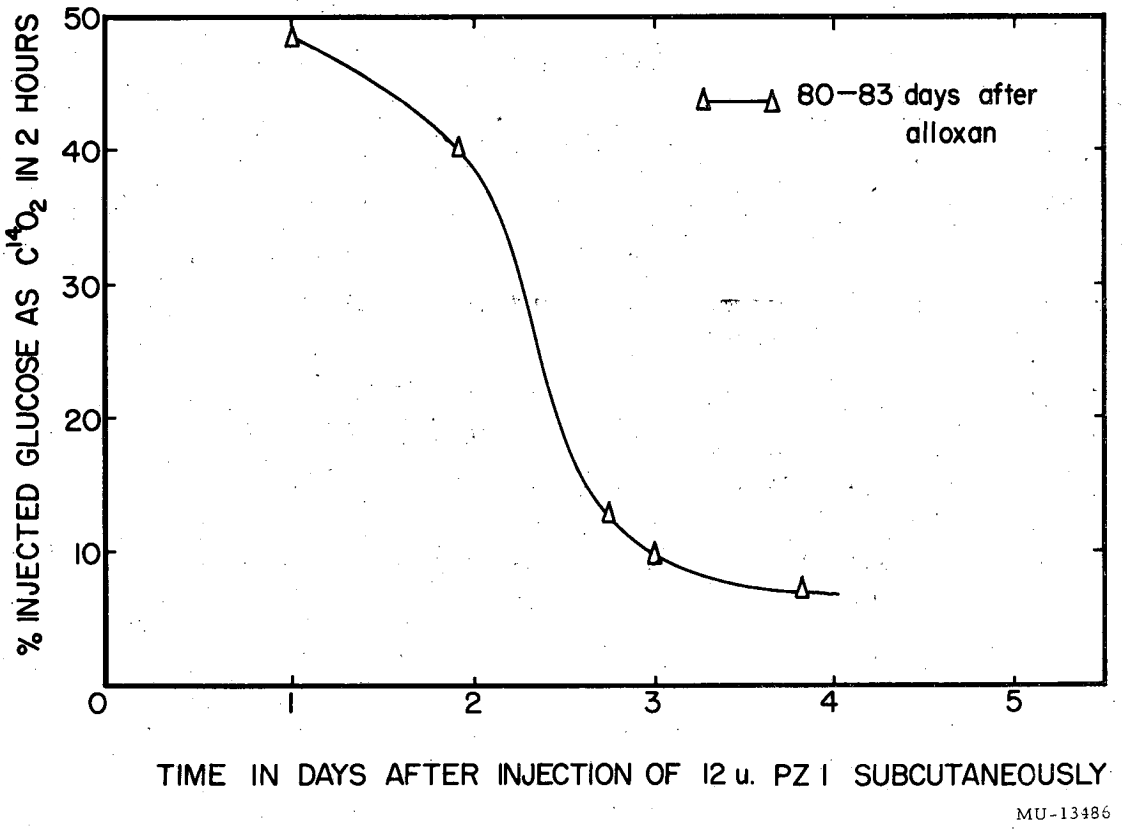

Fig. 8. Rat D2: duration of effect of protamine zinc insulin 80 to 83 days after alloxan.

DISTRIBUTION OF DIISOPROPYLFLUOROPHOSPHONATE IN RATS

Edward L. Bennett, Hilda M. Karlsson, and Joy Loe

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

In the preceding quarterly report initial experiments with diisopropylfluorophosphonate-P^{32} (DFP^{32}) were described in which the distribution of P^{32} in the blood and brain of rats at several time intervals after administration of DFP^{32} was studied.[1] An attempt was made from these preliminary data to estimate a minimum turnover number for the enzyme cholinesterase (ChE); the assumptions involved were discussed in the previous report. It appeared that further fractionation of the brain proteins might yield fractions with increased ratios of ChE activity to P^{32}, and that therefore a higher (and presumably more valid) turnover number might be estimated. If several different fractionation procedures did not change the ChE/P^{32} ratio, this would be evidence that the P^{32} was bound primarily to the ChE protein, and therefore the estimates of turnover number might be approximately correct.

A few preliminary experiments were made to test possible fractionation procedures. A fractionation procedure using ammonium sulfate was not satisfactory, as the ammonia was lost from the solution during the assay procedure, and, as a result, the solution became acid at a rate much higher than the usual rate of hydrolysis of acetylcholine by a brain homogenate. Procedures employing butanol or chloroform to extract lipids from the brain homogenates were not successful, owing to denaturation of the protein and loss of enzyme activity. It was found that ChE was much more soluble when the brain or cortex was homogenized in water than in saline; only 5% to 10% of the enzyme activity was sedimented upon centrifugation at 3000 rpm in an International refrigerated centrifuge. Therefore, in the experiment to be described, the only fractionation procedure employed was centrifugation.

Four littermate male rats (S_3 strain, age 3 months, approximate weight 200 g) were injected with DFP^{32} (1 mg in propylene glycol per body weight). Atropine (1 mg/kg) was injected several minutes prior to the DFP^{32} injection. A control animal was sacrificed at one day, and DFP^{32}-injected animals were sacrificed at 1, 4, 8, and 15 days after injection. The brain and cortex were homogenized in water instead of 0.9% saline; otherwise the procedures used for sacrificing, analyzing for ChE, and determining the P^{32} activity were the same as described in the preceding quarterly report.[1] Rather erratic results were again obtained with the lysed cells; determinations made at longer intervals after lysis were generally higher than earlier determinations.

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[1] Bennett, Loe, and Karlsson, in Chemistry Division Quarterly Report, UCRL-3710 March, 1957, p. 57

The distribution of P^{32} activity is summarized in Table IV. In this experiment, the specific activity of the DFP^{32} was considerably less than expected, so that the actual counting rates were very low and subject to considerable error. In general, somewhat lower values for "turnover number" were obtained in this experiment, but since the actual inhibition of ChE in the brain and cortex was smaller than expected, the values are probably not so reliable as in the first experiment.

In addition, aliquot portions of the brain and cortex homogenates were centrifuged at 3000 rpm for 10 min in an International refrigerated centrifuge, and then at 20,000 rpm/20 min in the No. 40.1 head of the Spinco preparative centrifuge to separate the soluble and insoluble fractions of the homogenates. In general, less than 20% of the P^{32} activity and ChE activity were precipitated by centrifugation at 3000 rpm in either the brain or cortex homogenates. However, a large amount of ChE activity was sedimented from the cortex homogenate at 20,000 rpm, but considerably less P^{32} activity was precipitated. This is evidence that P^{32} is bound to proteins other than ChE in the cortex. The sedimentation of P^{32} activity and ChE acitivity were approximately equal in the brain homogenates. The results are summarized in Table V.

Table IV

P^{32} Distribution and ChE activity of rats following DFP^{32} administration*

Time after injection, days	Fraction	Total activity (cts/min)	Percent of injected DFP^{32}	Act. γ DFP per gram tissue or whole blood	Act. Moles	ChE Activity** moles AcCh hydrolyzed per gram tissue or ml whole blood per min	Per-cent con-trol	Turn-over number
1	Cortex	1.8×10^3	0.019	0.14	7.7×10^{-10}	48×10^{-7}	73	2300
	Brain	7.3×10^3	0.075	0.13	7.1×10^{-10}	120×10^{-7}	73	6200
	Serum	2.3×10^5	2.4	0.51	2.8×10^{-9}	33×10^{-9}	22	42
	Cells	5.7×10^4	0.6	0.12	6.5×10^{-10}	45×10^{-8}	74	245
4	Cortex	1.8×10^3	0.02	0.15	8.1×10^{-10}	45×10^{-7}	68	2600
	Brain	5.9×10^3	0.07	0.12	6.5×10^{-10}	118×10^{-7}	72	7100
	Serum	2.5×10^4	0.3	0.054	2.9×10^{-10}	108×10^{-9}	72	145
	Cells	2.6×10^4	0.3	0.055	3.0×10^{-10}	54×10^{-8}	88	230
8	Cortex	1.9×10^3	0.02	0.14	7.6×10^{-10}	56×10^{-7}	85	1300
	Brain	5.5×10^3	0.06	0.09	4.9×10^{-10}	141×10^{-7}	85	4700
	Serum	3.7×10^3	0.04	0.007	3.8×10^{-11}	120×10^{-9}	80	790
	Cells	2.4×10^4	0.26	0.048	2.6×10^{-10}	31×10^{-8}	51	1290
15	Cortex	9.6×10^2	0.009	0.07	3.8×10^{-10}	59×10^{-7}	90	1850
	Brain	3.9×10^3	0.037	0.07	3.8×10^{-10}	155×10^{-7}	94	2350
	Serum	5.9×10^2	0.0056	0.0009	5.0×10^{-12}	255×10^{-9}	150	--
	Cells	3.8×10^3	0.035	0.0058	3.1×10^{-11}	68×10^{-8}	111	--

* The rats (approx. wt. = 200 g) were administered 1 mg DFP^{32} in propylene glycol per kg of body weight by intraperitoneal injection. The DFP^{32} had an activity of 4.4×10^7 cts/min/mg at the time of injection. All values have been corrected for radioactive decay. For calculating total activity in cells and serum it was assumed that the blood volume was 5 ml/100 g body weight.

**The ChE activity of the control rat was as follows: Cortex: 66×10^{-7}; brain: 164×10^{-7}; serum: 15×10^{-8}; and cells: 61×10^{-8}.

Table V

Distribution of P^{32} and ChE activity of rat brain and rat cortex homogenates upon centrifugation*

Time after injection (days)	Fraction	P^{32} activity (cts/min)	Percent of non-centrifuged	ChE activity moles hydrolyzed per gram tissue per minute	Percent of non-centrifuged
1	Cortex	1.8×10^3		48×10^{-7}	
	Sup., 3,000 rpm	2.0×10^3	110	45×10^{-7}	94
	Sup., 20,000 rpm	1.5×10^3	85	11×10^{-7}	24
	Brain	7.3×10^3		120×10^{-7}	
	Sup., 3,000 rpm	8.3×10^3	114	107×10^{-7}	89
	Sup., 20,000 rpm	6.0×10^3	83	58×10^{-7}	48
4	Cortex	1.8×10^3		45×10^{-7}	
	Sup., 3,000 rpm	2.0×10^3	110	39×10^{-7}	87
	Sup., 20,000 rpm	1.1×10^3	61	6.4×10^{-7}	14
	Brain	5.9×10^3		118×10^{-7}	
	Sup., 3,000 rpm	5.5×10^3	93	104×10^{-7}	88
	Sup., 20,000 rpm	3.7×10^3	64	60×10^{-7}	51
8	Cortex	1.9×10^3		56×10^{-7}	
	Sup., 3,000 rpm	1.4×10^3	74	40×10^{-7}	71
	Sup., 20,000 rpm	0.7×10^3	37	8×10^{-7}	14
	Brain	5.5×10^3		141×10^{-7}	
	Sup., 3,000 rpm	4.6×10^3	83	138×10^{-7}	98
	Sup., 20,000 rpm	3.1×10^3	57	75×10^{-7}	53

Table V continued

Distribution of P^{32} and ChE activity of rat brain and rat cortex homogenates upon centrifugation*

Time after injection (days)	Fraction	P^{32} activity (cts/min)	Percent of non-centrifuged	ChE activity moles hydrolyzed per gram tissue per minute	Percent of non-centrifuged
15	Cortex	9.6×10^2		59×10^{-7}	
	Sup., 3,000 rpm	7.7×10^2	80	57×10^{-7}	96
	Sup., 20,000 rpm	5.6×10^2	58	22×10^{-7}	38
	Brain	3.8×10^3		155×10^{-7}	
	Sup., 3,000 rpm	3.0×10^3	80	156×10^{-7}	101
	Sup., 20,000 rpm	1.5×10^3	40	60×10^{-7}	39

*Homogenates of the brain and cortex in water were centrifuged at 3000 rpm for 10 min in an International refrigerated centrifuge, and then at 20,000 rpm for 20 min in a No. 40.1 rotor of a Spinco preparative centrifuge. The supernatants obtained were assayed for ChE activity and P^{32} activity and compared with an uncentrifuged aliquot portion.

INCORPORATION OF ADENINE-C^{14} INTO NUCLEOTIDES AND NUCLEIC ACIDS OF RATS

Edward L. Bennett and Hilda M. Karlsson

In previous quarterly reports, we have described the results of experiments utilizing adenine-C^{14} in mice and rats. This work was concerned with the influence of various factors such as time of sacrifice after administration, amount of adenine administered, position of C^{14} in adenine, x-radiation, and A-methopterin and aminopterin, upon the utilization and distribution of the adenine in various tissues and nucleic acid fractions. Our previous studies of the effect of x-radiation upon the incorporation of adenine into nucleotides and nucleic acids of various tissues of rats, in particular thymus, have given results[1] not in agreement with those obtained by Harrington and Lavik in rather similar experiments.[2] Differential effects of x-radiation upon the incorporation of various precursors into desoxynucleic acid have led to various hypotheses concerning the biosynthesis of nucleic acids and possible sites of action of x-radiation upon the biosynthetic pathway. Therefore, a resolution of these experimental differences is desirable.

An examination of data we had obtained in previous experiments with rats indicated that the incorporation of adenine into the DNA of the thymus was significantly less than expected on the basis of its incorporation into other tissues such as liver, spleen, and small intestine, and on the basis of corresponding data obtained with P^{32}-phosphate. It appeared that at the low dosage of adenine used (approximately 1.2 mg adenine per 175 g rat) less adenine is incorporated into nucleotides and nucleic acids of the thymus than would be expected for this relatively rapidly metabolizing tissue. This made the determination of specific activity of the nucleotides and nucleic acids of the thymus and spleen difficult and less accurate than similar determinations in other tissues. In experiments with mice, it has been noted that the relative distribution of adenine in other tissues has been dependent upon the amount used, and it was thought that at low dosages very little adenine was available to the thymus because it is taken up very rapidly by other organs, in particular by the liver and small intestine.

The purpose of this experiment was to determine the effect of dosage upon the distribution of adenine in the rat, with particular reference to those organs--such as thymus, spleen, and brain--that ordinarily take up little adenine in order to determine the optimal dosage of adenine for future experiments on the effect of x-radiation on incorporation into spleen and thymus. Three female Long-Evans rats, age 2 months, weighing 155, 165, and 200 g, were injected intraperitoneally with 1.3, 5.0, and 11.1 mg of adenine in 1.0 ml of 0.9% NaCl, respectively. Each solution contained adenine-8-C^{14} with a total activity of 2.6 to 3.0 x 10^7 dis/min. Therefore, if an equal percentage of the adenine administered at each dosage is incorporated into a tissue fraction, the total activity incorporated will be the same, whereas if equal absolute amounts of adenine are incorporated, the activity will be

[1] Edward L. Bennett and Barbara Krueckel, in Chemistry Division Quarterly Report, UCRL-3068, July 1955, p. 10.

[2] H. Harrington and P. S. Lavik, Arch. Biochem. Biophys. 54, 6 (1955).

in the ratio 100:26:11. The animals were sacrificed at 24 hr, and the cold trichloroacetic (TCA) fraction, ribonucleic acid (RNA) fraction, and desoxynucleic acid (DNA) fraction have been isolated from the liver, rinsed small intestine, kidney, brain, spleen, thymus, two femurs (bone marrow), and a portion of the abdominal muscle. At the present time, only the semi-quantitative total activity data in these fractions are available. They are summarized in Table VI. In nearly all cases the amount of activity incorporated, when expressed in microgram equivalents of adenine, increases with increasing amount of adenine administered.

The percentage of the injected adenine in the nucleotide and nucleic acid fractions usually is approximately the same or decreases in the liver, small intestine, and muscle, and decreases markedly in the kidney. The percentage of adenine found in the cold TCA, RNA, and DNA fractions from the bone marrow, thymus, spleen, and brain increased markedly with an increase in administered adenine from 1.3 mg to 5.0 mg, and in general increased slightly with a further increase of administered adenine to 11 mg. This indicates that these tissues get an appreciable amount of adenine only after other tissues become "saturated." Thus, for x-radiation experiments in which the incorporation of adenine into thymus and spleen are of particular interest, the utilization of relatively large amounts of inactive adenine carrier with a given amount of adenine-C^{14} will lead to an enhanced incorporation of radioactivity into these tissues, and thus facilitate isolation and determination of specific activity.

Determination of the specific activity of the 5'-adenylic acid, RNA-adenine and guanine, and DNA-adenine and guanine in the various tissue fractions is in progress.

Table VI

Distribution of C^{14} in the acid-soluble fraction, RNA fraction, and DNA fraction of the rat after administration of adenine-8-C^{14}*

Tissue	Amount adenine (mg)	Cold TCA fraction % inj. activity	Cold TCA fraction adenine (μg equiv.)	RNA fraction % inj. activity	RNA fraction adenine (μg equiv.)	DNA fraction % inj. activity	DNA fraction adenine (μg equiv.)
Liver	1.3	12	120	1.3	13	0.039	0.38
	5.0	7.8	300	2.1	81	0.042	1.6
	11.1	7.0	570	1.8	148	0.034	2.8
Small intestine	1.3	4.4	44	2.6	26	1.5	14
	5.0	3.1	119	4.9	188	2.7	104
	11.1	2.3	189	2.2	180	1.2	98
Muscle (portion)	1.3	1.4	14	0.08	0.8	0.007	0.07
	5.0	0.6	22	0.05	2.1	0.007	0.27
	11.1	0.55	45	0.06	5.3	0.0055	0.45
Kidney	1.3	2.5	24	0.9	9.1	0.010	0.1
	5.0	1.3	49	0.32	12.3	Lost	Lost
	11.1	0.54	44	0.25	20.1	0.009	0.08
Femurs (2)	1.3	0.036	0.36	0.04	0.40	0.032	0.32
	5.0	0.08	3.0	0.08	3.2	0.14	5.4
	11.1	0.05	4.2	0.10	8.2	0.16	13
Thymus	1.3	0.042	0.41	0.013	0.13	0.016	0.16
	5.0	0.08	3.2	0.05	2.1	0.12	4.8
	11.1	0.09	7.2	0.045	3.6	0.14	12
Spleen	1.3	0.40	3.9	0.09	0.91	0.036	0.35
	5.0	0.41	16	0.2	7.7	0.10	4.0
	11.1	0.47	39	0.2	16	0.15	12.3
Brain	1.3	0.04	0.39	0.009	0.09	0.002	< 0.02
	5.0	0.16	6.1	0.025	1.0	0.0015	< 0.06
	11.1	0.24	20	0.045	3.6	0.001	< 0.1

*Three female Long-Evans rats, weight 155, 165, and 200 g, age 2 months, were intraperitoneally administered 1.35, 5.05, and 11.1 mg of adenine containing adenine-8-C^{14} with 3.0, 2.6, and 2.7 x 10^7 dis/min. The animals were sacrificed 24 hrs later. All values are preliminary and subject to revision.

THE EXCRETION OF CYANIDE-C^{14} IN MICE

Ann M. Hughes and B. M. Tolbert

The excretion pattern of radioactivity from C^{14}-labeled compounds has been studied in animals and man for a number of compounds that occur naturally in biological systems. However, no data are available for excretion of radioactive cyanide. Because cyanide is a nonbiological, rather reactive ion, it is possible that an appreciable fraction of the radioactivity from labeled cyanide could be incorporated in the body in a very long-lived form. To investigate this possibility, we have studied the excretion pattern of $NaC^{14}N$ in mice.

Ten mice were injected intraperitoneally with 0.05 mg of sodium cyanide-C^{14} (about 4 μC) in 0.15 ml of pH 8 aqueous solution. These injections were carried out within a few minutes. The radioactivity in the breath was measured for the first 6 hr for four animals individually, using the continuous ionization-chamber assay method for the determination of the exhaled C^{14}.[1] At the end of 1-, 3-, and 11-day intervals, breath radioactivity was again determined.

The urine-feces excretion data were obtained by placing four mice in a metabolism cage and collecting the combined product. The excreta and cage washings were homogenized in a Waring blender, diluted to a convenient volume (50 to 150 ml) and a 25-to100 λ aliquot was transferred to a platinum counting dish. This aliquot was evaporated to dryness under a heat lamp and the sample counted in a windowless flow G-M counter with an efficiency of about 50%. At the end of the 30-day experiment, sample activities were about double background. Appropriate corrections were made for backscattering, self-absorption, and coincidence, where necessary.

Two animals were killed on each of the following days: 8, 15, 22, and 30. The residual total-body radioactivity was determined by cutting the animal into approximately 0.25-cm^3 pieces, adding these to 100 ml of N KOH, and digesting at 50°-60°C for 24 hr. Fifty ml of 95% ethanol was added to the digestion mixture, which was then centrifuged. The supernatant was decanted, the volume measured, the mixture shaken to emulsify the fat, and a 50- or 100-λ aliquot pipetted into a platinum dish for assay as previously described. The precipitate, presumably mostly protein, was redissolved in 100 ml of N KOH, and 100 λ of the supernatant was assayed for C^{14}. Reprecipitation of this material and radioactivity assay showed two-thirds of the activity to remain in the protein fraction. In this procedure the bones are not dissolved, but samples of the bones, ground up and counted on direct plates showed that within the limits of the counting error there was no detectable radioactivity. Samples of fat, counted separately, before emulsification, likewise contained no activity.

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

The data on the excretion rate of C^{14} from mice given cyanide-C^{14} are presented in Table VII. The radioactive-material balance for the series of animals is given in Table VIII.

A final report is being prepared that includes an analysis of the data and the possible application of the determination of radiation dosage in humans.

Table VII

Daily excretion rate of carbon-14 from mice given cyanide-C^{14}

Interval (days)	Urine and feces (μC/day)	Breath (μC/day)
0 to 1/4	0.36	1.64
1/4 to 1	1.72	0.282
1 to 2	0.76	0.163
2 to 3	0.29	0.054
3 to 4	0.19	0.034
4 to 5	0.10	0.024
5 to 6	0.085	0.0186
6 to 7	0.021	0.0151
7 to 8	0.023	0.0121
8 to 9	0.019	0.0100
9 to 10	0.015	0.0085
10 to 11	0.013	0.0074
11 to 12	0.0070	0.0065
12 to 13	0.0087	0.0058**
13 to 14	0.0072	0.0055**
14 to 15	0.0057	0.0045**
15 to 18	0.0042*	0.0035**
18 to 23	0.0033*	0.0023**
23 to 26	0.0032*	0.0017**
26 to 30	0.0028*	0.0011**

*These values are averaged and interpolated from measurements made on the fourteenth, seventeenth, twenty-second, twenty-fifth, and twenty-ninth day after injection.

**These values are obtained by extrapolation.

Table VIII

Carbon-14 material balance for mice given cyanide-C^{14}

Time (days)	Carcass Analysis* Soluble fraction (μC)	Carcass Analysis* Alcohol ppt. fraction (μC)	Breath, urine, and feces (μC)	Summation of C^{14} excreted plus C^{14} in situ (μC)
8	0.130	0.010	3.79	3.93
15	0.110	0.073	3.92	4.10
22	0.110	0.015	3.97	4.11
30	0.092	0.020	4.00	4.11

*Each value is the average of the analysis of two animals.

PHOSPHORYLATED INTERMEDIATES IN FAT METABOLISM

Rosemarie Ostwald, Richard M. Lemmon, and Samuel Lepkovsky

A previous quarterly report described some investigations into differences in adipose-tissue metabolism.[1] This report concerns a continuation of that work with two particular aspects in mind. One is the question of the function of adipose tissue--whether it is only a storage depot for fat, or whether it also contributes to the synthesis of fat. The other is the larger question of the mechanisms of hunger and satiety, and whether a metabolite or metabolites can be found in strikingly different concentrations in a hungry animal and in a satiated animal.

As a contribution to both aspects of the problem, phosphorylated intermediates of carbohydrates and fat metabolism were investigated with the help of P^{32}. The amounts of P^{32} incorporated and the compounds in which it appeared were determined and compared for livers and adipose tissue in rats. In addition, the same data were determined and compared for the same tissues from rats in two different nutritional states: fasted and fed.

[1] Ostwald, Lemmon, and Lepkovsky, in Chemistry Division Quarterly Report, UCRL-3595, Oct. 1956, p. 72.

Experimental

The procedure for the extraction of the tissues, separation of compounds, and chromatography were described earlier.[2]

Results

In Table IX are summarized the differences that have been found in the livers and in the adipose tissues of fasted (24 hrs) and fed rats. In Table X are summarized the data that have been obtained on individual phosphorylated compounds.

Discussion

These data show that, as far as the incorporation of P^{32} and the distribution of phosphorus among the various compounds and fractions are concerned, the adipose tissues' behavior is very similar to that of the liver. Quantitatively, the fats, particularly the white fats, are much less metabolically active than the livers. These facts alone, however, do not indicate whether this is anabolic or catabolic activity or both. So the question of whether adipose tissue synthesizes fats or only stores and catabolizes them is not directly answered. Data obtained in in vitro experiments clearly indicate that the fat depots are sites of active fat synthesis.[3]

Most of the data presented here show that "fasted" tissues have a lower rate of metabolism than do "fed" tissues. The latter tissues incorporate more total activity, and more of the inorganic phosphorus is converted to organically bound phosphorus in the "fed" tissues. In addition, the paper chromatographic work has shown that more ATP, IMP, glycerol phosphate, and sugar phosphates are present in the "fed" tissues than are found in the "fasted" tissues. These data indicate that the presence of food in an animal's stomach initiates mechanisms that control the rates of metabolism in the liver and the adipose tissues. Such mechanisms are probably intimately involved in producing the states of hunger and satiety.

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[2] Ostwald, Lemmon, and Lepkovsky, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 12.

[3] E. Wertheimer and B. Shapiro, Physiol. Rev. 28, 451 (1948).

Table IX

Differences in the distribution of P^{32} activity and phosphorus-containing compounds in "fasted" and "fed" tissues

Analytical determinations for a given weight of tissue	Tissue	Greater in
Activity incorporated into TCA extract	liver	fed
	fat	fed
Inorganic P in TCA extract	liver	fasted
	fat	fed
Inorganic P in TCA extract, specific activity	liver	fasted
	fat	fed
Organic P in TCA extract, specific activity	liver	fed
	fat	fed
$\frac{\text{Specific activity of inorg. P}}{\text{Specific activity of organic P}}$	liver	fasted
	fat	fasted
Activity incorporated into non-nucleotide fraction	liver	fed
	fat	fed
$\frac{\text{Sp. act. of inorg. nonnucleotide P}}{\text{Sp. act. of org. nonnucleotide P}}$	liver	fasted
	fat	fasted
Activity incorporated into nucleotide fraction	liver	fasted
	fat	fasted
Organic P of nucleotide fraction	liver	fasted
	fat	fasted
Organic P of nucleotide fraction, specific activity	liver	fasted
	fat	fasted
$\frac{\text{Activity of nonnucleotide fraction}}{\text{Activity of nucleotide fraction}}$	liver	fed
	fat	fed
Total P in TCA extract	liver	fasted
	fat	fed
$\frac{\text{Inorganic P in TCA extract}}{\text{Organic P in TCA extract}}$	liver	fasted
	fat	fasted

Table X

Data concerning specific P-containing compounds

Compound	Tissue	% of total radioactivity on paper chromatogram	Greater in*
Inorganic P	Liver TCA	40 to 70	fasted
	Fat TCA	20 to 80	fasted
AMP	liver nucleotides	20 to 60	fasted
	fat nucleotides	2 to 6	fasted
ADP	liver nucleotides	15 to 40	fasted
	fat nucleotides	10 to 30	fasted
ATP	liver nucleotides	1 to 10	fed
	fat nucleotides	5 to 25	fed
IMP	liver nucleotides	2 to 13	fed
	fat nucleotides	2 to 6	fed
"T"(unknown)	liver nucleotides	2 to 10	fasted
	fat nucleotides	4 to 25	fasted
"S"(unknown)	liver nucleotides	3 to 13	fed
	fat nucleotides	8 to 31	fed
Glycerol-P	liver TCA	10 to 20	fed
	liver nonnucleotides	4 to 17	fed
	fat TCA	4 to 17	fed
	fat nonnucleotides	6 to 50	fed
Sugar-P	liver nonnucleotides	2 to 18	fed
	fat nonnucleotides	3 to 30	fed

*The differences between the fasted and fed tissues are not great, a factor of 1.2 to 2.2, but were rather definite on most papers that were counted.

EFFECT OF GAMMA RADIATION ON PHOTOSYNTHESIS AND VIABILITY OF CHLORELLA

Christian Van Sumere and Osmund Holm-Hansen

Work has been continued on the effect of gamma radiation from a Co^{60} source on the growth and metabolism of Chlorella pyrenoidosa. Preliminary results[1] indicated that the photosynthetic uptake of $C^{14}O_2$ was not very sensitive to radiation doses up to 500,000 rep. The work described below extends the radiation range to 1,000,000 rep and furnishes information on the rate and pattern of $C^{14}O_2$ uptake after varying lengths of time after the irradiation, as well as information on the cell-division rate.

The general procedure in all experiments is essentially similar and is as follows. Two ml of bacteria-free Chlorella were pipetted into small cotton-stoppered test tubes (3/16 by 3 in.). These tubes were then inserted directly into the brass holders of a Co^{60} source. Three-minute photosynthesis tests were usually made approximately 1 hr after the algae were removed from the Co^{60} source. As the algae were radiated while still in nutrient solution, they were first centrifuged and resuspended in distilled water before any $C^{14}O_2$ photosynthesis test. Usually small samples were removed from each irradiated tube, either by a small pyrex glass loop or by micropipette, and streaked onto an agar nutrient medium in a petri dish. To further test the viability of irradiated cells, either 1.0- or 2.0-ml portions of irradiated algae suspension was pipetted into a sterile 5-ml volumetric flask. These flasks were especially equipped with a ground-glass joint with cotton-stoppered aeration tubes in the top part. The flasks were then suspended on the shaker apparatus at 24° to 25° C., and 4% CO_2 in air passed through them at a very slow rate.

In Table XI is shown the photosynthetic $C^{14}O_2$ uptake by cells immediately after irradiation and after 2 days. Chromatograms were made from the alcoholic extracts in Samples A and B, but no significant differences were seen in the distribution of activity among the compounds. By manometric techniques we attempted to demonstrate respiration by the irradiated cells of exogenously supplied glucose. The basal respiration of algae that received 0, 900, 8,500, 100,000, 500,000, and 1,000,000 rep was measured and then glucose was added to each sample. Unfortunately, none of the samples showed any increased respiratory rate due to the addition of glucose. The samples of irradiated algae that had been streaked onto agar showed that those receiving 0, 900, and 8,500 rep grew very well, while those receiving 100,000, 500,000, and 1,000,000 rep showed no detectable growth on the nutrient agar.

In Table XII are shown results from a similar experiment. Here, as in the previous experiment, the total $C^{14}O_2$ fixation 1 hr after irradiation is not significantly different in any of the treatments. However, after 2 days the samples that received 1,000,000 rep did not fix any $C^{14}O_2$. Chromatograms were made on all the samples (except that one with no fixed activity) and again no significant changes were seen in the pattern of activity in the

[1] C. Van Sumere and O. Holm-Hansen, in Chemistry Division Quarterly Report, UCRL-3710, March 1957, p. 32.

Table XI

Effect of Co^{60} radiation on the photosynthetic $C^{14}O_2$ uptake by algae

Sample	Time after irradiation (hr)	Radiation (rep)	Total fixation (cpm)	Total activity in 80% EtOH fraction(cpm)	Total activity in 20% EtCH fraction(cpm)	Total activity in insoluble fraction (cpm)
A	1	0	2,800,000	1,700,000	200,000	900,000
B	1	500,000	2,800,000	1,700,000	240,000	920,000
C	1	1,000,000	2,200,000	1,300,000	260,000	680,000
D	48	0	1,200,000	410,000	160,000	590,000
E	48	500,000	1,300,000	470,000	180,000	670,000

Table XII

Effect of radiation on photosynthetic uptake and cell division

Sample	Radiation dose in reps.	Time after irradiation (hr)	Total fixation (cpm)	Total activity in 80% EtOH fraction (cpm)	Total activity in 20% EtOH fraction (cpm)	Total activity in insoluble fraction (cpm)	Cell count per ml	Total fixation per 129×10^6 cells. (cpm)
A	0	1	1,190,000	570,000	370,000	520,000		
B	900	1	1,600,000	600,000	380,000	530,000		
C	8,500	1	1,500,000	590,000	360,000	450,000		
D	100,000	1	1,500,000	670,000	300,000	520,000		
E	500,000	1	1,400,000	570,000	280,000	500,000		
F	1,000,000	1	1,200,000	430,000	290,000	400,000		
							At start 129×10^6	
G	0	48	250,000	140,000	64,000	32,000	217×10^6	74×10^3
H	500,000	48	180,000	100,000	52,000	27,000	115×10^6	103×10^3
I	1,000,000	48	0	0	0	0	68×10^6	--

compounds on the chromatograms. The algae from samples A, B, and C all grew very well on the agar plates (left for 2 to 3 weeks), while those from D, E, and F did not show any sign of growth. To get some idea of the growth capacity of the irradiated cells (the volumes are too small to permit centrifuged cell-pack measurements), cell counts of the algae suspension in the small volumetric flasks were made after the 2-day growth period. As is seen in Table XII, the cell count at the time of irradiation was 129,000,000 cells per ml, and after 2 days, the count in Sample G was 217,000,000 per ml; in Sample H, 115,000,000 per ml, and in Sample I, only 68,000,000 per ml. Thus the cells increased in number in the control, remained essentially constant in the sample that received 500,000 rep, and decreased in the sample irradiated with 1,000,000 rep. It is likely that the rate of division in the control sample was limited by partial depletion of the nutrient medium. It should also be noted that the cells in Sample I showed a strong tendency toward clumping, which probably decreased the number of observable cells. Also, there was considerable cell debris noticed in Sample I.

In Table XIII the $C^{14}O_2$ fixation is reported for 2 days and for 1 week after irradiation, as well as immediately following the irradiation. To eliminate the problem of nutrient depletion in the 2-day test, 1 ml of the irradiated algae was pipetted into a sterile volumetric flask that contained one ml of fresh Myers's medium. It is seen from the data that although the total fixation per equal number of cells is determined, the rate of $C^{14}O_2$ fixation remains roughly the same. To measure the growth of the cells after one week, 1 ml of the irradiated algal suspension was pipetted into 250-ml Erlenmyer flasks containing 40 ml of sterile Myers's medium. These were placed on the shaker apparatus and aerated with 4% CO_2 in air. After a 1-week growing period, the cell counts in Samples I, J, and K were all 100 to 200 times that in the beginning, while the cell counts in Samples L, M, and N were either the same as or lower than at the beginning. The centrifuged cell packs of Samples I, J, and K were all about 1.5 ml, while that of Sample L was slightly less than 0.1 ml. The cell packs of Samples M and N were too small to be estimated, but were in the range of 0.01 ml. In Samples I, J, K, and L (Table XIII) the algae in each flask were centrifuged and resuspended in distilled water, and 0.02-aliquot portions were taken for the $C^{14}O_2$-incorporation determinations. When this fixation is compared for an equal number of cells, it is seen that the fixation per cell is about 10 times as high in Sample L as in the control. However, when it is compared on equal centrifuged cell packs, the fixation is roughly the same for Treatments I, J, K, and L. This would indicate that the photosynthetic rate per unit of cell volume is about the same in all four samples, and that the cells in Sample L have merely enlarged without undergoing cell division. Microscopic examination has indicated that this is actually so; actual measurement of cell sizes is planned for future work. The algal sample streaked onto agar plates confirmed the observations made earlier.

Table XIII

Effect of radiation on photosynthetic uptake, cell division, and cell volume

Sample	Irradiation dose (rep)	Time after irradiation (hr)	Total fixation (cpm)	Total activity in 80% EtOH fraction (cpm)	Total activity in 20% EtOH fraction (cpm)	Total activity in insoluble fraction (cpm)	Cell count per ml	Total fixation per 129×10^6 cells	Total fixation per 0.03 ml centrifuged pack cells (Approx) (cpm)
A	0	1	1,500,000	1,100,000					
B	500,000	1	900,000	560,000					
C	0	48	870,000	570,000	160,000	150,000	655×10^6	85,860	
D	900	48	960,000	610,000	140,000	140,000	615×10^6	96,000	
E	8,500	48	480,000	210,000	48,000	53,000	347×10^6	86,400	
F	100,000	48	170,000	93,000	32,000	20,000	83×10^6	130,900	
G	500,000	48	95,000	63,000	6,000	12,000	75×10^6	81,700	
H	1,000,000	48	44,000	11,000	2,000	6,000	54×10^6	64,240	
							started at 3.4×10^6		
I	0	168	140,000*				523×10^6	44,800	140,000
J	900	168	110,000*				527×10^6	30,000	110,000
K	8,500	168	84,000*				398×10^6	36,100	84,000
L	100,000	168	8,300*				3.5×10^6	409,550	113,000
M	500,000	168	0				3.6×10^6	0	
N	1,000,000	168	0				2.1×10^6	0	

*Represents fixation from 1/50 of the total amount of algae in each sample.

PHOTOSYNTHESIS OF LIPIDS AND PIGMENTS

Ulrich Blass

Continuing the work described in a previous report,[1] we are characterizing the pigments in photosynthesizing algae. This work is described in the two sections below.

Carotenoids of algae in light and in dark

Better polyethylene samples for column chromatography and an improved washing method with EDTA (ethylene diamine tetraacetic acid) for the columns made it possible to carry out some reproducible determinations of carotenoid concentrations in algae. Because we were particularly interested in the influence of light upon the carotenoid concentrations, we made simultaneous determinations of the pigments in algae samples from which one half was kept in the dark while the other half was exposed to light (1300 ft-c). The experimental conditions have already been reported,[2] therefore this report is confined to results with polyethylene chromatography and the concentration of carotenoids so measured.

As adsorbent polyethylene powder of low molecular weight does not allow a separation of the main epoxy-carotenoids in which we are especially interested, violaxanthin and xanthophyll-epoxide. Therefore, the use of a polyethylene with a molecular weight of at least 100,000 was desirable. Fifteen grams of such a polyethylene powder were suspended in 80% aqueous methanol by means of an electric blender and poured into a chromatogram tube as a slurry. The solvent was moved through the powder under a pressure of 0.03 to 0.05 atmos., causing a fairly tight packing of the column. The column was then washed with a solution of 1 g EDTA-Na_4 (tetra sodium salt) in 1:1 methanol-water. Then the solvent was changed to 80% aqueous methanol again and the column was ready for use. Figure 9 shows the development of typical chromatograms of methanolic extracts of Chlorella and Scenedesmus on polyethylene columns. The identification of the colored zones was based on spectral data for the eluted fractions.[2] Table XIV represents the carotenoid concentrations of Chlorella and Scenedesmus as they were determined in various experiments. The data show that the concentration of violaxanthin in Chlorella is considerably changed by light within 30 min, whereas values from shorter exposure times and from Scenedesmus show only changes within the experimental error.

The decrease in the violaxanthin concentration during 30-min light exposure could be due to (a) decomposition by light irradiation, (b) rearrangement following a light-induced drop in the pH, or (c) enzymatic conversion into other metabolites. The first reason is unlikely because the plant is known to use much higher light intensities for growth and cell division. This means that it can build up pigments at higher light intensities than those used here. The second reason, the pH effect, accounts for the extreme sensitivity of violaxanthin towards acids, which

[1] Ulrich Blass, in Chemistry Div. Quarterly Report, UCRL-3710, March 1957 p. 28.

[2] Ulrich Blass, in Chemistry Div. Quarterly Report, UCRL-3629, Jan. 1957, p. 34.

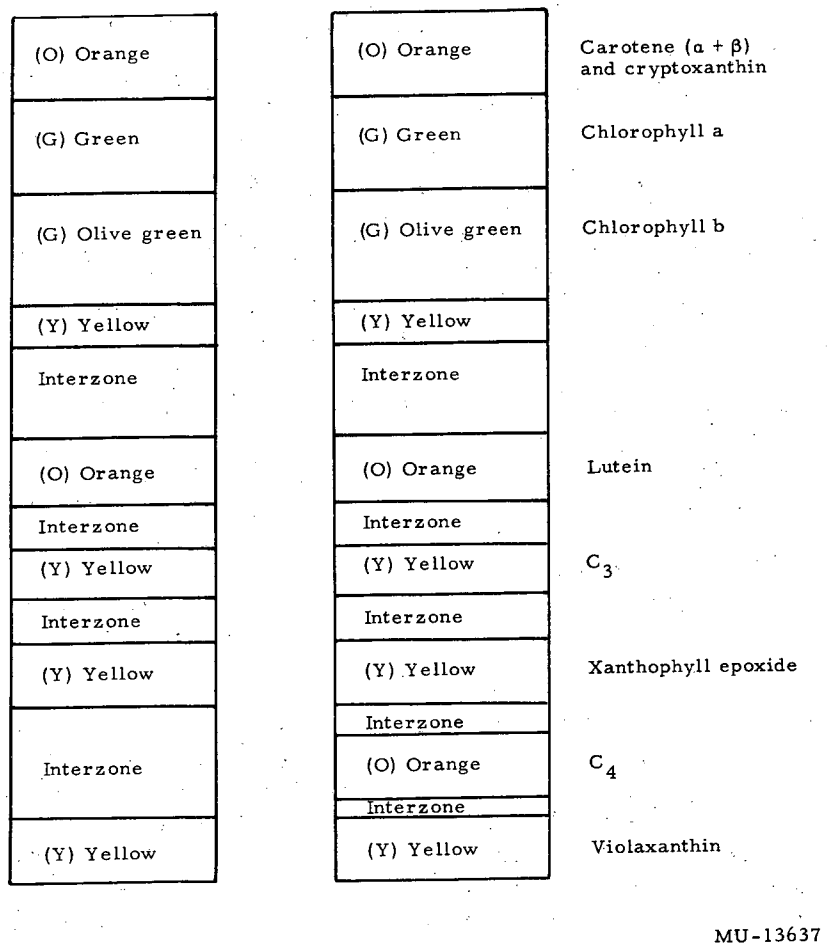

Fig. 9. Separation of the pigments of Chlorella (left) and Scenedesmus (right) in polyethylene powder columns with 80% aqueous methanol.

Table XIV

Carotenoid concentrations in light and dark periods, in 10^{-8} mole per ml wet packed algae

Algae	Chlorella		Chlorella		Scenedesmus		Scenedesmus		Chlorella		Chlorella	
Light exposure time	30 min		30 min		30 min		30 min		15 min		15 min	
Light (1300 ft-c)	dark	light	dark	light	dark	light	dark	light	dark	light	dark	light
Carotenoid Carotene (α + β)			65.6	58.6								
Lutein	158	152	191	160								
C_3												3.6
Xanthophyll-epoxide	51.2	49.2	50.4	44.4	51.2	47.2	47.6	53.6	62.1	67.8	41.2	37.3
C_4					84.0	61.2	65.8	68.8				
Violaxanthin	42.6	22.0	52.0	24.0	56.2	48.4	52.8	43.6	52.1	60.0	49.3	37.2
Adsorbent	← Polyethylene →											

Algae	Chlorella		Chlorella	
Light exposure time	30 min		30 min	
Light (1300 ft-c)	dark	light	dark	light
Carotenoid α-Carotene	39.0	41.0	33.6	39.2
β-Carotene	24.1	17.0	30.6	29.0
Adsorbent	Calcium hydroxide			

cause rearrangement to furanoid carotenoids. The latter pigments occur only in very low concentrations (1/50 to 1/100 of that of violaxanthin) in Chlorella and Scenedesmus and do not show an increase in amount after illumination. From this and the fact that algae can be adapted to acid media, one might conclude that the algae can prevent the reaarrangement regardless of the acidity of the medium. These conclusions leave us still the possibility of enzymatic conversion to other metabolites. So far, we have not been able to find a substance whose concentration increase in the light would correspond to the decrease in the violaxanthin concentration.

The specific radioactivity of Scenedesmus pigments

An attempt was made to determine the specific radioactivity of algae pigments by comparing the radioactivity of chromatogram fractions with their light absorption. The experimental details are as follows:

Algae (Scenedesmus) in a 1% suspension in 50 ml nutrient solution were allowed to photosynthesize for 1 hr in the presence of 100 μC of radioactive sodium bicarbonate (1 ml 0.036 N $NaHC^{14}O_3$). Air containing 1% carbon dioxide was bubbled through the suspension. The light intensity was 3000 ft-c. The algae were then centrifuged and extracted twice with 15 ml of boiling methanol. The extract was applied to a chromatographic column (11 g polyethylene in 80% aq. methanol) and developed as an elution chromatogram with 80% methanol. Fractions of 10 to 15 ml were collected successively, aliquots of which were evaporated on aluminum plates and counted for their radioactivity. From every colored fraction, we determined the visible-light absorption spectrum and calculated the carotenoid concentrations from the spectra.[1] The chromatographic distribution of light absorption and radioactivity is shown in Fig. 10. This diagram reveals that the absorption maxima correspond mostly with minima of radioactivity, which makes it doubtful whether the pigments become radioactive at all. To prove this supposition we made paper chromatograms and radioautograms of each fraction with pure light-absorption spectra (Fractions No. 19, 25, 30, 34, 46, 52, 58, 63, 67). The radioautograms of these chromatograms are also shown in Fig. 10. They demonstrate that most of the radioactivity in each fraction belongs to colorless compounds, while only lutein and chlorophyll among the pigments show any radioactivity. The calculations for the lutein fraction suggests a value of 12×10^{10} cpm/mole (220,000 cpm/mg) as upper limit. The radioautograph of the lutein paper chromatogram shows this value to be 2 to 10 times as high. Within an hour of photosynthesis the algae (about 100 mg dry weight) fixed 1.8×10^{7} cpm. The lutein fraction was determined spectroscopically as 65×10^{-8} mole, i.e. 0.35 mg, which fixed 35,000 cpm as a maximal value. The ratio of fixation in lutein over total fixation (2×10^{-3}) shows the same order of magnitude as the weight ratio (3.5×10^{-3}). These data indicate that radiocarbon is incorporated in the algae carotenoids as rapidly (within 1 hour) as into most other algae metabolites.

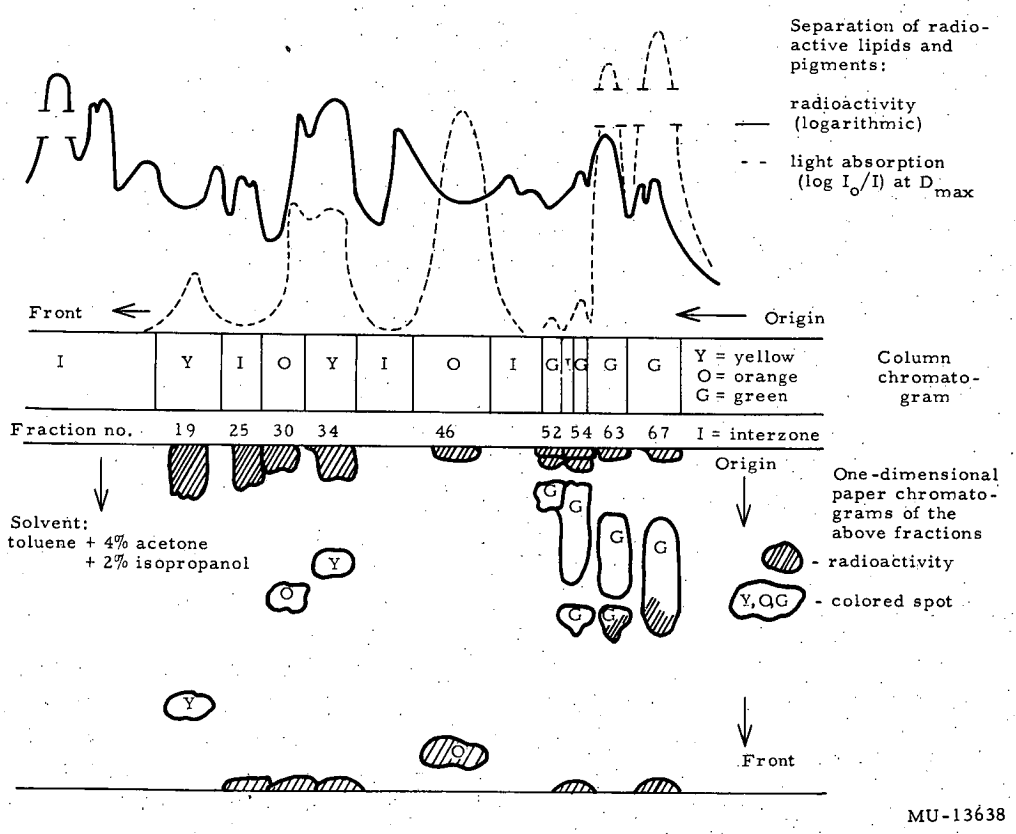

Fig. 10. Comparison of the visible-light absorption, C^{14} incorporation, column chromatography and paper chromatography of algae pigments and lipids.

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

RADIATION CHEMISTRY

Amos S. Newton and Aldo F. Sciamanna

The radiation chemistry studies of trichlorethylene, started last year, have been resumed. The irradiation of the pure liquid trichlorethylene with 48-Mev alpha particles yields a rather simple set of products as shown below.

Product	Yield (mM)
H_2	≤ 0.005
CH_4	≤ 0.0015
CO	≤ 0.0001
C_2H_2	~0.007
HCl	~0.8
HC=CCl	~1.6
ClC=CCl	~0.6
$CHCl_3$	Trace
CHCl=CHCl	Trace
"Polymer"	~10 [Calculated as $(C_2HCl_3)_n$]
Cl_2	(not determined).

These figures are written as approximate because the yields vary somewhat with the radiation dose. The H_2, CH_4, and CO contributions are variable and are apparently produced from impurities. They are much less from highly purified samples of trichlorethylene. The products in the heavier liquid have not been positively identified, and some change is possible in the identification of these materials. We are collecting such products from gas chromatographic runs to provide a positive identification.

The "polymer" has an average molecular weight of about 350 and an empirical composition close to that of the original trichlorethylene. Thus, it corresponds to an average polymerization of two or three molecules of trichlorethylene (a dimer or trimer). One possible explanation of the limitation of the polymerization is the formation of a "buried" radical. With the cooperation of Mr. Power Sogo of the Bio-Organic Group, a check was made for free electrons in the polymer. None was seen in the polymer in concentration greater than 10^{-5} to 10^{-6} M. Thus, it does not appear that the polymer has any appreciable number of long-lived radicals present in its composition. A similar check with the polymer from isopropenyl acetate also gave negative results.

DEUTERON-INDUCED FISSION OF URANIUM-234, URANIUM-235, AND URANIUM-236

Richard M. Lessler

U^{234}, U^{235}, and U^{236} have been bombarded on the 60-in. cyclotron with deuterons. Figures 11 through 14 show the fission yield curves obtained. The values are given in Table XV in which

E_d = energy of bombarding deuteron,

σ_{meas} = measured cross section (in millibarns),

σ_{corr} = cross section (in millibarns) for the given mass number after it has been corrected for chain yields.

These cross-section values are good to ± 50% in most cases.

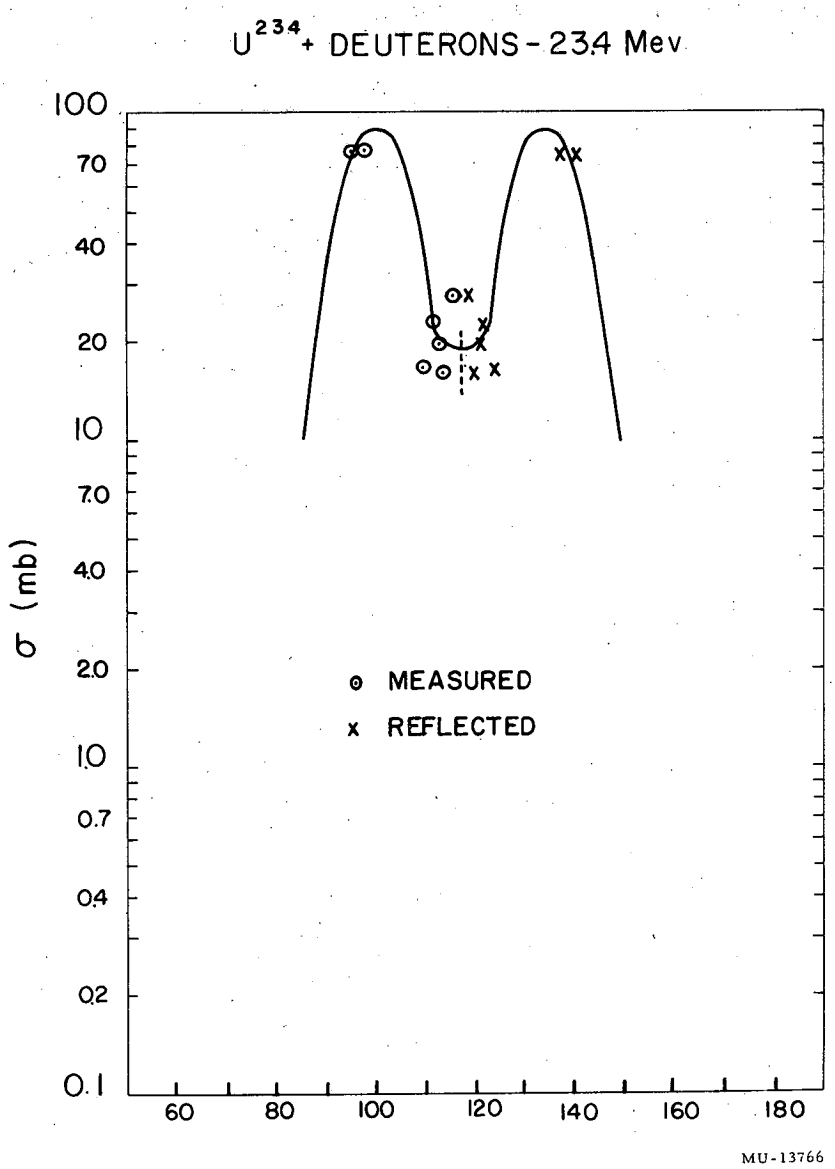

Fig. 11. Fission yield curves for U^{234} bombarded with deuterons at 23.4 Mev.

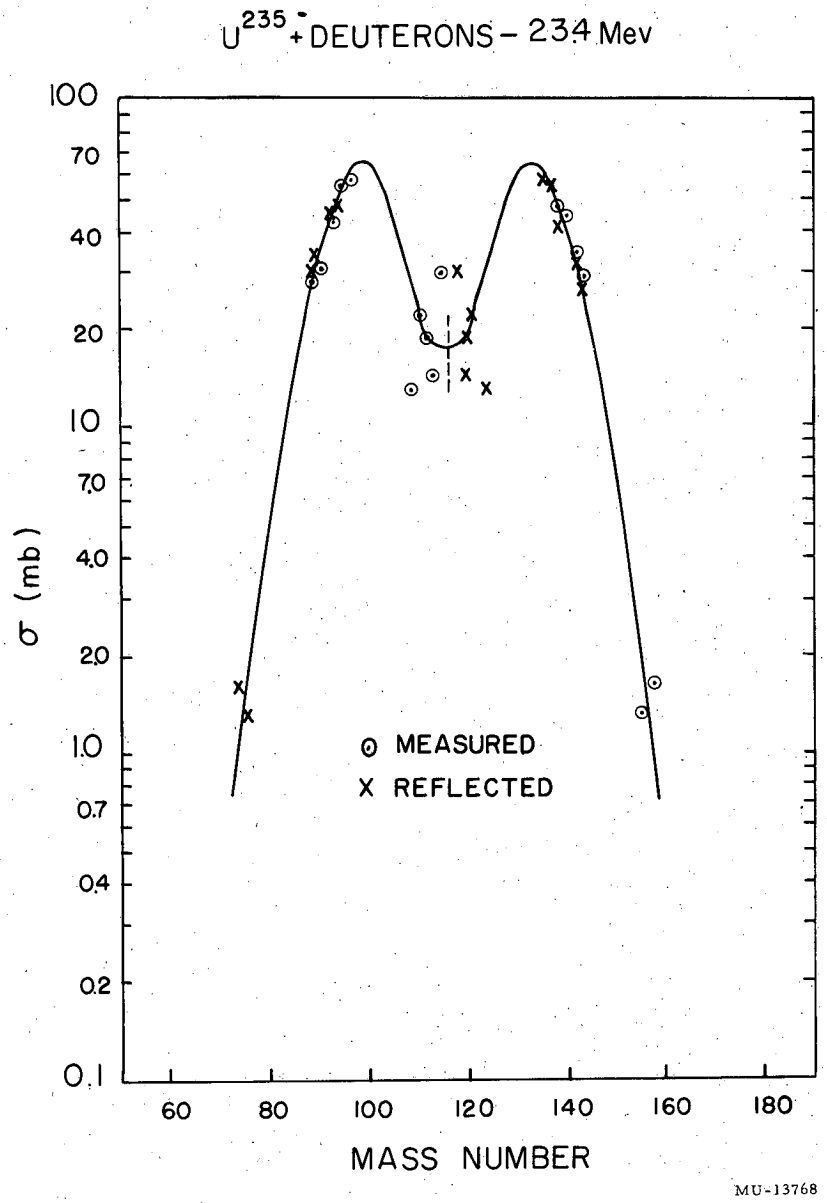

Fig. 12. Fission yield curves for U^{235} bombarded with deuterons at 23.4 Mev.

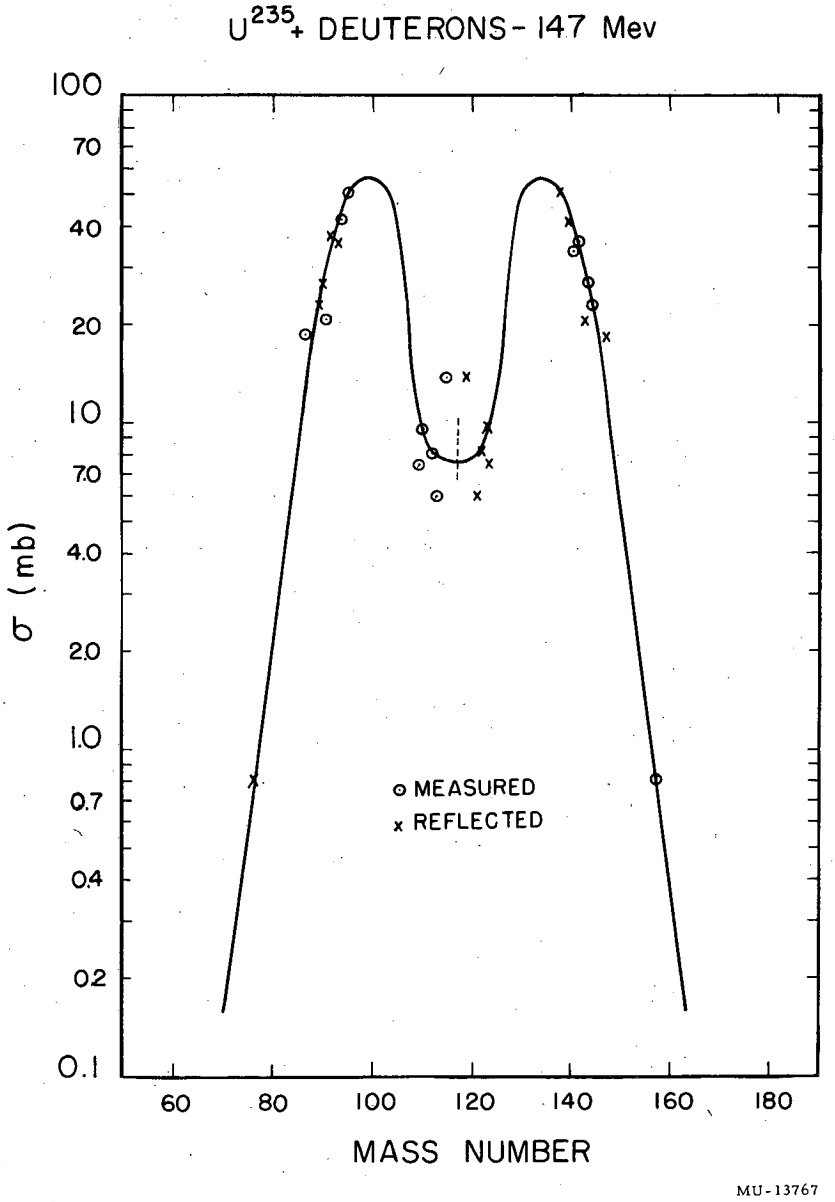

Fig. 13. Fission yield curves for U^{235} bombarded with deuterons at 14.7 Mev.

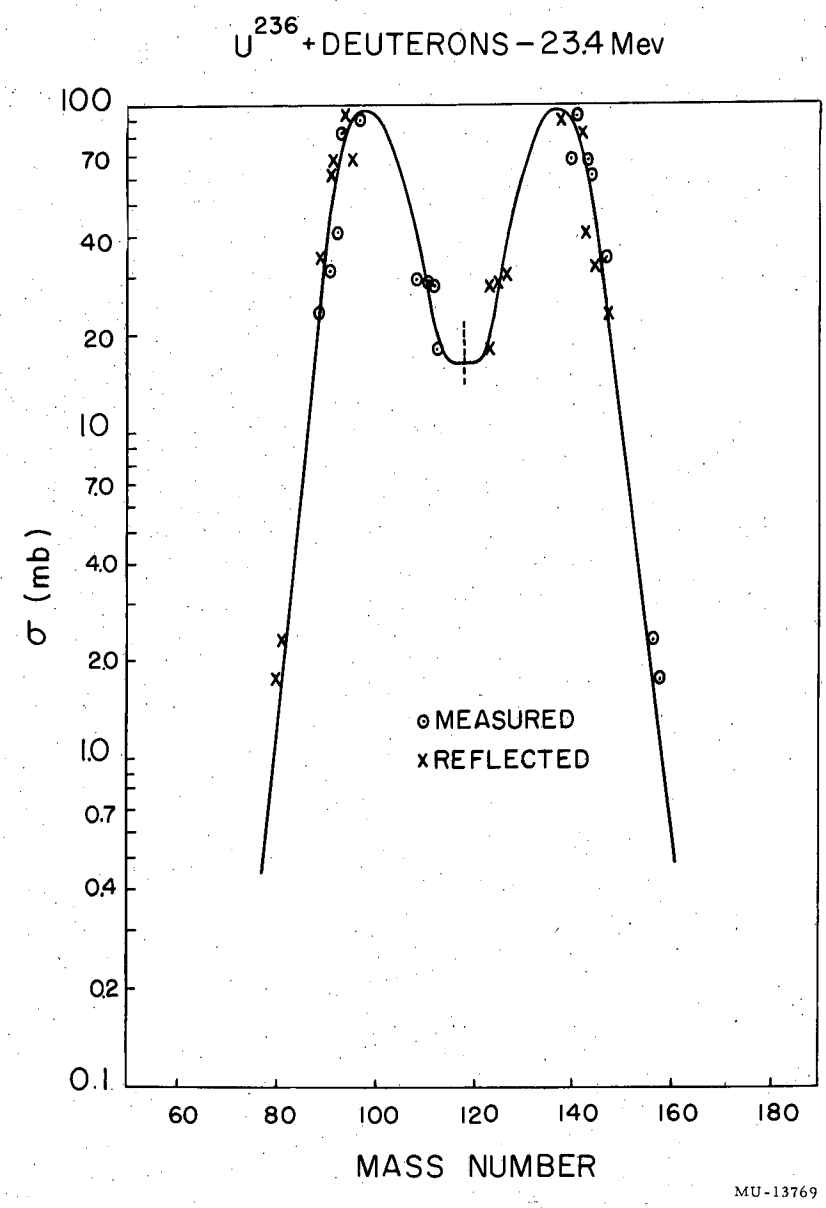

Fig. 14. Fission yield curves for U^{236} bombarded with deuterons at 23.4 Mev.

Table XV

Fission cross sections (millibarns)

Target	U^{235} + d		U^{235} + d		U^{236} + d		U^{234} + d	
E_d	14.7 Mev		23.4 Mev		23.4 Mev		23.4 Mev	
Isotope	σ_{meas}	σ_{corr}	σ_{meas}	σ_{corr}	σ_{meas}	σ_{corr}	σ_{meas}	σ_{corr}
Sr^{89}	18.4	18.6	28.1	28.4	22.8	23.0		
Sr^{91}	20.3	20.9	27.4		23.7			
Y^{91}	0		3.8		7.2			
$Sr^{91}+Y^{91}$			31.2	31.5	30.9	31.2		
Y^{93}			41.7	42.8	38.8	39.7		
Zr^{95}	40.3	41.2	53.8	54.9	83.0	83.8	73.9	76.0
Zr^{97}	49.8	52.0	55.4	57.6	85.5	88.2	69.6	74.9
Pd^{109}	7.42	7.51	12.9	13.0	28.3	28.3	16.3	16.7
Ag^{111}	9.30	9.40	21.7	22.0	27.7	28.0	22.1	22.6
Pd^{112}	9.42	9.52	17.8	18.6	26.5	27.4	18.2	19.6
Ag^{113}	5.83	6.00	14.1	14.5	17.3	17.5	15.4	16.1
Cd^{115}	12.7		27.7				24.0	
Cd^{115m}	≤0.941		≤1.52				≤2.58	
$Cd^{115}+Cd^{115m}$	13.6	13.9	29.2	29.8			26.6	27.4
Ba^{140}	25.8	34.4	30.1	46.4	60.5	68.2		
Ce^{141}	34.5	35.5	44.0	45.3	90.8	91.7		
Ce^{143}	25.1	26.4	30.3	34.5	64.0	66.0		
Ce^{144}	20.1	22.1	24.3	29.3	57.2	60.3		
Nd^{147}					33.1	33.8		
Eu^{156}	0.720	0.810	1.08	1.30	2.17	2.24		
Eu^{157}			1.17	1.62	1.63	1.72		
Total Fission	940		1315		1690		1640	
			Primary fission yields					
Y^{90}	<0.147		<0.609		<0.236			
La^{140}	1.56		3.49		5.56			
Pr^{142}	<0.704		≤0.510		1.91			

Seaborg, Perlman

(d, γ) REACTIONS OF URANIUM-234 AND URANIUM-236

Richard M. Lessler

U^{234} and U^{236} have been bombarded on the 60-inch cyclotron with deuterons. The resulting neptunium fractions were chemically separated. The neptunium activity from U^{236} was counted on the nucleometer (windowless proportional counter). In the case of U^{234}, the Np^{236} daughter, Pu^{236}, was counted on the alpha pulse analyzer. Table XVI shows tentative (d, γ) cross sections in millibarns, in which

E_d = energy of bombarding deuteron,

σ_{meas} = measured cross section in millibarns,

σ_{corr} = cross section in millibarns after the cross sections due to the isotopic impurity of the bombarded uranium has been subtracted from the measured cross section.

Table XVI

Cross sections for (d, γ) reactions of uranium

E_d(Mev)	σ_{meas} (mb)	σ_{corr}(mb)
U^{234} (d, γ) Np^{236}		
14.8	0.283	0.080
16.4	0.570	0.355
17.5	0.702	0.461
18.9	0.720	0.440
20.0	0.794	0.380
U^{236} (d, γ) Np^{238}		
16.7	1.53	0.56
18.9	1.94	1.10
20.7	1.84	1.11
22.6	1.11	0.49
23.4	0.645	0.075

REACTIONS INDUCED IN URANIUM-233 BY HELIUM-ION BOMBARDMENTS

T. Darrah Thomas

Work has been completed on a study of the competition between spallation and fission in the reactions induced in U^{233} with helium ions of energies between 20 and 46 Mev. The striking features of the excitation functions are the lowness of the (α, xn) cross sections (less than 7 mb) compared with cross sections for the same reactions in other very heavy elements, and the prominence of the (α, pxn) reactions (with cross sections as high as 20 mb).

The results can be interpreted in terms of reaction mechanisms involving either the formation of a compound nucleus or direct interactions between the projectile and the nucleons on the surface of the nucleus. Fission competes more successfully against the compound-nucleus spallation reactions in the reactions of U^{233} than in those of other very heavy elements; in general, the products of the direct interaction reactions are able to survive fission equally well for all targets. There is evidence for the emission of complex charged particles by direct interaction.

REPORT TO BE PUBLISHED

Frank Asaro and Isadore Perlman

The following paper has been submitted to the editors of the Physical Review for publication:

Frank Asaro and Isadore Perlman, An Isomeric State of Uranium-235.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

Correlation of Limiting Currents at Horizontal Electrodes under Free-Convection Conditions

Eugene J. Fenech and Charles W. Tobias

Attempts at visual observation of the liquid currents within horizontal electrode cell are being planned. Several schemes are to be tried when equipment is ready. They are observation by (a) change in refractive index as the concentration changes, (b) suspension of very fine mica particles, and (c) polarized light. It is doubtful that (a) will furnish any good results; however, a combination of (a) and (c) may give a good picture of the convection process. Method (b) appears very attractive because of the possibility of obtaining velocities of fluid elements by the distance a particle moves in a given time interval. Both movies and stills will be taken to study the convective process.

Stability of Perforated-Plate Trays

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

It has been found that the use of an inlet weir is required to remove any liquid-momentum effects. Thus the liquid feed rate affects the dumping rate only through its effect as the liquid head. This effect can be calculated by use of the Francis weir formula.

A semitheoretical equation has been proposed, and equipment is now being built to photograph the phenomenon and thus verify the equation and give better insight into the actual plate operation.

Multicomponent Distillation Studies Using an IBM-701 Two-Frame Digital Computer

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

Distillation columns have been simulated with the above machine, and variables have been studied by this means.

Planned work calls for comparing performance of an actual industrial column with machine-calculated performance.

Electrochemical Studies in Nonaqueous Solvents

W. S. Harris and C. W. Tobias

Further quantitative measurements have been made of the solubility of inorganic salts and of their conductivities in propylene carbonate at 25°C.

Liquid-Liquid Extraction and Agitation Coalescence Rates in Two-Phase Agitated Systems

J. H. Vanderveen and T. Vermeulen

Empirical analysis of the data obtained previously on droplet breakup and droplet coalescence has been continued. In a comparison of gas-liquid and liquid-liquid results, it now appears that gas-bubble diameters depend upon the dynamic balance of breakup and coalescence rates, to the near-exclusion of the equilibrium-limit term that largely controls liquid-drop diameters.

Performance of Sphere-Packed Extraction Columns

G. Jacques and T. Vermeulen

Longitudinal-dispersion rates have been measured for two-phase countercurrent flow in a randomly packed bed of 3/4-inch spheres. The eddy dispersivity of the continuous phase increases with the flow rate of dispersed phase, and dispersivities up to 10 times the values in single-phase flow have been observed. Flooding is found to occur at, or near, the transition from laminar to turbulent flow of the continuous phase.

Extraction Rates for Single Drops

T. Miyauchi and T. Vermeulen

Nitric acid extraction to and from penta-ether droplets has been measured in a continuation of this investigation. The main resistance to mass transfer is on the penta-ether side. A small amount of internal circulation occurs, but this appears due to convection induced by density differences rather than to skin-friction effects.

Gas-Liquid Partition Chromatography (GLPC)

Robert H. Houston and C. R. Wilke

The work has been completed and a report is being written.

Zone Melting

William R. Wilcox and C. R. Wilke

The general objective of this study is to determine the effect of system properties and experimental variables on separations obtained in zone melting, and to develop a general design theory. An apparatus of the moving-boat type is under construction. Binary solutions of organic solids will be employed in initial phases of the experimental program.

GENERAL CHEMISTRY

Leo Brewer, Robert E. Gonnick, and Kenneth S. Pitzer in charge

Absolute-Lifetime Apparatus

Richard Brewer and Fred Stafford

The electronic equipment has been completed and is now being tested. Work on molecular-beam sources is continuing. It is hoped that measurements of gases such as iodine can be carried out during the next quarter.

Thermodynamic Calculations

Elizabeth Brackett

Work on the thermodynamic properties of the elements has been completed. The evaluation of the data related to the vaporization of univalent and divalent halides is now in progress.

New Light Sources

John Engelke and Earl Worden

Study of arcs and other discharges as possible light sources is continuing.

Electronic States of the C_2 Molecule

John Engelke

An apparatus is under construction to be used for study of C_2 electronic emission bands in the infrared in an effort to fix the positions of low-lying electronic levels of C_2.

Ruthenium Chemistry

Howard Cady

This work has been completed and written up as a separate report: Howard Cady, Aqueous Chemistry of Ruthenium in the +2, +3, and +4 Oxidation States: Species, Spectra, and Potentials (Thesis), UCRL-3757, April 1957.

The Ferrate and Selenide Ions

Robert Wood

The experiments on the heat, free energy, and entropy of the ferrate(VI) and selenide ions, are described in a report: Robert H. Wood, The Heat, Free Energy, and Entropy of the Ferrate(VI) and Selenide Ions (Thesis), UCRL-3751, April 1957.

The Entropies of Dilution of Strong Electrolytes

Robert Wood

An empirical correlation of the entropies of dilution of strong electrolytes is being developed. In those cases where ion-pair formation is not the dominant factor a single parameter for each ion is sufficient to give a fairly good correlation.

Kinetics of Rapid Reactions

Claude Coppel

A study of the kinetics of the ferric chloride complexing reaction is being completed; an account of this work will be presented in the next Quarterly Report.

Determination of the Molecular Structure of Aluminum Hydride

Robert F. Nickerson

In the course of the quarter just ended one line of investigation was completed and two additional ones were started.

The studies being made on perfluoroethers as possible hydrogen-free solvents to be used to study aluminum hydride showed that such compounds would not be useful as solvents for the chemicals involved.

During this quarter high-resolution nuclear magnetic resonance was used to study the reaction leading to the formation of aluminum hydride from lithium aluminum hydride and aluminum chloride in ethyl ether. The results were interesting but inconclusive, and additional work will be necessary in order to obtain any useful information.

Also initiated during this quarter was a systematic investigation on the preparation of ether-free aluminum hydride in order to determine the technique necessary for success. The information given in the literature for such a preparation is apparently insufficient, and repeated attempts following the procedures given have yielded only aluminum hydride containing ether. All attempts so far have been unsuccessful.

Heat Capacities of Metals between 0.1° and $4.2^{\circ}K$

Norman E. Phillips

The apparatus has been modified to permit the attainment of lower temperatures with the liquid helium bath, and also with the adiabatic demagnetization stage.

Hydrogen Bonding in Acids

Jefferson C. Davis, Jr.

Nuclear magnetic resonance studies of hydrogen bonding in acid systems have continued. Dilute solutions of benzoic acid and formic acid in benzene have been studied in an attempt to determine the equilibrium between the monomeric and dimeric species of the acid. It has not been possible to extend the range of the equipment so as to make measurements in the dilute region where the equilibrium can be observed directly. Indirect calculations are now being attempted.

The effect of temperature on the chemical shift of the OH proton has also been studied in the region from 20° to 100°C. A special probe insert was constructed for this purpose, utilizing a stream of air to maintain the desired constant temperature in the sample. Temperature shifts were measured for pure formic acid, water, and benzene solutions of formic acid.

Thermal Properties of Silver Oxide

Roger Gerkin

There is an anomaly of unknown cause in the heat capacity of Ag_2O near 35°K. Previous studies used a sample of extremely small crystals. A sample is now being prepared in which the crystals have been grown and annealed under oxygen pressure at high temperature. This sample will be studied thermally as a first step in determining the nature and cause of the anomalous behavior.

Energy Levels of Nearly Linear Molecules

Robert Curl

Quantum mechanical calculations using the I.B.M. 650 have been carried out for the vibration-rotation energy levels of a triatomic molecule that is just slightly nonlinear. The problem was set up in a fashion to cover the range from strictly linear to finitely nonlinear molecules continuously in terms of values of a potential term. The most likely example is a more complex molecule, disiloxane. Spectral measurements for disiloxane and calculations for that model are in progress.

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