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CHEMISTRY DIVISION QUARTERLY REPORT

June, July, August 1956

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CHEMISTRY DIVISION QUARTERLY REPORT

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CHEMISTRY DIVISION QUARTERLY REPORT

June, July, and August 1956

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

October 15, 1956

BIO-ORGANIC CHEMISTRY

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

CARBON-14-ION IRRADIATIONS OF BENZENE

Richard M. Lemmon, Fred L. Reynolds, and Franco Mazzetti

INTRODUCTION

In collaboration with the Nuclear Chemistry Group, we are investigating the possibility of labeling organic compounds by irradiation with a C^{14}-ion beam. The labeling of organic compounds by C^{14} atoms derived from the $N^{14}(n,p)C^{14}$ reaction has been reported by several workers.[1] In these experiments the compound to be labeled is irradiated with neutrons in the presence of a nitrogen-containing compound. The recoiling C^{14} atom then displaces a C^{12} atom in the original molecule to give a C^{14}-labeled organic molecule. A disadvantage of this procedure is that it is necessary to have very high neutron fluxes of the intensities obtainable only with an atomic pile. Thus, the organic compound being labeled is also subjected to a high intensity of gamma rays and this, combined with the neutron-flux effects, causes radiation decomposition to become a serious problem. It is therefore of interest to determine whether a C^{14}-ion beam in a mass spectrometer can be used to give appreciable amounts of labeling in organic molecules. Work along this line has already been reported by Croatto and Giacomello.[2] Our approach to this problem was to irradiate solid benzene with such a beam and to determine what fraction of the beam appears as C^{14}-labeled benzene. Benzene was chosen because a large fraction of all organic compounds contain phenyl groups and, if benzene can be successfully labeled, presumably all aromatic compounds can be labeled with some success. In addition, benzene is very resistant to radiation decomposition. Finally the separation and purification of benzene may be carried out with extremely high efficiency by means of the technique of vapor-phase chromatography.

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1 e.g., Yankwich, Rollefson, and Norris, J. Chem. Phys. 14, 131 (1946).
G. Giacomello, Ricerca sci. 21, 1211 (1951).
A. P. Wolf and R. C. Anderson, J. Am. Chem. Soc. 77, 1608 (1955).
A. G. Schrodt and W. F. Libby, ibid. 78, 1267 (1956).

2 Abstracts of the Naples 1954 Meeting of the Italian Society for Scientific Progress (S. I. P. S.). See also, Ricerca sci. 26, 529 (1956).

METHODS AND APPARATUS

Mass Spectrometer

The instrument used for this work is a 60°-sector, 15-cm-radius spectrometer modified in the following manner:

The carbon ions were made by a conventional electron bombardment except that the width and length of the electron beam were increased by opening the slit in the field-free ion box to a maximum. The cathode was a nickel core coated with barium and strontium oxides. No source magnet was used.

Labeled carbon dioxide (23% C^{14}) was introduced from a cylindrical brass container into the ion source through a flattened copper tube variable-leak valve. For the experiments described here, the singly charged carbon ions were used for the irradiations. All the source slits were increased in width from the conventional 0.020 and 0.010 in. to 0.062 in.; in this way a maximum beam intensity (approximately 10^{-9} amp) could be obtained. The receiver slits were also widened to 0.062 in. and a monitor detector in the form of a 10-mil tungsten wire intercepted a portion of the total beam.

The receiver end of the spectrometer was capped by a flange upon which was mounted a stainless steel "cold finger" vapor trap. Benzene vapor was directed continuously but slowly onto the surface of the trap during the irradiations. The vapor was introduced through a delivery tube and its flow was controlled by suitable needle valves.

The temperature of the trap was maintained at -155 ± 5°C during irradiations. The trap was filled with isopentane and a glass tube was inserted into the isopentane. The temperature was maintained by periodic additions of small quantities of liquid nitrogen into the glass tube. The temperature was recorded with a thermocouple which was connected to a recording potentiometer. At -155° the vapor pressure of the solid benzene is less than 10^{-6} mm, while solid carbon dioxide has a vapor pressure of about 10^{-2} mm. As the internal pressure of the mass spectrometer is about 10^{-6} mm the occlusion in the benzene of $C^{14}O_2$ from the ion source is minimized.

After the beam left the monitor-wire region, it was deflected by a split-slit system. Impressed on one of these slits was a saw-tooth voltage having a period of about 10 sec. This arrangement permitted movement of the beam back and forth over the surface of the benzene.

The exact beam intensity was not known to better than a factor of two because of our imperfect knowledge of the beam's geometry. It varied from 10^{-9} to 10^{-10} amp. The beam had to be monitored periodically because during the time the beam-spreading circuit was used, the charging voltages to the split-deflection plates made the detection electrometer unusable. During an irradiation the beam centering and intensity were measured every half hour. The accelerating voltage was 2 kv; the carbon ions therefore had an energy of 2,000 ev. The magnetic field of the spectrometer was monitored by a nuclear magnetic-resonant proton signal.

The collection of the benzene sample after irradiation was accomplished by closing off the spectrometer pumping system by a magnetically operated valve, and allowing the collector trap at the target area to warm up to room temperature. The benzene was pumped out through copper and glass tubing into a small glass trap held at liquid nitrogen temperature. The trap was then sealed off and removed from the spectrometer.

Vapor-Phase Chromatography

The trap containing the irradiated benzene was opened and 100 to 200 λ of toluene was added (methyl-labeled toluene is one possible product[3]). This mixture was then separated and purified by vapor-phase chromatography.[4] The chromatograms, which were the gas-liquid partition type and employed either silicone or carbowax, were recently described.[5] About 150 λ of the benzene-toluene mixture was passed through the column at one time. The benzene and toluene fractions were collected separately and pooled.

After an irradiation the benzene contained an appreciable quantity of $C^{14}O_2$. However, it was found that this radioactivity could be removed below detectable levels either by bubbling inactive CO_2 through the benzene for about one-half hour or by one passage of the benzene through a vapor-phase chromatogram.

Radioactivity Determinations

The benzene and toluene fractions were assayed for their C^{14} content by direct addition to a liquid scintillation solution. The scintillation counter employed was described in an earlier report.[6]

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[3] A. G. Schrodt and W. F. Libby, J. Am. Chem. Soc. 78, 1267 (1956).

[4] We wish to thank Drs. K. P. Dimick and J. Corse of the Department of Agriculture, Western Utilization Research Branch, Albany, California, for the use of their vapor-phase chromatography apparatus.

[5] K. P. Dimick and J. Corse, J. Food Tech. 10, 360 (1956).

[6] E. M. Baker, in Chemistry Division Quarterly Report, UCRL-3157, Sept. 1955, p. 51.

RESULTS

Two experiments have been performed. Their results are as follows (all activities are total values):

First Experiment

Ion beam: approx. 2×10^{-10} amp

Time of irradiation: 10 hr

Total number of C^{14} ions striking the benzene: 4.3×10^{13}

μc of C^{14} in the ion beam, total: 4.6×10^{-3}

C^{14} activity in the total ion beam: 10,000 disint/min

Benzene activity[7] after two passages through a silicone column:
657 disint/min = 6.6% of the total activity of the ion beam (Activity of the C^{14} ion).

Benzene activity after three passages through a silicone column:
526 disint/min = 5.2% of the activity of the C^{14} ion.

Toluene activity after one passage through a silicone column:
292 disint/min = 2.9% of the activity of the C^{14} ion.

Second Experiment

Ion beam: between 6×10^{-10} and 10^{-9} amp

Time of irradiation: 9 hr

Total number of C^{14} ions striking the benzene:
1.2×10^{14} (assuming a 6×10^{-10}-amp beam)

μc of C^{14} in the ion beam, total: 1.3×10^{-2}

C^{14} activity in the total ion beam: 28,600 disint/min.

Activity in the irradiated benzene solution after passing in inactive CO_2 for one hour: 15,000 disint/min

Benzene activity after one passage through a silicone column:
5,000 disint/min = 18% of the activity of the C^{14} ion.

Benzene activity after one passage through silicone followed by one passage through a carbowax column:
960 disint/min = 3.4% of the activity of the C^{14} ion.

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[7] The calculated activities for the benzene and toluene fractions for both experiments are based on 100% recoveries from the chromatograms. Actually some 25% of a fraction was usually lost during the chromatography.

Benzene activity after successive passages through silicone, carbowax, and silicone columns:

672 disint/min = 2.4% of the activity of the C^{14} ion.[8]

Toluene activity after one passage through silicone:

560 disint/min = 2.0% of the activity of the C^{14} ion.

Toluene activity after one passage through silicone followed by one passage through carbowax:

253 disint/min = 0.9% of the activity of the C^{14} ion.

Toluene activity after successive passages through silicone, carbowax, and silicone columns:

250 disint/min = 0.9% of the activity of the C^{14} ion.[8]

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[8] The fore and back parts of the fraction emerging from the column were collected separately at this point. Within the limits of the measurements, both parts of the fraction had the same specific activity.

EFFECTS OF IONIZING RADIATION ON CHOLINE ANALOGS

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

Beginning in our Quarterly Report for September, October, and November 1953 (UCRL-2455) and continuing in subsequent reports, we described a research program to determine the effects of changes in the choline chloride molecule on its radiation sensitivity. Up to the present time, nineteen analogs of choline chloride have been prepared and the radiation sensitivities of the pure crystalline salts have been determined. All these analogs are far more stable towards radiation than is crystalline choline chloride, and only one of them (choline bromide) shows anything unusual in radiation sensitivity. Consequently, it now appears unlikely that any analog of choline chloride will exhibit the same high degree of sensitivity; for this reason the work on the systematic exploration of choline analogs has ended. It therefore appears appropriate at this time to summarize the data which has been obtained.

Table I is a list of the compounds that have been irradiated, the type of radiation used, the method of analysis, and the G values (molecules permanently altered per 100 ev of energy input) that were obtained at the point of 10% decomposition. This definite percentage was picked because the G values change with differing amounts of decomposition. A lesser amount of decomposition gives a lower accuracy in the analytical procedures; a greater amount gives radically altered G values. An ideal G value would be that obtained after only an infinitesimal amount of decomposition had taken place.

Table I

Radiation decomposition of choline analogs

Compound	Type of radiation*	Method of analysis**	G value at 10% decomposition***
$[(CH_3)_3NCH_2CH_2OH]^+Cl^-$	e^-	R	98
"	γ	R	354
$[(CH_3)_3NCH_2CH_2OH]^+Br^-$	e^-	R, TPB	30
"	γ	R, TPB	92
$[(CH_3)_3NCH_2CH_2OH]^+I^-$	e^-	R	1.0
"	γ	R	2.5
$[(CH_3)_3NCH_2CH_2OH]^+NO_3^-$	e^-	R	1.4
"	γ	R, TPB, PC	4.9
$[(CH_3)_3NCH_2CH_2OH]^+SO_4^-$	e^-	R	14
"	γ	R	29
$[(CH_3)_3NCH_2CH_2OH]^+CH_3CO_2^-$	e^-	R, TPB	8.2
"	γ	R	11
$[(CH_3)_3NCH_2CH_2OH]^+CN^-$	e^-	R	19
"	γ	R	16
$[(CH_3)_3NCH_2CH_2OCOCH_3]^+Cl^-$	e^-	R, PC	2.7
"	γ	R, PC	3.5
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	e^-	PC	1.9
"	γ	TPB	4.8
$[(CH_3)_3NCH_2CH(OH)CH_3]^+Cl^-$	e^-	TPB	6.7
"	γ	TPB	6.9
$[(CH_3)_3NCH_2COOH]^+Cl^-$	e^-	R, TPB	16
"	γ	R, TPB	15
$[(CH_3)_3NCH_2CH(OH)CH_2COOH]^+Cl^-$	e^-	R	14
"	γ	R, PC	14
$\left[\begin{matrix}C_2H_5\\(CH_3)_2\end{matrix}NCH_2CH_2OH\right]^+Cl^-$	e^-	R	6.1
"	γ	R	26
$\left[\begin{matrix}(C_2H_5)_2\\CH_3\end{matrix}NCH_2CH_2OH\right]^+Cl^-$	e^-	R	6.0
"	γ	R	7.9
$[(C_2H_5)_3NCH_2CH_2OH]^+Cl^-$	e^-	R	5.5
"	γ	R	14
$\left[\begin{matrix}C_6H_5\\(CH_3)_2\end{matrix}NCH_2CH_2OH\right]^+Cl^-$	e^-	R	4.8
"	γ	TPB	1.9
$[(CH_3)_2N(CH_2CH_2OH)_2]^+Cl^-$	e^-	PC	5.6
"	γ	PC	14.
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	e^-	R, PC	4.3
"	γ	R	2.6
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	e^-	R	1.6
"	γ	R	1.7
$[(CH_3)_3PCH_2CH_2OH]^+Cl^-$	e^-	TPB	4.9
"	γ	TPB	4.9

* e^- is 3 to 5 Mv electrons, γ is Co^{60} γ's (1.1 and 1.3 Mev)

** R is Reineckate analysis, TPB is tetraphenyl boron analytical procedure, and PC is determination by means of paper chromatography. These procedures have been described in earlier reports.

*** The estimated accuracy for the choline chloride and choline bromide analyses is ± 25%. For all other compounds the G values given may be in error by as much as a factor of 2. The values are all averages of from 2 to 11 experiments.

IMPROVED METHODS OF ION-CHAMBER ASSAY OF CARBON-14

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

The preparation of samples for ion-chamber assay of carbon-14-labeled compounds has been accomplished by many procedures,[1] including the use of the Van Slyke-Folch oxidizing mixture, the sealed-tube copper oxide oxidation, and the conventional Pregl microcombustion, followed by isolation of the resulting CO_2. Most of these procedures suffer from some defect or weakness, and the following experiments have been undertaken to find improvements of existing methods, or to devise simplified new methods.

In a previous report[2] the authors have shown a method of extending the sealed-tube combustion technique of Wilzbach and Sykes[3] to salts of organic acids by using $CuSO_4$ as an oxidizing acid. This modified method has now been further extended to allow the assay of $BaC^{14}O_3$ by the same procedure.

$BaCO_3$ decomposes to BaO and CO_2 at 1450°C, a temperature which is inconveniently high for ordinary laboratory work. As $CuSO_4$ decomposes into CuO and SO_3 at 650°C, it was thought to be a source of acid to react with the $BaCO_3$ in a sealed-tube reaction and thus permit a quantitative generation of CO_2:

$$CuSO_4 + BaC^{14}O_3 \xrightarrow[1/2\ hr]{650^\circ} CuO + BaSO_4 + C^{14}O_2.$$

The results of ten combustions of a single batch of $BaCO_3$ using about a 50% excess of $CuSO_4$ and ignited for a minimum time of 1/2 hr and at a minimum temperature of 650°C are shown in Table II. This $BaCO_3$ was finely divided. A previous batch of less well divided $BaCO_3$ gave unreproducible results, presumably because the SO_3 reacted on the surface of the lumps and formed an impermeable layer of $BaSO_4$ over some of the $BaCO_3$. The measurements of radioactivity were all made with a vibrating-reed electrometer by the rate-of-drift technique.[1] The measurements may also be made by the high-resistance leak method.[1] This would allow the determination of very high specific activities if a resistor of appropriate value was used.

Another analytical technique that has been investigated is a combination of Pregl microcombustion[4] and continuous-flow ion-current measurements.[5] A small (3 to 5 mg) sample of the radioactive organic compound is burned in a microfurnace. The oxidized gases are passed

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1 B. M. Tolbert, Ionization-Chamber Assay of Radioactive Gases, UCRL-3499, Aug. 1956.

2 Whittemore and Tolbert, Oxidation of Sodium Salts of Organic Acids, UCRL-3415, June 1956, p. 45.

3 K. E. Wilzbach and W. Y. Sykes, Science 120, 494-496 (1954).

4 A. Steyermark, Quantitative Organic Microanalysis, Blakiston, New York, 1951

5 Tolbert, Hughes, Kirk, and Calvin, Arch. Biochem. Biophys. 60, 301 (1956).

Table II

Specific activity of $BaC^{14}O_3$ sample as determined by sealed-tube combustion

Run	Specific activity (μc/mg)
1	0.0151
2	0.0150
3	0.0153
4	0.0157
5	0.0151
6	0.0153
7	0.0154
8	0.0156
9	0.0152
10	0.0153
Average specific activity and standard deviation	0.0153 ± .0002

Calvin

through a dehydrating agent as usual. The CO_2-absorbing unit is replaced with a small (20 cc) ionization chamber of a flow-through type. The chamber is connected to a vibrating-reed electrometer with a high-value resistor leak. The output of the electrometer goes to a recording potentiometer, and a trace representing radioactivity in the chamber is permanently recorded. As the sample is burned, the $C^{14}O_2$ in the oxygen stream is dried in the desiccant and passes into the ionization chamber, where a signal is produced that is proportional to the activity. The $C^{14}O_2$ then passes out of the chamber. The total activity passing through the chamber may then be determined by integrating the area under the curve with a polar planimeter. This method is capable of giving quite precise results, particularly with good instrumentation. It has been used, so far as the essential elements go, in the measurements of breath excretion of $C^{14}O_2$ from animals given labeled compounds. It is capable of ± 1 to 2% precision if the system is accurately calibrated and the flow is properly regulated. A series of samples was analyzed and good precision was obtained until a difficulty arose in producing and maintaining a flow rate that was constant and nonpulsing within limits of 1% or better. This is necessary as the signal is inversely proportional to the flow rate. The flow rate need not be absolutely known as long as it is constant and reproducible.

An alternate method of using the Pregl combustion and ionization chamber was developed which obviates the need for accurate, constant flow rates. The method is now being tested and good preliminary results have been obtained. An evacuated 250-cc chamber is placed on the end of a combustion train (see Fig. 1). The gases from the train are then allowed to enter the chamber as the combustion proceeds. This flow rate is about 8 to 10 cc/min. After the combustion is complete the chamber is allowed to fill with oxygen which is sweeping out the combustion tube. The chamber must be of sufficient size to allow ample time for burning the sample and sweeping the train. When the chamber is at atmospheric pressure it is closed off, and the activity in it is measured by either rate-of-drift or high-resistance-leak methods.

Calvin

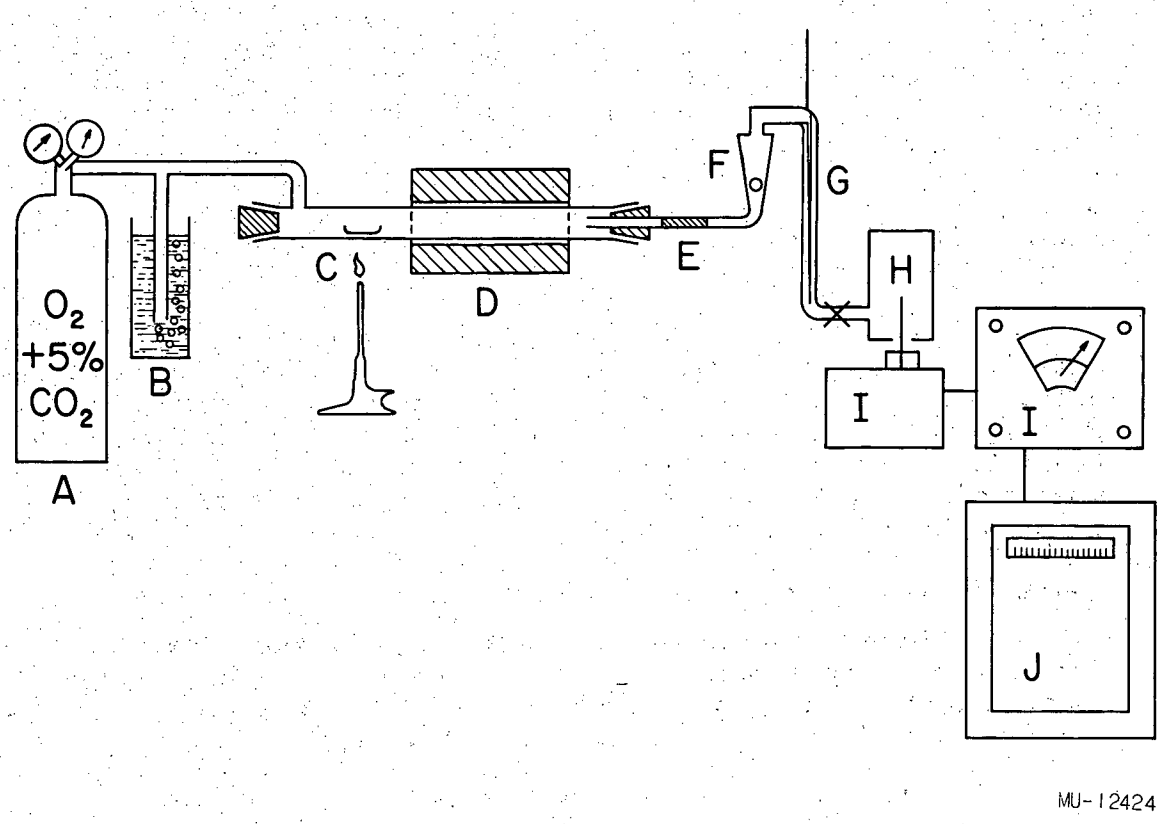


Fig. 1. Pregl combustion of organic samples for ion-chamber assay. A, oxygen cylinder; B, oil-filled pressure-relief bubbler; C, combustion tube with broken-quartz filling; D, furnace; E, magnesium perchlorate drying tube, 6 mm by 3 cm loosely packed; F, rotameter flow meter; G, capillary tubing 1/2 mm by 15 in.; H, ion chamber; I, vibrating-reed electrometer; J, potentiometer recorder.

NMR SPECTROSCOPY AND SOME CHEMICAL PROBLEMS

H. Montague Frey and Power B. Sogo

Some chemical problems have been investigated by means of a Varian Model V-4300B spectrometer.

The Exchange of Hydrogen with Deuterium in Organic Compounds.

The NMR spectrum of deuterium occurs in a completely different frequency region from that of hydrogen (in the same magnetic field). It is thus easily possible to observe the replacement of hydrogen atoms by deuterium atoms in organic compounds. If the spectrum of the normal compound is first obtained and then that of the deuterated product, one can immediately determine the number of hydrogen atoms exchanged by a comparison of resonance intensities. If the original spectrum has been completely analyzed, the actual position of the exchanging atoms will also be known. (The reverse process can also be used, i.e., deuterated compounds may be used in helping to analyze spectra.)

In an attempt to determine the structure of "azulinium ion," we examined the NMR spectra of azulene in concentrated sulfuric acid and in deutero-sulfuric acids and the spectrum of azulene in carbon tetrachloride. Because of the complexity of the spectrum of azulene in CCl_4, it was not possible to determine definitely the structure of the ion. It was, however, possible to show that only 2 hydrogen atoms are exchanged by the azulene in concentrated deutero-sulfuric acid; these are exchanged very rapidly.

Rates of Exchange of Hydrogen with Deuterium.

High-resolution NMR spectroscopy offers some advantages for rate studies in deuteration reactions. The total required sample is very small and of the order of 0.3 ml. In addition, the reaction can be followed continuously without affecting it or interrupting it in any way.

The disadvantages of the method are the required high concentration of the reactants and the difficulty of obtaining highly accurate results.

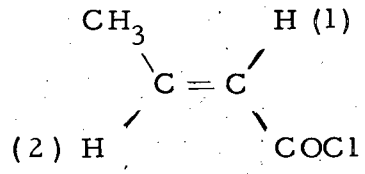
At present the reaction can be carried out continuously only at room temperature, though a modification of the apparatus is contemplated that will allow the use of elevated temperatures. The rate of deuteration of m-dimethoxybenzene in CH_3COOD was measured by means of this technique, and rate constants were obtained at ~25°C. From the spectra of the original and the deuterated product, it is evident that three hydrogen atoms are replaced by deuterium.

Examination of the Spectra of Organic Molecules.

Much work has been carried out on correlating the NMR spectra of organic liquids with their structural formulas. Indeed, it is now possible in certain favorable cases to suggest probable structures (or at least rule some out) merely by observing the NMR spectrum of the compound. The advent of Super-High-Resolution NMR Spectrometry will no doubt increase

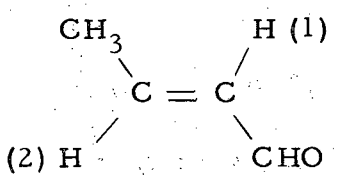
this field of the subject. Work has also been carried out and is in progress in relating the more subtle properties, such as ionicity of a band and NMR spectra. At present no satisfactory theory is available that will enable one to predict the various spin-spin interactions that will occur. To this extent much of the work has tended to be of an empirical nature.

The spectra of a large number of compounds containing an ethylenic double bond have been examined. These spectra are frequently rather complex because of spin-spin interaction between protons on neighboring carbon atoms and, in come cases, on carbon atoms further removed. In such spectra the assignment of peaks to particular hydrogen atoms in the molecule is often made possible by the examination of a number of related compounds. The method may be illustrated by reference to the spectra for crotonaldehyde and crotonyl chloride shown in Fig. 2. In the crotonaldehyde spectrum, the positions of the -CHO and $-CH_3$ peaks are quite certain, for, in a large number of compounds, these peaks always occur in the part of the spectrum shown. The ethylenic hydrogen peaks cannot be ascribed to the individual hydrogen atoms from this spectrum alone. From the spectrum of crotonyl chloride it appears that the two peaks occurring between 0 and -1 must be ascribed to proton (1) of the molecule



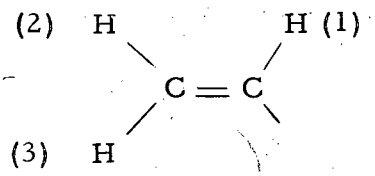
because this is split only by proton (2). (The peak intensities are approximately those which are to be expected for $J/\delta = 1/2.5$). The peaks occurring between -1 and -3 must be due to proton (2), which is split by both proton (1) and the protons in the methyl group.

In the spectrum of crotonaldehyde it would appear that the four peaks immediately to the right of the dotted line are because proton (1) of the molecule



is split by both proton (2) and the -CHO group. As before, the peaks immediately to the left of the dotted line are attributed to proton (2). (Note that these peaks are unaltered in both spectra.)

In many of the spectra of compounds containing the group



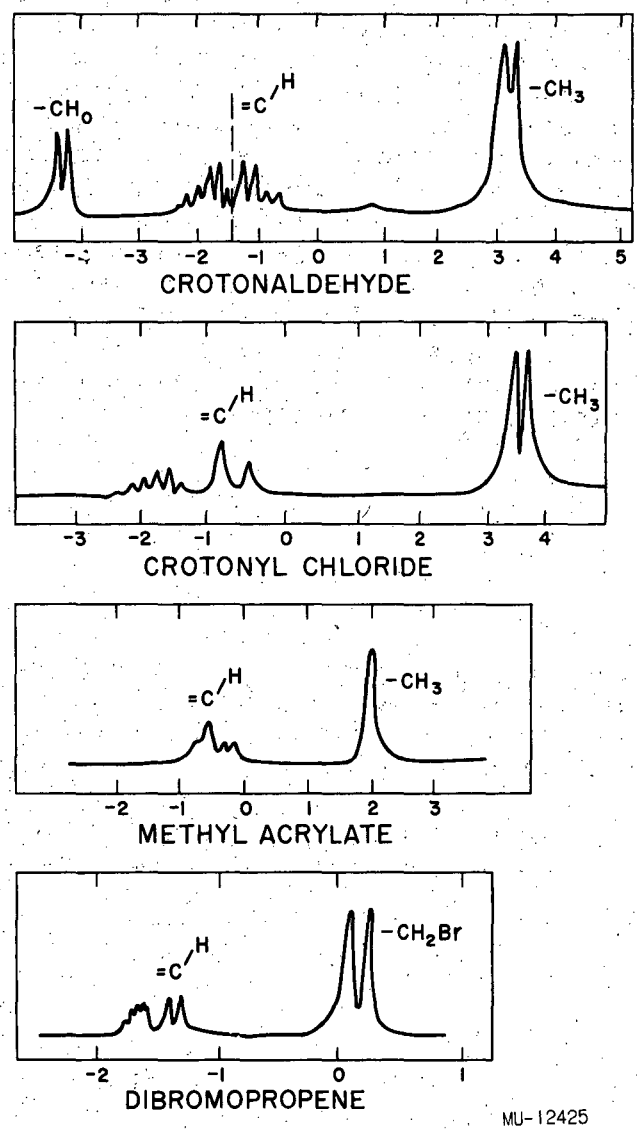
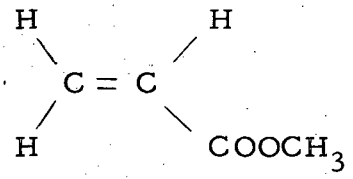


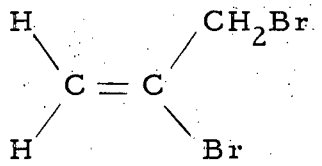
Fig. 2. NMR proton spectra of several organic compounds.

that have been examined, it is found that these spectra can be easily understood only if it is assumed that hydrogen (1) interacts with only one of the two other hydrogen atoms. (In some cases the spectra are further complicated by interactions between hydrogen (2) and (3), e.g., the vinyl halides). Methyl acrylate,



is an example of the above type of spectrum.

A similar discrimination is also shown in the spectrum of 2,3-dibromopropane,

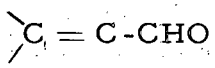


where the $-CH_2Br$ group interacts with only one of the ethylene hydrogens.

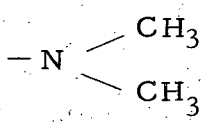
Hindered Rotation in Organic Compounds

Gutowsky and his co-workers have used the techniques of NMR spectroscopy to detect and measure quantitatively the presence of hindered rotation in dimethylformamide, dimethylacetamide, and some related compounds. Using the same techniques, we should be able to measure such hindered rotation if its energy of activation is in the range of about 5 to 15 kcal/mole (assuming a normal entropy factor).

Attempts to find such hindered rotation about the carbon-carbon single bond in the system



have failed, and it appears that the energy barrier to rotation is less than 5 kcal. Other compounds examined in this fashion were various substituted benzaldehydes. Hindered rotation was also looked for in compounds containing the group



directly attached to the aromatic nucleus, e.g., α-N, N-dimethylnaphthylamine and various substituted N-methyl and N-dimethyl anilines. In no case could hindered rotation with an energy of activation within the appropriate limits be observed. This is also true for such compounds as CH_2Br-CH_2CN.

The NMR spectrum of a compound containing a hydrogen bond is somewhat different from that of a similar compound not so bonded. The normal position of the -OH group is considerably shifted towards lower field (i.e. less shielding of the proton) by hydrogen bonding. It is thus relatively easy to follow the effect of dilution by an inert solvent on hydrogen bonding. A future project will be to use this technique to investigate the effect of temperature on hydrogen bonding.

IDENTIFICATION AND SPIN RESONANCE OF AN ORGANIC FREE RADICAL

Antonino Fava and Power B. Sogo

Previous workers[1, 2] have studied the spin resonance of sulfuric acid solutions of phenyl and p-tolyl disulfides, the corresponding thiols, and diphenylene sulfide (thianthrene). A stable free radical was observed but its identity was not established. The object of the work described here is the identification of the stable free radical.

The optical spectra were observed with a Cary Model 14 spectrophotometer and the electron-spin resonance spectra were taken at a frequency of 9.25 kmc/s on a recording differentiating spectrometer using a transmission cavity and bolometer detection.

A comparison of the optical and spin-resonance spectra of diphenyl disulfide and thianthrene solutions in H_2SO_4 showed that they produced the same free radical. Subsequent experiments were therefore carried out on thianthrene and its derivatives. Several experiments demonstrated the necessity for using nonoxidizing solvents in vacuo in order to avoid difficulties in interpretation. Thus the principal measurements were made in vacuo with trifluoroacetic acid as a solvent. Three oxides of thianthrene were studied – the monosulfoxide and the cis and trans disulfoxides. Solutions of these oxides are initially colorless and do not exhibit spin resonance. However, very slowly at room temperature and more rapidly at 80°C, the color appears and the solutions show paramagnetic resonance. Equilibrium is reached at 80°C in about 20 hr. The formation of radicals is much faster and the amount of the monosulfoxide is larger at equilibrium than for the two disulfoxides. These results suggested that the free radical is an intermediate state of oxidation between two oxides. This hypothesis was tested by observing the intensity of the spin resonance exhibited at equilibrium by mixed solutions of mono- and disulfoxide. The results supported the view that mono- and disulfoxide are in equilibrium with one another and with the radical, the latter being an intermediate state of oxidation between the two.

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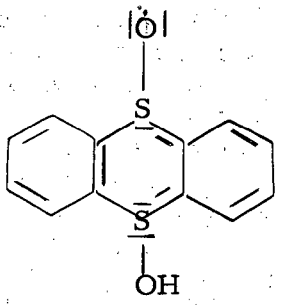
1 Hirshon, Gardner, and Frainkel, J. Am. Chem. Soc. 75, 4115 (1953).

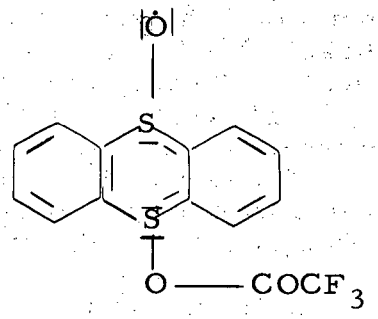
2 J. E. Wertz and J. Vivo, J. Chem. Phys. 23, 2193 (1955).

Calvin

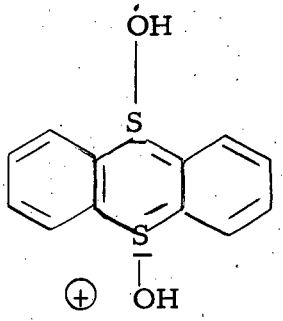
By writing down the more probable intermediates, the following set of radicals was proposed:



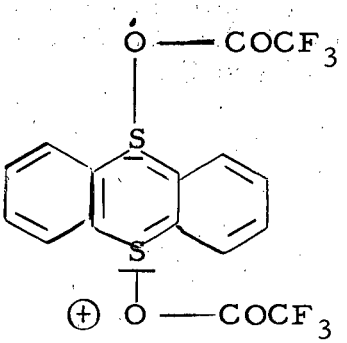
I



II



III



IV

The relative merit of these structures was decided on the basis of the spin-resonance spectrum which consisted of 5 lines separated by 1.5 gauss with the intensity ratios 1:3:5:3:1. The g value for the center of the pattern was found to be 2.009 ± 0.002.

Assuming that the fine structure is caused by the interaction of the magnetic moment of the unpaired electron with nuclear magnetic moments, we deduce that the electron only interacts with 4 protons. As at least 8 protons are present in each of the radicals proposed above, at least 9 lines should be expected, if all of the protons were equivalent.

However, if the structure of the radical is asymmetric, such as II, it is easily seen, by writing down all the resonance formulae not involving separation of charge, that the electron may be found on only four of the carbon atoms that are bound to hydrogen atoms.

By this interpretation of the electron-spin resonance spectra, an asymmetric radical such as II with no additional protons is the only one present in large amounts.

According to the proposal of the way in which this principal radical is formed, it should be in equilibrium with the oxides of thianthrene and radical I, III, and IV. A search for free radicals of low abundance was made with the result that three additional groups of lines were observed. The intensity of these signals were about 1/500 of that obtained for the principal radical. Two groups consisting of 5 lines each were found to be symmetrically displaced on either side of the principal resonance by a field of 16 gauss. The intensity ratio, hyperfine splitting, and general appearance of the individual lines in the two groups is the same as that for the principal resonance. We suggest that these two groups of lines originate from the free radical I with the 32 gauss splitting between the groups arising from the hyperfine interaction between the proton on the oxygen and the electron.

The third group of lines has a g value only about 0.025% higher than the principal resonance with the result that almost all of it is obscured. Three lines are observed on the low-field side and 2 lines appear on the high-field side of the principal resonance. The intensity of these lines is too small to allow their inclusion as part of the principal resonance, so we have ascribed them to another free radical. The number of lines suggests a radical of type IV.

ATTEMPTS TO SHOW INHIBITION OF TRANSAMINATION BY AZASERINE IN CELL-FREE PREPARATIONS

U. Carol Quarck, Petronella Y. F. van der Meulen, and James A. Bassham

In the preceding report[1] a description of azaserine inhibition during photosynthesis by Scenedesmus was given and the suggestion was made that one of the major sites of azaserine action is in reactions involving transamination. This report is concerned with the effect of azaserine on the conversion of C^{14}-labeled pyruvate to alanine in cell-free preparations.

Because of the difficulties involved in breaking up Scenedesmus cells, it was first decided to study the transamination reaction by using the powdered residue from a crude acetone extract of horse liver (obtained through the courtesy of Dr. Akira Ichihara and Professor D. M. Greenberg). One gram of this extract was suspended in 10 ml of 0.05 M phosphate buffer, pH 8, the suspension was centrifuged at 40,000 rpm for 30 min to remove the insoluble material, and the supernatant solution was dialyzed against distilled water for 4 hr in the cold and used without further purification. Each experiment was performed using 0.05 mg of glutamine and 0.05 mg of sodium pyruvate-2-C^{14}.[2] The reaction mixtures were incubated at 37° and then boiled for 2 min to inactivate the enzymes. The denatured material was removed by centrifugation and the supernatant solution was applied to washed Whatman No. 4 papers and separated, first in phenol-water and then in butanol–propionic acid. After radioautographs of the papers had been made, the main components detected on paper were counted and the papers sprayed with ninhydrin reagent to locate the ninhydrin-positive compounds. Table III summarizes the data from these experiments. In the controls, phosphate buffer was substituted for the enzyme solution.

It can be seen from Table III that although azaserine appears to have interfered to some extent with the formation of alanine from pyruvate, the concentration of azaserine in the enzyme solution necessary to bring about this effect was very large. Moreover, the amount of inhibition was not very great. The amino group involved in the transamination reaction must have come from one or more substances present in the enzyme solution, as the addition of glutamine to the incubation mixture had no effect on the amount of alanine formed. In the controls, where no enzyme solution was added, no alanine was formed and no effect of azaserine on pyruvate was observed. Alanine appeared to be the only amino acid containing radioactivity on the chromatogram.

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1 Bassham, Barker, and Quarck, Intermediates in Algae Metabolism, UCRL-3415, June 1956, p. 8.

2 The activity of a solution containing 0.05 mg of the sodium pyruvate was 4.4×10^5 cpm, as measured on an aluminum disc. However, chromatography of the solution alone showed a great number of radioactive impurities.

Table III

Transamination experiments with horse liver extract

Amount of enzyme solution (μl)	Incubation time (min)	Azaserine added (mg)	Activity of alanine formed (cpm, on paper)
500[a]	60[b]	none	23,700
500[a]	60	none	21,900
500[a]	60	1	7,550
500	60	none	23,700
500	60	1	6,550
250	5	none	4,400
250	30	none	11,100
250	30	0.5	4,900
250	15	none	10,000
250	15	0.1	6,900

[a] Enzyme solution preincubated with 1 μg pyridoxal phosphate for 45 min at 37°.

[b] No glutamine was added to the incubation mixture.

In view of the negative results obtained with the horse liver extract, it was decided to investigate the effect of azaserine on a cell-free extract from Chlorella. The freshly harvested Chlorella (3.3 g of wet packed cells) was washed once and suspended in 33 ml of a 0.05 M phosphate buffer, pH 8. A cell-free preparation was obtained by a 30-min treatment at 2° to 5° in a 9-kc Raytheon oscillator and subsequent removal of cell wall material and remaining whole cells by centrifugation for 30 min at 40,000 rpm. The pale-gree supernatant solution was used in the experiments without further purification. Except where otherwise indicated, the enzyme solution was incubated with pyridoxal phosphate (1 μg per 100 ml of enzyme solution) for 45 min at 37° before the substrates and the azaserine were added. Each experiment was carried out using 0.05 mg of C^{14}-labeled sodium pyruvate. All mixtures were incubated at 37° for 75 min and then boiled for 2 min to inactivate the enzymes. The solutions were then applied to papers and the components were separated and detected by the procedure described for the experiment with the horse liver extract. Table IV summarizes the data from these experiments.

It can be seen from Table IV that the azaserine has no effect on the formation of alanine from pyruvate under the conditions of this experiment except in the case where 0.05 mg of azaserine was used for the extract from 10 mg of wet cells. However, even in this case the extent of inhibition was very small. It is interesting to note that whereas the addition of glutamine to the incubation mixture had no effect on the amount of alanine formed, the addition of glutamic acid brought about a significant increase in the amount of alanine formed.

After these negative results an attempt was made to break up Scendesmus cells. One cc of packed cells was suspended in 0.1 M sodium phosphate buffer, pH 7.4, and ground with "Monterey" sand in a mortar. Some of the cells were ruptured. The suspension was then centrifuged at 40,000 rpm for 30 min to remove cell debris and whole cells, and the supernatant was diluted with phosphate buffer to 10 ml. The operations were done at a temperature of 4°C or lower.

In each experiment the following was used: 300 λ crude extract (extract of 30 mg wet packed cells); 0.05 mg sodium pyruvate-C^{14} in 50 μl H_2O; and additions as shown in Table V.

Each reaction mixture was incubated at 37°C for 60 min, then boiled for 2 min, applied to acid-washed Whatman No. 4 paper, and chromatographed first in a phenol–water solution and then in a butanol–propionic acid–water solution. Radioautographs were made and the activity of the alanine spot counted as before.

Inhibition of 40% to 60% resulted here with the equivalent of 0.06 mg azaserine per batch of extract of 10 mg wet packed cells. However, only a small fraction of the cells was smashed (an estimated 5% to 10%), so that the relative amount of azaserine is higher by a factor of 10 to 20.

Calvin

Table IV

Transamination experiments with Chlorella extract

Amount of enzyme solution (μl)	Azaserine added (mg)	Amino acid added (0.05 mg)	Activity of alanine formed (cpm, on paper)
100[a]	none	none	5.0×10^4
100	none	glutamine	5.7×10^4
100	0.1	glutamine	4.7×10^4
100	0.1[b]	glutamine	4.8×10^4
100	0.5	glutamine	2.9×10^4
200	none	glutamine	7.1×10^4
100[c]	none	glutamine	5.6×10^4
100[c]	0.1	glutamine	5.1×10^4
100	none	monosodium glutamate	8.6×10^4
100	0.1	monosodium glutamate	8.2×10^4

[a] This amount represents the extract from 10 mg of wet packed cells.

[b] Enzyme solution preincubated with 0.1 mg of azaserine.

[c] No pyridoxal phosphate added.

Table V

Transamination experiments with Scenedesmus extract

Exp. No.	Azaserine (mg)	Pyridoxal phosphate (mg)	Glutamine (mg)	Glutamic acid (mg)	Activity of alanine formed (cpm, on paper)
1	none				12,961
2	0.02				12,946
3	0.2				7,026
4	none	0.001			14,594
5	0.02	0.001			14,171
6	0.2	0.001			7,814
7	none		0.05		16,808
8	0.2		0.05		7,811
9	none			0.05	36,260
10	0.2			0.05	19,018

From these experiments we can conclude that partial inhibition of the forming of alanine from pyruvic-C^{14} acid under these circumstances is brought about only by a very high concentration of azaserine, of the order of 10 mg azaserine per batch of extract of 0.2 ml wet-packed cells. In the 5-min photosynthesis experiments, 4 mg azaserine per batch of 0.2 ml wet packed cells suspended in 20 ml buffer had a very marked effect.

In experiments with both Chlorella and Scendesmus the addition of glutamine produced a very slight increase in the amount of alanine formed. However, with the same amount of glutamic acid, the alanine was doubled. Azaserine caused a 50% inhibition of the reaction.

INFLUENCE OF AZASERINE ON SCENEDESMUS IN THE STEADY STATE

James A. Bassham and Petronella Y. F. van der Meulen

In a previous report,[1] the effect of azaserine on the metabolism of Scenedesmus during photosynthesis was described. In these earlier experiments the algae were allowed to photosynthesize in a stream of air plus 4% CO_2 for 1 hr in the presence of the inhibitor before the 5-min photosynthesis with $NaHC^{14}O_3$ was carried out. The algae exposed to azaserine showed a decrease of incorporation of activity in glutamic acid, aspartic acid, serine, alanine, and lipid-phospholipid areas and an increase in the activity of glutamine, malic acid, citric acid, α-ketoglutaric acid, glycolic acid, and sucrose.

In the current experiment described here, the algae photosynthesized in a stream of air plus 1.8% CO_2 containing C^{14}, until they were presumed to have reached a steady state. The azaserine then was injected and small aliquots were taken at different times after the injection.

An experimental setup similar to that described by A. T. Wilson and M. Calvin[2] was used. Sixty ml of a 1% suspension of Scenedesmus cells containing 1 ml potassium phosphate buffer (2.5×10^{-3} M) was left in the light while a stream of air, plus 1.8% $C^{14}O_2$ from a 5-l reservoir, was bubbled through and returned. During the experiment the CO_2 concentration went down from 1.8% to 1.55%. After 50 min, 15 mg of azaserine in 1 ml H_2O was injected. Several aliquots were taken before and after the injection, each of about 1 ml. The aliquots were dropped into 5 ml cold methanol and their size determined by weighing. After the cells were boiled and centrifuged, they were re-extracted with 20% methanol. The total extract was then concentrated under vacuum, using the "octopus."[2] The concentrated extract was quantitatively applied to acid-washed Whatman No. 4 papers, chromatographed in phenol-water as a first direction, and in butanol-propionic acid-water as a second direction, and radioautograms were made. The compounds were counted on paper and the activity/extract from 1 ml suspension calculated.

Figures 3 and 4 show the most striking results. Unfortunately, one of the three points taken before injection of the azaserine was lost, and some compounds do not seem to have been in a steady state as to the amount of C^{14} incorporated, so that the change in radioactivity cannot be considered to reflect exactly the same change in the total amount. The graphs show that the primary and most pronounced effect is an immediate and almost linear build-up of glutamine. At the same time, there appears to be a decrease of aspartic and glutamic acids, in agreement with the results of Bassham, Barker, and Quarck.[1] The activity of aspartic acid stayed below 200 cpm, whereas glutamic acid showed a slow but definite increase of activity after

[1] Bassham, Barker, and Quarck, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 41.

[2] A. T. Wilson and M. Calvin, J. Am. Chem. Soc. 77, 5948 (1955).

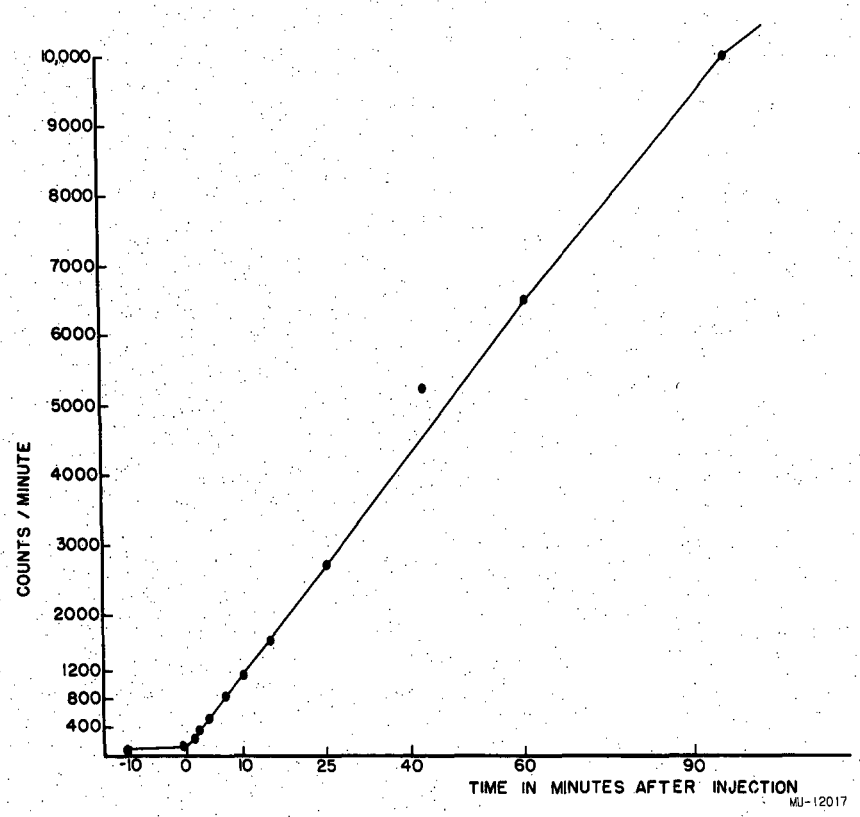

Fig. 3. Increase of radioactivity of glutamine after injection of azaserine.

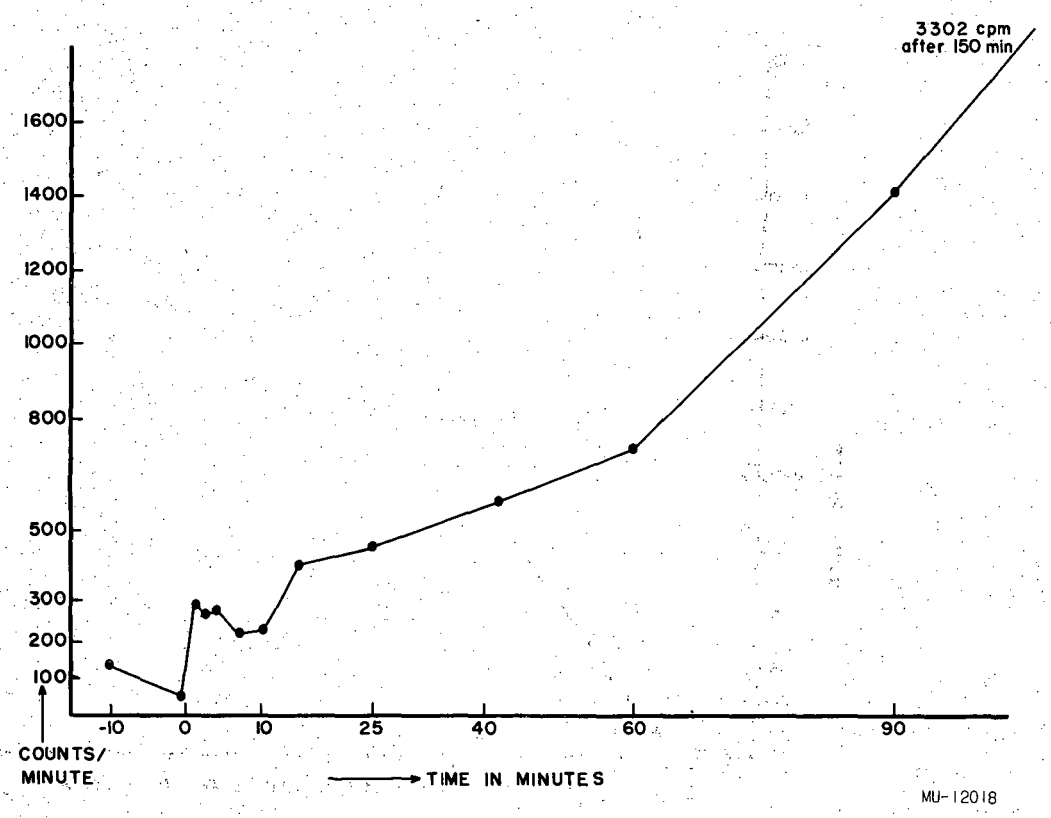

Fig. 4. Change in radioactivity of the glucose-1, 2-phosphate area after injection of azaserine.

about 1 hr. The activities of citric acid and α-ketoglutaric acid stayed below 200 cpm during the first hour after injection of the azaserine and as a result were very inaccurate. After 1 hr they showed a slow but steady increase similar to that of glutamic acid. The activity of malic acid almost doubled during the first 15 min, but from then on slowly decreased. The activities of alanine and serine were too low to allow much accuracy in the counting. The serine activity decreased; that of alanine slowly increased after about 20 min. There was no clear effect on the sucrose. The glucose-1,2-diphosphate area rapidly gained activity after about 1 hr.

The specific activities of glutamine and glutamic acid were determined by means of the ninhydrin method (see Table VI). Because of the small quantities the results are only accurate within 50%.

These results indicate that azaserine inhibits a reaction (or reactions) in which glutamine is involved.[3,4] The synthesis of aspartic acid and glutamic acid are also blocked, but glutamic acid apparently can be synthesized by another route. If azaserine inhibits transaminations generally, some build-up of α-ketocarboxylic acids could be expected. However, after 1 hr only a slow build-up of acids could be detected. No conclusion can be drawn, therefore, because of their low activity and because of their possible involvement in several metabolic reactions.

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[3] B. Levenberg and J. M. Buchanan, J. Am. Chem. Soc. 78, 504 (1956).

[4] A. Meister and S. V. Tice, J. Biol. Chem. 187, 173 (1950).

Table VI

Influence of azaserine on the specific activity of glutamine and glutamic acid

Time after injection of azaserine, in min	Specific activity of glutamine in cpm/μmole	Specific activity of glutamic acid in cpm/μmole
42	40,000	20,000
120	120,000	20,000
150		20,000
190	120,000	
360	140,000	20,000

The specific activity of the CO_2 was approximately 260,000 cpm/μmole

INFLUENCE OF 6-DIAZO-5-OXONORLEUCINE ON THE METABOLISM OF SCENEDESMUS AND CHLORELLA DURING PHOTOSYNTHESIS

Petronella Y. F. van der Meulen and James A. Bassham

In a previous report[1] the influence of azaserine on the metabolism of Scenedesmus was described. Chlorella were found to be unaffected. The same kind of experiments have now been carried out with 6-diazo-5-oxonorleucine (DON) a tumor-inhibitory substance.[2]

The experiments were carried out with a suspension of washed cells (packed volume 0.4 cc) in a solution of 0.4 ml KH_2PO_4 buffer (2.5×10^{-3} M) in 40 ml H_2O. The suspension was equally divided between two lollipops, and to one of them was added DON (see Tables VII and VIII). They were left in the light for 1 hr with air and 4% CO_2 bubbling through. Then 60 µl HCl (0.102 N) and 300 µl $NaHC^{14}O_3$ solution (400 µc/ml) were added (the HCl was necessary to bring the pH of the bicarbonate to 7.6). They were allowed to photosynthesize for 5 min, then poured in boiling ethanol (80 ml). After centrifugation the cells were re-extracted with 20% ethanol, and the activity of 50 µl of the combined extracts was determined. The extracts were concentrated and aliquots containing 10^6 cpm were applied on acid-washed Whatman No. 4 papers. These papers were chromatographed, first in phenol-water and then in butanol–propionic acid–water, and radioautograms were made. The various compounds were counted on paper and their activities are given in Tables VII and VIII.

The interference with the metabolism of Scenedesmus (Table VII) shows the same over-all picture as with the azaserine. The decrease in the activity of the amino acids is even more pronounced. Whether or not the new compound that appeared in the glucose-1,2-diphosphate area of the chromatogram from azaserine-inhibited Scenedesmus is formed on DON inhibition is still to be examined.

An experiment with 10 mg DON and Scenedesmus showed the same results.

The influence of DON on Chlorella (Table VIII) produces a much smaller increase in glutamine, a smaller decrease of amino acids, and no change in the glutamic acid. Changes in the carboxylic acids are also less pronounced. The activity of the sucrose decreases whereas, in Scenedesmus, it is doubled under the influence of DON. Two spots (new Spots 1 and 2 of Table VIII), moving slower than sucrose in both directions, show a marked increase in activity. Both are phosphates and give, upon phosphatasing, compounds that run far in normal solvent systems, to the neighborhood of dihydroxyacetone. Near glutamine a spot arises (activity 489 cpm) which probably is citrulline. Identification of these compounds is being sought.

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1 Bassham, Barker, and Quarck, Intermediates in Algae Metabolism UCRL-3351, March 23, 1956, p. 8.

2 H. W. Dion and S. A. Fusari, J. Am. Chem. Soc. 78 3075 (1956).

Table VII

The effect of 6-diazo-5-oxonorleucine (DON) on Scenedesmus

	Control (cpm)	DON (2 mg) (cpm)
Total activity of extract	18.5×10^6	17×10^6
Glutamine	192	2284
Glutamic acid	1975	777
Aspartic acid	5582	485
Serine	3458	384
Glycine	1151	383
Threonine	408	204
Alanine	1689	531
α-Ketoglutaric acid	219	1055
Citric acid	1250	2480
Sucrose	4014	8093

Table VIII

The effect of 6-diazo-5-oxonorleucine (DON) on Chlorella

	Control (cpm)	DON (4 mg) (cpm)
Total activity of extract	16.5×10^6	26×10^6
Glutamine	602	1385
Glutamic acid	9662	9468
Aspartic acid	1648	760
Serine and glycine	5811	6613
Alanine	21375	9479
α-Ketoglutaric acid	1519	578
Citric acid	406	648
Malic acid	1172	2140
Sucrose	57568	32056
New spot 1	719	3142
New spot 2	1464	2978

Calvin

INHIBITION OF PHOTOSYNTHESIS IN ALGAE BY 6-ETHYL-8-THIOLOCTANOIC ACID

James A. Bassham, Nancy Winter, and Walter Kelsen

Inhibition of valine and isoleucine synthesis in yeast by thioctic acid analogy has been reported by Broquist and Stiffey.[1] Because of previous interest in the possible role of thioctic acid in photosynthesis, studies have been undertaken on the effects of 6-ethyl-8-thioloctanoic acid (ETO) on the carbon-fixation pattern of unicellular algae during photosynthesis with $C^{14}O_2$.

Preliminary studies were carried out in a closed gas-circulating system described in previous reports in which the mixture of CO_2 in air, circulating through an illuminated transparent cell containing algae, passes through an oxygen analyzer and a CO_2 analyzer. The signals from these instruments are plotted continuously by a recorder, and the slopes of these gas tensions against time give the rate of photosynthesis and respiration.

Algae were centrifuged from their nutrient solutions and resuspended in phosphate buffer in the cell described above. After a steady rate of photosynthesis or respiration was established, inhibitor was added, as indicated in Tables IX, X, and XI.

As can be seen from Tables I and II, photosynthesis in Scenedesmus is markedly inhibited by ETO at these levels, but respiration is either unaffected or actually stimulated. Addition of β-mercaptopropionic acid at a somewhat higher level (twice as much on a molar basis) gave some stimulation of respiration but no inhibition of photosynthesis. In other experiments, ETO was added while the plants were photosynthesizing. Some inhibition became apparent after a minute, and the degree of inhibition gradually increased. It appears that ETO produces at least two effects on the metabolism of Scenedesmus, one more or less specific to ETO and to photosynthesis, and one which affects respiration and which can be produced by other thiol fatty acids.

The effects observed with Chlorella were somewhat different. Addition of ETO to Chlorella at the same ratio of inhibitor to algae produced an immediate effect in the light, as shown in Table XI.

In other experiments, this level in inhibitor was added in the dark during respiration, and 20 min was allowed before turning on the light. Stimulation of respiration was observed as in the case of Scenedesmus, but complete inhibition of photosynthesis occurred, with the respiration continuing at an essentially unchanged rate in the light.

Preliminary experiments showed that a partial inhibition of photosynthesis could be achieved by adding 3 mg ETO to 0.3 cc Chlorella in 60 ml H_2O plus 1 ml phosphate buffer (described above). This amount of

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[1] H. P. Broquist and A. V. Stiffey, Fed. Proc. 15, 224 (1956).

Table IX

Scenedesmus obliquus, 1.5 cc (wet packed volume), suspended in 60 ml H_2O plus 5 ml 2.5×10^{-3} M KH_2PO_4-K_2HPO_4 buffer

	CO_2	O_2
Photosynthesis, rate during 10 min	-273	+350
Respiration, 90 min	+18.5	-21.5
Photosynthesis, 10 min	-252	+305
Respiration, 40 min	+18.8	-22.0
Respiration, 34 min (added 15 mg ETO in 300 µl ethanol neutralized to pH 7)	+24.2	-30.6
Photosynthesis, initial rate	-147	+190
Photosynthesis, after 30 min	-74	+104
Respiration, 76 min	+25.2	-37.1
Photosynthesis, 20 min	-70	+76

Table X

Scenedesmus, 1.5 cc suspended in same solution as in Table IX

	CO_2	O_2
Photosynthesis, rate during 8 min	294	345
Respiration, 20 min	18.8	25.7
Photosyntheses, 8 min	---	---
Respiration, 20 min	17.6	24.4
Respiration, 20 min (added 22.8 mg mercaptopropionic acid, pH 7, in 400 µl ethanol-water solution)	19.6	30.2
Photosynthesis, 8 min	305	342
Respiration, 28 min	26.6	34.4
Respiration, 10 min (added 20 mg ETO in 400 µl ethanol, pH 7)	20.7	34.4
Photosynthesis, 15 min	134	174

Table XI

Chlorella, 0.6 cc suspended in
60 ml H_2O plus 1 ml 2.5×10^{-3} M KH_2PO_4-K_2HPO_4 buffer

	CO_2	O_2
Photosynthesis	-163	190
Added 7.7 mg ETO, light left on	0	0
after 2 min	0	0
After 8 min algae began to recover from 12 min to 36 min approx rate	-53.4	+61.4
Then the rate slowly dropped, becoming zero, and after another 10 min	+19.3	-18.0

Calvin

ETO was added to Chlorella that had been allowed to photosynthesize for 1 hr in the closed system which was connected with a 5 l flask. The entire system contained 1.8% CO_2 labeled with $C^{14}O_2$. The large capacity of the system prevented the CO_2 concentration from changing significantly during the course of the experiment. Aliquot samples of the algae suspension were taken before and after addition of the inhibitor and subsequently analyzed by paper chromatography and radioautography. The radiocarbon incorporated in the various labeled compounds was determined by counting. The most striking effect was a rise in the diphosphate area (about 30% in 2 min), a decrease in PGA (50% in 4 min), and a huge increase in a compound tentatively identified as glycolic acid (twofold in 4 min, fortyfold in 25 min). These results are reminiscent of those obtained by lowering the CO_2 pressure,[2] and suggest that at least one action of ETO may be an effect on the carboxylation of ribulose diphosphate to give PGA. However, Ning Pon in this laboratory has tested the effect of ETO on carboxydismutase at levels comparable to those employed in these experiments and has found no inhibition. Further studies are in progress.

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[2] A. T. Wilson and M. Calvin, J. Am. Chem. Soc. 77, 5948 (1955).

Calvin

METABOLISM OF THIOCTIC ACID IN ALGAE

Masao Nakazaki, Patricia T. Adams and James A. Bassham

The alcohol-soluble fraction of chloroplasts from Chlorella under photosaturated conditions was studied for metabolites of thioctic acid.

1. Fractionation of Chlorella

Five cc of wet packed cells of Chlorella was obtained from 700 ml of culture solution of Chlorella by centrifugation and washing with distilled water. The cells were suspended in 200 ml of nutrient solution and aerated with 4% CO_2 under photosaturation for 1.5 hr to obtain steady-state conditions. Five ml of radioactive thioctic acid solution (4.20 mg/10 ml of 0.067 M phosphate buffer at pH 6.74; specific activity of thioctic acid, 30 μc/mg) were added to this suspension, which was then shaken and aerated under photosaturation. After 3 hr the cell suspension was centrifuged 10 min at 2000 rpm to obtain the packed cells (5 cc), which were washed twice with distilled water and recentrifuged. The packed cells thus obtained were resuspended in 90 ml of phosphate buffer and ruptured by treatment for 10 min in a 9-kc sonicator. After centrifugation for 10 min at 2100 rpm, the unbroken cells and cell walls were separated from the supernatant, which was then centrifuged at 40,000 rpm for 15 min to obtain the chloroplasts. The chloroplasts were extracted with hot 90% ethanol and the insoluble material removed by centrifugation. The alcoholic solution was evaporated to dryness under reduced pressure and taken up in n-hexane. The distribution of radioactivity in the fractions of Chlorella is shown diagrammatically in Fig. 5.

2. Chromatographic Separation of Thioctic Acid Lipid on Cellulose Powder

After several trials using powdered sugar and cellulose powder, the conditions described below were shown to be the best for good separation.

The column (25 by 46 cm) packed with cellulose powder was washed with 150 ml of n-hexane, and 25 ml of n-hexane solution of thioctic acid lipid fraction from the chloroplasts was introduced on the top of the column and followed by elution with n-hexane, hexane plus benzene, benzene plus ether, and ether.

The fractions obtained are shown in Table XII, with the percentage distribution of radioactivity.

3. Further Separation of Thioctic Acid Lipid by Countercurrent Distribution

Fraction No. 4 in the above chromatographic separation was evaporated under reduced pressure and fractionated by countercurrent distribution using a 25-transfer Craig stainless steel machine (capacity of the lower tube is 8 ml; of the upper layer, 10 ml). The solvent system was n-hexane–95% methanol. The distribution of radioactivity is shown in Fig. 6.

The same type of radioactivity distribution curve was obtained from the combined fractions No. 3, 5, and 6 from the cellulose powder chromatogram.

Calvin

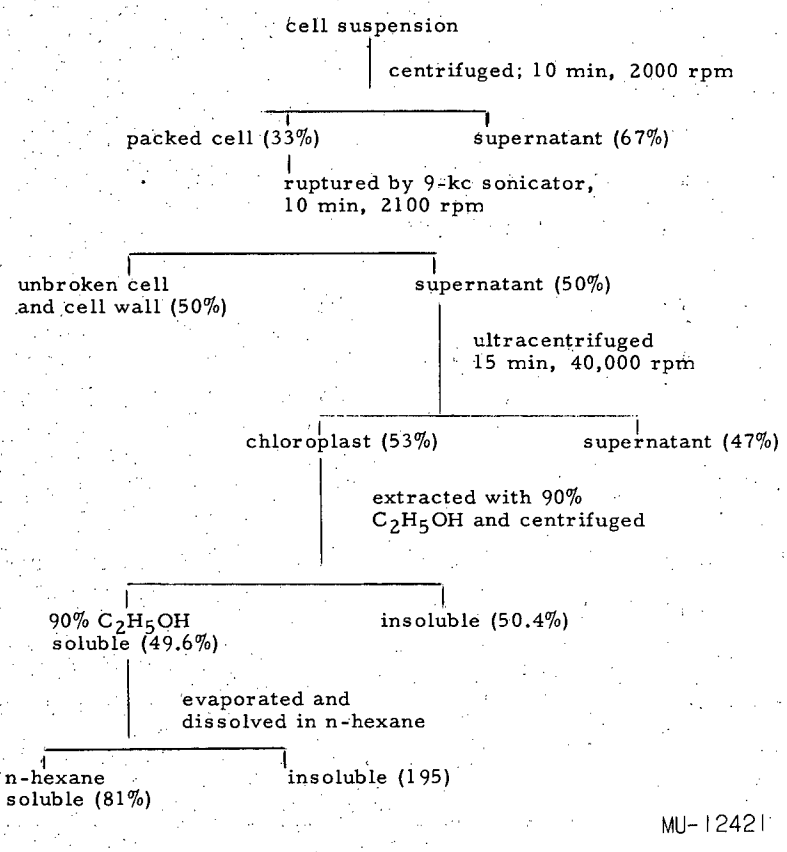

Fig. 5. The distribution of radioactivity in the fractions of Chlorella.

Table XII

Chromatographic separation of the thioctic acid lipid on cellulose powder

Fraction No.	Solvent(s) and volume in ml				Eluate (ml)	Character-istics	Percentage distribu-tion
1	n-hexane	200			122	colorless	0.00
2					21	colorless	0.08
3					55	yellow(carotene)	10.9
4	n-hexane	100			66	pale yellow	9.0
5	n-hexane	100	benzene	5	230	pale green	17.3
	n-hexane	80	benzene	20		(grey band on column)	
6	n-hexane	70	benzene	30	225	yellow	9.3
	n-hexane	50	benzene	50		(xanthophyll)	
7	n-hexane	50	benzene	70	95	yellow-green band	3.5
8	benzene	100			80	yellow-green band	31.8
9	benzene	100	ether	1	140	chlorophyll a	9.5
	benzene	100	ether	2			
10					150	chlorophyll b	5.6
11	benzene	50	ether	50	280	light green	21.0
	ether	100					recovery 29%

Calvin

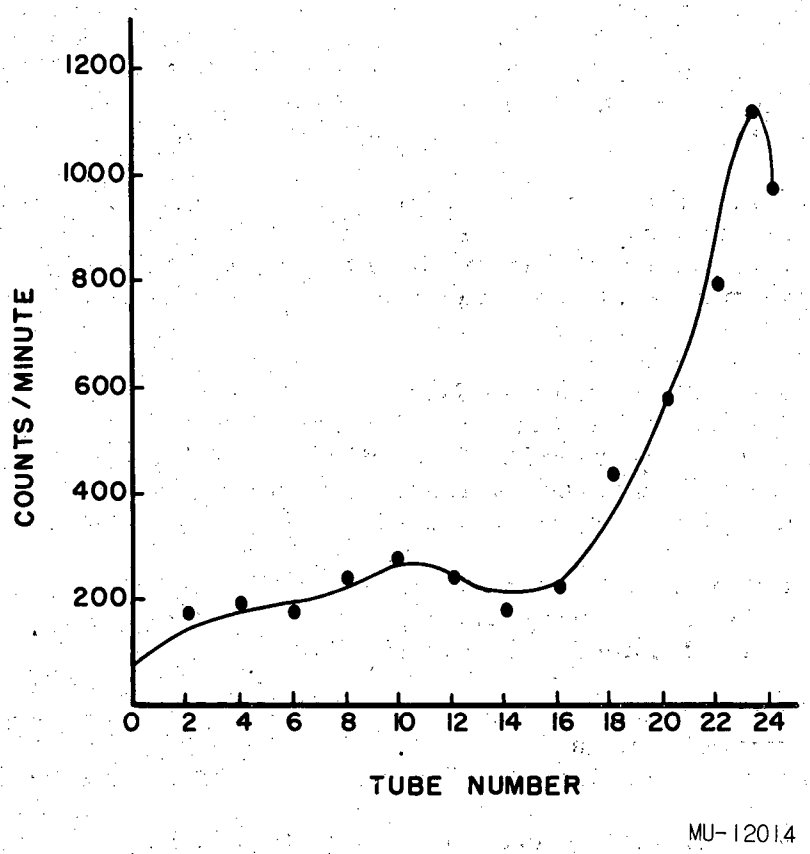

Fig. 6. Distribution between 95% methanol and n-hexane of fraction No. 4 from the cellulose powder chromatogram.

The fraction in tube No. 22 was evaporated under reduced pressure and hydrolyzed with 6 N hydrochloric acid at 120°C for 3 hr. This solution was evaporated to dryness under reduced pressure and the residue was dissolved in 80% ethanol. The ethanol solution was chromatographed on the same paper as the original fraction and radioactive thioctic acid, with butanol–0.5 N ammonia as solvent. All of the radioactivity of the original fraction came to the solvent front, and no thioctic acid or sulfoxide of thioctic acid was found. In the hydrolyzed material, thioctic acid and sulfoxide of thioctic acid were detected, but about 50% of the radioactivity was still found on the solvent front.

The combined solution from Nos. 21, 22, 23, and 24 was evaporated under reduced pressure and dissolved in 15 ml of 85% ethanol. After the addition of 10 ml of 5% ammonium persulfate solution, the mixture was kept at room temperature for 3 hr. The fact that under the same condition thioctic acid is completely converted into the sulfoxide was confirmed using radioactive thioctic acid from a paper chromatogram.

The reaction mixture was concentrated under vacuum and extracted with 50 ml of benzene, and the benzene extract was washed with water. After the benzene was removed from the extract, the residue was distributed between 95% methanol and n-hexane. The distribution of radioactivity is shown in Fig. 7.

The fractions of tubes Nos. 20, 22, and 24 were combined and hydrolyzed as described above. The paper chromatogram of this hydrolysate showed the spot of sulfoxide of thioctic acid and a very weak spot of thioctic acid, but about 50% of the radioactivity was still found on the solvent front. In the original solution, only one spot was found on the front.

Fraction No. 8 from the separation using cellulose powder was concentrated under vacuum and countercurrent-distributed between 95% methanol and n-hexane. The distribution of radioactivity is shown in Fig. 8.[1]

The fractions in tubes Nos. 0, 1, and 2 were evaporated and hydrolyzed with 6 N hydrochloric acid as described above, and paper chromatographed using butanol saturated with 0.5 N ammonia as the solvent.

In the original solution, almost all radioactivity was found on the solvent front and there were no traces of thioctic acid and the sulfoxide. After hydrolysis, the spots of thioctic acid and sulfoxide were detected, but about 60% of the radioactivity remained on the solvent front.

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[1] Thioctic acid and its sulfoxide (in phosphate buffer pH 6.75) remained on tube No. 0 after 25 transfers.

Calvin

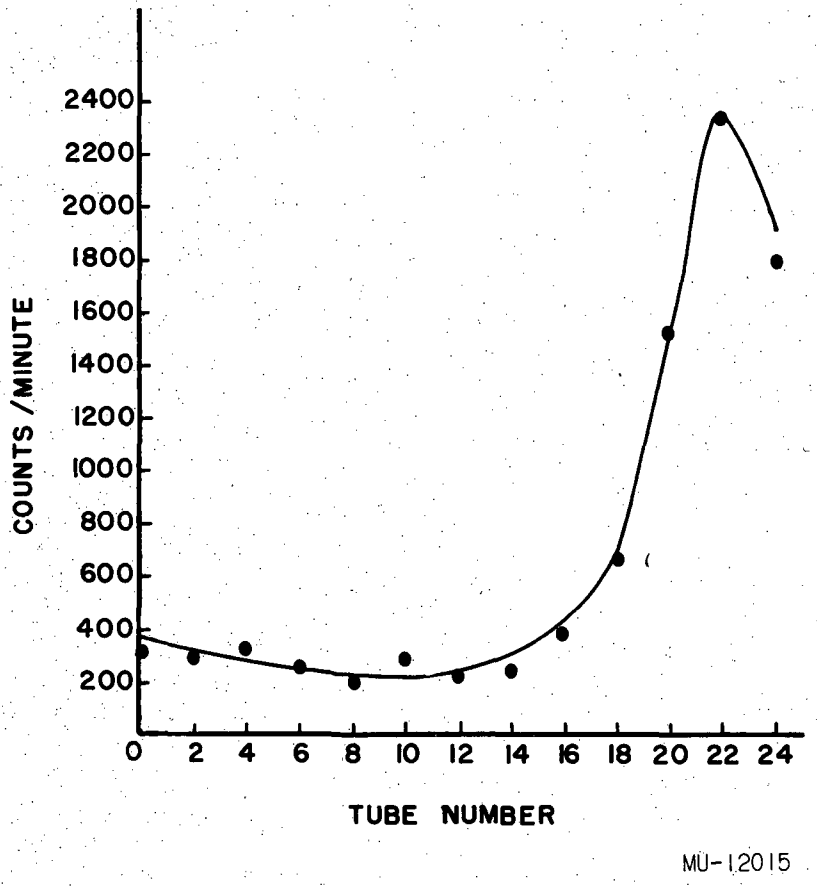

Fig. 7. Distribution of oxidized thioctic acid lipid between 95% methanol and n-hexane.

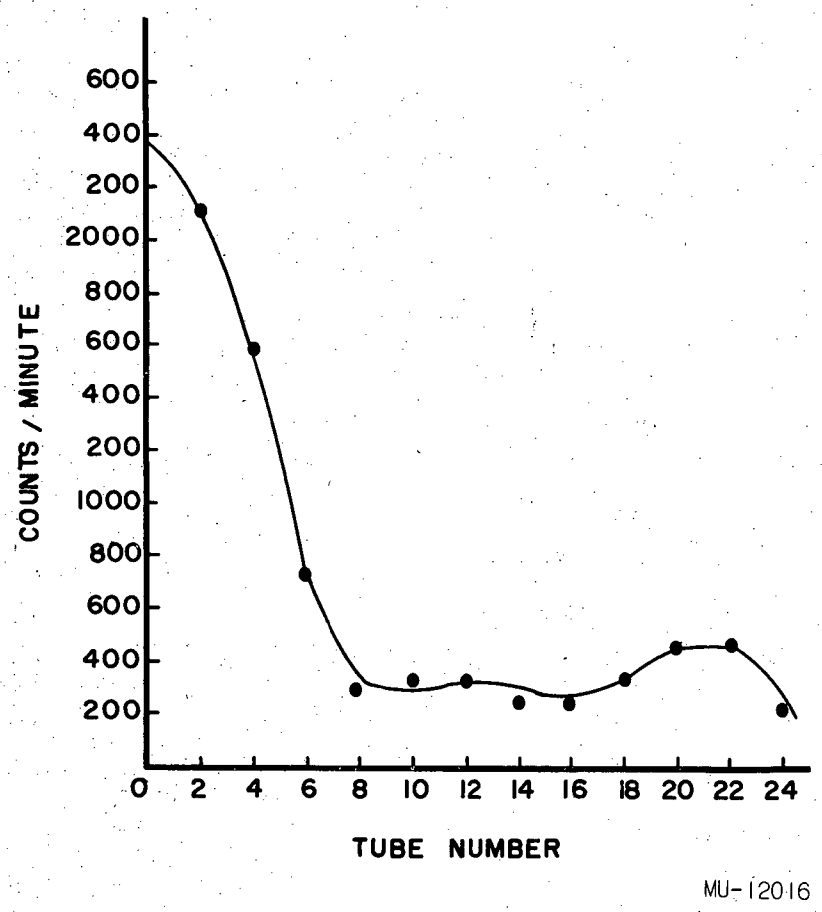

Fig. 8. Distribution of the fraction of No. 8 of cellulose powder chromatogram between 95% methanol and n-hexane.

4. Attempts at Paper Chromatographic Separation of Thioctic Acid Lipid

Many trials were made of paper chromatographic separation of thioctic acid lipid using several solvent systems (for example: ligroin–5% toluene, ligroin–2% isopropyl alcohol, ligroin–5% acetone and 65% ethanol), and two-dimensional paper chromatograms using the combinations of above solvents, and these sometimes showed rather complicated patterns of radioactivity distribution. However, these patterns showed clearly the two principal fractions that were found in the cellulose powder chromatography described above.

Because the paper chromatography using these solvent systems was found to be very sensitive to overload and because of the lability of the compounds of thioctic acid on paper, further study by this method was abandoned.

Calvin

THE PHOTOSYNTHESIS OF LIPIDS: TRACER STUDIES WITH SCENEDESMUS

Ulrich Blass

Photosynthesis studies with $C^{14}O_2$ revealed that green algae incorporate radioactive carbon into their lipid compounds in as short a time as 40 sec.[1] This report deals with attempts to separate extracts of radioactive metabolic lipids of Scenedesmus into their components.

Extraction

The algae, as a 1% suspension in aqueous phosphate buffer, were allowed to photosynthesize in a lollipop in the presence of sodium bicarbonate-C^{14}. They were centrifuged after the desired time. The cells were extracted al:1 mixture of benzene and methanol, and again centrifuged. The extract was washed with a 1% aqueous solution of sodium chloride, separated, and kept frozen until use. Even frozen extracts appear considerably changed in their composition when they become older than 1 to 2 weeks.

Paper Chromatography

A fairly good separation of lipids and pigments in algae extracts can be achieved on Whatman No. 3 MM paper sheets by use of toluene as solvent. Test runs in this method with the ascending technique need no more time than 30 to 40 min. However, the radioautograms of those chromatograms show more than 90% of the radioactivity in the spots at the origin and in front of the solvent, whereas two-dimensional chromatograms with different solvent systems indicate that any of these radioactive spots consists of complex mixtures with at least five different compounds. Chromatograms with a large number of solvent systems on sheets of paper, plastic fibres, and reversed-phase papers did not allow a sufficient separation of these mixtures. This is partly due to decomposition reactions of water-containing solvents with the pigments and phospholipids. In order to avoid decomposition by light, we performed all the chromatograms in complete darkness.

Labeled Lipids and Pigments

The technique described made it possible to study the distribution of radioactive carbon in a series of extracts of algae that were exposed to $C^{14}O_2$ for 3, 5, or 10 min and 1, 5, or 12 hr. These experiments prove that most of the pigments in Scenedesmus become radioactive only slowly during photosynthesis. Only the yellow spot of the oxygen-free carotenoids shows some radioactivity after very short exposure times, but chromatograms in varying solvent systems made it clear that this spot is always accompanied by colorless radioactive material. Especially slow is the fixation of radiocarbon in the chlorophylls. Even after 12 hr of exposure of Scenedesmus to light and $C^{14}O_2$, it is difficult to detect any radioactivity in the chlorophyll b. Figure 9 shows, as an example, the distribution of radioactive carbon in chromatograms of extracts from algae, which were exposed to $C^{14}O_2$ for periods of 10 min and 5 hr.

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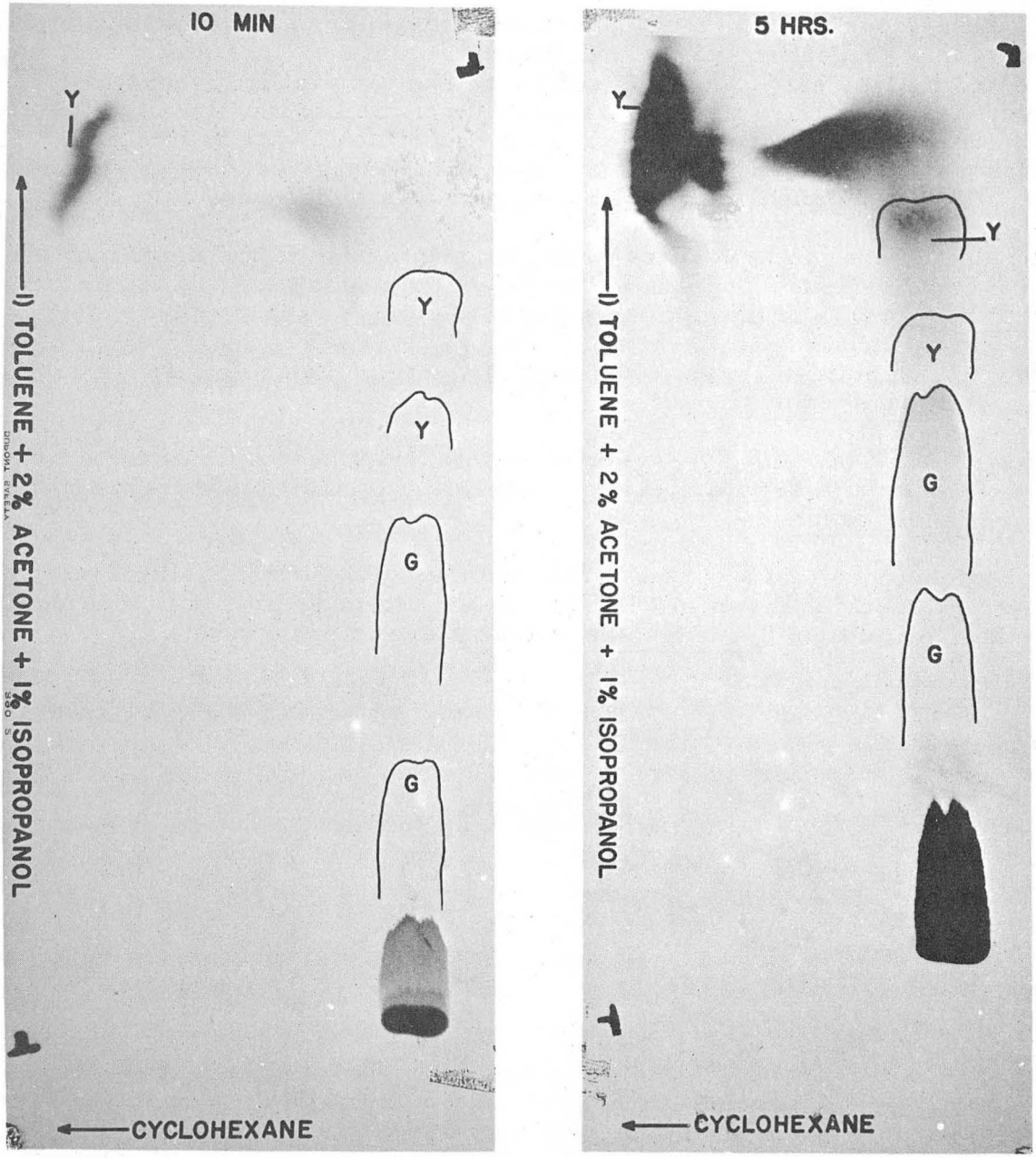
[1] K. A. Clendenning, Arch. Biochem. 27, 75 (1950).



ZN-1596

Fig. 9. Lipid structure from Scenedesmus after 10 min and 5 hr exposures to $C^{14}O_2$. (y indicates yellow, g indicates green.)

Other experiments proved that the lipids of Scenedesmus fix 1000 times as much radioactive carbon in the light as in the dark during 1 hr.

Identification

Previous work on the paper chromatography of chloroplast pigments[2,3] offers some suggestions about the chemical nature of the colored chromatogram spots from algae extracts. The colorless radioactive lipids have also been studied analytically.[1] However, we could not predict the chemical nature of any of the radioactive spots. Tracer experiments with radioactive phosphorus, radioactive sulfur, and radioactive triglycerides proved useful here. The technique for these experiments was as follows:

P^{32}: The algae were allowed to photosynthesize in the usual manner for 2 hr in the presence of P^{32}-labeled phosphates. A lipid extract was prepared from these algae as described above.

S^{35}: Thioctic-S^{35}_2 acid was admixed with an unlabeled lipid extract of Scenedesmus.

Fats: C^{14}-labeled glyceryl tripalmitate and glyceryl tristearate were admixed in separate experiments with labeled lipid extracts of Scenedesmus.

The radioautogram of a parallel-run chromatogram of these differently labeled extracts is shown in Fig. 10. Other attempts to isolate and identify radioactive lipids of Scenedesmus extracts are in progress.

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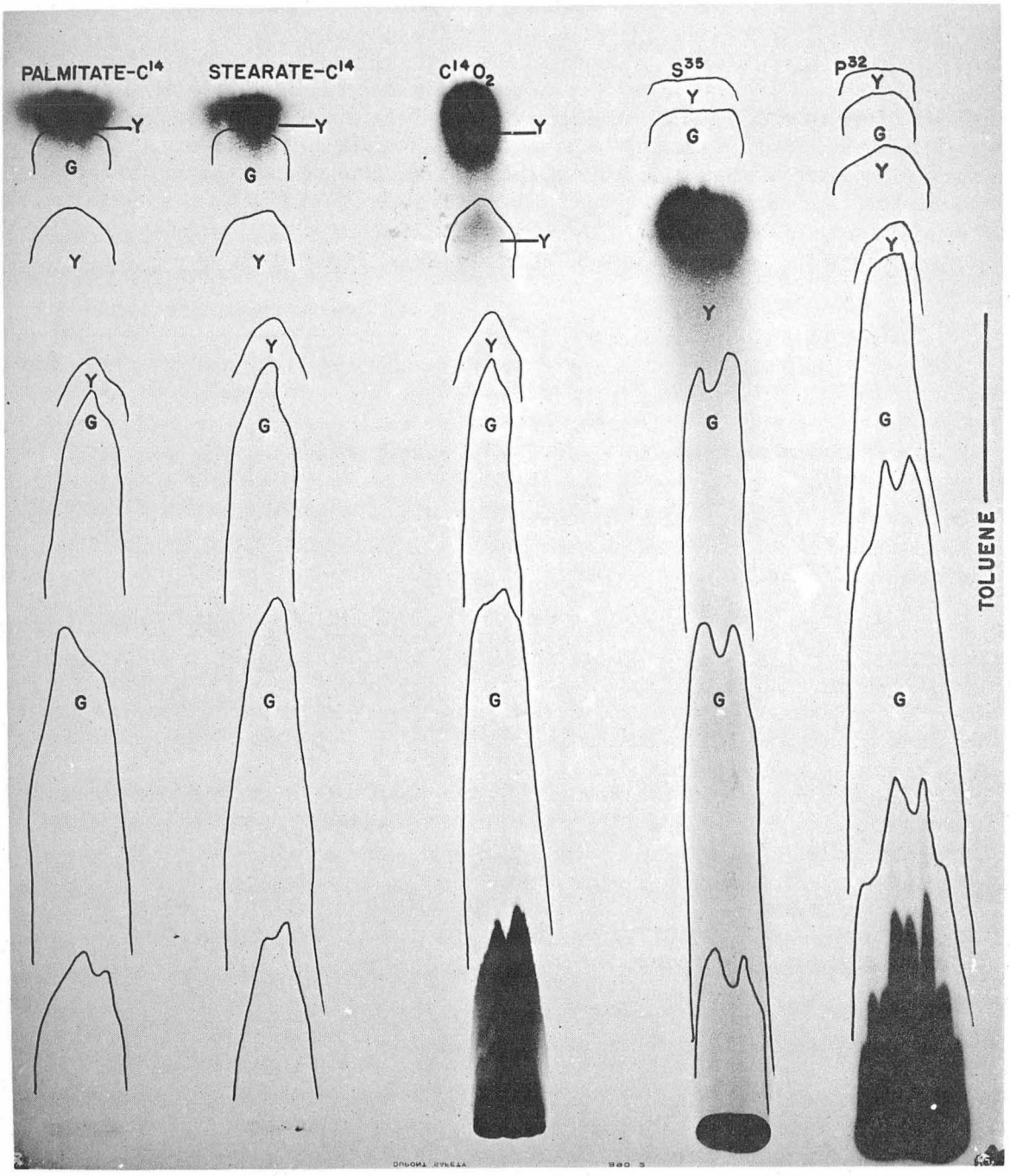
2 L. Bauer, Naturwissenschaften 39, 88 (1952).

3 H. H. S. Strain, J. Phys. Chem. 57, 638 (1953).



ZN-1595

Fig. 10. Lipid structure from Scenedesmus labeled with palmitate-C^{14}, stearate-C^{14}, $C^{14}O_2$, S^{35}, and P^{32}. (y indicates yellow, g indicates green.)

REPRODUCIBILITY AND THE CARBOXYDISMUTASE ACTIVITY

Ning G. Pon

In the study of an enzyme, the problem of its assay is of paramount importance. For example, the carbon dioxide fixing activity per unit weight of enzyme must be specified in order to establish each stage of purification. This specific-activity study leads to the necessity for reproducibility of results. Therefore, the first things that should be tested are the experimental techniques involved in the assay.

The reaction for the carboxylation is postulated as

$$HCO_3^- + \text{ribulose-1,5-diphosphate (RuDP)} \xrightarrow[\text{cofactor } Ni^{++} \text{ and no other metal ions}]{\text{enzyme carboxydismutase}} [\text{2 molecules of 3-phosphoglycerate (PGA)}] + H^+$$

The assay can be realized by the measurement of the amount of radioactive PGA formed from the carboxylation of unlabeled RuDP with $HC^{14}O_3^-$. Variations in the determination of the radioactivity should thus be considered.

Experiments were undertaken to test for these variations. It was found that for a given reaction mixture (i.e., incubation mixture), the variations in counts per minute were of the order of ±5% or less. However, when the radioactivity is compared from one reaction mixture to another within a given day, the variations may be as large as ±33% even though the conditions of the two experiments appear to be identical. It should be noted that when no metal ion is added to the incubation mixture, the enzyme has a low activity (residual activity), which seems to yield reproducible results to within ±5%.

These experiments indicated that (a) the enzyme is not stable over long periods, and (or) (b) it requires a preincubation period with the co-factor, namely, the metal ion.

Tests were conducted along this line to clarify the situation. Carboxydismutase was derived from two sources:

(a) Tetragonia expansa; the 35% to 39% saturated ammonium sulfate precipitate was used for studies. The preparation of this fraction, designated as the "purified" fraction, is described elsewhere.[1] Only dialyzed fractions were used.

(b) Chlorella pyrenoidosa; the complete extract, designated as a "crude" extract, was used. It was prepared by sonication of the Chlorella in pH 6.8 phosphate buffer, followed by centrifugation of the cell debris, etc. and

[1] Ning G. Pon, in Chemistry Division Quarterly Report, UCRL-3157, Sept. 1955, p. 18.

dialysis of the supernatant solution. Both the crude Chlorella extract and the purified Tetragonia fraction were subjected to stability tests. The results are summarized in Table XIII. It can be seen that the variations observed during the assays cannot be primarily explained on stability grounds.

Some side experiments were done on the stability of the purified Tetragonia fraction towards heat, ethyl alcohol, and basic lead acetate, which is a precipitating agent. In the first case, the enzyme solution was heated for 4 min at 56° to 58°C with constant stirring. The precipitate was spun down and the supernatant was tested for activity. In the ethyl alcohol treatment, the operation was carried out at 0°C or lower, and the precipitate obtained between 20 and 30% ethanol was retained for activity studies. In both the heat and ethyl-alcohol-treated enzymes, the activity was nearly completely recovered. Addition of cysteine (final concentration 5×10^{-3} M) to the incubation mixture in both cases resulted in a loss of >80% of the original activity.

The treatment of the enzyme with basic lead acetate was as follows: The enzyme was precipitated by a lead acetate solution, pH 7. The resultant precipitate was collected by centrifugation and resuspended in $NaHCO_3$ solution. The excess Pb^{++} was removed by bubbling the suspension with CO_2. The supernatant was tested for activity and was found to have only 15% of the original activity. Possibly not all of the Pb^{++} had been removed, or the bubbling of CO_2 might have denatured the protein by foaming.

In an effort to establish reproducible results in the assay technique, it was found that the order of addition of the enzyme markedly affected biological activity of the enzyme. This observation led to the conclusion that the enzyme required preincubation with the metal ion in order to attain maximum activity.

Assays of both the crude Chlorella extract and the purified Tetragonia fraction were done in the following manner: H^+, Ni^{++}, $HC^{14}O_3^-$, enzyme, and H_2O were mixed to make up a desired volume, with the bicarbonate utilized both as substrate and as buffer set to pH 7.0. The mixture was kept at 0°C for a predetermined period. At the end of this period, RuDP was added and the complete system was incubated for 10 min at 25°C. The reaction was stopped with either steam heat or with acid, or both.

The results are:

(a) in all cases the activity of the enzyme rises sharply to a maximum and gradually falls off as preincubation time is increased;

(b) the activity maximum varies as a function of the temperature of the preincubation mixture, the metal ion concentration, and the total incubation volume.

Table XIII

Stability of carboxydismutase

Source of enzyme	Chlorella	Tetragonia	Tetragonia	Chlorella	Chlorella	Tetragonia	Chlorella	Tetragonia	Chlorella semi-purified*
State of enzyme	Solution	Solution	Solution	Frozen solution thawed 7 times	Frozen solution thawed once	Frozen solution thawed once	Lyophilized powder	Lyophilized powder	Lyophilized powder
Storage temperature (°C)	0	0	8	-24	-24	-24	-24	-24	Room temperature
Period of storage of enzyme (days)	12	8	>90	>90	>90	~38	2 to 3	~38	~730
% of original activity at end of storage period	50-	40	4	60	100	40	100	20	active

* The 30% to 40% saturated ammonium sulfate precipitate

Thus, as the temperature of preincubation is raised from 0° to 25°C the activity peak shifts from a 10-min preincubation period to a 2-min preincubation period. However, the maximum activity attained at 0°-preincubation was ~2500 cpm as compared with only 750 cpm under equivalent conditions at 25°C preincubation. This indicates that the rate of inactivation of the enzyme also varies with temperature.

When the metal ion concentration is increased, two main effects are observed, namely, the increasing activity of the enzyme and the shifting of the activity maximum towards shorter preincubation times. Variation of the Ni^{++} concentration was carried out over a range of 10^{-5} M to 10^{-2} M.

When the total incubation volume is decreased from 200 µl to 40 µl while all the components (i.e. RuDP, $HC^{14}O_3^-$, enzyme, Ni^{++}, H_2O) are kept in the same proportions, the maximum activity changes from 5000 cpm to 1500 cpm, indicating surface effects on the enzymatic activity. Perhaps these occur by means of competitive adsorption of the metal ion or as a result of the change in pH of the incubation mixture. Experiments are now in progress to test the preincubation effects with other metal ions.

CURRENT METHODS OF ALGAL CULTURE

Osmund Holm-Hansen, Paul Hayes, and Patricia Smith

At present there are two different methods in use for the cultivation of algae: a shaker apparatus that represents a batch-style method of growth, and a newly developed continuous-culture column apparatus that maintains a constant algal density. During the past few years several references have been made to these methods.[1-5] Because many modifications have been made in the design of the apparatus, the methods currently in use are briefly described below.

Figure 11 shows the essential details of the shaker-flask apparatus; a picture of the shaker trough may be found in Reference 1. The flasks, which have a capacity of 2500 ml, contain 1100 ml of an algal suspension and are mechanically shaken in a water-cooled bath which is thermostatically held at a temperature of 24 to 25°C. A stream of 4% CO_2 in air is passed through the flasks at approximately 50 to 100 ml per min. Illumination is provided from below by six 100-watt fluorescent lights (daylight type) at an intensity of about 2000 foot-candles. The algae are harvested daily by withdrawal of 900 ml of culture into the sterile graduate (I in Fig. 1), leaving 200 ml in the flask as an inoculum. A volume of sterile nutrient solution equivalent to the withdrawal aliquot is then siphoned from the 16-l carboy into the shaker flask.

The drawbacks of this batch-style method are chiefly in the impossibility of maintaining a truly constant metabolic state in the algae and the large time expenditure required for daily maintenance. These difficulties are largely overcome by the continuous-culture apparatus sketched in Fig. 12. This consists of a temperature-controlled column containing about 900 ml of algal suspension that is aerated with 4% CO_2 in air, a bank of eight 20-watt fluorescent lights, an overflow receptacle to permit the maintenance of a constant volume in the column, and an optical-density measuring device that governs the automatic dilution of the culture with fresh nutrient solution to maintain a constant density of algae.

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1 Benson, Calvin, Haas, Aronoff, Hall, Bassham, and Weigl, "C^{14} in Photosynthesis," in Photosynthesis in Plants, J. Franck and W. Loomis, eds (Iowa State College Press, Ames, Iowa, 1949), pp. 382-384.

2 M. Calvin, "The Path of Carbon in Photosynthesis," in The Harvey Lecture Series, (Chas. C. Thomas, Springfield, Illinois, 1950-51) Vol. XLVI, p. 226

3 Calvin, Bassham, Benson, Kawaguchi, Lynch, Stepka, and Tolbert, The Path of Carbon in Photosynthesis; XIV, UCRL-1386, June 1951, p. 9.

4 R. Norris and P. Hayes, in Chemistry Division Quarterly Report, UCRL-2841, Jan. 1955, p. 5.

5 O. Holm-Hansen, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 36.

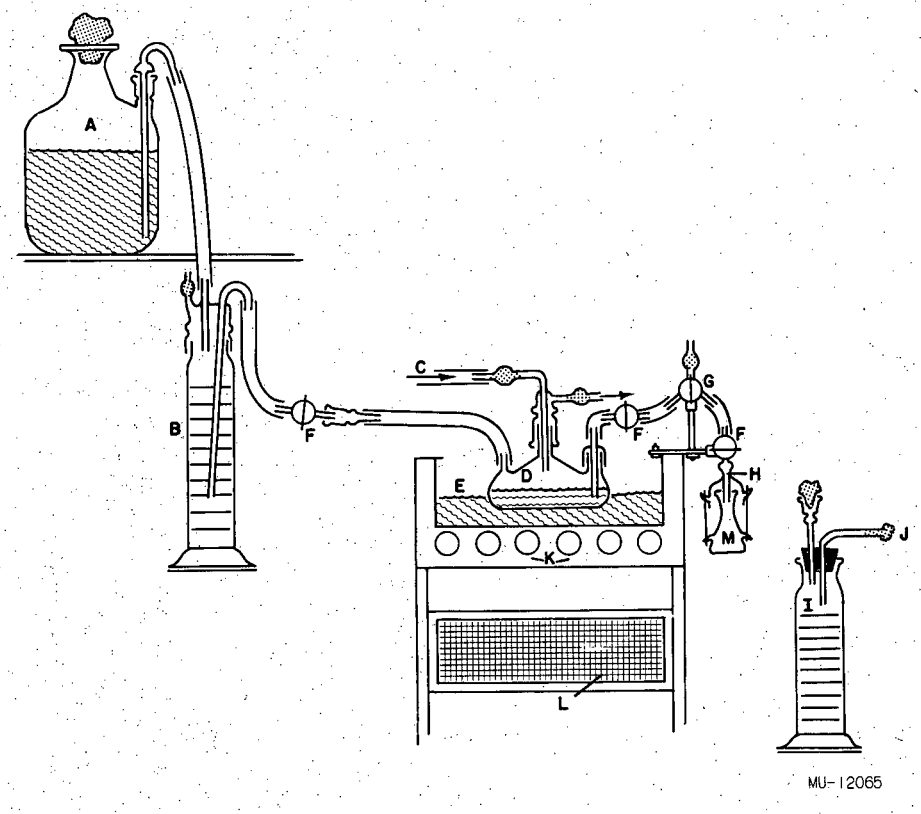

Fig. 11. Shaker flask apparatus. A, 16-liter carboy of medium; B, graduate; C, air inlet; D, algae flask; E, cooling water; F, 2-way stopcocks; G, 3-way stopcock; H, ground-glass joint; I, graduate; J, suction tube; K, fluorescent lights; L, refrigerating unit; M, flask with ground-glass joint.

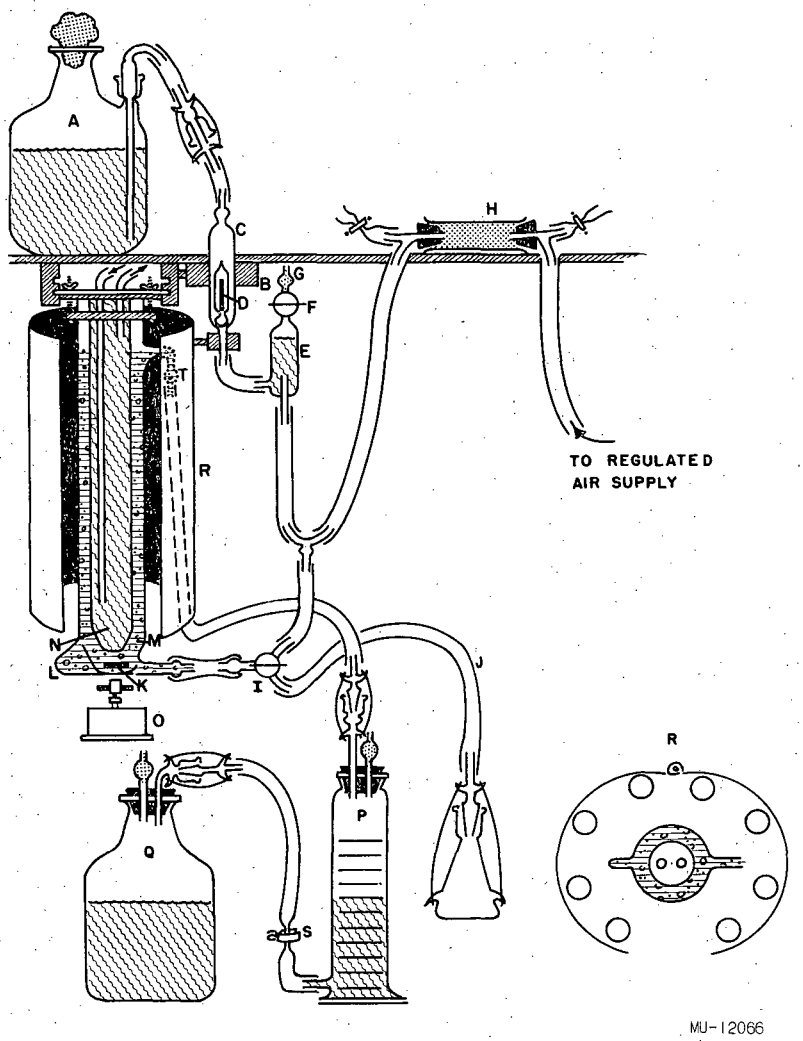

Fig. 12. Continuous-culture column apparatus. A, 16-liter carboy of medium; B, solenoid; C, glass tube with D, magnet; E, bubble trap; F, stopcock; G, cotton-packed air outlet; H, cotton-packed air filter; I, 3-way stopcock; J, draining and inoculating tube; K, magnet; L, fin; M, encased algae culture; N, water bath; O, magnetic stirrer; P, collecting graduate; Q, reservoir; R, jacket with 8 fluorescent lights; S, pinch clamp; T, overflow outlet.

The column itself is a double-walled tube with the algae in the outer layer, which has a nominal thickness of about 0.75 cm, while the inner tube contains circulating water to maintain any desired temperature. The temperature regulation of the circulating water is accomplished in a separate water-bath unit that is equipped with its own pump, heater, refrigeration coil, and thermostat. The optical system regulating the algal density (Fig. 13) consists of a light source, a set of lenses, mirrors, a light chopper, a sliding reference grating, and phototubes, together with all necessary switches, relays, and allied equipment. The light beam that is transmitted through the algal suspension passes through an infrared filter, a color filter (to transmit red light only), and an opal glass before being received by the photocell.

Samples from the culture are withdrawn (via tube J, Fig. 12) at regular intervals and used to determine the centrifuged cell pack, dry weight, cell number, and absorption spectrum for chlorophyll. Results so far have been promising in that cultures have been maintained up to 8 wk with a fairly constant amount of growth per day. When Scenedesmus is grown at a density of about 0.50 cc packed cells per 100 ml culture solution, the overflow measures approximately 1200 ml per day, which represents a generation time of about 17 to 18 hr. This generation time is considerably longer than that obtained in the shaker flasks (about 10 hr), and indicates that the light and CO_2 relationships should be examined for the culture column.

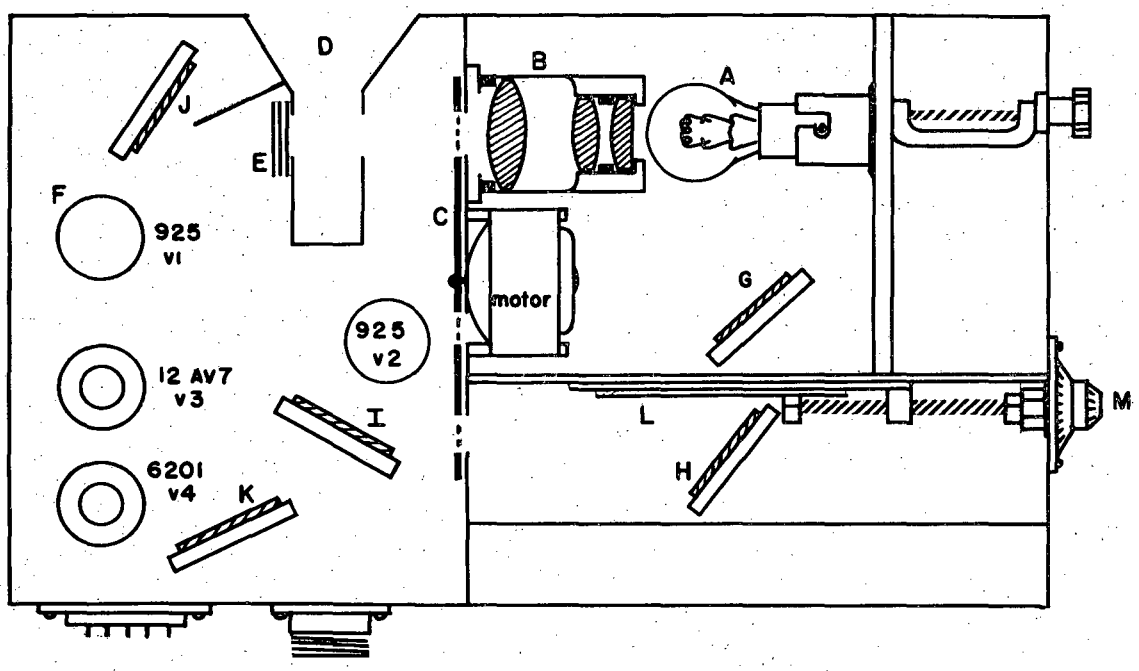

Fig. 13. Optical-density regulating mechanism. A, light source; B, lens system; C, light chopper; D, fin-insertion area; E, filters, F, photocell; G to K, mirrors; L, sliding grating; M, control for grating.

THE EFFECT OF AZASERINE ON THE RESPIRATORY METABOLISM OF ALANINE

Ann M. Hughes and Bert M. Tolbert

Azaserine (O-diazoacetyl-L-serine) has recently been studied rather extensively as an inhibitor of tumor growth. First obtained as a crude extract from the culture broth of a Streptomyces, it was later isolated, purified, and finally synthesized by Parke, Davis, and Company. It has been shown to inhibit the growth of sarcoma-180 in mice[1] and to inhibit the incorporation of formate into the nucleic acids of tumor cells.[2] Clinical trials in patients with various neoplastic diseases showed some reduction of leukocyte count in leukemic patients but no therapeutic effects in other types of cancer. Adverse reactions included digestive disturbances, decreased platelet counts, systemic intoxication, and liver damage.[3]

It is believed that one of the modes of action of azaserine is by transamination reactions. We therefore hoped to add some information concerning its mode of action by studying its effect on the respiratory metabolism of alanine-2-C^{14}.

Long-Evans rats, 50 to 75 g, were injected with either 8 mg or 12 mg of azaserine 30 min before the metabolic experiments. The respiratory metabolism of alanine-2-C^{14} was then determined, by use of the breath-line apparatus currently in use in this laboratory for determining $C^{14}O_2$. Results are shown in Figs. 14 and 15.

There is a slight--probably not significant--depression in the total $C^{14}O_2$ excretion in the 8-mg-dose group and a definite depression in the animals receiving 12 mg of azaserine (Fig. 13). The alteration in the metabolic rate pattern is evident in both groups (Fig. 14). At the time of the azaserine injection and throughout the metabolic experiment, the animals in both groups appeared to be perfectly normal and healthy. However, 3 to 4 days after the azaserine injections, animals of the 8-mg-dose group died. Autopsy showed evidence of extreme anemia, the livers appearing as pallid as that of a well-perfused animal. Animals in the 12-mg-dose group all died within 24 hours of the azaserine injections. Although there is no report of erythrocyte counts in the clinical trials of azaserine, it appears that this compound is a very effective inhibitor of red-cell formation.

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1 Stock, Reilly, Buckley, Clarke, and Rhoads, Nature 173, 71 (1954).

2 Skipper, Bennett, and Schabel, Fed. Proc. 13, 298 (1954).

3 Ellison, Karnofsky, Sternberg, Murphy, and Burchenal, Cancer 7, 801 (1954).

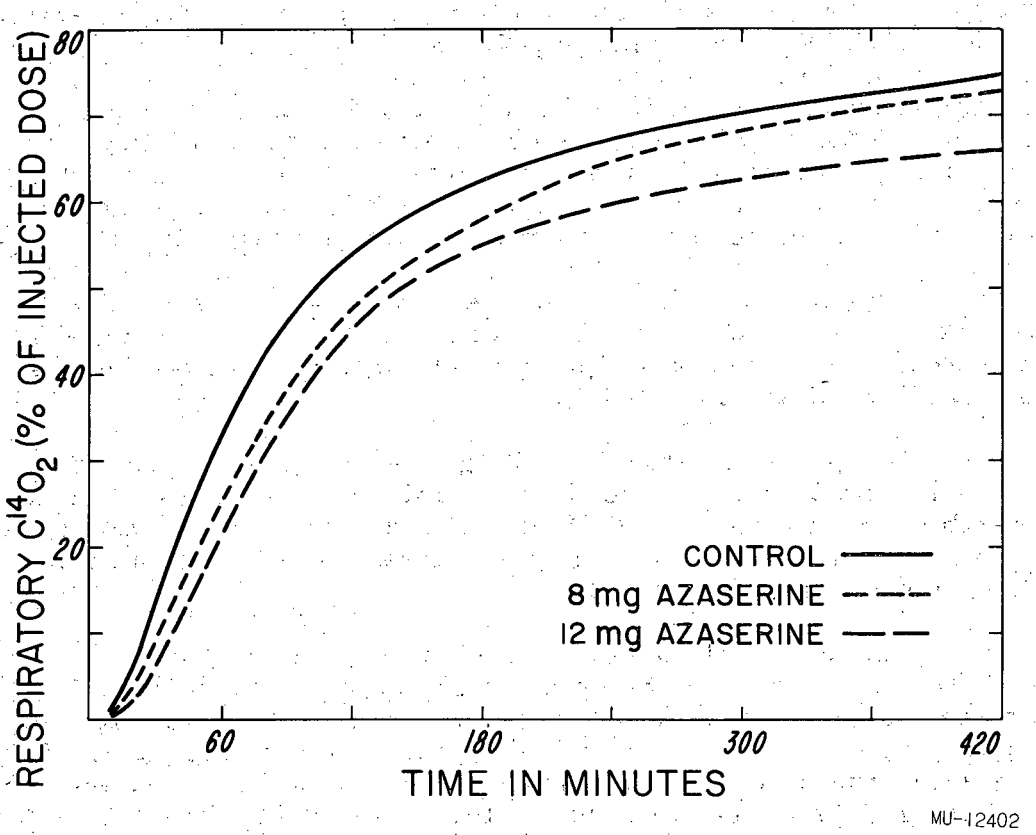

Fig. 14. The effect of azaserine on the cumulative excretion of $C^{14}O_2$ in rats following the administration of alanine-2-C^{14}.

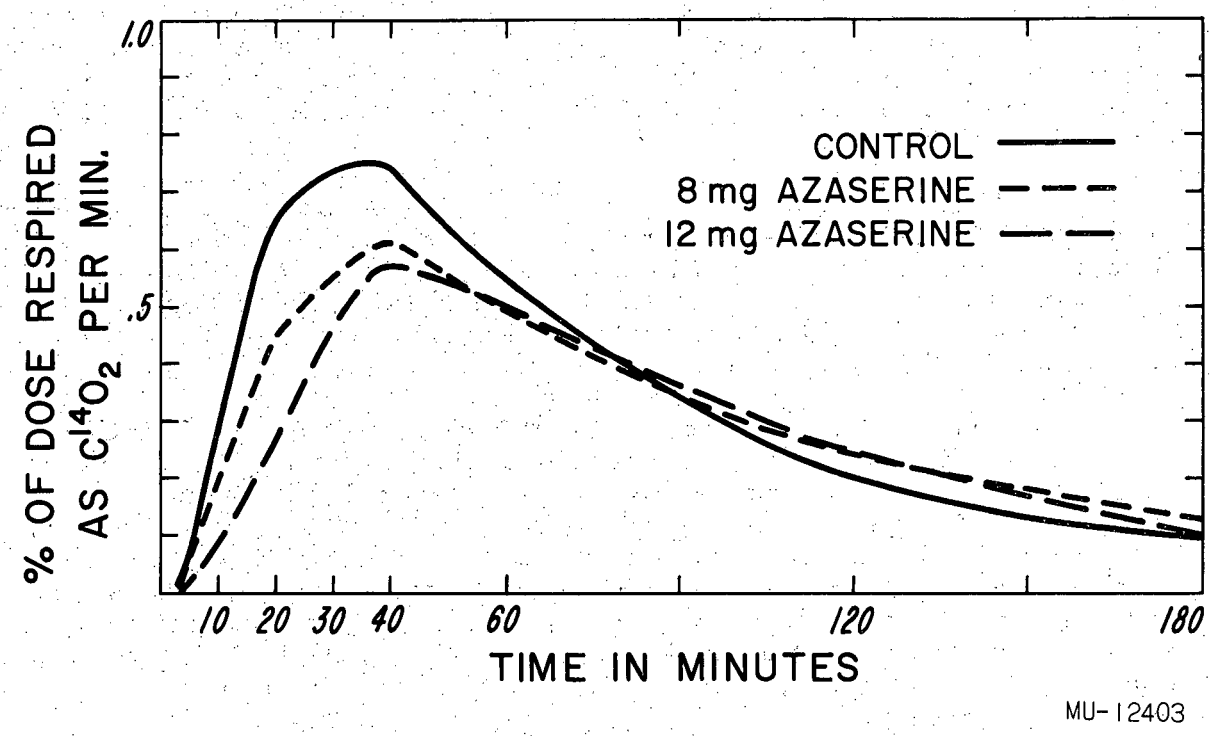

Fig. 15. The effect of azaserine on the rate of excretion of $C^{14}O_2$ in rats following the administration of alanine-2-C^{14}.

THE EFFECT OF 6-ETHYL-8-THIOLOCTANOIC ACID ON THE RESPIRATORY METABOLISM OF LACTIC ACID

Ann M. Hughes and Bert M. Tolbert

In a previous quarterly report,[1] one of us reported the results of studies on the metabolic effects of 8-methyl thioctic acid, a compound which is supposed to be a thioctic acid antagonist. It was thought that evidence of its anti-thioctic-acid activity could be demonstrated by showing an inhibition of lactic acid decarboxylation, a reaction believed to be catalyzed by thioctic acid. However, the compound proved to be so toxic that no conclusions could be reached concerning its antithioctic activity. Recently another thioctic acid analog, 6-ethyl-8-thioloctanoic acid (ETO), was received by this laboratory. It was hoped that this would be less toxic than the methyl thioctic acid. Accordingly, the experiments on the in vivo decarboxylation of lactic acid have been repeated, with the new compound administered as a possible inhibitor.

"A" strain mice, each weighing about 20 g, were given a total of 15 mg ETO in divided doses over a period of either 16 hr (Group I) or 30 hr (Group II) prior to the metabolic experiments. The respiratory metabolism of lactic acid 1-C^{14} was then studied, using the breath-line apparatus currently in use in this laboratory for the measurement of $C^{14}O_2$. Results are shown in Figs. 16 and 17.

Animals in Group I appeared sick before the series of ETO injections was completed, and were almost moribund during the metabolic experiments. All were dead within 48 hours. Animals in Group II looked normal throughout the injection period and the metabolic experiments. There were no deaths in this group. Examination of Figs. 16 and 17 shows that there was no significant metabolic effect of ETO in a concentration which was not toxic to the animal (Group II). The depression in Group I of both the total $C^{14}O_2$ excreted and the rate of excretion can probably be attributed to the moribund condition of the animals, rather than to any direct effect of ETO on lactic acid metabolism.

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[1] A. M. Hughes, in Chemistry Division Quarterly Report, UCRL-3068, July 1955, p. 16.

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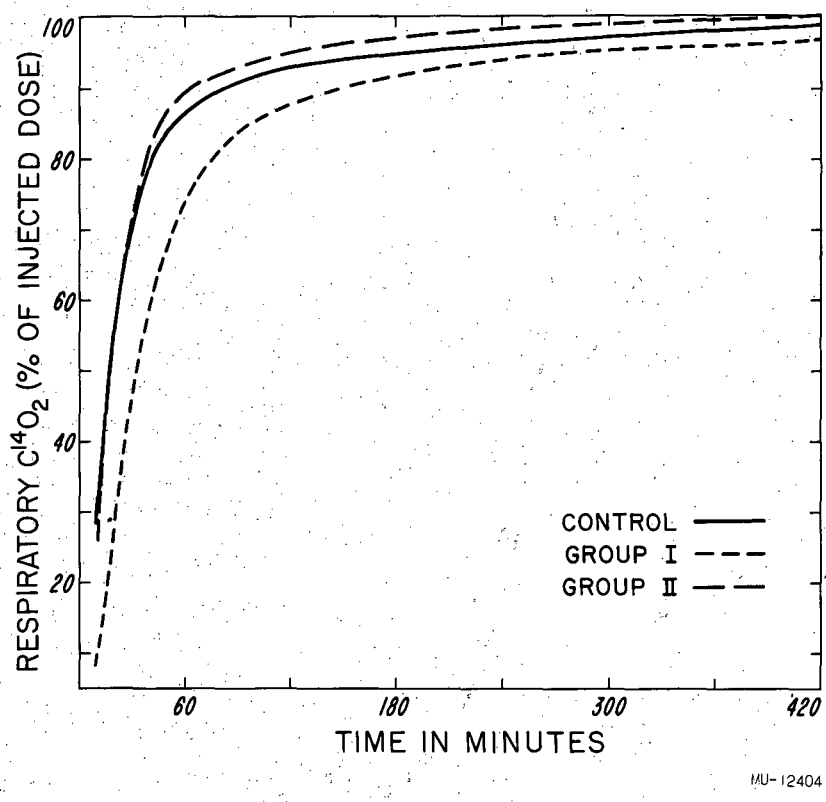

Fig. 16. The effect of 6-ethyl-8-thioloctanoic acid on the cumulative excretion of $C^{14}O_2$ in mice following the administration of lactic acid-1-C^{14}.

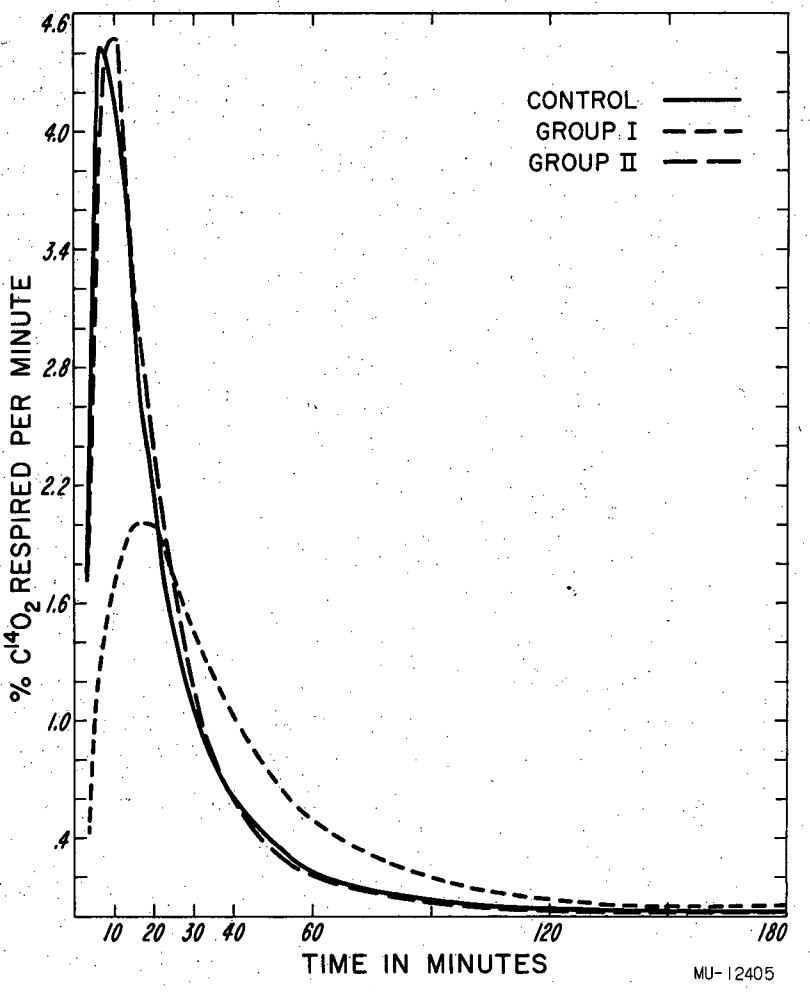

Fig. 17. The effect of 6-ethyl-8-thioloctanoic acid on the rate of excretion of $C^{14}O_2$ in mice following the administration of lactic acid-1-C^{14}.

THE EFFECT OF TOTAL-BODY IRRADIATION ON THE METABOLISM OF SOME ORGANIC COMPOUNDS IN MICE

Ann M. Hughes and Bert M. Tolbert

The metabolism of C^{14}-labeled sodium acetate, glucose, glycine, and leucine by mice following total body irradiation has been previously reported.[1,2] The study has been expanded to include the metabolism of methionine and sodium formate, two compounds closely associated with nucleic acid metabolism. The technique for irradiating the mice was the same as that previously reported. A total-body irradiation of 1000 r was obtained in 75 min. The apparatus for measuring respiratory metabolism of carbon-14-labeled compounds was used for the measurement of the metabolism of L-methionine-methyl-C^{14} and sodium formate-C^{14}. Swiss mice weighing 20 to 25 g were used. Metabolic experiments were conducted immediately after irradiation and 24 hr after irradiation, as it has been reported that the interference with formate incorporation into nucleic acids occurs about 24 hr after irradiation. The results are shown in Figs. 18 to 21.

With methionine (Figs. 18 and 19) the changes in metabolism immediately after irradiation are possibly significant: the total excretion of $C^{14}O_2$ is increased by about 10% to 12% after irradiation, and the specific activity of the $C^{14}O_2$ is depressed about 25%. These differences are not apparent 24 hr after irradiation.

With formate (Figs. 20 and 21) there is no significant change in the total excretion of $C^{14}O_2$, although the peak specific activity of the CO_2 from the formate 24 hr after irradiation is down about 15%.

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1 M. Kirk and B. Tolbert, in Chemistry Division Quarterly Report, UCRL-2647, July 1954, p. 38.

2 M. Kirk and B. Tolbert, in Chemistry Division Quarterly Report, UCRL-2709, Sept. 1954, p. 19.

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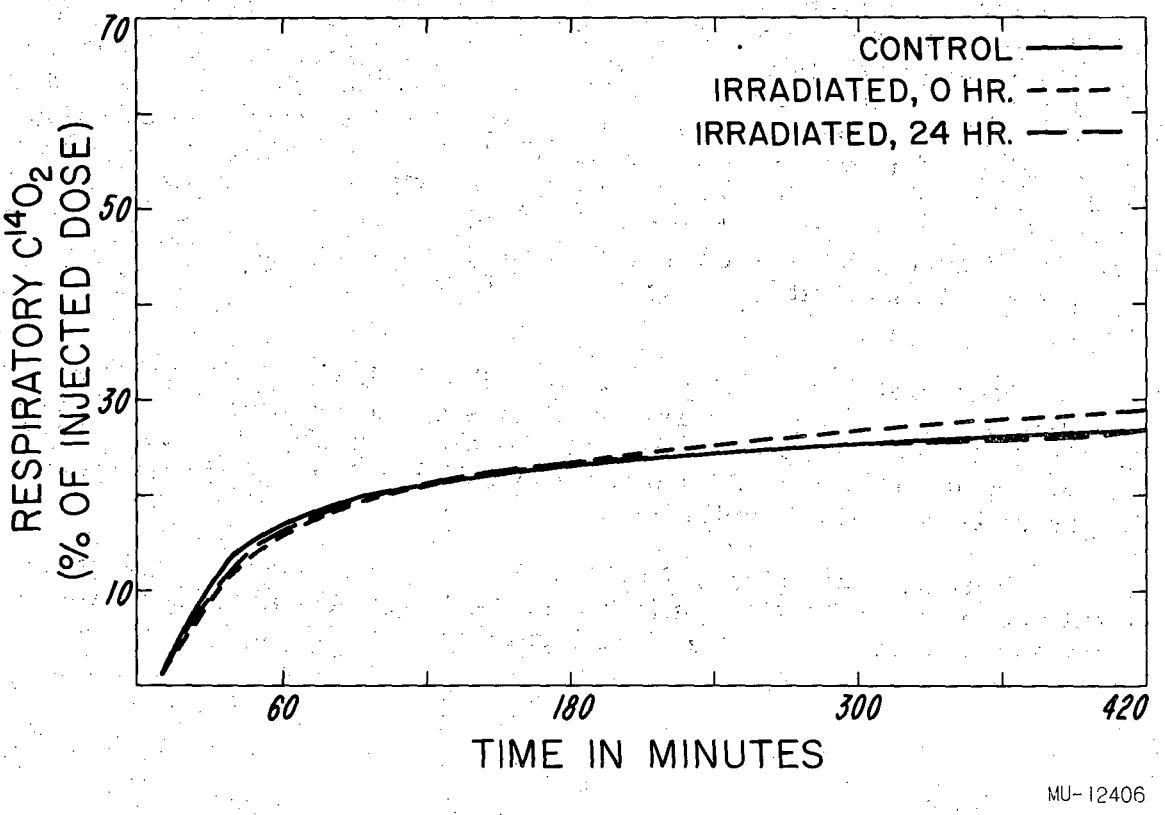

Fig. 18. Cumulative excretion of $C^{14}O_2$ from irradiated mice following the injection of L-methionine-methyl-C^{14}.

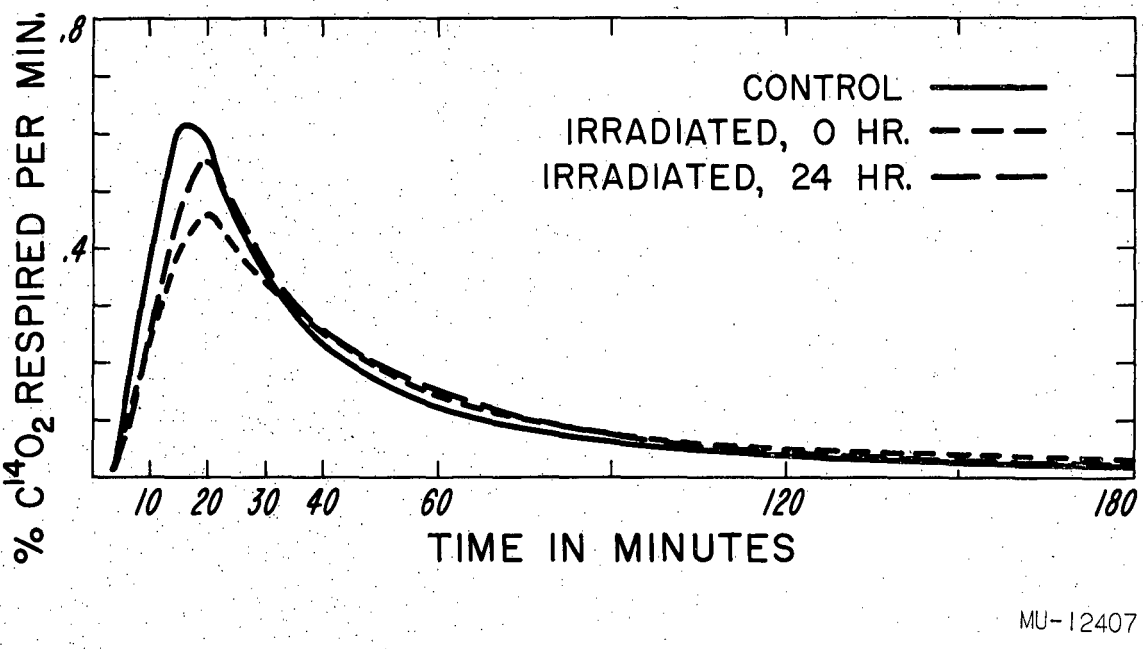

Fig. 19. Rate of excretion of $C^{14}O_2$ from irradiated mice following the injection of L-methionine-methyl-C^{14}.

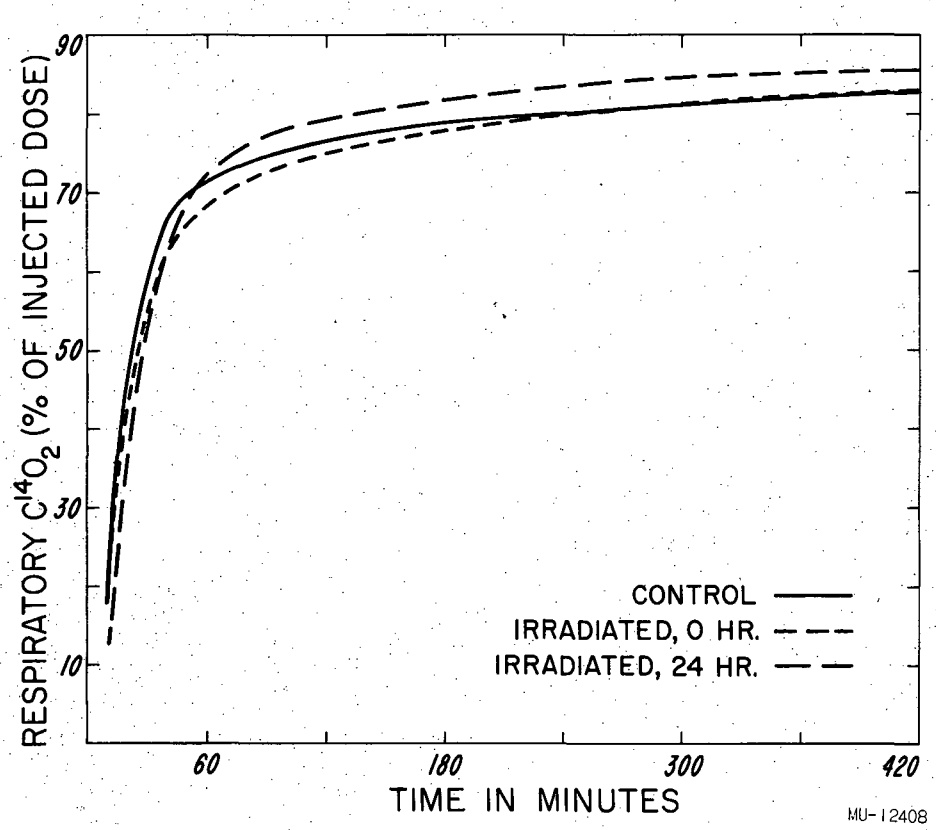

Fig. 20. Cumulative excretion of $C^{14}O_2$ from irradiated mice following the injection of sodium formate-C^{14}.

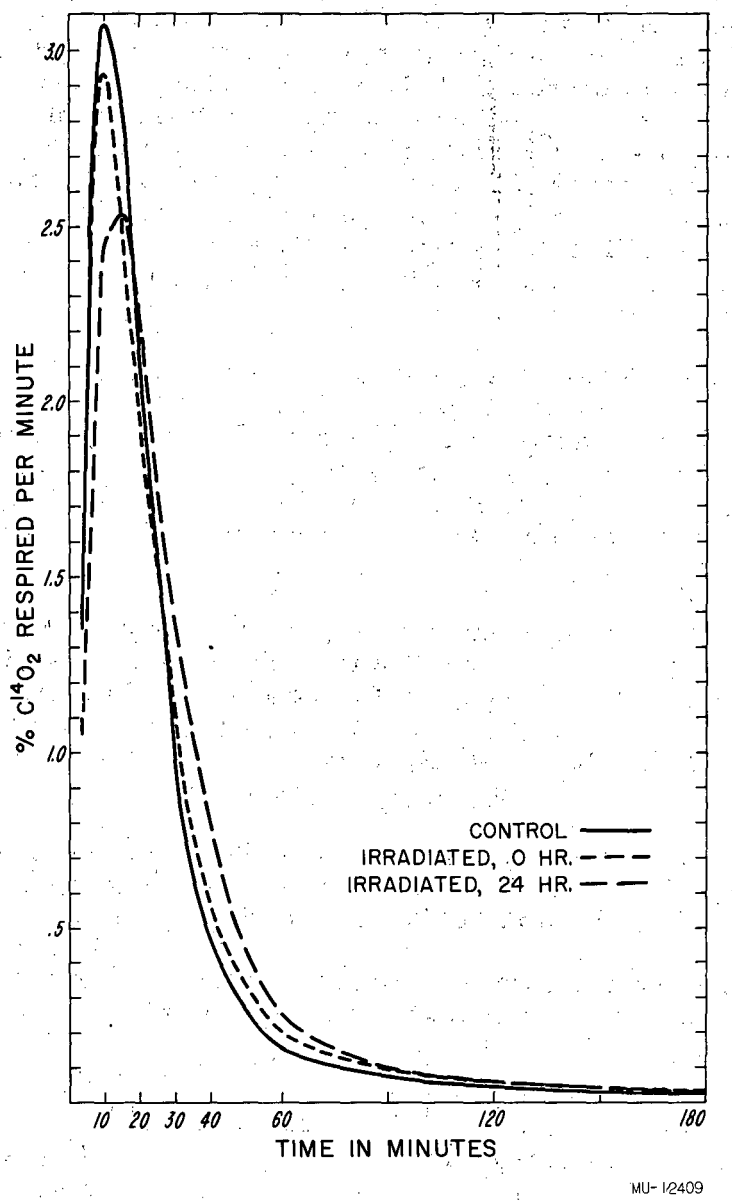

Fig. 21. Rate of excretion of $C^{14}O_2$ from irradiated mice following the injection of sodium formate-C^{14}.

PHOSPHORYLATED INTERMEDIATES IN FAT METABOLISM

Rosemarie Ostwald, Richard M. Lemmon, and Samuel Lepkovsky

We have previously reported the results of experiments to determine certain aspects of fat metabolism.[1] The work has since progressed in two main directions. One is the identification of compounds found by paper chromatography in extracts of liver and adipose tissues after injection of the animal with P^{32}. The other is the investigation of differences in the kinds and amounts of these compounds found in rats in various nutritional states. The two states chosen were in animals fasted for 24 hr and in animals fed immediately before the injection with sodium phosphate-P^{32}. Both groups were sacrificed 2 hr after the injection. The procedures for the extraction of the tissues, separation of compounds, and chromatography were described earlier.[1]

The data show (a) a greater incorporation of radioactivity into tissues from fed animals than into tissues from fasted animals, (b) a higher ratio of the specific activity of inorganic P to the specific activity of organic P in tissues from fasted animals than in tissues from fed animals, and (c) a higher ratio of the activity of the nonnucleotide fraction to the activity of the nucleotide fraction in tissues from fed animals than in tissues from fasted animals.

As was previously described, paper chromatograms of the liver and fat extracts and their nucleotide and nonnucleotide fractions showed a number of radioactive spots. In addition to the previously reported inorganic P, AMP, ADP, IMP, and TPN,* the following compounds have been identified: ATP, GMP, ITP, glucose-6-P, DPN, and glyceryl-P. There remain two major and four to six minor spots unidentified.

In general the patterns of compounds from both the brown and white fats and from the livers look similar, though they are not identical. Particularly the nonnucleotide fractions of the fats and of the livers show differences.

There are quantitative differences in the amounts of certain compounds in extracts from tissues of fasted and of fed animals. The following differences have been noted:

Tissue	Compound	Group having greater amount of compound
Liver	Inorganic P	Fasted
	ADP	Fasted
	Glyceryl-P	Fed
	Sugar phosphates	Fed
	IMP	Fed
Fat	ADP	Fasted

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

* The abbreviations used are: AMP, ADP, ATP, for adenosinemono-, di-, and triphosphate; GMP, for guanosinemonophosphate; DPN and TPN, for di- and triphosphopyridine nucleotide; IMP, IDP, for inosinemono- and diphosphate.

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

Melvin Look and Henry Rapoport

INTRODUCTION

Studies of morphine metabolism in humans are desirable using carbon-14-labeled morphine with a nonlabeled N-methyl group. Previous work[1] has shown that part of the methyl group of morphine is eliminated as carbon dioxide in the breath. In a continuation of our work of synthesizing morphine from normorphine using unlabeled morphine derivatives,[2] the compound formed from the reaction of normorphine with ethyl chloroformate was positively identified as O^3, N-dicarbethoxynormorphine. Reduction of the latter with lithium aluminum hydride in tetrahydrofuran gave morphine in 76% or better yield. Prior to the successful reduction, O^6-acetyl-O^3, N-dicarbethoxynormorphine was also synthesized because the complexing of O^3, N-dicarbethoxynormorphine by lithium aluminum hydride was anticipated.

EXPERIMENTAL AND RESULTS

Proof of the Structure of O^3, N-dicarbethoxynormorphine

The results of quantitative analysis of the compound formed by the reaction of normorphine with ethyl chloroformate were compatible with that of O^3, N-dicarbethoxynormorphine: Calculated for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.64; H, 5.96; N, 3.49. Further proof that the carbethoxy group was on the O^3 oxygen and not on the O^6 oxygen was derived from the ultraviolet date of Table XIV.

From the spectrum of the solution, it seems that the phenolic group (O^3) of morphine is covered, as the band at 256 mμ attributed to the phenolate ion did not appear instantaneously but followed a saponification period. Acidification produced a compound having a spectrum similar to that of morphine. From the above data and the fact that the compound was nonbasic, it was concluded that the carbethoxy groups were on the O^3-oxygen and the nitrogen of normorphine.

Attempted Reduction of O^3, N-dicarbethoxynormorphine with Lithium Aluminum Hydride in Ether

A solution of lithium aluminum hydride in ether added to an ether solution of O^3, N-dicarbethoxynormorphine formed an immediate precipitate. Subsequent workup and isolation showed that the major portion of the starting material had not reacted after being removed from solution.

Synthesis of O^6-acetyl-O^3, N-dicarbethoxynormorphine

A mixture of 0.522 g of O^3, N-dicarbethoxynormorphine, 0.5 g fused sodium acetate, and 10 ml of acetic anhydride was heated under reflux for 1 hr.

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1 Elliott, Tolbert, Adler, and Anderson, Proc. Soc. Exp. Biol. Med. 85, 77 (1954).

2 M. Look and H. Rapoport, in the Chemistry Division Quarterly Report, UCRL-3415, June 1956, p. 4.

Table XIV

Ultraviolet Data on O^3, N-dicarbethoxynormorphine

Solution medium	ϵ_{max}(mμ)	λ_{max}(A)
(1) 95% ethanol	281	2200
	278 (shoulder)	2050
	221	6760
(2) 95% ethanol with excess NaOH	298	3240
	256[a]	6400
	235	8240
(3) Solution No. (2) above acidified with HCl	286	1870
	225	7300

[a] Formation of band was gradual after addition of base. Formation was essentially completed 20 min after the addition.

The reaction product was chromatographed rapidly through an alumina column with 2:1 petroleum ether (30° to 60°) and benzene, yielding 218 mg of a colorless oil which sublimed at 15 to 20 microns at 130° to 140° to give a solid. Recrystallization from petroleum ether (65° to 110°) and benzene gave a solid melting at 110.5° to 111.5°: Analysis: Calculated for $C_{24}H_{27}O_8N$: C, 63.01; H, 5.95. Found: C, 63.01; H, 5.95. Ultraviolet and infrared spectra were also compatible with the compound. The latter showed three carbonyl bands and no hydroxy or nitrogen-hydrogen bands, whereas O^3, N-dicarbethoxynormorphine possessed two carbonyl bands and a hydroxy band.

Reduction of O^3, N-dicarbethoxynormorphine with Lithium Aluminum Hydride in Tetrahydrofuran

No precipitation was encountered in a reduction of 250 mg of O^3, N-dicarbethoxynormorphine with lithium aluminum hydride in tetrahydrofuran. Hydrolysis of the reaction mixture with hydrochloric acid and Rochelle salt solution, basification of the solution with ammonium hydroxide, continuous extraction with methylene chloride, and recrystallization of the residue from water and ethanol gave 138 mg (76%) of morphine hydrate. The product was compatible with the melting point, qualitative tests, and ultraviolet spectra (in neutral, basic, and acidic solutions) of morphine hydrate. Further extraction and examination of the extract with ultraviolet spectroscopy indicated a yield of morphine amounting to as much as 162 mg (90%).

Reduction of O^6-acetyl-O^3, N-dicarbethoxynormorphine with Lithium Aluminum Hydride

In a reaction similar to that described above, 240 mg of acetyl-dicarbethoxynormorphine was reduced with lithium aluminum hydride to give 123 mg (78%) of morphine hydrate.

The Conversion of Morphine to Normorphine to Morphine Without Isolation of Intermediates

The degradation of 100.2 mg of morphine to normorphine and reconversion of the latter to morphine without isolation of the intermediates gave 71 mg (71%) of morphine.

STUDIES IN THE BIOSYNTHESIS OF MORPHINE

Several growth chambers are being designed for growing Papaver Somniferum (opium poppy) under radioactive carbon dioxide and for subsequent work related to photosynthesis. Previous reports of

growth chambers[3,4,5] described assemblies too elaborate or simplified for our studies. Papaver somniferum plants subjected to a standard 5-min photosynthesis experiment[6] with radioactive carbon dioxide yielded radioautograms similar to those from other green plants. Paper-chromatographic mapping of the many opium alkaloids and water-soluble components[7] of the poppy is being continued. To date, standard solvents of phenol-water and n-butanol–propionic acid–water are being used. Because most of these components have R_F's in the region of 0.7 to 10 with these solvents, the solvents are somewhat unsatisfactory. Accordingly, other solvent systems will be tried.

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3 Geiling, McIntosh, and Kelsey, J. Am. Pharm. Assoc. 39, 512 (1950).

4 A. M. Kuzin and V. I. Merenova, Biokhimiya 19, 616 (1954).

5 Scully, Chorney, Kostal, Watanabe, Skok, and Glattfeld, International Conference on the Peaceful Uses of Atomic Energy, Paper No. 274, Geneva, 1955. Proceedings, United Nations, New York, 1956, 12, 377.

6 A. A. Benson and M. Calvin, Intermediates of Photosynthesis: Isolation and Degradation Methods, UCRL-2682, Aug. 1954, p. 8.

7 H. Schmid and P. Karrer, Helv. Chim. Acta 28, 722 (1945).

STUDIES ON NUCLEIC ACID METABOLSIM. THE INCORPORATION OF ADENINE-2-C^{14} AND ADENINE-4,6-C^{14}

Edward L. Bennett and Hilda Karlsson

The comparative experiment of the metabolism of adenine-2-C^{14} and adenine-4,6-C^{14} which has been discussed and partially reported in previous quarterly reports has been essentially completed.

The aim of this experiment has been to compare the metabolism of adenine-2-C^{14} and adenine-4,6-C^{14} in mice. Our knowledge of the biosynthesis of adenine suggests a possible lability of either the C-2 or C-8 position of the purine ring. A previously reported experiment[1] indicated little or no lability of the C-8 position relative to the C-4,6 positions of adenine, but suggested a possible lability of the C-2 position. Therefore, a more carefully controlled experiment was designed to compare the metabolism of adenine-2-C^{14} with that of adenine-4,6-C^{14}. Six male C_{57} mice were administered 1.1 mg of adenine-2-C^{14} and 6 mice were administered adenine-4,6-C^{14}. Three mice (litter mates) from each group were sacrificed at 24 hr and the remainder were sacrificed at 15 days after injection of the adenine. The soluble nucleotide adenine (5-AMP), RNA-adenine, RNA-guanine, DNA-adenine and DNA-guanine were isolated and the specific activities were determined for several tissues. The results for the 5-AMP analyses were reported in UCRL-3351, [1] and the nucleic acid specific activities are compared in Tables XV and XVI.

No evidence was obtained for lability of the C-2 of the adenine ring as no consistent or large differences were noted in the specific activities of the nucleic-acid adenine or guanine after adenine-2-C^{14} administration as compared with those obtained after adenine-4,6-C^{14} administration.

The average specific-activity value for a given fraction after administration of adenine-2-C^{14} seldom differed by more than 10 to 15% from that obtained after administration of adenine-4,6-C^{14} (with the exception of kidney DNA values where low specific activities make the values less reliable). As has been previously noted, the adenine-to-guanine ratio decreases with time, which may be indicative of a more rapid renewal of adenine than of guanine, or of an interconversion of adenine to guanine either at the nucleic acid level or (more likely) at the nucleotide level.

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1 Kirk, Tolbert, and Bennett, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 28.

Table XV

Specific activity (dis/μg) of RNA-adenine and RNA-guanine of mice injected with adenine-2-C^{14} or adenine-4,6-C^{14} [a]

Time after injection (days)		Tissue: Small intestine					
		Adenine-4,6-C^{14}			Adenine-2-C^{14}		
		Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)	Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)
1		648	104	6.2	660	109	6.1
		661	101	6.5	679	94	7.2
		547	86	6.4	607	87	7.0
	Average	619	97	6.4	649	97	6.7
	Ratios [b]				1.05	1.00	
15		20.5	10.1	2.0	24.0	12.1	2.0
		29.0	14.1	2.1	23.8	12.5	1.9
		27.7	14.8	1.9	23.4	11.6	2.0
	Average	25.7	13.0	2.0	23.7	12.1	2.0
	Ratios				0.92	0.93	
		Carcass					
1		231	38.0	6.1	193	41.0	4.7
		227	47.3	4.8	204	39.2	5.2
		219	42.6	5.2	204	40.0	5.1
	Average	226	42.6	5.3	200	40.1	5.0
	Ratios				0.88	0.94	
15		146	46.0	3.2	160	58.6	2.7
		142	52.5	2.7	124	45.4	2.7
		106	43.8	2.4	109	46.7	2.3
	Average	131	47.4	2.8	131	50.2	2.6
	Ratios				1.00	1.06	

Table XV continued

Specific activity (dis/μg) of RNA-adenine and RNA-guanine of mice injected with adenine-2-C^{14} or adenine-4,6-C^{14} [a]

Time after injection (days)		Tissue					
		Liver					
		Adenine-4,6-C^{14}			Adenine-2-C^{14}		
		Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)	Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)
1		256	38.6	6.6	312	39.2	8.0
		351	61.2	5.7	417	65.5	6.4
		377	58.8	6.4	370	41.6	8.9
	Average	328	52.9	6.2	366	48.8	7.5
	Ratios				1.11	0.92	
15		154	93.7	1.6	140	83.5	1.7
		154	88.4	1.7	182	90.6	2.0
		170	75.5	2.2	191	95.7	2.0
	Average	159	85.9	1.9	171	89.9	1.9
	Ratios				1.08	1.05	
		Large intestine					
1		830	157	5.3	880	189	4.7
		975	205	4.8	900	166	5.4
		626	123	5.0	830	141	5.9
	Average	810	162	5.0	870	165	5.3
	Ratios [b]				1.07	1.02	
15		29.8	21.8	1.4	32.4	22.4	1.4
		39.4	29.2	1.3	27.4	18.2	1.5
		23.0	17.7	1.3	31.0	19.9	1.6
	Average	30.7	22.9	1.3	30.3	20.2	1.5
	Ratios				0.99	0.88	

[a] Male C_{57} mice, age 3 mo, weight about 25 g, were administered 1.1 mg of adenine-2-C^{14} with a specific activity of 2.0×10^4 dis/μg. The specific activities of the guanine have been converted to the gamma equivalent of adenine. They were sacrificed at 1 or 15 days after administration of the adenine.

[b] Ratios of the average specific activities of adenine and guanine in adenine-2-C^{14} to those in adenine-4,6-C^{14}.

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Table XVI

Specific activity (dis/μg) of DNA-adenine and DNA-guanine of mice injected with adenine-2-C^{14} or adenine-4,6-C^{14} [a]

Time after injection (days)		Tissue					
		Small intestine					
		Adenine-4,6-C^{14}			Adenine-2-C^{14}		
		Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)	Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)
1		362	62.0	5.8	282	48.0	5.9
		306	58.1	5.3	352	50.5	7.0
		255	40.8	6.2	244	44.5	5.5
	Average	308	53.6	5.7	293	47.7	6.1
	Ratios[b]				0.95	0.89	
15		12.6	4.2	3.0	11.4	4.1	2.7
		10.7	4.0	2.7	10.8	3.7	2.9
		15.3	5.4	2.9	15.0	5.2	2.9
	Average	12.9	4.5	2.8	12.4	4.3	2.8
	Ratios				0.96	0.95	
		Carcass					
1		138	28.1	4.9	138	22.8	6.1
		154	32.8	4.7	148	24.6	6.0
		142	28.5	5.0	122	24.6	5.0
	Average	145	29.8	4.9	136	24.1	5.6
	Ratios				0.94	0.81	
15		12.7	8.2	1.5	13.3	4.8	2.8
		10.3	5.5	1.9	11.7	4.6	2.6
		12.6	4.5	2.8	13.1	6.0	2.2
	Average	11.9	6.4	1.9	12.7	5.1	2.5
	Ratios				1.06	0.80	
		Kidney					
1		11.3	4.2	2.7	8.2	3.0	2.7
		13.1	3.6	3.7	10.9	3.0	3.6
		8.3	4.1	2.0	8.4	3.4	2.4
	Average	10.9	4.0	2.7	9.2	3.1	2.9
	Ratios[b]				0.84	0.78	
15		26.2	15.0	1.7	21.8	7.3	3.0
			11.2	-	14.4	7.0	2.1
		17.4	7.7	2.3	16.8	9.3	1.8
	Average	21.8	11.3	1.9	17.7	7.8	2.3
	Ratios[b]				0.81	0.69	

Table XVI continued

Specific activity (dis/μg) of DNA-adenine and DNA-guanine of mice injected with adenine-2-C^{14} or adenine-4,6-C^{14} [a]

Time after injection (days)		Tissue					
		Large intestine					
		Adenine-4,6-C^{14}			Adenine-2-C^{14}		
		Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)	Adenine spec.act.	Guanine spec.act.	Ratio (Ad/Guan)
1		441	74.6	5.9	410	67.0	6.1
		338	66.6	5.1	390	77.7	5.0
		295	41.7	7.1	309	61.0	5.1
	Average	358	61.0	5.9	370	68.6	5.4
	Ratios				1.03	1.13	
15		11.1	7.5	1.5	14.5	7.5	1.9
		14.5	8.0	1.8	16.2	7.8	2.1
		14.5	6.0	2.4	19.5	8.7	2.2
	Average	13.4	7.2	1.9	16.7	8.0	2.1
	Ratios				1.25	1.11	
		Liver					
1		11.1	2.6	4.3	15.2	2.8	5.4
		14.0	1.9	7.4	11.4	1.9	6.0
		14.3	2.5	5.7	9.4	2.2	4.3
	Average	13.1	2.3	5.7	12.0	2.3	5.2
	Ratios				0.92	1.00	
15		21.0	8.0	2.6	22.0	7.6	2.9
		15.1	6.5	2.3	23.5	10.8	2.2
		42.2	17.9	2.3	40.0	16.8	2.4
	Average	26.1	10.8	2.4	28.5	11.7	2.4
	Ratios				1.09	1.08	

[a] Male C_{57} mice, age 3 mo, weight about 25 g, were administered 1.1 mg of adenine-4,6-C^{14} or adenine-2-C^{14}, spec. act. 2.0×10^4 dis/μg. The specific activities of the guanine have been converted to the gamma equivalent of adenine. They were sacrificed at 1 or 15 days after administration of the adenine.

[b] Ratios of the average specific activities of adenine and guanine in adenine-2-C^{14} to those in adenine-4,6-C^{14}.

REPORTS ISSUED

The following paper has been published as a UCRL report:

Carbon-14 Respiration Patterns in Normal and Diabetic Rats, by Bert M. Tolbert and Martha R. Kirk, UCRL-3503, August 24, 1956.

Calvin

NUCLEAR CHEMISTRY

Glenn T. Seaborg and Isadore Perlman in charge

NEUTRON-DEFICIENT ISOTOPES OF IRIDIUM

Kenneth S. Toth

Light isotopes of iridium have been made by alpha bombardments on rhenium in the 60-inch cyclotron.

Natural rhenium (37.1% Re^{185}, 62.9% Re^{187}) in the metallic form was bombarded with 33.4-Mev and 39.4-Mev particles. The unpurified target portions as well as the pure iridium fractions were counted over a period of time on a Geiger counter. Activities seen were:

a. 11 to 14 hr Ir^{187}
b. 38 to 42 hr Ir^{188}
c. 12 to 15 d Ir^{189}
d. A 2-hr activity

The purified iridium fractions were also followed on the 50-channel gamma analyzer, using a thallium-activated sodium iodide crystal detector. The following are the peaks seen in both bombardments:

Energy (kev)	Rough half life	Probable assignment
135 to 150	---	Ir^{187} and Ir^{188}
280	14 hr	Ir^{187}
420	14 hr	Ir^{187}
480	---	Ir^{188}
510	2 hr	New activity
620	46 hr	Ir^{188}
620	3 hr	New activity
750	14 hr	Ir^{187}
900	14 hr	Ir^{187}

Rhenium enriched in Re^{185}(85%) was bombarded at 49 Mev. The Geiger counter curves gave the same half lives as above. Peaks seen on the 50-channel analyzer were the following.

Energy (kev)	Rough half life	Probable assignment
420	--	Ir^{187}
510	2 hr	New activity
1350	14 hr	Ir^{187}
1650	41 hr	Ir^{188}
2150	14 hr	Ir^{187}

The 2-hr activity has not been previously reported. It may be either an isomer, unknown until now, or a new isotope, Ir^{186}.

HALF LIFE OF RHENIUM-183

Donald Strominger and Charles J. Gallagher

Experiments are in progress to redetermine the half life of the electron-capture isotope Re^{183}.

Wilkinson and Hicks first assigned a half life of 240 days to Re^{183} obtained from a helium-ion bombardment of tantalum.[1] Stover reported a half life of 120 days in a rhenium fraction milked from neutron-deficient osmium isotopes.[2] Turner and Morgan reported a half life of 155 days in a rhenium fraction prepared from a helium-ion bombardment of tungsten.[3] In the last case the authors discussed the possibility of formation of the long-lived Re^{189} and concluded that their sample probably contained both Re^{183} and Re^{189}.

In our work a sample of Re^{183} was chemically separated from a tantalum target which had undergone bombardment from 48-Mev helium ions at the 60-inch cyclotron.[4] The sample was allowed to decay for approximately 1 yr, at the end of which time the sample was essentially free of Re^{184}($t_{1/2}$ = 50 days).

The decay of the remaining activity is being followed on a NaI(Tl) scintillation spectrometer coupled to a fifty-channel differential pulse-height analyzer.[5] The most intense electromagnetic radiation peaks observed on the spectrometer are at energies of 55 kev (K x-rays), 102 kev, and 126 kev.

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1 G. Wilkinson and H. G. Hicks, Phys. Rev. 77, 314 (1950).

2 B. J. Stover, Phys. Rev. 80, 99 (1950).

3 S. E. Turner and L. O. Morgan, Phys. Rev. 81, 881 (1951).

4 Thulin, Rasmussen, Gallagher, Smith, and Hollander, Phys. Rev. (in press) (1956).

5 A. Ghiorso and A. E. Larsh, in Chemistry Division Quarterly Report UCRL-2647, July, 1954.

The decay of the K x-rays and the 126-kev gamma ray has been followed for 130 days. The 126-kev gamma ray decays as a simple exponential with a half life of 70 days, which value for the half life of Re^{183} is in disagreement with the previous work described above.[1,2,3] The decay of the K x-rays, however, is complex, and this may be explained by the presence of Re^{181} in the original rhenium fraction.[6] This isotope decays to 140-day W^{181}. To verify this point we are now following the decay of a rhenium sample prepared from a 28-Mev helium-ion bombardment of tantalum. At this energy the formation of Re^{181} is not energetically feasible[6] and the sample should not show any long-lived tail due to W^{181}.

We are grateful to John O. Rasmussen and Sigvard Thulin for the loan of the rhenium samples.

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6 Gallagher, Canavan, Strominger, and Rasmussen, in Chemistry Division Quarterly Report UCRL-3415, June 1956.

Seaborg, Perlman

A NEW ALPHA-EMITTING DYSPROSIUM ISOTOPE

Kenneth S. Toth and John O. Rasmussen

Work is progressing on the neutron-deficient isotopes of dysprosium. Gadolinium, enriched in Gd^{152}, was bombarded with full-energy (49 Mev) alpha particles on the 60-inch cyclotron.

A pure dysprosium fraction was eluted from a Dowex-50 column with alpha-hydroxy isobutyric acid. The sample was followed on the alpha pulse analyzer.

Two alpha emitters were seen. The first, with an energy of 3.66 Mev and a half life of approximately 3 hr, is probably the previously reported 2.3 hr dysprosium isotope.[1]

The other alpha emitter peaked at 3.48 Mev and decayed with a half life of approximately 6 hr. This alpha emitter has not been previously reported.

At the bombarding energy used, the two most favored reactions would be (α, 4n) and (α, 3n). A present best guess might assign the 2.3-hr isotope as Dy^{152} and the 6-hr one as Dy^{153}.

An excitation-function experiment is planned to determine these mass assignments more definitely.

THE NUCLIDE PLUTONIUM-233

T. Darrah Thomas

The nuclide Pu^{233} has been produced by the (α, 4n) reaction on U^{233} with 45-Mev helium ions. A peak corresponding to an alpha energy of 6.30 Mev was observed in the alpha pulse analysis of the plutonium fraction isolated from such bombardments, and this peak was observed to decay with a 20-min half life. The amount of electron-capture branching was determined by milking of the Np^{233} daughter and from this value the partial alpha half life was found to be 18 days.

Extensive experiments were performed to show that the observed activity could not be due to Th^{226}, a member of the Pu^{234} decay chain that has a half life of 31 min and an alpha energy of 6.33 Mev.

The mass assignment is based on a rough excitation function, the appearance of alpha particles attributable to the U^{229} daughter of Pu^{233}, and on the agreement of the alpha energy and half life with alpha systematics.

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

SPALLATION OF URANIUM-234 WITH 9 TO 25-Mev DEUTERONS

Richard M. Lessler

Bombardments of U^{234} with deuterons have been started. Analysis of product yields have resulted in the following preliminary cross sections.

	Cross section (in millibarns)		
E_d (Mev)	(d, n)[a]	(d, 2n)	(d, 3n)
8.9	0.48	0.87	--
11.8	--	2.25	--
14.8	4.56	11.3	1.43
16.5	--	18.3	4.97
17.4	--	--	7.69
17.8	--	18.6	--
20.8	--	12.9	8.88
23.7	5.92	8.00	4.44

[a] The (d, n) cross sections are upper limits, because the assumption was made that the proportional-counter activity remaining on the sample plates after decay of 4.4-day Np^{234}, and 35-min Np^{233} was due to 410-day Np^{235}.

Proportional-counter efficiencies were assumed to be 100% for all samples. Corrections will be made when the counting efficiencies are determined for electroplated samples. Work on this problem is continuing.

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THE REACTIONS OF MIXED-CRYSTAL RARE-EARTH TRIFLUORIDES WITH FLUORINE GAS

William P. Bryan

I. Lanthanum-Cerium Trifluorides

A previous report[1] has summarized the reactions of lanthanum–cerium trifluoride mixed crystals with F_2 gas. Further work in the region of high cerium content makes it necessary to modify some statements previously made. The reactions will be summarized in the light of these new results.

Region 1. 100% Ce to 78% Ce

The reaction observed is:

$$La_\delta Ce_{1-\delta}F_3 + \frac{1-\delta-\alpha}{2} F_2 = \underset{(CeF_4 \text{ structure})}{Ce^{IV}_{1-\delta-\alpha}} Ce^{III}_\alpha La_\delta F_{4-\delta-\alpha} \cdots \quad (1)$$

Previous results indicated that α might be as high as ~0.2, and, in fact, the reaction did appear to stop at this point. However, more prolonged heating in F_2 at a temperature of 200° to 250° indicates that oxidation is complete (i.e., $\alpha = 0$). Thus the mechanism of this reaction appears to involve an initial rapid reaction that involves oxidation of enough Ce to the +4 state to stabilize the CeF_4 structure. Once stabilized, the new phase then takes up the remaining F_2 at a slower rate. The entire reaction is completed in 2 to 3 hr at 200° to 250°.

Region 2. 78% Ce to 56% Ce

In this region two phases are produced by a rapid reaction:

$$La_\delta Ce_{1-\delta}F_3 + \frac{1-\delta}{2} F_2 \rightarrow$$

$$= \frac{1-\delta-\beta}{\gamma-\beta} \underset{(CeF_4 \text{ structure})}{Ce^{IV}_\gamma La_{1-\gamma}F_{\gamma+3}} + \frac{\gamma+\delta-1}{\gamma-\beta} \underset{(LaF_3 \text{ structure})}{Ce^{IV}_\beta La_{1-\beta}F_{\beta+3}} \cdots \quad (2)$$

The values for β and γ are: $\beta = 0.56$ and $\gamma = 0.78$ to 1.00. The fact that γ may be greater than 0.78 indicates some uncertainty in the statement that only one phase is produced when the reaction in Region 1 has gone to completion.

Region 3. 56% Ce to 0% Ce

Here the reaction is slow (40 hr at 250°) and produces only one final phase:

$$La_\delta Ce_{1-\delta}F_3 + \frac{1-\delta}{2} F_2 = \underset{(LaF_3 \text{ structure})}{La_\delta Ce^{IV}_{1-\delta}F_{4-\delta}} \quad \cdots\cdots \quad (3)$$

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[1] W. P. Bryan, in Chemistry Division Quarterly Report UCRL-3415, June 1956, p. 55.

Unit-cell dimensions have been determined for these final phases. They show no significant change from the unit-cell dimensions of the starting materials. Results also indicate that more F_2 may be taken up by the solid than is equivalent to the Ce present.

In the light of these results, it is not necessary to postulate any crossing of $-\Delta F$ curves as was done in the previous report.

II. Yttrium-Cerium Trifluoride

The reactions of yttrium–cerium trifluoride mixed crystals with F_2 have been studied by measuring the change in weight of the sample upon heating in F_2 by:

1. Weighing the sample and pan before and after reaction.

2. Observing the deflections of a nickel helix from which the pan was suspended during the course of the reaction.

Agreement between these two methods was satisfactory, and it was found that the Ce was completely oxidized to the +4 state throughout the entire composition range. In general, the rates of the various reactions were similar. About 2 to 5 hr at 250° with P_{F_2} = 1 atmos was necessary for the reactions to go to completion.

As YF_3 has an orthorhombic structure and CeF_3 has a hexagonal structure, the mixed crystals will have the YF_3 structure in the yttrium-rich region and the CeF_3 structure in the cerium-rich region. In some intermediate region, both structures should be present, corresponding to the two phases representing YF_3 saturated with CeF_3 and vice versa. X-ray results indicate that the YF_3 structure is saturated with CeF_3 when 27 to 37 atom % Ce is present and that the CeF_3 structure is saturated with YF_3 when 57 to 67 atom % Ce is present.

X-ray examination of the fluorinated materials should reveal a similar situation. Thus, in the range 78 to 89 atom % Ce the CeF_4 structure becomes saturated with YF_3. Two phases are observed down to 27 to 37 atom % Ce, at which point there is apparent saturation of the YF_3 structure with CeF_4. However, as soon as two phases appear in the starting material, two phases must of necessity appear in the fluorinated product. Thus, no definite statement can be made as to the limits for the saturation of YF_3 with CeF_4 other than to set a lower limit (27 to 37 atom % Ce) on the amount of cerium present in the YF_3 structure at saturation.

At present attempts are being made to study the YF_3 structure containing CeF_4 in order to get some idea of the nature of the defects present.

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QUADRUPOLE RESONANCE MEASUREMENTS

A. H. Reddoch

At present we are interested in finding the nuclear quadrupole resonance of chlorine in thorium tetrachloride in order to get some direct measure of the character of the bonding, which Zachariasen[1] and Mooney[2] believe to be primarily covalent. We are also interested in the lutetium resonance in lutetium oxide, Lu_2O_3, in order to study the structural behavior of the compound.

So far neither resonance has been observed. A search for each has been made in the frequency range from 11 Mc/sec to about 22 Mc/sec with a superregenerative oscillator. Although the negative results may not be conclusive, we believe the resonances may occur at still lower frequencies.

The circuit of the superregenerative oscillator was very similar to the regenerative circuit by Livingston.[3] This circuit is very simple; it is of the self-quenching type. It shows a high sensitivity in that it has a good signal-to-noise ratio and also in that the presence of sidebands gives a multiple line structure that is easier to distinguish from noise than a single line. This sensitivity is illustrated by the fact that the Cl^{37} resonance in 0.1 g of $NaClO_3$ at room temperature has been seen with this equipment.

It was hoped that this apparatus could be used to search for the resonances at the lower frequencies where they might be expected, i.e. below 10 Mc/sec. Since the oscillator depends on small stray capacitances for its feedback, it will not oscillate at low frequencies. When the approximate capacitance was added, it could be made to oscillate, but this was found to reduce its frequency range.

This reduced range necessitates frequent changing of oscillator coils during an extended search. The sidebands are also a disadvantage because it is sometimes difficult to determine which is the true centerline and which are sidebands. Finally, the most serious objection to this equipment was the critical adjustment necessary for optimum sensitivity and the great dependence of this adjustment on the oscillator frequency, making necessary frequent readjustments while searching.

For this reason it was felt that a new apparatus was necessary, for while it was easy to observe a known resonance such as Cl in $Al Cl_3 \cdot Et_2O$ at 11.3 Mc and N in $(CH_2)_6N_4$ at 3.3 Mc, searching for an unknown resonance was uncertain as well as tedious.

Upon the advice of Professor E. Hahn of the Physics Department, and following a circuit supplied by him, a Pound-Knight oscillator[4] was built. The circuit differs from that in Reference 4, primarily in that the

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1 W. H. Zachariasen, Actinide Elements (McGraw-Hill, New York, 1954), p. 787.

2 R. C. L. Mooney, Acta Cryst. 2, 189 (1949).

3 R. Livingston, Ann N.Y. Acad Sci 55, 800 (1952).

4 R. V. Pound and W. D. Knight, Rev. Sci. Instr. 21, 219 (1950).

tuned rf amplifier has been replaced by a video amplifier which has relatively constant gain from 1 to 11 Mc.

This equipment appears to have a lower sensitivity than the super-regenerative oscillator. However, its adjustment is not critical and it is much easier to search with. The sensitivity appears to change with frequency and can be kept up by keeping the capacitance of the tank circuit as high as possible. Since tuning is effected by a variable condenser, it is desirable to change coils more frequently than otherwise necessary in order to maintain a high sensitivity.

Numerous difficulties have been encountered due to noise, pickup, vibration, and drift, all of which are quite noticeable in the Pound-Knight circuit. However, these difficulties now seem to be fairly well under control. Some erratic behavior has been traced to the use of carbon resistors and coaxial cables in the oscillator stage and to the use of liquid nitrogen as a refrigerant with the particular design of sample holder used.

In order to check the performance of the equipment, two known resonances have been observed so far. The first is nitrogen in hexamethylene tetramine, previously reported by Watkins and Pound.[5] Their frequency at room temperature has been checked to within the present experimental error of about 300 cps. The other resonance is the four-line resonance of Cl in titanium tetrachloride that Dehmelt[6] reported to have an average frequency of 6.05 Mc at liquid-nitrogen temperatures. The frequencies observed here are 5.9790, 6.0366, 6.0795 and 6.1103 Mc at liquid-nitrogen temperatures, while at dry-ice temperatures (-75°C) they have shifted to 5.9329, 5.9772, 6.0105 and 6.0465 Mc respectively.

These results seem to indicate satisfactory operation at these frequencies. We hope that after we check the equipment at 11 Mc, it will be possible to resume searching for the resonances we are interested in.

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5 G. D. Watkins and R. V. Pound, Phys. Rev. 85, 1062 (1952).

6 H. G. Dehmelt, J. Chem. Phys. 21, 380 (1952).

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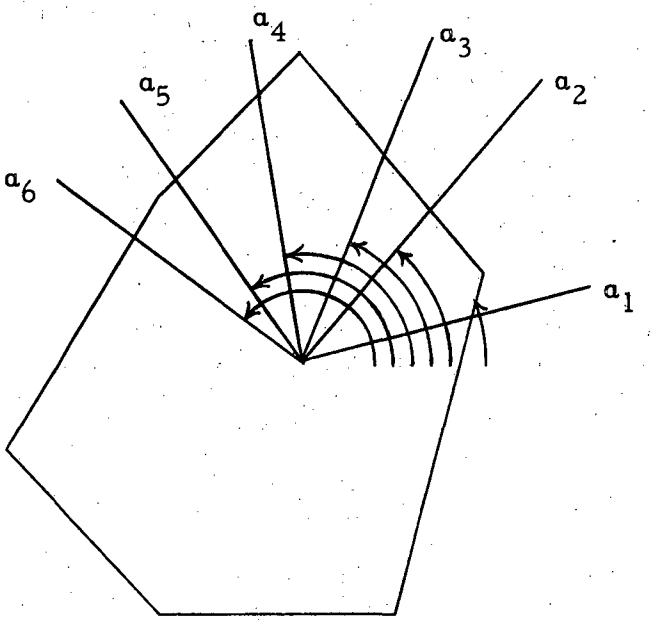
X-RAY ABSORPTION CORRECTIONS FOR PRISMATIC CRYSTALS

Carol H. Dauben

Using the intensity-of-reflection values obtained from zero-layer Weissenberg photographs, we have written a program to calculate on the IBM-650 the absorption correction for an irregular prism.

In the specific case for which this program was written, the crystal was rotated about its short axis and was long in comparison with its diameter, so that the end effects were ignored. The cross section of the crystal can be described as being enclosed by 6 lines, the equations of which are stored in the machine. The crystal is divided into roughly 300 segments of equal area, and the coordinates of these segments are stored also. Six tangents, 1/sin, and 1/cos of possible beam angles for which the absorption is to be computed are stored in the machine. A table of the exponential e^{-ux} must be stored in the machine for each calculation. The machine computes the distance traveled by the incoming x-ray beam at an angle $\alpha = \alpha_1$ through the crystal to the first segment, stores this distance and computes the distance



traveled by the outgoing beam at angle $\beta = \alpha_1$. It then adds these distances together, looks up the exponential, and stores it. After computing the distance traveled by the outgoing beam at angle $\beta = \alpha_2$, the machine adds this value to the incoming $\alpha = \alpha_1$, stores this, etc. It does this for six incoming and outgoing angles, making 21 separate calculations in all. It then goes to the next segment and repeats the process. In addition to calculating the distance traveled by the x-ray beam entering at angle α, it also stores the distance traveled by the x-ray beam entering at angle $\alpha + 180^\circ$, and therefore stores four exponentials for each pair of angles considered.

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For the segments that are on the faces of the crystal and therefore do not have an area equal to the whole segment, the calculation of the exponential is made and then divided by 2 before being added into the sums. For these the coordinates are given as being at the center of mass of the segments. Care must be taken to avoid listing points outside the crystal. Very small segments are ignored, and large ones are computed as if they were whole ones. If there are many segments contributing, it is hoped that this "half-point" calculation will be sufficient. Edge effects are ignored.

The longest distance through this crystal is about 11 absorption lengths (0.11 mm). The machine throws out all distances greater than 5 absorption lengths (0.05 mm) as too large and substitutes $e^{-\infty} = 0$ for these exponentials. Because of this, some of the points at the center of the crystal (for which it is known in advance that the distance will be larger than 5 absorption lengths for any beam angle) are not listed.

From the data punched out of the machine at the end of the calculation, a graph of the absorption correction can be plotted on a Weissenberg net, and the corrections read off by placing the film on top of the graph.

The calculation takes in the neighborhood of 18 to 20 sec per segment, as it is not fully optimized.

BETA SPECTROSCOPY

R. G. Albridge and Jack M. Hollander

The conversion-electron spectrum of Pu^{239} was studied in the 100-gauss permanent-magnet electron spectrometer. Accurate energy values were obtained for the two known gamma rays, and an upper limit of 0.1% was set on the possible abundance of a conversion-electron line in the neighborhood of 100 kev.

Samples of Np^{237} and Th^{232} have been prepared for irradiation in the Materials Testing Reactor for future study of the decay of Np^{238} and Pa^{233} by means of the permanent-magnet spectrographs.

A study of the levels of Am^{241} from decay of Cm^{241} is under way (with S. Amiel, C. Gallagher, F. Asaro). A sample of Pu^{241} was prepared by the Pu^{239} (α, 2n) reaction in the 60-inch cyclotron.

Work has begun on the design of an electron accelerator to be used with the permanent-magnet spectrographs for the study of low-energy conversion and Auger electrons.

ENERGY LEVELS OF NEPTUNIUM-237

J. O. Rasmussen and J. M. Hollander

A study of the energy levels of Np^{237} by the beta decay of U^{237} is being completed. Measurements of conversion-electron spectra have been made in the permanent-magnet spectrographs and the double-focusing spectrometer (with F. Canavan). Beta-spectral studies have been made in the double-focusing spectrometer by F. Canavan. A study of γ-γ coincidences is being carried out by F. Asaro.

DESIGN OF PERMANENT MAGNET SPECTROGRAPHS

Jack M. Hollander

A design has been completed for four new permanent-magnet spectrographs of field strengths 50, 100, 150, and 250 gauss to augment our present equipment, which includes instruments of 50, 100, 200, and 340 gauss.

RECORDING OF CONVERSION-ELECTRON LINES

Jack M. Hollander and Walter H. Barkas

A program has begun to investigate the feasibility of replacing no-screen x-ray film with electron-track plates as a means of recording conversion-electron lines in the permanent-magnet spectrographs. This method may find limited application for accurate measurement of line intensities and for detection of lines in very weak samples that otherwise could not be run in these instruments.

PRECISION ALPHA COUNTING

Herman P. Robinson and Eleanor Potter

Two samples of Am^{241} that were counted with the precision low-geometry alpha counter were sent to Hanford for interlaboratory comparison.

The results are as follows:

Source number	HAPO d/m	UCRL d/m	HAPO/UCRL % difference
10	4.705×10^6	4.713×10^6	0.17
12	3.731×10^7	3.728×10^7	0.08

LOW-BACKGROUND ALPHA-COUNTING CHAMBERS

Albert Ghiorso and Almon E. Larsh

The sliding alpha-counter chambers used in the Nuclear Chemistry Department's fission-alpha-beta counters are made of aluminum and steel. The natural Ra content of aluminum and all of its alloys[1] gives rise to an alpha background in the chamber of 0.5 cpm and 30 cph.

It was decided that synthetic organic material might cover up the natural activity, and certain materials were tested. Lining the chamber with Teflon reduced the background, but this material is mechanically impractical. An Epon coating on the inside of the chamber reduced the background but was laborious to apply. The synthetic paint, 4A, used by the Health Chemistry group to recondition equipment can easily be sprayed into the chambers. The 4A paint reduces chamber background to 0.06 cpm 4 cph.

The insides of the painted chambers are coated with aquadag for two reasons. First, the proper collecting field is maintained; second, a contaminated chamber is extremely easy to clean with water, which takes out the aquadag and contamination together.

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[1] C. E. Miller, Nucleonics 14, No. 4, 42 (1956).

A TRANSISTOR PULSE-HEIGHT ANALYZER

Almon E. Larsh

In the process of building circuits to study transistor characteristics, a differential pulse-height analyzer was constructed.

In the circuit of Fig. 1, a biased diode is used for primary discrimination. A 1- to 10-μsec signal pulse triggers the discriminator univibrator which generates 60-μsec channel and inhibit pulses. An inspect pulse is used to prevent possible trouble from variations in inhibit-pulse width from different channels. The inspect pulse provides a convenient means of gating the analyzer for coincidence analysis.

The channel, inspect, and inhibit pulses are mixed in a diode matrix. If there is a channel pulse and no-inhibit pulse, the register univibrator will produce a 40 msec register drive pulse to drive the Sodeco register.

Although the register used is rated at 60 v, it can be driven at 10 pps by a transistor, from a 22.5-v supply. A 45-v supply is used here to get a register rate of 15 pps. When a regulated E_{CC} is used, the channel-edge stability is within a few mv over a period of 24 hr.

To read the information out of a transistor binary scaler operating with E_{CC} = -4.5 v to -22.5 v, we found the circuit of Fig. 2 convenient.

THE SUPERSNAPPER

Almon E. Larsh, Alfred A. Wydler, and Albert Ghiorso

The snapper pulse-height analyzer[1] has been redesigned to accept higher counting rates for analysis. The new circuit is represented schematically in Fig. 3. Required input pulses are illustrated in Fig. 4. A scale-of-ten glow-transfer tube has been built into each channel. The in-channel dead time is 200 μsec. The adjacent-channel dead time is a minimum of 2 μsec, but is determined by the gate pulse width. The new analyzers are being built as 10-channel units that will usually be stacked in banks of five to use as 50-channel analyzers. Four years' experience has shown that it would be hard to improve on the circuit reliability of the original snapper. The weakest point of the snapper is the Veeder-Root mechanical register. The supersnapper uses Sodeco registers, which require less driving power, run faster, and seem to have a longer mechanical life than the Veeder-Root register.

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[1] A. Ghiorso and A. E. Larsh, in Chemistry Division Quarterly Report, UCRL 2647, July 1954.

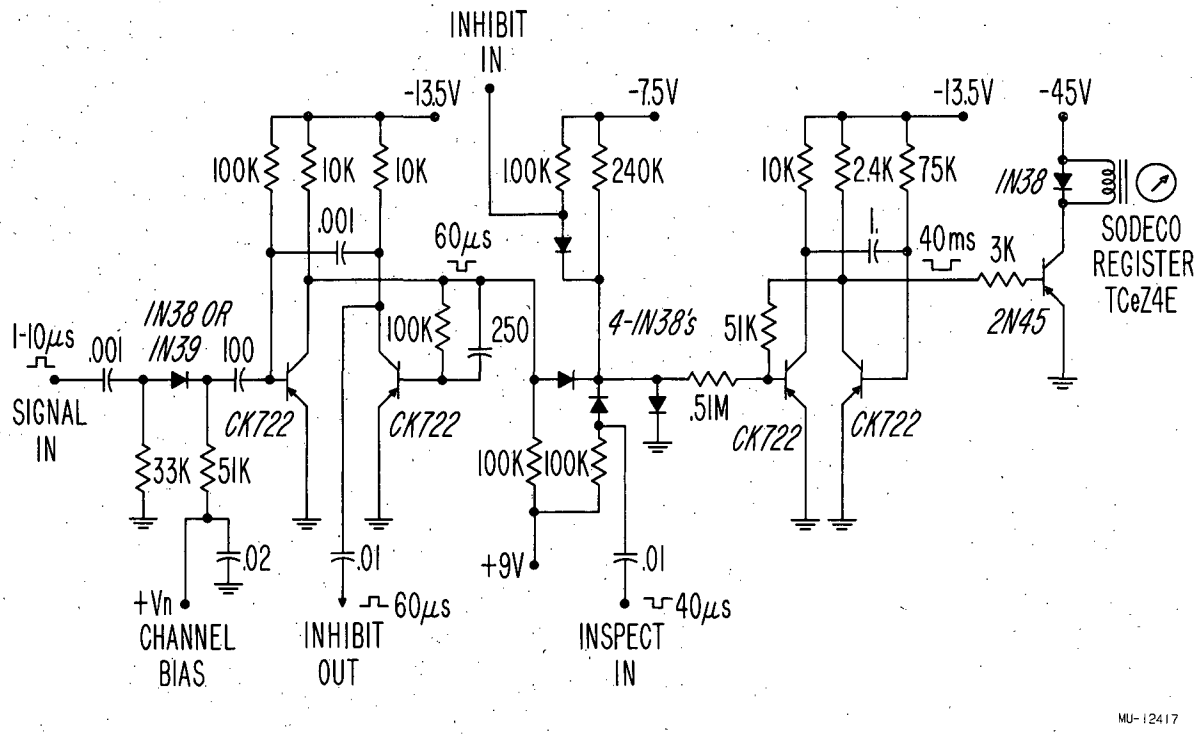

Fig. 1. Schematic diagram of the transistor pulse-height analyzer.

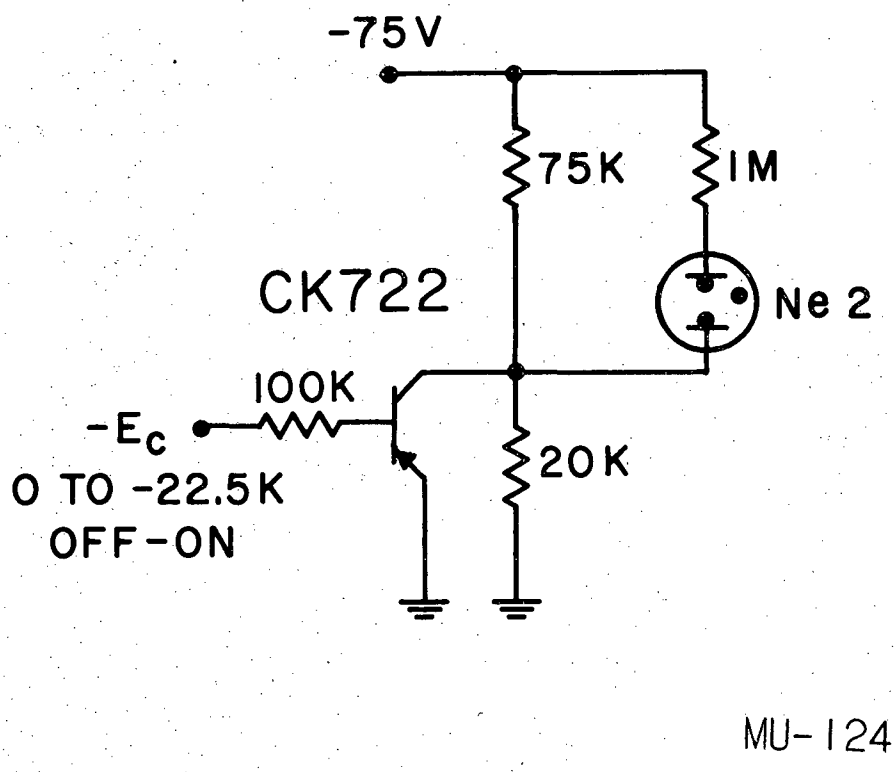

Fig. 2. Neon-2 switching circuit for the transistor pulse-height analyzer.

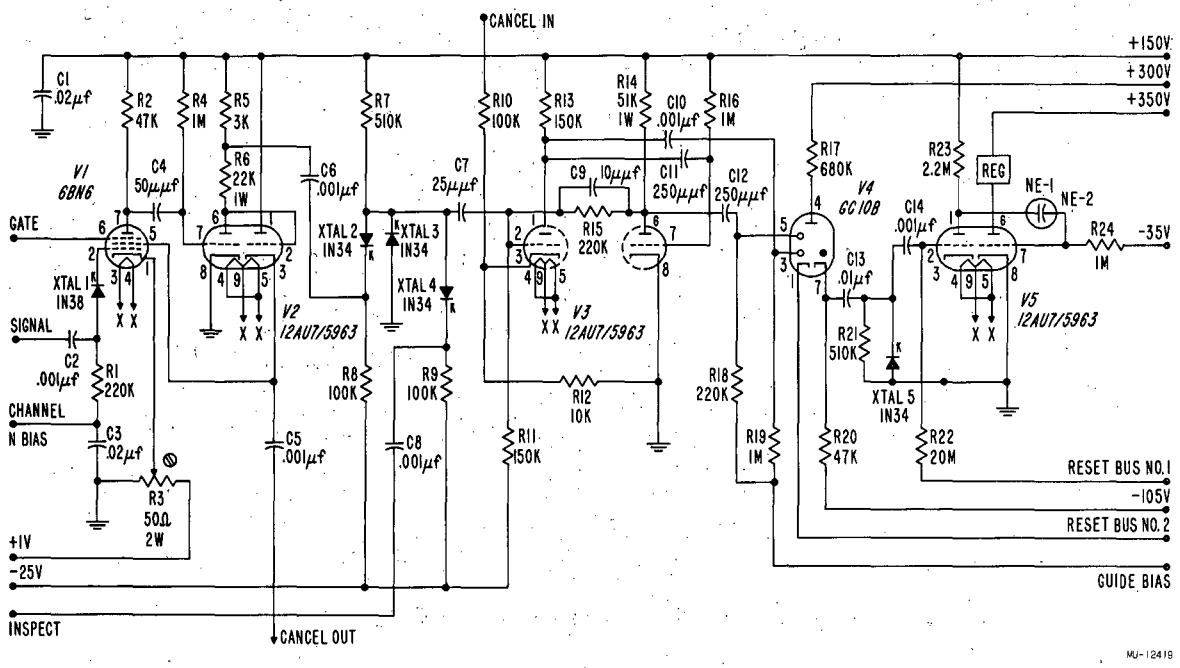

Fig. 3. Schematic diagram of a typical channel of the supersnapper.

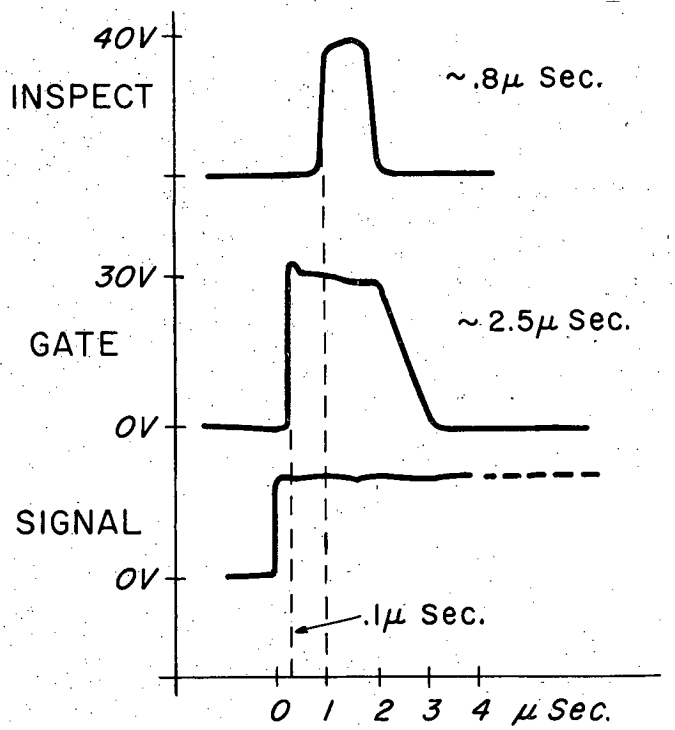

Fig. 4. Required input pulses for the supersnapper.

CHEMICAL ENGINEERING (PROCESS CHEMISTRY)

LIQUID-LIQUID EXTRACTION AND AGITATION

James Vanderveen and Theodore Vermeulen

Coalescence rates are now being investigated in several mixtures by the following measurements: (a) variation of interfacial area with height in the mixing vessel; (b) rate of approach to steady-state dispersion, in start-up; and (c) colorimetric determination of mixing rates in the dispersed phase, using components insoluble in the continuous phase.

ACTIVITY COEFFICIENTS IN MULTICOMPONENT SYSTEMS

Theodore Vermeulen

A high-speed program has been developed for the IBM-650 Computer that will be used to determine thermodynamic constants in several four-component mixtures.

PERFORMANCE OF SPHERE-PACKED EXTRACTION COLUMNS

Gabriel Jacques and Theodore Vermeulen

Mass-transfer rates in packed columns are believed to be reduced markedly by longitudinal mixing. This investigation is under way to measure such mixing as a function of packing arrangement and flow conditions, and to determine its effect on over-all performance. The experimental columns (6 in. in diameter, with 3/4-in.-diameter packing) are provided with a series of miniature conductivity cells. During the last 3 months, electronic equipment has been provided for recording the specific resistance of these cells.

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

Eugene J. Fenech and Charles W. Tobias

Final evaluation of data, and correlation in the $Nu' = \text{const.}\ (Gr, Sc)^n$ form has been completed using the IBM-650 machine for routine calculations.

On the basis of this work, limiting currents on horizontal electrodes can be predicted with very satisfactory accuracy for concentrations larger than 0.05 M and electrode widths above 0.5 cm. A technical report (Master's thesis) will be completed during September.

STABILITY OF PERFORATED-PLATE TRAYS

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

Dry-plate pressure-drop data for different plates have been taken and are in complete agreement with Hunt's data.[1] Therefore it can be concluded that the gas dynamics have not been changed by using a rectangular column in place of a circular column.

Some stability data have been taken with the air-water system. It was found that the slope of the plate has a significant effect on the dumping rate.

GAS-LIQUID PARTITION CHROMATOGRAPHY

Robert H. Houston and Charles R. Wilke

Equipment is in operation using a 6-ft Celite 545- DC-550 silicone oil column. Helium is being used as inert carrier gas. Detection of separated products in the gas stream is accomplished through an unbalance of the thermal conductivity bridge. We are conducting initial trials using aromatic hydrocarbons.

The theory reported in a prior report has been modified to give a more exact solution of the differential equation. Graphical solutions will be compared with experimental results.

MASS TRANSFER FROM PACKED BEDS INTO SEVERAL GASES AT VARIOUS PRESSURES

Robert J. Fallat and Charles R. Wilke

Experimental work has been completed and a formal written report is now in progress. The report will include, in addition to the present high-pressure work, the low-pressure studies completed previously by Dr. Edward Lynch, whose work is an integral part of the investigation that has not been formally reported.

THERMAL CONDUCTIVITY OF GASES AT HIGH TEMPERATURES

Henry Cheung and Charles R. Wilke

Measurement of thermal conductivity of 32 mixtures and 9 pure components at 100°C has been completed. Runs at 300°C are in progress.

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[1] Charles d'A. Hunt, Capacity Factors in the Performance of Perforated-Plate Columns (Thesis), UCRL-2696, Oct. 1954.

GENERAL CHEMISTRY

Robert E. Connick and Leo Brewer in charge

METALS- AND HIGH-TEMPERATURE THERMODYNAMICS

Absolute Lifetime Apparatus

C. Geoffrey James and Leo Brewer

The shops have continued their work on the apparatus and it should be completed this fall. A furnace for preparation of CN has been completed.

Ground State of C_2

William T. Hicks and Leo Brewer

Errors due to self-absorption and fast scanning speeds have been corrected and the final result is that the ground state of C_2 is the $^3\pi$ state.

Stability of Solid SiO

Frank T. Greene and Leo Brewer

The high-frequency-heated differential thermal-analysis apparatus has been used to demonstrate that solid SiO is thermodynamically unstable at all temperatures.

Study of MgOH Spectrum

Frank T. Greene, C. Geoffrey James, and Leo Brewer

The dependence of the ultraviolet bands of MgO upon hydrogen pressure is being studied to determine if the bands are actually due to MgOH. No conclusive results have been obtained yet.

BASIC CHEMISTRY

Ruthenium Chemistry

Howard H. Cady and Robert E. Connick

Ruthenium(II) has been prepared by the reduction of RuO_4 with V^{++} in perchloric acid. The reaction is slow and the product is not exactly the same as that formed using Ti^{+3} as a reducing agent. The most significant difference is that the absorption peak for Ru(II) is shifted from 375 mμ (V^{++}) to 385 mμ (Ti^{+3}). Also the molar-extinction coefficients indicate either a different species or a different amount of reaction. (The ϵ_{375} value is 5.4×10^3 for the V^{++} reduction and the ϵ_{385} value is 2.3×10^3 for the Ti^{+3} reduction.) Both of these ϵ values are low because of the presence of Ru(III) in the solutions.

Work has been started on methods of measuring the charge on an ion, utilizing ion-exchange resins. One system uses the change of the distribution ratios upon dilution of a solution containing a known and an unknown ion (e.g., Mg^{++} and Ru(III)). Another system involves column operation in which the ion of unknown charge is replaced by an ion of known charge.

Kinetics and Thermodynamics of +1 Iodine

Yuan-Tsan Chia and Robert E. Connick

The absorption spectrum of the +1 iodine species was investigated. This species could be prepared either by adding standard sodium hydroxide to a triiodide solution or by adding an equivalent amount of hypochlorite to a basic iodide solution. An absorption peak was found at 370 mμ in both cases. The +1 iodine species thus formed was unstable with respect to disproportionation. An investigation of this rate law is in progress.

During the investigation of the effect of the concentrations of iodide ion and hydroxide ion on the rate law of the disproportionation, it was found that under conditions of moderate iodide and hydroxide concentrations (0.1M), an appreciable amount of triiodide is present. The dependence of the equilibrium between triiodide and the +1 iodine species on the concentration of hydroxide ion was found to be approximately first power. The study of the dependence on the concentration of iodide ion is in progress.

Kinetics of Rapid Reactions

Claude P. Coppel and Robert E. Connick

The preliminary work on the new baffle, which was discussed in the preceding report (UCRL-3415), was extended somewhat. A high-speed photography apparatus was made available at the Richmond Engineering Field Station. With the aid of this apparatus the actual firing of the mixer was seen, and from these photographs it was learned that there is very

little or no cavitation behind the turbulator plate as it rises through the solution. Although this result did not substantiate our earlier views on cavitation, it did not contradict any experimental observations previously made. The results also gave an independent method of measuring the firing time that was in approximate agreement with the earlier spectroscopic measurement.

With the completion of the preliminary work, the mixer is at present being applied to the study of reaction rates. The work described here is an attempt to check some temperature effects on the ferric thiocyanate complexing reaction which was studied earlier by John Below.

Composition of Carbide Vapors

Stanley G. Davis and Alan W. Searcy

Further study of the weight losses from SiC heated in the modified Knudsen effusion cell at a temperature near 1840°C indicates:

(a) essentially no dependence upon the area of loss per unit orifice area;

(b) very little condensation near the orifice;

(c) very little dependence, with the small but reproducible weight losses used, upon possible depletion of silicon from surface of charge.

These results indicate close approach to equilibrium composition for the effusate.

Radiation-Induced Reactions in Nonaqueous Solutions

Ladd Griffith

This work has been completed and a report (UCRL-3422) has been written.

Chemical Shifts in the F^{19} Nuclear Magnetic Resonance In Aqueous Solutions of Inorganic Fluorides

Richard E. Poulson and Robert E. Connick

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

Determination of the Molecular Structure of Aluminum Hydride

Robert F. Nickerson and Edwin F. Orlemann

During the last quarter, several designs for a low-temperature nuclear induction probe were investigated. The design finally decided upon consists of two orthogonal coils. The transmitter coil is a rectangular

Helmholtz coil cemented onto a quartz tube. The axis of the tube and the coil are orthogonal. The orthogonal receiver coil was wound and cemented onto another quartz tube capable of being inserted into the transmitter tube. Another coil was wound around the transmitter coil for introducing power from a saturation oscillator. An assembly made of aluminum was constructed to hold the two coil forms rigid. The entire assembly is to be inserted in a dewar of a special design and the unit is cooled with precooled nitrogen gas. Balancing of the probe is to be accomplished both geometrically and by means of an external electrical bridge.

Heat of Formation of Aqueous Ferrate(VI)

Robert H. Wood

The heat of formation of aqueous ferrate(VI) solutions has been determined by adding 50 ml of 0.01 to 0.02 M K_2FeO_4 solutions to 1 liter of 0.500 M $HClO_4$ in a calorimeter. The ferrate VI solutions were checked for the presence of +5 or +4 iron by a gas volumetric technique. The volume of O_2 evolved was from 99.5 to 100.0% of the theoretical. The three calculated values for the heat of formation of aqueous ferrate(VI) are -118.2, -115.0, and -116.0 kcal/mole. Additional measurements on a different sample of K_2FeO_4 are in progress.

Acetaldehyde Extinction Coefficients

Earl F. Worden

In order to confirm the observed lowering of the extinction coefficient in the ultraviolet absorption spectrum of acetaldehyde on deuteration, acetaldehyde was prepared by the same method as acetaldehyde $d_1(CH_3CDO)$. The spectrum of the prepared acetaldehyde was identical with that of purified commerial acetaldehyde. The extinction coefficient was greater than that of the deutero compound by approximately 15%. Propionaldehyde and butyraldehyde showed the same reduction in extinction coefficient on deuteration.

The lower extinction coefficient of the deutero compound indicates that the tritiated compound will have an extinction coefficient lower than normal acetaldehyde by an unknown amount. The relative rates of the following primary process reactions were to be determined:

$$CH_3CHO + h\nu \rightarrow CH_4 + CO,$$

$$CH_3CTO + h\nu \rightarrow CH_3T + CO.$$

As the relative amount of light absorbed by the two species is not the same and is unknown, it will not be possible to determine the isotope effect on the primary process.

Connick, Brewer

Information Division
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