UCRL 8204

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BIO-ORGANIC CHEMISTRY QUARTERLY REPORT
December 1957, January, February 1958

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*Preceding Quarterly Reports: UCRL-8141, UCRL-3950.
Note: Material formerly included in Chemistry Division Chemistry Report will henceforth appear in two separate series, Bio-Organic Chemistry Quarterly Report and Chemistry Division Semiannual Report.

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Edited by Richard M. Lemmon

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

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PROTON MAGNETIC RESONANCE OF COMPOUNDS CONTAINING NITROGEN

Erminio Lombardi and Power B. Sogo

A study of the proton magnetic resonance of compounds containing nitrogen in different forms has been undertaken in order to determine if there is spin-spin interaction between protons bound to nitrogen and other protons present in the molecule. From the results it seems that there is no evidence for such spin-spin interaction (only one line has been observed for protons bound to a nitrogen atom). The principal results are listed on the following page. The chemical shift δ is given as

$$\delta = \frac{H-Hr}{Hr} \times 10^6,$$

where Hr is the resonating field for the -OH proton in ethanol, and H is the resonating field for the nitrogen-bound proton of the compound measured.

The principal features of the results are:

(a) A correlation is indicated between the basicity of the nitrogen compounds and the chemical shift, i.e., the more basic the nitrogen atom, the more shielded are the protons bound to it.

(b) It is possible to shift the observed resonance position of the protons bound to a nitrogen atom to approximately zero by means of the exchange reaction with D_2O.

We intend to study the rate of this exchange reaction at different temperatures and get a more accurate correlation between basicity and chemical shift.

AMINES	δ	AMIDES	δ
t-butylamine	+3.5	formamide	-2.6
cyclohexylamine	+3.2	formamide in D_2O	+0.4
isobutylamine	+2.8	acetamide in D_2O	+0.2
methyl-p-toluidine	+2.2	propionamide in D_2O	+0.6
2, 4-dimethylaniline	+2.0	n-butyramide in D_2O	+0.5
butylaniline	+1.9	isobutyramide in D_2O	+0.5
benzylamine	+1.8	n-valeramide in D_2O	+0.4
o-toluidine	+1.8	isovaleramide in D_2O	+0.4
o-phenetidine	+1.5	malonamide in D_2O	+0.5
aniline	+1.2	lactamide in D_2O	+0.0
o-phenylemdiamine. 2 HCl in D_2O	+1.0	acrylamide in D_2O	+0.8
p-anisidine in D_2O	+0.8	glycine amide. HCl	+0.2
ethylamine. HBr in D_2O	+0.2	benzamide in acetone	-1.8
diphenylamine in ether	+0.0	benzenesulfonamide in acetone	-1.2
methylamine. HCl in D_2O	-0.5	benzanilide in acetone	-1.5
		salicylamide	-1.8

AMINO ACIDS	δ	HYDRAZINES	δ
glycine in D_2O	0	α-methyl, α-phenylhydrazine	2.1
α-alanine in D_2O	0	α-ethyl, α-phenylhydrazine	2.2
β-alanine in D_2O	0	α-isoamyl, α-phenylhydrazine	2.0
α-aminobutyric acid in D_2O	0	α-benzyl, α-phenylhydrazine	1.0
γ-aminobutyric acid in D_2O	0	m-tolylhydrazine	1.2
2, 4-diaminobutyric acid. 2 HCl in D_2O	0	phenylhydrazine	1.2
α-aminovaleric acid in D_2O	0	hydrazine hydrate	0.0
δ-aminovaleric acid in D_2O	0	phenylhydrazine. HCl in D_2O	0.0
α-amino isovaleric acid in D_2O	0	methylhydrazine sulfate in D_2O	-0.8
DL-proline in D_2O	0	hydrazobenzene in ether	-1.0
DL-threonine in D_2O	0	acetone phenylhydrazone	-1.8

CARBAMATES	
methylcarbamate in D_2O	0.2
ethylcarbamate in D_2O	0.6
ethyl N-ethylcarbamate	-0.5
phenylurethane in CCl_4	-1.8

ELECTRON-SPIN RESONANCE STUDIES OF IRRADIATED CHOLINE CHLORIDE AND ITS ANALOGS

Robert O. Lindblom and Richard M. Lemmon

In a previous report[1] the syntheses of selectively deuterated choline chlorides were described. One of the reaction steps, the esterification, has been found to be very sensitive to the order of addition of the reagents. If the esterification is attempted by adding the bromoacetyl bromide to ethanol (at 0° or 78°), no ethyl bromoacetate can be isolated. Reversing the addition, i.e., adding the alcohol to the bromoacetyl bromide, gives a 60% yield. Using the same order of addition and diluting the reagents with benzene gave a yield of 84%. The reaction in benzene was refluxed for an hour to assure completion.

The deuterated choline chlorides have been irradiated and their G values

$$\left(\frac{\text{molecules decomp}}{\text{100 ev absorbed}} \right)$$

determined. Table I records these results.

Table I

Radiation sensitivity of normal and deuterated choline chloride

Compound		G value*
Choline chloride,	$[(CH_3)_3NCH_2CH_2OH]^+Cl^-$	75
α-d analog,	$[(CH_3)_3NCH_2CD_2OH]^+Cl^-$	30
β-d analog,	$[(CH_3)_3NCD_2CH_2OH]^+Cl^-$	67
Me-d analog,	$[(CD_3)_3NCH_2CH_2OH]^+Cl^-$	80

*Each sample received 2.5×10^6 rad of Co^{60} γ-radiation during a period of 13 minutes.

The data of Table I show that the α-deuterated compound is significantly less radiation-sensitive than the other analogs. This indicates an involvement of the α position in the transition state of either the propagating or terminating reaction. Kinetic measurements should permit a decision as to which type of reaction is involved.

The electron-spin resonance (ESR) derivative spectra of some irradiated analogs of choline chloride are shown in Fig. 1. The spectra have been divided into three groups on the basis of their hyperfine structure. The choline chloride and the propyl analog $[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$ have similar hyperfine structure (Fig. 1a). The resolution of the propyl analog is somewhat better than that of choline. The same type of structure as proposed for the radical ion of choline--that is, a substituted, electron-deficient ethylene

[1] Robert O. Lindblom and Richard M. Lemmon, in Chemistry Division Quarterly Report, UCRL-8141, Dec. 1957, p. 69.

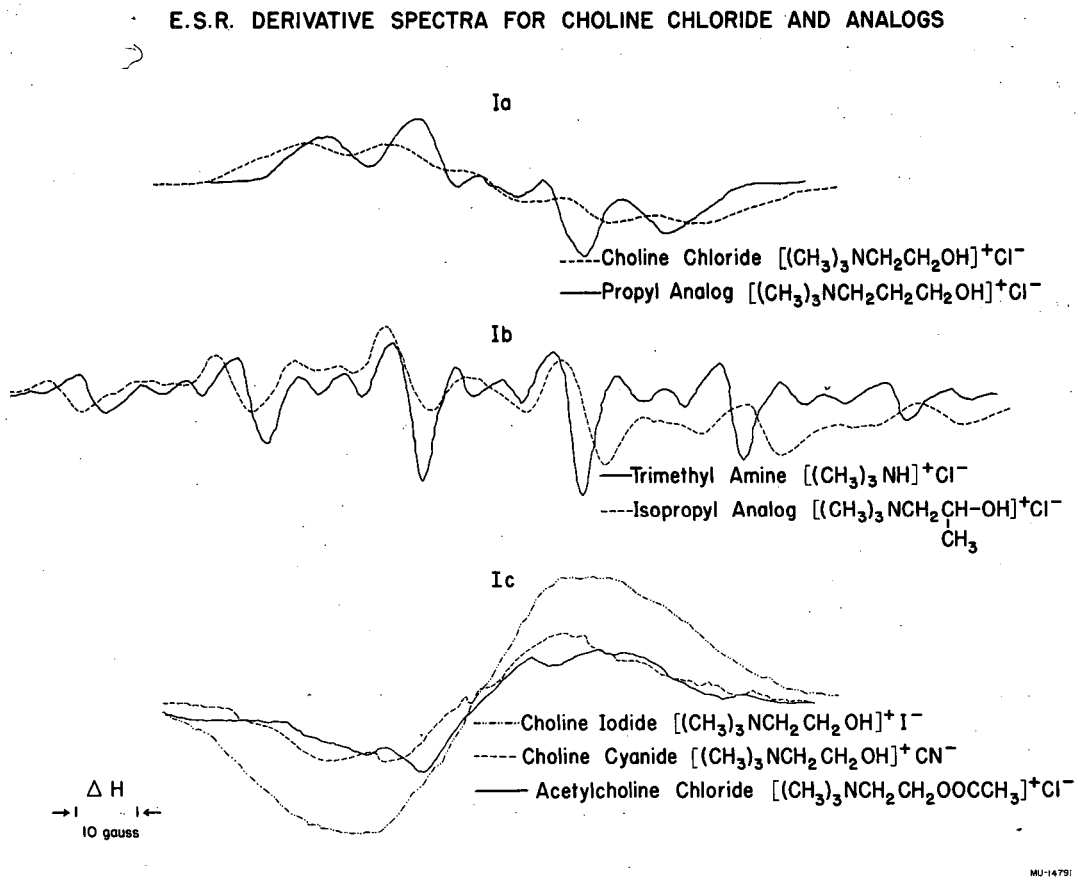

Fig. 1. E.S.R. derivative spectra for choline chloride and analogs.

oxide--is compatible with the propyl analog signal. Another interesting feature of the propyl-analog ESR signal is its long half life. Whereas the choline chloride signal has a half life of 2 to 3 hours, the propyl analog has a half life of about 24 hours. Figure 1b shows the ESR derivative spectra of irradiated trimethylamine hydrochloride and the isopropyl analog

$$[(CH_3)_3NCH_2\text{-}\overset{H}{\underset{CH_3}{C}}\text{-}OH]^+Cl^-.$$

Although the isopropyl analog shows somewhat greater splitting, the hyperfine structures have striking similarities. Both have six major peaks with minor peaks occurring before and after each major peak. The half life of trimethyl ammonium chloride is several weeks, and that of the isopropyl analog is 4 to 5 hours. It is not obvious what radical gives this structure. However, in order to account for the large number of lines and the relatively small amplitude ratios between lines, we must conclude that it involves a nitrogen atom.

Figure 1c shows the ESR spectra of the iodide and cyanide salts of choline, and of acetylcholine chloride. Of these only the acetylcholine chloride shows any suggestion of hyperfine structure. The over-all splitting is about the same as for choline chloride. The lack of fine structure makes speculation on the radical structure difficult.

An interesting dose-saturation effect has been observed in our ESR studies. Table II summarizes our observations to date.

Table II

Dose saturation effect

Sample	Dose (megarad)	Magnetic sweep modulation (gauss)	ESR derivative signal height	Ratio of ESR signals
Choline chloride	7.4	0.7	3.6	1.5
	22.2	0.7	5.4	
Isopropyl analog	7.4	7.0	6	1.16
	22.2	7.0	7	
Propyl analog	7.4	7.0	7.6	1.32
	22.2	7.0	10	

The choline- and isopropyl-analog spectra were determined shortly after irradiation. The propyl analog was determined 2 weeks later. If a linear relationship exists between dose and radical production, there should be a ratio of 3.0 for the ESR signal heights. The highest value found was 1.5. Dose-saturation effects have been reported for irradiation-induced F centers in alkali halide crystals.[2]

[2] F. Seitz, Revs. Modern Phys. 18, 384, (1946).

The saturation effect can be explained by a crystal model in which crystal imperfections act as electron traps. In crystals like choline, in which anions and cations differ appreciably in size, Frenkel defects are the common types. This name is applied to a crystal defect in which an ion is in an interstitial instead of a lattice position. With choline chloride the chloride ion is the small ion, and when it reaches an interstitial position it leaves behind in the lattice a negative-ion vacancy, or positive hole, which can act as a electron trap. In terms of this model the dose-saturation effect occurs when all the traps are saturated and further electrons can only return to and neutralize ion radicals. This model also can explain the variation of G values with the rate of irradiation. Table III summarizes some data from previous work[3] plus some present work with a more intense Co^{60} γ ray.

Table III

G Values for choline chloride irradiation

Radiation	Time of irradiation	G value
C^{14} β's	Several months	1780
$Co^{60}\gamma$'s	10 hours	160
$Co^{60}\gamma$'s	13 minutes	70
3-Mev e^-'s	5 minutes	23

These data can also be explained by the effect of the electron traps. The lower rate of applied radiation allows the traps to operate well below saturation and make efficient use of the radiation. A further test of this model through correlation of the conductivity and ESR radical signals is planned.

[3] Lemmon, Parsons, and Chin, J. Am. Chem. Soc. 77, 4139 (1955).

ISOTOPE EFFECT IN THE DECARBONYLATION OF BENZOYLFORMIC ACID

Bernice Fingerman and Richard M. Lemmon

In a previous report we have described the experimental techniques and preliminary results in the determination of a C^{12}-C^{14} isotope effect in the decarbonylation of benzoylformic acid.[1] We have employed the method of collecting successive fractions of product CO (as CO_2) and observing their specific activities in comparison with the initial specific activity of the starting benzoylformic acid. In this way, we have observed a "positive" or "normal" isotope effect; i.e., in the rate-determining step, bonds involving C^{12} of the carboxyl group are breaking more easily than bonds involving C^{14}. As also might be expected for such an effect, successive fractions of CO_2 showed increasing specific activities.

Several small modifications of experimental technique previously described[1] have enhanced the reproducibility of the data. First, the helium flow was introduced into the reaction flask through a side arm just above the level of the reaction mixture. Secondly, the flow of helium was increased from 4 cc/min to 65 cc/min, yet CO_2 still efficiently condensed in the liquid nitrogen-cooled traps. Both techniques assured that CO_2 would be carried off immediately as it was produced by the reaction mixture. Thirdly, the CO_2-sample volumes being measured in the manometer were first passed through a spiral trap immersed in dry ice–isopropyl alcohol for drying. This was necessary because even a small amount of water vapor (pressure of a few millimeters of Hg) would cause significant inaccuracies in quantitative measurement of small samples of CO_2.

Nine decarbonylation experiments were performed and the data from this work are shown graphically in Fig. 2. The "% Decarbonylation" figures presented are the median percentage figures, e.g., the specific activity of the fraction collected between 10 and 30% decarbonylation is plotted at 20% on the abscissa. The straight line that appears on the graph was obtained, from only the data out to 65%, by the method of least squares. This line meets the ordinate at a specific activity of 0.328 μC/mmole. This value represents the specific activity of the first minute fraction of carbon monoxide, and contrasts with the value of 0.366 for the specific activity of the benzoylformic acid. The measured C^{12}-C^{14} isotope effect is therefore $(0.366-0.328)(100)/0.366 = 10.4\%$. This means that the probability that $C^{12}O$ will be evolved from $C_6H_5COC^{12}O_2H$ is 10.4% higher than the probability that $C^{14}O$ will be evolved from $C_6H_5COC^{14}O_2H$.

At present, theoretical calculations of isotope effects remain only roughly approximate, owing to the lack of information about vibrational-energy levels in the transition states of these reactions. Most of the C^{14}, C^{12} isotope effect studies, both theoretical and experimental, have involved the breaking of a C-C bond as the rate-determining step. However, the

[1] B. Fingerman and R. M. Lemmon, in Chemistry Division Quarterly Report, UCRL-3950, Sept. 1957, p. 76.

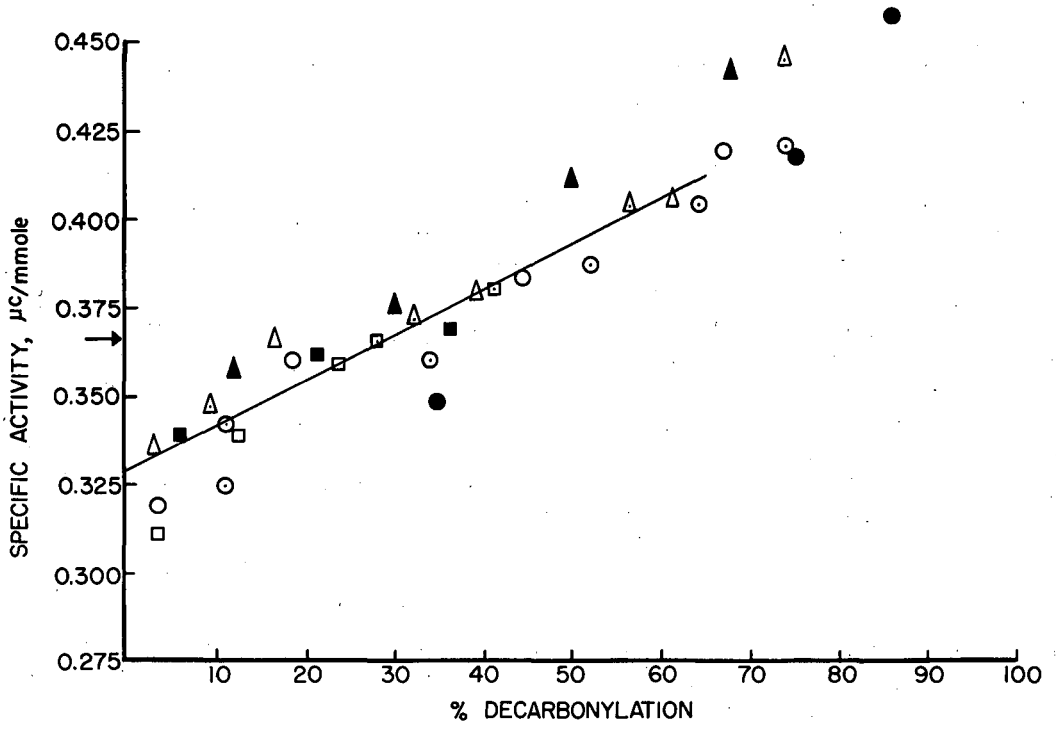

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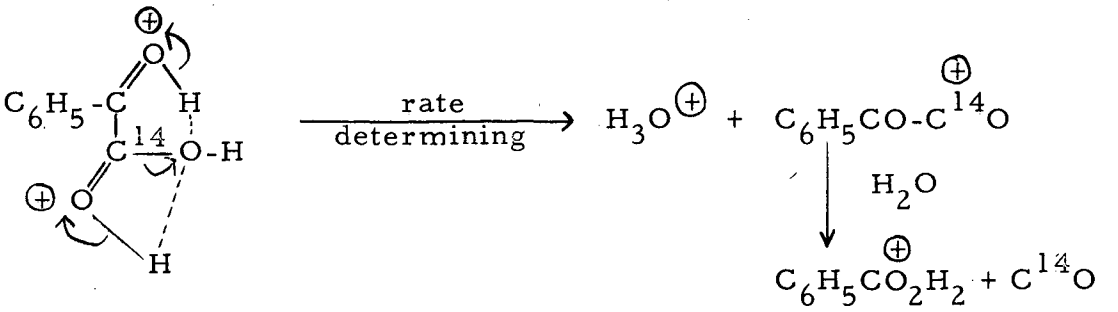
Fig. 2. Isotope effect in the decarbonylation of $C_6H_5COC^{14}O_2H$ (arrow indicates the initial specific activity of the benzoylformic acid).

work of Elliot and Hammick[2] and of Banholzer and Schmid[3] has shown the rate-determining step in this reaction to be as follows.



Significantly, it is the breaking of a C-O bond, not a C-C bond, at the labeled site that must account for the observed isotope effect.

[2] W. W. Elliot and D. Hammick, J. Chem. Soc. 1951, 3402.

[3] K. Banholzer and H. Schmid, Helv. Chim. Acta 39, 548 (1956).

THE DISTRIBUTION OF RADIOACTIVITY IN TOLUENE FORMED FROM BENZENE AND PHOTOLYZED DIAZOMETHANE-C^{14}

Richard M. Lemmon and Walter Strohmeier

A number of reports have appeared recently describing the irradiation of organic compounds with recoiling C^{14} atoms, derived from the reaction, $N^{14}(n,p)C^{14}$,[1] or with C^{14} ions in a mass spectrometer.[2] These reports have heightened interest in and speculation on the kind of interaction that is taking place when the "hot" atom or ion is reduced in energy to the point (probably 25 to 50 ev) at which formation of a chemical bond can take place.

Wolf and co-workers have shown that when toluene is formed from the irradiation of benzene with recoiling C^{14} atoms, not all the C^{14} activity is present in the methyl group.[3] About 86% of the activity was found in the methyl group and the remaining 14% in the ring. A similar result was obtained for o-xylene formed from the C^{14}-atom irradiation of toluene.

Doering and Knox photolyzed diazomethane in the presence of benzene and formed toluene and cycloheptatriene.[4] Wolf has concluded that the recoiling C^{14} atom may behave similarly to Doering and Knox's "carbene" (CH_2:) but that the recoiling atom, having a much larger energy associated with it, may be able to form additional products.[3] The purpose of the work reported here was to photolyze C^{14}-labeled diazomethane (using both visible and uv light) in the presence of benzene, and to determine the distribution of radioactivity between the methyl group and the ring.

---

[1] Wolf, Redvanly, and Anderson, J. Am. Chem. Soc. 79, 3717 (1957) have thoroughly reviewed this type of experiment.

[2] Lemmon, Mazzetti, Reynolds, and Calvin, J. Am. Chem. Soc. 78, 6414 (1956).

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

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

Experimental Procedure

N-methyl-N-nitrosourea-methyl-C^{14}

Labeled methylnitrosourea was prepared according to the reactions

$$BaC^{14}O_3 \xrightarrow[640^\circ]{K,\ NH_4Cl} KC^{14}N, \qquad (1)$$

$$HC^{14}N \xrightarrow[Pt,\ H^+]{H_2,\ 3\ atm.} C^{14}H_3NH_2, \qquad (2)$$

$$C^{14}H_3NH_2 + H_2NCONH_2 \xrightarrow{HONO} C^{14}H_3(ON)N\text{-}CO\text{-}NH_2 . \qquad (3)$$

The labeled cyanide was prepared according to the method of Sixma et al.[5] The conversion of the cyanide to diazomethane followed, in general, the methods described by Jones and Skraba.[6] The specific activity of the labeled nitroso-methylurea used in the benzene experiments was 124 μC/mmole.

Benzene-$C^{14}H_2N_2$ Irradiations

To 250 ml of reagent-grade benzene in a 500-ml flask was added about 40 ml of a 45% aqueous solution of KOH. The flask was cooled in an ice bath and a mixture of 36.5 mg of the labeled methylnitrosourea and 15.3 g of inactive N-methyl-N-nitroso-N'-nitroguanidine was added slowly. The yellow benzene solution of diazomethane was decanted into another flask and the aqueous residue washed with 60 ml of benzene. The combined benzene solutions were dried for one hour with KOH pellets. The concentration of diazomethane was determined by titrating against benzoic acid. The yields of diazomethane varied from 75 to 80% and the average concentration in benzene was approximately 0.25 mole per liter.

Approximately 300 ml of the C_6H_6-$C^{14}H_2N_2$ solution was placed in a 500-ml Pyrex flask. Most of the air was removed from the flask by a stream of nitrogen. Nitrogen generated during the reaction was allowed to escape through a mercury valve which protected the flask's contents from atmospheric oxygen. The flask was irradiated with two 275-watt General Electric Reflector Sunlamps, one on each side. The distance between the lamps and the flask was about 20 cm. During the irradiation the flask and its contents were kept at approximately 20° by a stream of water running over the outside of the flask. After eighteen hours of irradiation the solution was colorless and no further evolution of nitrogen could be observed.

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[5] F. L. J. Sixma, H. Hendricks, K. Helle, U. Hollstein, and R. Van Ling, Rec. trav. chim. 73, 161 (1954).

[6] A. R. Jones and W. J. Skraba Science 117, 252 (1953).

The ultraviolet irradiations were performed in the same way except that the solution was contained in a quartz flask, and the two sunlamps were replaced by a water-cooled 1-kw General Electric A-H-6 mercury vapor ultraviolet lamp. This lamp was also 20 cm from the reaction flask.

Separation of Reaction Products

After the irradiation the bulk of the benzene was distilled through a 15-plate vacuum-jacketed column (Glass Engineering Laboratories). From the initial 300 ml of solution, all but about 10 ml were distilled at a boiling temperature of 80°. The residue was then separated into toluene and cycloheptatriene fractions by distillation on a Podbielniak "whirling heli-band" fractionating column. The fractions were nearly quantitatively pure at this point. The fractions were further purified, and their identities unequivocally established, by gas-liquid partition chromatography (Wilkens "Aerograph", 5-ft column, polyethylene oxide substrate, 86°, He flow 30 to 50 cc per minute).

Determination of C^{14} Activity

The amounts of radioactivity in the purified toluene and cycloheptatriene fractions were determined by direct addition to a liquid scintillation solution (2,5-diphenyloxazole in toluene). The scintillation counter was described earlier.[7]

Distribution of Radioactivity in Toluene

The purified, labeled toluene was diluted with inactive, reagent-grade toluene to give a specific activity of 1.04×10^5 disintegrations/min/mmole. The toluene was then converted into benzoic acid by the addition of aqueous permanganate.[8] In a reaction flask equipped with a heater, thermometer, magnetic stirrer, and reflux condenser were placed 3.1 ml of the toluene and 200 ml of water. The contents were heated to 95° and, with vigorous stirring, 9.2 g of $KMnO_4$ was added in small portions. At the end of 10 hours, the solution was practically colorless. The MnO_2 was filtered from the hot solution, and benzoic acid was precipitated by cooling the solution to 10° and adding about 30 ml of conc. HCl. The benzoic acid was collected by filtration and purified by crystallization from about 60 ml of water. Its melting point was 122° and its elementary analysis was:

Calcd. for $C_7H_6O_2$: C, 68.84, H, 4.95. Found: C, 68.72; H, 5.09.

The specific activity was determined by combustion to $C^{14}O_2$ and counting in an ionization chamber.[9, 10] It was found to be 1.04×10^5 disintegrations/min/mmole. The benzoic acid was then recrystallized in the same fashion and the specific activity redetermined. Its activity was unchanged.

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

[8] F. Ullmann and J. B. Uzbachian, Ber. deut. chem. Ges. 36, 1797 (1903).

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

[10] B. M. Tolbert, Ionization Chamber Assay of Radioactive Gases, UCRL-3499, March 1956, p. 32.

About 600 mg of the benzoic acid was quantitatively decarboxylated by heating at 400° for 3 hours with 200 mg of cadmium powder in an evacuated tube.[11] The benzene and CO_2 were separted, the CO_2 volume measured, and the CO_2 transferred into an ionization chamber by the use of the apparatus shown in Fig. 3. The decarboxylation tube A was attached to the vacuum line and cooled to -196°; the entire system was then evacuated. Traps B and D were cooled by liquid nitrogen and trap C was cooled in a dry ice-isopropyl alcohol bath. Stopcocks 1, 2, 3, and 5 were closed, the decarboxylation tube was broken by moving the ball joint, the liquid nitrogen bath was removed from Tube A, and the benzene and CO_2 were collected in Trap B. Stopcock 5 was opened and pumping was continued for a few minutes (to remove traces of CO formed during the decarboxylation). Stopcock 5 was closed, the liquid nitrogen bath removed from Trap B, and benzene collected in Trap C while CO_2 collected in Trap D. Trap C was closed and the CO_2 volume was measured by removing the bath from trap D and allowing the CO_2 to expand into the previously measured volume between Stopcocks 3 and 5. The CO_2 was then recondensed back into Trap D; this trap was then removed from the line and the CO_2 transferred into an ionization chamber for the radioactivity determination. The benzene in Trap C was then transferred into Trap E, which was removed from the vacuum line. The benzene was purified by two passages through a gas-liquid partition chromatographic column ("Aerograph," 5-ft column, paraffin substrate) and its radioactivity measured by liquid-scintillation counting.

Rearrangement Tests

In order to determine whether cycloheptatriene would rearrange into toluene under the conditions of the reaction, the following experiments were performed:

(a) Chromatographically pure cycloheptatriene (0.3 ml) was sealed in a Pyrex tube and heated at 130° for 50 hours. An aliquot portion was then chromatographed (polyethylene oxide) under conditions that would permit the detection of 0.5% of toluene. No toluene could be detected.

(b) Two other portions of cycloheptatriene were sealed in separate tubes; one tube (Pyrex) was irradiated with sun lamps for 60 hours, the other (quartz) with the ultraviolet lamp for the same time. In neither case could any toluene be detected (i.e., any toluene formed was less than 0.5% of the cycloheptatriene).

Results and Discussion

Table IV presents the results obtained from typical experiments using either the sun lamps or the ultraviolet lamp. The data were obtained from 600 ml of a solution of diazomethane (0.25 molar) in benzene. This solution was divided into two equal parts for the two irradiations; the irradiations were performed concurrently.

[11] W. Moser, Helv. Chim. Acta 14, 971 (1931).

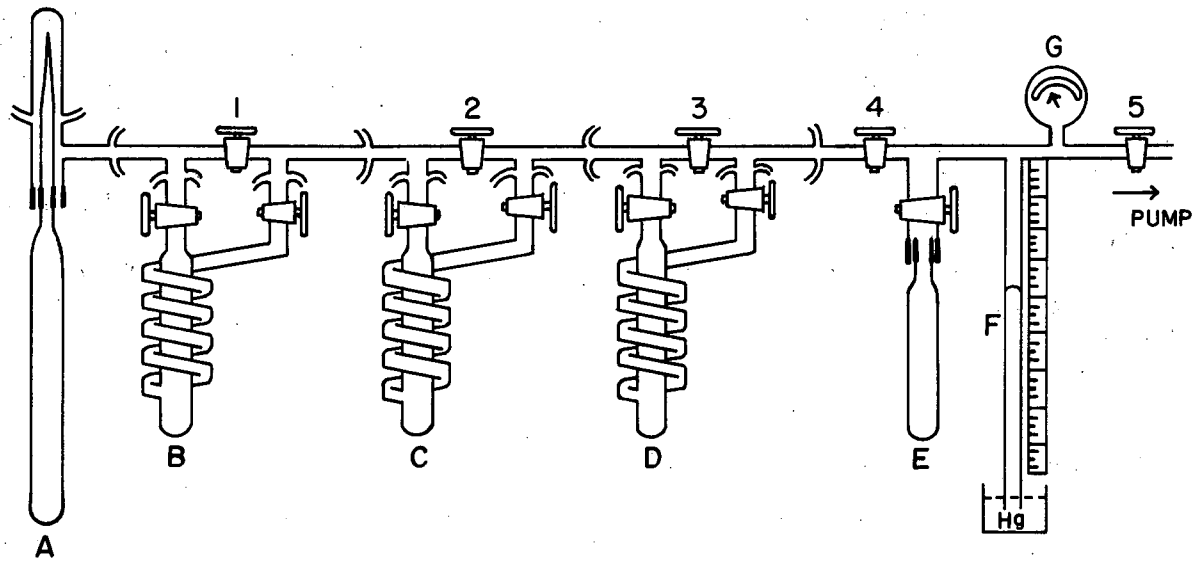

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Fig. 3. Vacuum line for transfer of products from benzoic acid decarboxylation.

A. Combustion tube
B. Benzene and CO_2 trap
C. Benzene trap
D. CO_2 trap
E. Benzene trap
F. Manometer
G. Vacuum gauge

Table IV

Results of visible- and ultraviolet-light irradiations of solutions of diazomethane-C^{14} in benzene

	Sunlamp	Ultraviolet lamp
Percentages of Total Activity:		
$C^{14}H_2N_2$-C_6H_6 soln., before irrad.	100	100
$C^{14}H_2N_2$-C_6H_6 soln., after irrad.	82	83
Residue after removal of C_6H_6	66	66
Cycloheptatriene/toluene ratio	3.5	3.5
Sp. act. cycloheptatriene, dpm/mmole	9.4×10^5	9.4×10^5
Sp. act. toluene, dpm/mmole	9.3×10^5	9.3×10^5
Toluene decarboxylation:		
Sp. act. toluene (after dilution), dpm/mmole	1.43×10^5	1.04×10^5
Sp. act. benzoic acid, dpm/mmole	1.42×10^5	1.04×10^5
Sp. act. CO_2, dpm/mmole	1.39×10^5	1.01×10^5
Sp. act. benzene, dpm/mmole	$<2.0 \times 10^2$	$<1.5 \times 10^2$
C^{14} in methyl group	> 99.8%	>99.8%
C^{14} in ring	< 0.2%	< 0.2%

The data of Table IV show no detectable difference between the visible and ultraviolet light irradiations.

The loss of about 18% of the radioactivity during the irradiations probably reflects a combination of "carbene" radicals to form ethylene and other low-molecular-weight hydrocarbons. More of this volatile material was removed during the benzene distillation, the bulk of it coming over in the first 50 to 100 ml. Gas chromatography (on polyethylene oxide) of the distilled benzene showed the presence of at least two compounds with retention times less than that of benzene. The same compounds appear to be present in both types of irradiations. No compounds other than toluene and cycloheptatriene were detectable (by gas chromatography) after the removal of the benzene.

In the ultraviolet irradiations reported here, the "carbene" from the photolyzed diazomethane may have an imparted energy as high as 5 ev (corresponding to the radiation of around 2000 Å transmitted through the quartz). Such an energy appears to be not high enough to permit the "carbene" to accomplish what a recoiling C^{14} atom, or radical, can accomplish--namely, to introduce

activity into the benzene ring of toluene. These experiments therefore indicate a lower limit of energy, i.e., the recoiling C^{14} species probably has an energy above 5 ev when it interacts "chemically" with benzene.

LUMINESCENCE OF GREEN-PLANT MATERIALS

Gordon Tollin and Eiji Fujimori

We have recently been attempting to revise the apparatus used in the measurement of green-plant luminescence.[1] The purpose of this revision was threefold:

(a) To achieve a system capable of observing the delayed light emission a few milliseconds after the flash excitation (rather than after the 0.1- to 0.2-second lapse previously necessary between flash and observation).

(b) To achieve a system capable of observing simultaneously the millisecond range, the tenth-of-a-second range, and the second range.

(c) To achieve a better signal-to-noise ratio to permit more accurate decay-curve measurements.

The basic concept behind the revision involved the use of a flash tube with a much shorter pulse duration than the General Electric FT-503 tube used previously. It was then planned, in the manner described below, to close off the photomultiplier during the flash. In addition, filters would be used to minimize the light incident on the photomultiplier and thus prevent any saturation phenomena. The flash tube chosen was the G. E. FT-230, which--according to the specifications supplied by the manufacturer--has a light output that decays to about 5% of its peak intensity in about 5 microseconds. However, even after many revisions in the flash-tube circuitry to achieve optimum conditions, the shortest pulse we could obtain still required about 300 μsec to decay to 5% of its peak output. Furthermore, when the flash is observed with a photomultiplier under high gain conditions, a substantial continuing output (or "tail") of light can be observed out to about 30 milliseconds. Thus, the main problem was to reduce the light scattered into the photomultiplier in order to minimize the amount of "tail" observable under experimental conditions. To do this we used two blue filters (Corning Nos. 5113 and 4303) and a Corning special infrared filter between the flash and the sample, and a red filter (Corning No. 2030) plus another infrared

[1] Gordon Tollin and M. Calvin, The Luminescence of Chlorophyll-Containing Plant Material, UCRL-3863, July 1957.

filter between the sample and the photomultiplier. In addition, all possible scattering surfaces were blackened. In this way, with a proper gate duration, it was possible to begin observation of the luminescence 2.5 msec after the beginning of the flash.

The flash is operated at 2000 volts with a 32-μf load capacitor. The triggering system is standard. All electrical leads were kept as short as possible to achieve optimum pulse-transmission characteristics.

The photomultiplier is closed or gated by applying a 400-v pulse to the first dynode, thus making it negative with respect to the photocathode. This serves to prevent most of the electrons liberated at the cathode from being multiplied in the tube. The duration of this gating pulse is continuously variable from a few μsec to about 5 msec. The gate is also used to start the sweep of a Tetronix-514 oscilloscope. A delay of about 500 μsec is introduced between the start of the gate and the triggering of the flash, to make certain that the gate is fully operative before the light commences. This arrangement permits elimination of the camera shutters used in the previous apparatus.

The photomultiplier anode is supplied with a 2-megohm load resistor and is fed directly into a Sanborn dc preamplifier. This arrangement eliminates the dc electrometer used previously. This achieves about a fivefold improvement in signal-to-noise ratio, as most of the noise in the previous arrangement was introduced in the electrometer via microphonics. The preamplifier supplies the oscilloscope, the Sanborn recorder, and a Leeds and Northrup "Speedomax" recorder (which has been provided with a separate zeroing control and a variable gain control). It is thus possible to record the decay curve of the luminescence from 2.5 msec to 60 sec after the flash.

A block diagram of the apparatus is shown in Fig. 4.

The sequence of events is as follows. A pushbutton activates the photomultiplier gate, which gates off the photomultiplier and simultaneously triggers the oscilloscope sweep. Five hundred microseconds later the flash is fired and 2.5 msec later the gate turns off and the photomultiplier is then ready to observe light from the sample.

To date we have been able to detect two luminescences in Chlorella which we were not able to observe previously. These emissions have half lives of 2 to 3 msec and 30 to 50 msec, respectively. The 2-to-3- msec emission seems to be independent of temperature down to about -150°C. This suggests that it may reflect the lifetime of one of the very early physical intermediates in the quantum-conversion process.

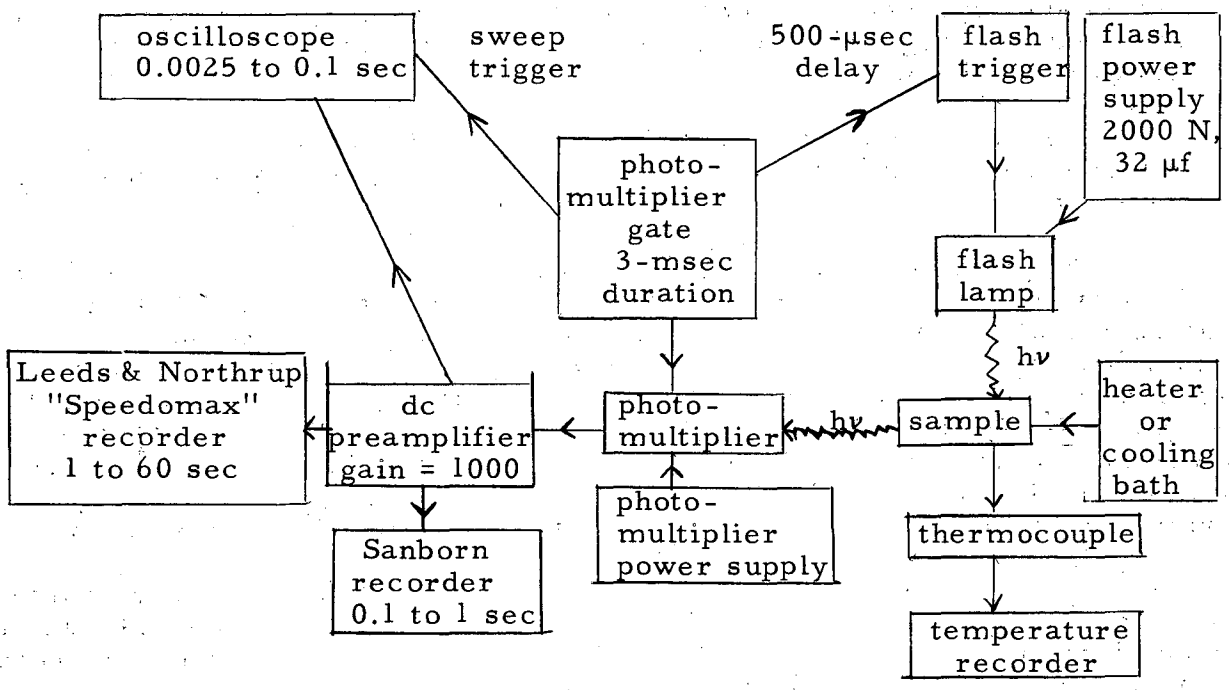


Fig. 4. Apparatus for measuring green-plant luminescence.

BIOSYNTHETIC STUDIES ON MORPHINE ALKALOIDS

Henry Rapoport, Don R. Baker, and Melvin Look

Papaver somniferum (opium poppy) plants are being cultivated under radioactive carbon dioxide for the purpose of studying the path of carbon to the morphine alkaloids in the poppy and of securing randomly labeled radioactive morphine for human metabolism studies. Two different approaches are being used: Short-term cultivation and long-term cultivation, both under radiocarbon.

The morphine alkaloids were extracted and identified in both approaches by the following procedures: The alkaloids were removed from the plants by extraction with methylene chloride. This was followed by extraction of the methylene chloride solution with 0.1 M phosphoric acid. The phosphoric acid solution was adjusted to pH 8.5 and again extracted with methylene chloride to yield, on evaporation to dryness, the crude alkaloid extract. The alkaloids were separated by paper chromatography using 0.2 M potassium dihydrogen phosphate buffered paper and n-propanol-ether-water (2:1:1 by volume) as the developing solvent.

Short-Term Studies

A plant chamber capable of containing nine standard 7-inch flower pots has been described previously.[1] This chamber is shown in Fig. 5. The size of the chamber, however, made it impractical for small-scale one-pot growth studies. For this reason a smaller chamber (Fig. 6), constructed from a battery jar, 18 by 8-3/4 inches, was utilized. Lighting and humidity control are similar to those in the larger chamber.

A total of 191 μC of $C^{14}O_2$ in 50 ml of carbon dioxide was completely taken up by four plants in the plant chamber within a 6-hour period. One plant was then harvested and the three remaining plants were placed in the greenhouse in a special chamber for radioactive plants. One plant each was harvested at the end of 2 days, 5 days, and 9 days.

At the end of the 6-hour period the chromatographic spot of thebaine contained more radioactivity than those of morphine or codeine. The activity in the thebain appears to decrease after the 6-hour period whereas the amount of activity found in the morphine and codeine steadily increases.

Experiments are now being done to determine the specific activities of the alkaloids at various times after exposure to radioactive carbon dioxide.

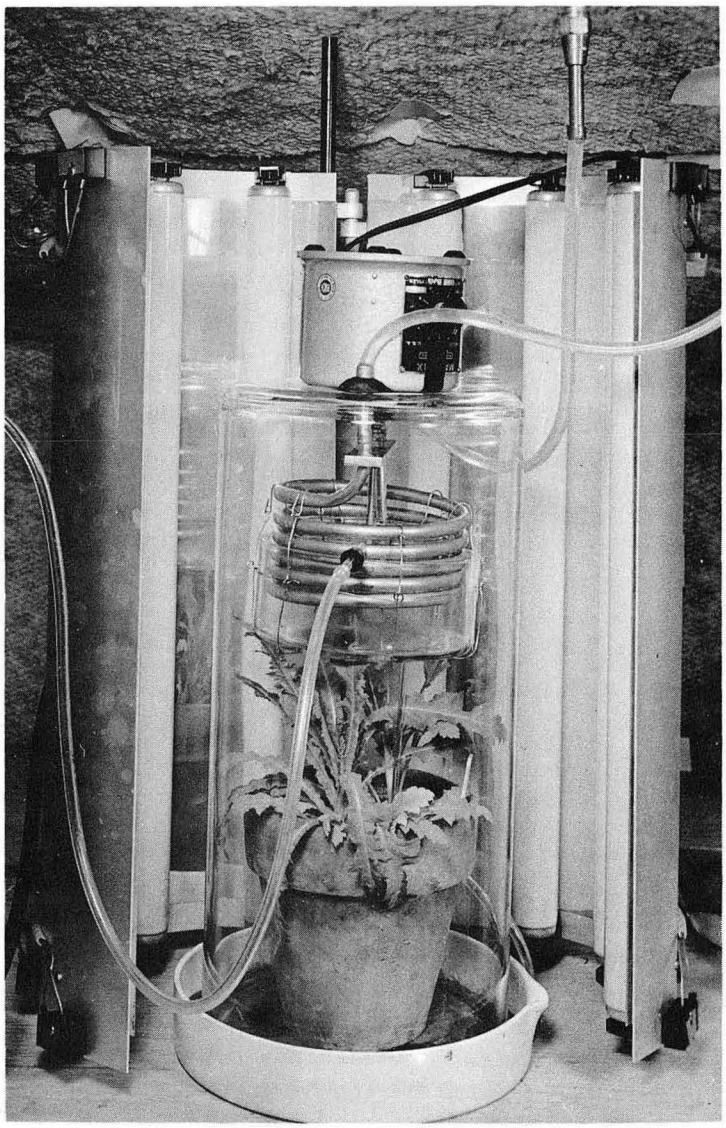
Long-Term Studies

Six 3-month-old plants were grown under a controlled atmosphere of radioactive carbon dioxide for 34 days.[1] Of the original 500 μC to which the plants were exposed, they took up about 300 μC. The plants were extracted (as above), giving an alkaloid fraction containing about 0.67 μC. An aliquot

[1] Don R. Baker and Henry Rapoport, in Chemistry Division Quarterly Report, UCRL-8141, Sept. 1957.

ZN-1897

Fig. 5. Chamber for exposing plants to $C^{14}O_2$.



ZN-1898

Fig. 6. Single-plant chamber for exposure to $C^{14}O_2$.

portion of the alkaloid fraction was chromatographed on paper. Three radioactive alkaloids were detected: morphine, codeine, and thebaine. Thebaine was estimated to be highest in specific activity, codeine was next, and morphine was the lowest. Demethylation studies are in progress on the isolated alkaloids. A second plant cultivation with 4.8 mC of radioactive carbon dioxide is in stages of isolation.

THE EFFECTS OF HYDROXYLAMINE ON THE $C^{14}O_2$ FIXATION PATTERN DURING PHOTOSYNTHESIS

James A. Bassham and Martha Kirk

Evidence for an unstable product or products formed in the early stages of carbon fixation during photosynthesis by algae has been presented by Metzner et al.[1] In an attempt to stabilize, isolate, and characterize such unstable products, Metzner et al. added hydroxylamine to the algae just before, during, or after a short period of photosynthesis with $C^{14}O_2$.[2]

Analysis of the products of such fixation by the usual techniques of paper chromatography and radioautography led to observation of an apparently new radioactive compound on the chromatogram. This compound or "spot" usually appeared in the two-dimensional papers as a thin streak (its long dimension in the phenol direction) somewhere between aspartic and malic acids. Observations that the spot appeared to coincide with one of two fluorescent streaks (sometimes there was a radioactive compound coinciding with each of them), and that it had an ultraviolet absorption peak of 285 mμ, led to the hypothesis that the new compound was a pterine complexed in some way with newly incorporated $C^{14}O_2$.

We have attempted to identify this substance and to elucidate its role in photosynthesis. We have consistently obtained enhancement of C^{14} fixation by the addition of hydroxylamine to the photosynthesizing algae (both Chlorella and Scenedesmus) and have observed the fluorescent spots and a new radioactive spot (hereafter called F') which we believe to be the same as those reported by the previous workers, at least in the two-dimensional chromatograms.

[1] Metzner, Metzner and Calvin, Proc. Natl. Acad. Sci. U.S. 43, 892 (1957).

[2] Metzner, Metzner and Calvin, Proc. Natl. Acad. Sci. U. S. (to be published).

Our results may be summarized as follows:

1. Addition of hydroxylamine hydrochloride—to a concentration of 0.01 M—to the suspension of photosynthesizing algae just before or during their fixation of $C^{14}O_2$ for 5 seconds in the light stimulates the incorporation of C^{14} into compounds that are stable to our usual techniques (paper chromatography, etc.) The increase in radioactivity is seen in the phosphate esters, particularly phosphoglyceric acid.

2. A similar increase in fixation of C^{14} is seen if hydroxylamine hydrochloride is added to the algae suspension prior to or during dark fixation of $C^{14}O_2$ immediately following a period of illumination.

3. Addition of hydrochloric acid alone in an amount producing the same pH(4.8) in the algae suspension resulted (in one experiment) in as much stimulation as addition of hydroxylamine hydrochloride. In the same series of experiments addition of neutralized hydroxylamine caused an inhibition of $C^{14}O_2$ uptake.

4. Addition of hydroxylamine or hydroxylamine hydrochloride with the alcohol at the time of killing sometimes results in a slight increase in fixation and at other times in no increase at all. However, if hydroxylamine hydrochloride is added for as short a time as one second before the algae are killed, there is sometimes a definite increase (around 10% higher than in the controls) in $C^{14}O_2$ fixation.

5. In Cases 1 and 2 there appears to be a decrease in C^{14} incorporated into malic acid.

6. In Cases 1, 2, and 4 the radioactive spot or streak (F') formed in the presence of hydroxylamine and running between aspartic acid and malic acid often but not always coincides with one or the other of the fluorescent spots that run in the same area. Because the radioactive compound and the fluorescent compound do not coincide in every case, it appears that they are not the same substance and that their frequent coincidence is a chromatographic accident, caused perhaps by their both being pushed along in the butanol-propionic acid direction by a third substance, which may be hydroxylamine itself.

7. The radioactive spot, on rechromatography in the same solvents, gives a spot which is phosphoenolpyruvic acid. This identity has been established by cochromatography with phosphoenolpyruvic acid (from California Foundation for Biochemical Research) followed by spraying with the Haynes-Isherwood phosphate spray. It was also established by cochromatography (with pyruvic acid) of the radioactive compound obtained after treating the original spot with phosphatase. The position of the pyruvic acid on the paper was located by treatment with silver nitrate in acetone followed by spraying with ethanolic sodium hydroxide.

8. When the radioactive spot, identified as phosphoenolpyruvic acid and obtained from a photosynthesis experiment with $C^{14}O_2$, is treated with 0.01 M hydroxylamine hydrochloride at room temperature for 1 hour and the resulting solution is concentrated and chromatographed, a radioactive spot is obtained which has the same appearance and location on the chromatogram as the spot F'.

These results lead to the following conclusions:

(a) The stimulation of fixation of $C^{14}O_2$ by hydroxylamine which is most noticeable at very short exposures of the algae to C^{14} is an actual acceleration of one or more steps of the photosynthetic incorporation of CO_2 rather than the stabilization of an unstable intermediate. The entry of $C^{14}O_2$ into the cell seems a likely place at which stimulation may take place.

(b) Hydroxylamine may form a compound or a stable salt with phosphoenolpyruvate, and this compound is stable only in the presence of excess hydroxylamine. Alternatively, hydroxylamine affects the chromatographic behavior of phosphoenolpyruvate in some manner to cause the experimentally observed movement on the chromatogram. Once the excess of hydroxylamine is removed (rechromatography) phosphoenolpyruvate runs to its usual position on the chromatogram.

(c) The decrease in radioactivity found in malic acid may indicate that the hydroxylamine reacts with phosphoenolpyruvic acid in the algae during the period of photosynthesis and thereby inhibits carboxylation of a three-carbon compound.

(d) The fluorescence that often coincides with the radioactive spot is due to a different substance from the radioactive spot.

THE PREINCUBATION OF CARBOXYDISMUTASE WITH METAL IONS

Ning G. Pon, Brian R. Rabin, and Yasuo Natori

Introduction

The preincubation of carboxydismutase with Ni^{++} ions has been described previously.[1] The salient features of these experiments are:

(a) in most cases the activity of carboxydismutase rose sharply to a maximum and gradually decreased as a function of increasing preincubation time;

(b) the activity maximum was dependent on the temperature of preincubation, the nickel ion concentration, and the total volume of the incubation mixture.

Because Mg^{++} ion was found to activate carboxydismutase,[2] it was of particular interest to investigate the effect of preincubation of carboxydismutase with this metal ion. Furthermore, because of the large effect of preincubation on the carboxydismutase activity, the reliability of the general method of assay for this enzymic activity was reinvestigated.

Experimental Procedure

The procedure using the preincubation effect has been described elsewhere.[1,3] However, the studies made in these experiments were carried out under such a variety of conditions that a more detailed description is given here.

Preincubation Experiments

In all experiments the total incubation volume was 200 μl. The carboxydismutase system was made up of $HC^{14}O_3^-$ and ribulose-1,5-diphosphate (RuDP) as substrates, carboxydismutase as the enzyme, and a metal ion as the cofactor. In some experiments cysteine was also added. The enzyme was mixed with the metal ion at the same time as $HC^{14}O_3^-$ if the pH of the complete incubation mixture was near 8. $NaHCO_3^-$ was added first if the pH was nearer 7. At the lower pH the $HC^{14}O_3^-$ was used not only as a substrate, but also as a buffer; at pH 8 tris-(hydroxymethyl) amino-methane (otherwise known as tris) was used as a buffer. The temperature of preincubation was either 0° or 25°C.

The preincubation consisted of this sequence of operations:
At the end of a predetermined time the reaction was started by the addition

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[1] Ning G. Pon, in Chemistry Division Quarterly Report, UCRL-3595, Oct. 1956, p. 52.

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

[3] Ning G. Pon and Brian R. Rabin, in Chemistry Division Quarterly Report, UCRL-3710, March 1957, p. 33.

of RuDP ($HC^{14}O_3^-$ was added where necessary). The incubation mixture was allowed to stand at 25° for 5 or 10 min. The reaction was stopped with acetic acid or with steam heat (or both) for 40 sec. A suitable aliquot portion of this mixture was plated and counted.

For the true "zero-time preincubation" all the components of the carboxydismutase system were mixed together except for the enzyme (i.e., $HC^{14}O_3^-$, RuDP, buffer, and metal ion). The incubation reaction was started by the addition of the enzyme and the reaction was allowed to proceed according to the conditions given above.

The complete details of incubation and preincubation conditions for each experiment are listed in the results (given in Figs. 7 through 10).

Assay of the Carboxydismutase Activity

In order to obtain a reliable assay for any enzyme, one must minimize the number of variables. For this reason the preincubation effect was eliminated by conducting the assay under zero-time preincubation conditions. The reproducibility of such an assay was tested in the following manner:

Reagents: Mg^{++}-tris: 0.2 M $MgCl_2$; 1.5 M tris, pH 8.29

$HC^{14}O_3^-$: 0.0264 N $NaHCO_3$; 400 μc/ml.

RuDP : 10 mg/ml; 55% pure

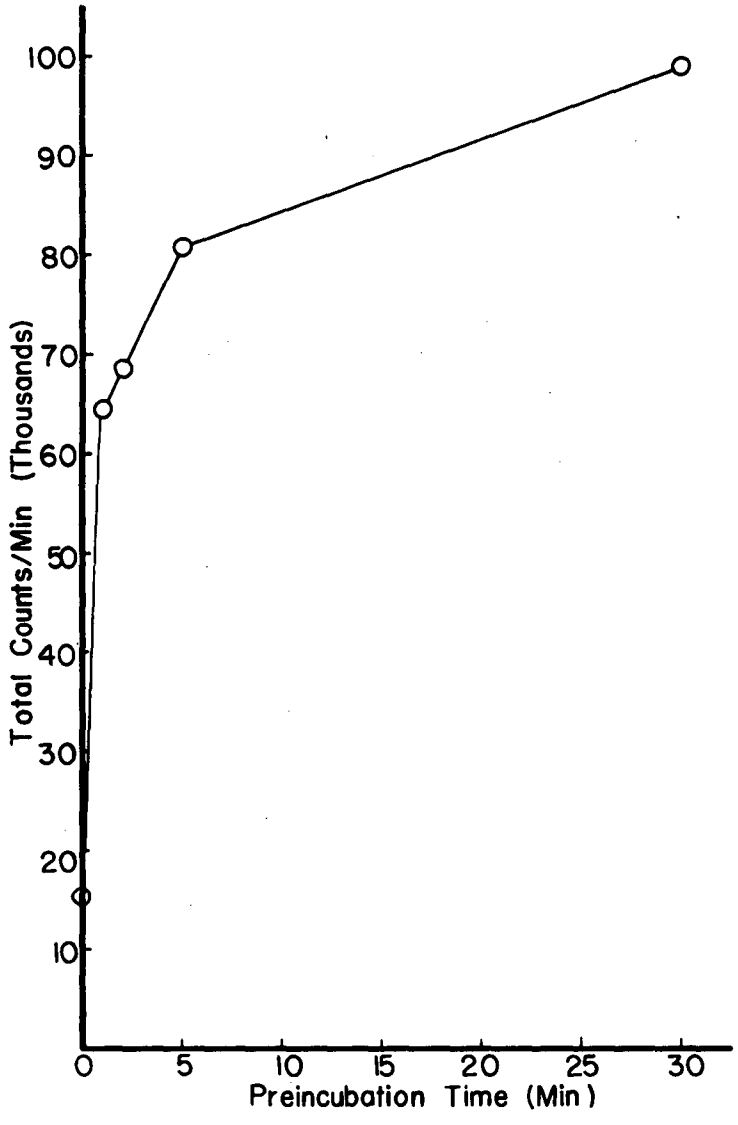
Enzyme : 34-38% saturated ammonium sulfate precipitate (SAS ppt.) of the extract of Tetragonia expansa.[2,4] Conc. ca. 6 mg/ml

Incubation mixture: All components were added in the order listed below.

Components	Volumes Control (μl)	Variable (μl)
H_2O	135	130
Mg^{++}-tris	10	10
$HC^{14}O_3^-$	50	50
RuDP	0	5
Enzyme	5	5

All incubations were carried out at 25° ± 0.2°C for 10 min. At the end of this period 50 μl of 6 N HOAc was added to stop the enzymic activity and to drive off the excess $C^{14}O_2$. Triplicate aliquot portions of 15 μl from each acidified mixture were plated on planchets and counted in a Nuclear-Chicago automatic counter. The counting was performed through three cycles. Incubations were also done in triplicate for both the control and the variable.

[4] Mayaudon, Benson, and Calvin, Biochim. Biophys. Acta 23, 342 (1957).

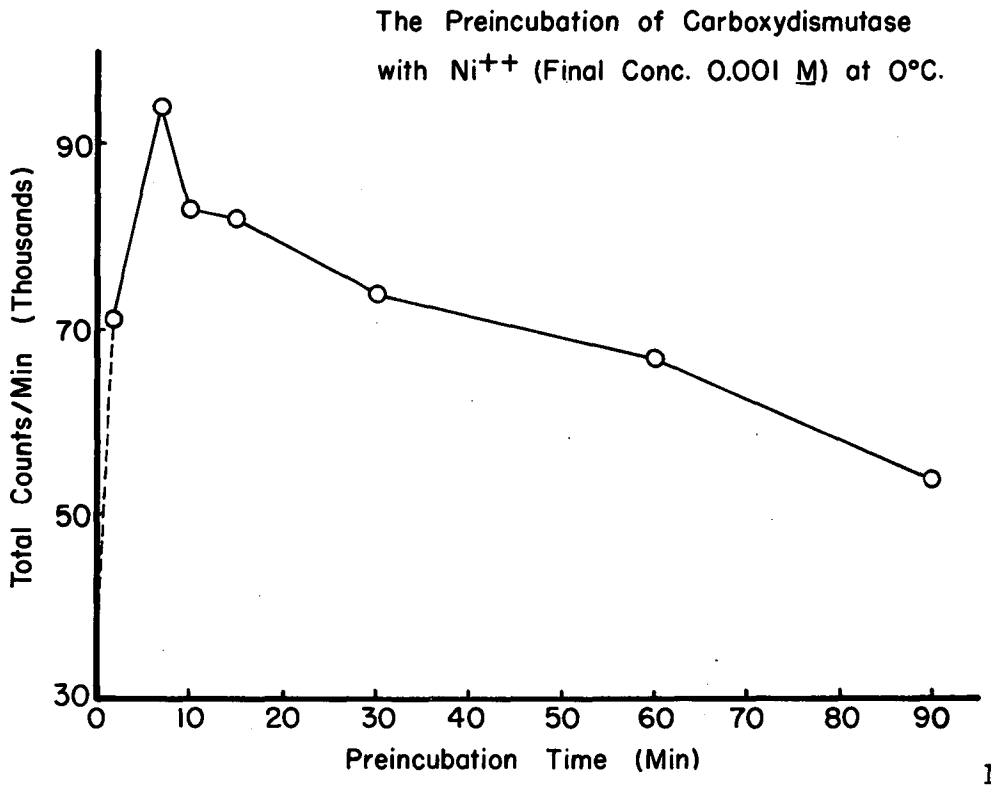


MU-14793

Fig. 7. The preincubation of carboxydismutase with Mg^{++} at $0^{o}C$. Incubation at pH 7.0, $25^{o}C$, for 5 min.

Order of addition of components (and final concentrations):

$HC^{14}O_3^-$ (0.0088 M);
Mg^{++} (9.75×10^{-4} M);
enzyme (0.125 μC/μl) (35 to 50% SAS ppt of chloroplast extract of Tetragonia expansa);
RuDP (2.3×10^{-4} M).



MU-14794

Fig. 8. Preincubation of carboxydismutase with Ni^{++} at $0^{o}C$. Incubation at pH 7.5 to 7.6, $25^{o}C$, for 10 min.

Order of addition of components (and final concentrations):

Ni^{++} (0.001 M);
$HC^{14}O_3^-$ (0.0065 M);
enzyme (0.104 μg/μl) (34 to 38% SAS ppt of crude *Tetragonia expansa* extract[2,4])
RuDP (1.9×10^{-4} M).

The zero-time preincubation point was obtained from a separate experiment (10 days earlier) using the same enzyme preparation. The dotted line connects that point to the first point for this experiment.

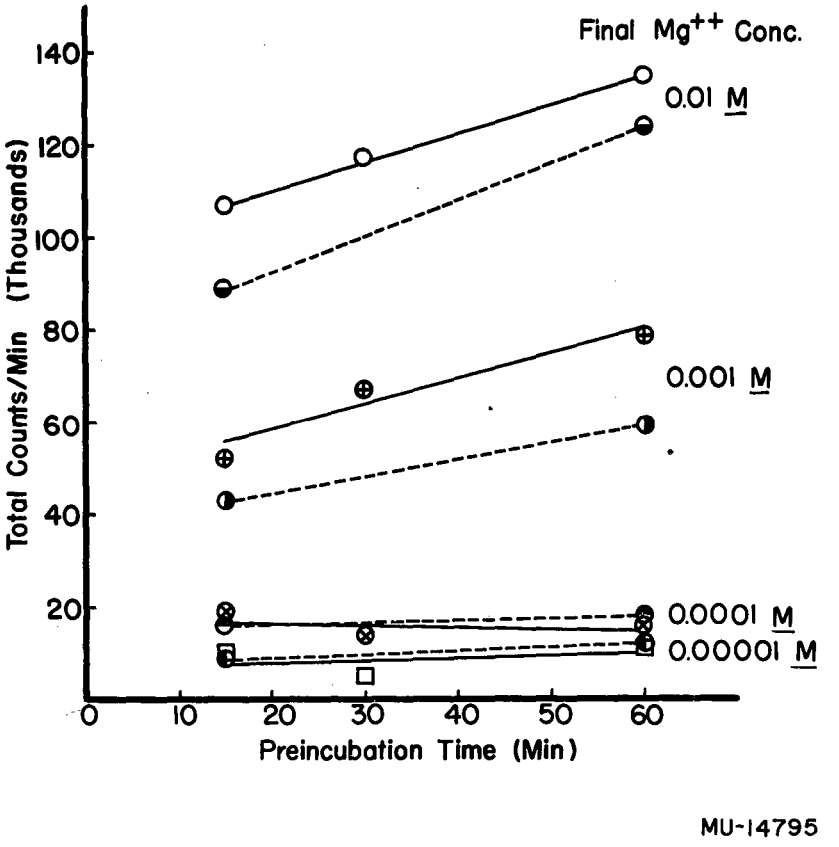


Fig. 9. Preincubation of carboxydismutase with different concentrations of Mg^{++}, with cysteine (solid line) and without cysteine (broken line), at 0°C. Incubation at pH 8.0, 25°C, for 5 min.

Order of addition of components (and final concentrations): H_2O [or cysteine (0.001 M)]; enzyme (35 to 50% SAS ppt of chloroplast extract of *Tetragonia expansa*[3]); Mg^{++} plus tris (0.0025 M in tris; Mg^{++} as shown in graph); RuDP plus $HC^{14}O_3^-$ (2.3×10^{-4} M and 0.0088 M).

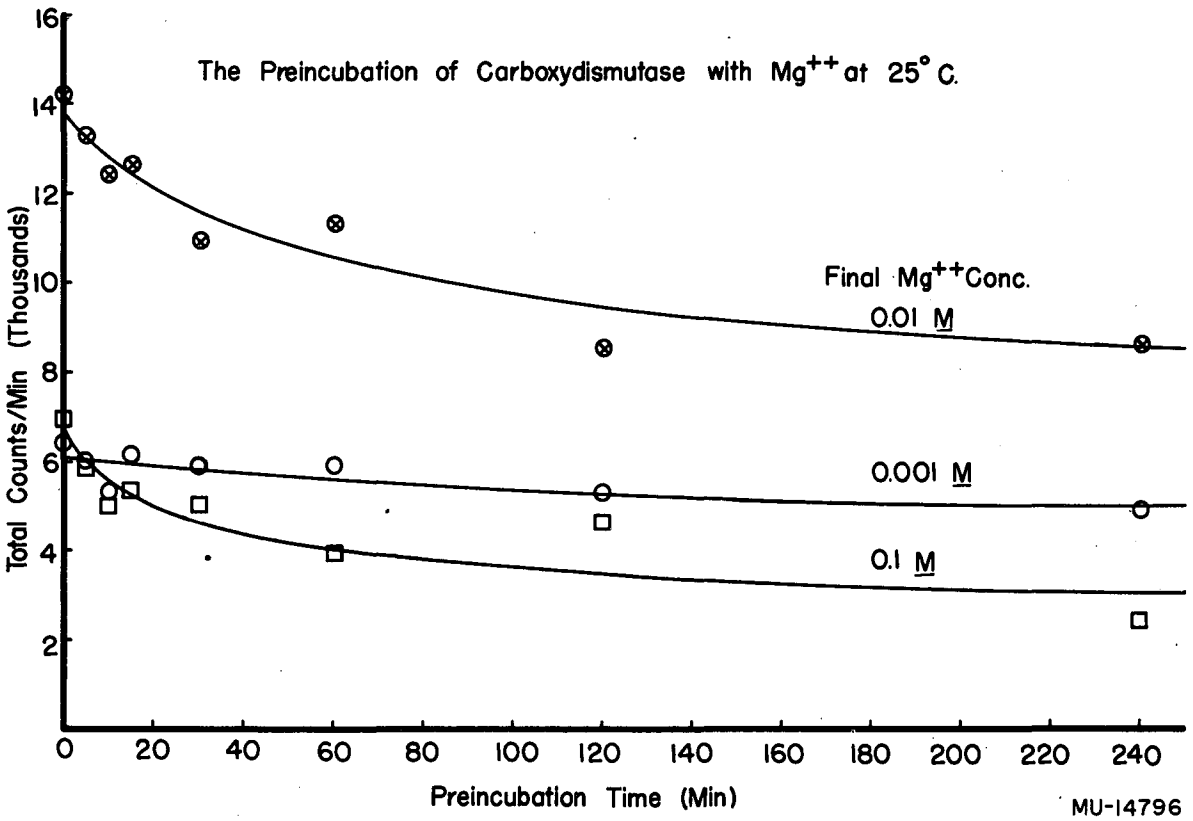


Fig. 10. Preincubation of carboxydismutase with Mg^{++} at 25°C. Incubation at pH 8.0, 25°C, for 10 min.

Order of addition of components (and final concentrations): H_2O;
enzyme (0.025 μg/μl) (35 to 50% SAS ppt of chloroplast extract of Tetragonia expansa[3]);
Mg^{++} plus tris (0.01 M in tris; Mg^{++} as shown on graph);
RuDP plus $HC^{14}O_3^-$ (5×10^{-4} M and 0.012 M).

The incubation mixtures for the controls and the variables were incubated in a more or less random fashion so that any systematic error that might have arisen from such effects as "aging" of substrate or enzyme was eliminated.

Results and Discussion

The results are given in Figs. 7 to 10 and in Tables V to VII.

Preincubation Effect

Figure 7 shows the effect of the preincubation of carboxydismutase with Mg^{++} at 0°. In contrast to the preincubation of the enzyme with Ni^{++} at the same temperature (see Fig. 8), Mg^{++} does not appear to inactivate the enzyme during the observed period of preincubation. The dependence of the activity of carboxydismutase during the preincubation on different concentrations of Mg^{++}, and with and without cysteine, is shown in Fig. 9. The prominent features of this experiment are: 1) the activation by cysteine is apparent only at Mg^{++} concentrations of 0.001 M and 0.01 M, 2) the preincubation at these concentrations of metal ion still increases the activity of the enzyme even after 60 min., and, 3) little activation, if any, is obtained during the preincubation at very low concentrations of Mg^{++}. Since the activity of the enzyme still increases after 60 min. preincubation at 0°C., the preincubation was carried out at 25°C. The results are given in Fig. 10. There appears to be no activation; instead a slow inactivation of the enzyme is evident at all concentrations of Mg^{++}. It should be noted here that the point at "zero minute" preincubation was not actually at zero time, but at 15 sec. The possibility that maximum activation might have been reached within the first 15 sec. of preincubation at 25°C. was considered. To show this effect a true zero-time preincubation effect was compared against the 30- and 60-min. preincubation effects (see Table V). From the results in this table it is evident that there is an activation of the enzyme which is due to preincubation. The data shown from Fig. 4 and Table I suggest that the time required for maximum activity is of the order of seconds.

Reproducibility of the Enzyme Assay

The results of the test for the reliability are summarized in Tables VI and VII. When an over-all average is taken of either the control series or the variable series, the mean error of the assay is not more than ± 3% from the mean value. In fact, a close scrutiny of the counting data indicated that a large error was due to counting (see, for example, the control series). This error can be reduced by increasing the total number of counts to be recorded. Thus, if one compares the deviations obtained during the counting of the control series with those of the variable series, it is found that the maximum deviation of the mean value is ± 12% for the former series whereas for the latter series it is ± 2%.

Table V

The preincubation effect of Mg^{++} (0.001 final conc.) on the carboxydismutase system. Incubation time, 10 min. Activity expressed in total counts per min.

Preincubation time (min.)	Preincubation temperature 0°C	25°C
0	110,000	110,000
30	170,000	172,000
60	182,000	153,000

Experimental conditions described under Assay of the Carboxydismutase Activity ; preincubation as in Preincubation Experiments.

Table VI

Reproducibility of the carboxydismutase assay--control series (no RuDP). Activity expressed in cpm.

Incubation Mixture No.	I			II			III		
Planchet No.	1	2	3	4	5	6	7	8	9
First count	47	45	44	44	41	43	49	43	49
Second count	48	47	44	46	49	53	47	41	48
Third count	41	41	42	42	60	46	49	43	54
Mean value	45	44	43	44	49	47	48	42	50
Mean error of mean value	±3	±2	±1	±1	±6	±4	±1	±1	±2

Table VII

Reproducibility of the carboxydismutase assay--variable series (complete system). Activity expressed in cpm.

Incubation Mixture No.	I			II			III		
Planchet No.	1	2	3	4	5	6	7	8	9
First count	3450	3520	3430	3570	3680	3830	3520	3480	3510
Second count	3340	3590	3440	3590	3660	3810	3500	3410	3510
Third count	3390	3430	3480	3490	3710	3640	3360	3400	3400
Mean value	3390	3510	3450	3560	3680	3760	3460	3440	3470
Mean error of mean value	±34	±60	±23	±37	±13	±83	±70	±35	±47

Conclusions

It is difficult to draw definite conclusions from these preincubation experiments. The rate of entrance of the Mg^{++} into the active sites of the enzyme, as is evident from the effect of preincubation, appears to be no faster than that for Ni^{++}. However, the lower rate of inactivation of the enzyme in the presence of Mg^{++} than in the presence of Ni^{++} can lead to some interesting speculations. Thus, it is possible that Mg^{++} is a "true" co-factor of the enzyme, whereas Ni^{++} is not. There then remains the problem of explaining the activation due to Ni^{++}. One possible mechanism is that Ni^{++} can activate the enzyme by displacement of the true metal co-factor from the inactive sites of the enzyme to active sites. It may be argued that there is no metal ion present in the enzyme because during the fractionation procedure the preparation was dialyzed.[2,4] However, it has been demonstrated that dialysis may not remove all the metal ions from crude extracts of Tetragonia expansa, even when they are dialyzed against ethylenediaminetetraacetic acid. The basis of this conclusion is that there is no loss of activity of the crude extract after dialysis.[2,4] One other fact is consistent with this mechanism. Ni^{++} inactivates the enzyme at a more rapid rate than Mg^{++} during preincubation. This phenomenon can be explained by the subsequent displacement of the activating metal ion from the active sites by Ni^{++}. It should be borne out that other metal ions such as Co^{++}, Mn^{++}, and Cr^{++} will activate carboxydismutase.[5,6] Until the preincubation characteristics of these cations have been investigated, the role of Mg^{++} as a true co-factor remains on tenuous grounds.

In summary, the preincubation effect on carboxydismutase in the presence of Mg^{++} has been studied. A rapid activation and a slow inactivation of the enzyme were found to be characteristic of this preincubation. On this basis the role of Mg^{++} as a true co-factor was discussed.

The reliability of the carboxydismutase assay was reinvestigated. The assay was found to be reproducible to within ± 3%.

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[5] Ning G. Pon, in Chemistry Division Quarterly Report, UCRL-3351, March 1956, p. 50.

[6] Weissbach, Horecker, and Hurwitz, J. Biol. Chem. 218, 795 (1956).

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