positively charged species, and the prior equilibrium involving the proton results in an increase in charge of the one participant. It would seem that a simple electron transfer between the two ions would not be facilitated by the increase in repulsive forces, and there is no obvious role for the proton in a simple electron transfer mechanism. Two mechanisms which do take into account the presence of the proton are

$$OUO^{+}—HOUO^{++} \longrightarrow OUOH^{+}—OUO^{++}$$

which symbolizes the removal of hydrogen atom from $UOOH^{++}$ by UO_2^+ and:

$$OUOH^{++}—UO_2^{+} \longrightarrow OU^{++}—HOUO_2^{+}$$

which means oxidation of UO_2^+ by the removal of neutral OH from $UOOH^{++}$.

In view of the success attained by the use of deuterium in the investigation of mechanisms, it was decided to determine the relative rates of this disproportionation in deuterium oxide and water.

Experimental

Materials.—A perchloric acid solution of $UO_2(ClO_4)_2$ was prepared by boiling down $UO_2(NO_3)_2$ several times with 70% $HClO_4$. The uranium solution was standardized gravimetrically as U_3O_8. Stock solutions of 0.5 *M* $NaClO_4$ were prepared by dissolving a weighed quantity of the anhydrous salt in light or heavy water.

Apparatus.—The polarographic method of Kern and Orleman[2b] was used. A simple manual instrument was constructed and provided with a switching arrangement so that the circuit could also be used as a potentiometer for the measurements of *p*H. A combined electrolysis and polarograph cell was employed, similar to the model of Kern and Orleman[2b] except that it was of reduced dimensions and provided with a water jacket to maintain a temperature of 25.0 ± 0.2°.

Procedure.—A preliminary examination was made in which it was found that the polarographic wave for the couple $UO_2^{++} = UO_2^{+} + e^-$ was almost identical in heavy water to that ordinarily obtained. Hence the same potentials were employed for electrolysis and analysis in D_2O as in H_2O. The values of the diffusion coefficients of UO_2^+ were assumed to be the same, and the value of Kern and Orleman was used.

In a typical run, 20 ml. of salt solution (light or heavy water) and 1 ml. of stock $UO_2(ClO_4)_2$ solution (light water) were electrolyzed for 20–30 min. at a current of about 3 ma. With the smaller cathode area used this was sufficient to maintain a potential suitable for the reduction. The mercury cathode was stirred rapidly and nitrogen was bubbled through both the main cell and anode compartment throughout the electrolysis. Then 1 ml. of dilute $HClO_4$ (in ordinary water) was added and the polarograph was used to follow the diffusion current for 40 min.

The hydrogen ion activity was determined with the quinhydrone electrode against a satd. calomel cell. In the case of the heavy water solutions (mole fraction D_2O, 0.91) the reference potential used was 0.4865. This value was obtained by interpolation from the data of Rule and LaMer,[3] and is here referred to the satd. calomel cell.

Interpretation of Results

The values of k''/a_{H^+} reported here are somewhat higher than those obtained by Kern and Orleman.[2b] The greatest source of error involved is in the determination of $a_{H^+} + a_{D^+}$, due to an uncertain liquid junction potential between the calomel cell and solution. However, if we may assume that the same liquid junction exists for the heavy water solution as the ordinary, the error involved will cancel out in the ratio of the two rates k_D/k_H.

It is seen that the rate in D_2O is about 1.7 times as fast as in water. This may be the result of two effects, which are inseparable in these experiments. The value of the equilibrium constant of reaction (1) may be changed, and the magnitude of the specific rate constant of reaction (2) may be affected. The ratio of equilibrium constants of (1) in D_2O and H_2O should be somewhat less than 3, since we may reckon $UOOH^{++}$ to be a strong acid.[2b] This effect alone can explain the increased rate in D_2O. As for the effect on reaction (2), if any is present, it would be expected from previous experimental results in which it is possible to isolate the isotope effect that the specific rate constant observed in heavy water should be lower than that in light water. The transfer of a deuteron from carbon to oxygen is said to be 3.5 to 10 times as slow as that of a proton.[4]

TABLE I

Mole fraction D_2O	k''[a]	*E* quinhydrone *vs.* S.C.E.	$a_{H^+} + a_{D^+}$	$k''/(a_{H^+} + a_{D^+})$
(ionic strength = 0.5)				
0	1.17	0.3281	7.60×10^{-3}	154
0	1.24	.3288	7.80×10^{-3}	159
0	2.17	.3238	1.41×10^{-2}	154
Av. $k''/a_{H^+} = 156 = k_H$				
0.91	1.56	.3547	5.90×10^{-3}	264
0.91	3.89	.3791	1.53×10^{-2}	255
Av. $k''/a_{H^+} = 260 = k_D$				
$k_D/k_H = 1.7$				

[a] The symbols used here are chosen to agree with those adopted by Kern and Orleman.

Another effect which cannot be ignored is that resulting from the change in solvent. Ionic mobilities are lower in deuterium oxide. A few experiments are listed which have some bearing on the question.[5] The general conclusion is that, except for acid–base equilibria, the solvent effect of a change to deuterium oxide is to lower slightly the rate of reaction.

From the results, a mechanism involving proton transfer appears to be highly improbable. An electron exchange mechanism which depends in some unknown manner on the presence of a proton may apply; or the proton may be required so that a neutral OH can be transferred rather than an O^-.

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[3] Rule and LaMer, THIS JOURNAL, **60**, 1974 (1938).

[4] Bonhoefer, *Trans. Faraday Soc.*, **34**, 252 (1938).

[5] (a) Hughes, Yudken, Kemp and Rideal, *J. Chem. Soc.*, 1105 (1934); (b) Champetier, *Compt. rend.*, **201**, 1118 (1934); (c) Yamasaki, *Bull. Chem. Soc. Japan*, **11**, 431 (1936).

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RECEIVED SEPTEMBER 25, 1950

The Path of Oxygen in Photosynthesis[1]

BY G. D. DOROUGH[2] AND M. CALVIN

It now appears established[3] that the source of oxygen evolved in photosynthesis is the water

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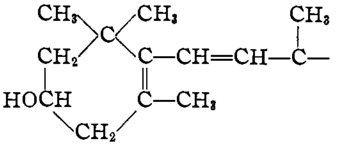
[1] The work described in this paper was sponsored by the Atomic Energy Commission.

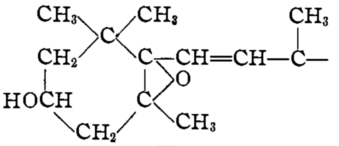
[2] While on leave from the Department of Chemistry, Washington University, St. Louis, Missouri.

[3] Ruben, Randall, Kamen and Hyde, THIS JOURNAL, **63**, 877 (1941). Holt and French, "Photosynthesis in Plants," Iowa State College Press, Ames, Iowa, 1949, Chap. 14.

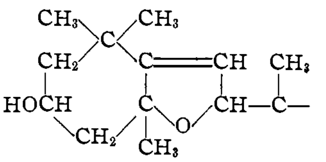
molecule. However, no information has been obtained concerning the manner in which this oxidation takes place, for no oxygen-containing compound has ever been established as an intermediate between water oxygen and molecular oxygen. The isolation by Karrer[4] of a number of epoxide (II) and furan (III) structures among the oxygen-containing carotenoids suggests the possibility that these pigments might be such intermediates. (The furans are formed readily by a rearrangement of the epoxides.) The type of mechanism in which these structures might operate would involve first the hydration of a carotenoid structure such as I (addition of water across the ring double bond), followed by oxidation to an epoxide structure II. The elimination of oxygen to restore pigment I (and thus complete the cycle) might be something of the type



I



II



III

$$\text{II} \xrightarrow[\text{light?}]{\text{enzymes}} \text{III}[5]$$

$$\text{II (or III)} + H_2O \xrightarrow[\text{light?}]{\text{enzymes}} 1 + H_2O_2$$

$$\text{or: II (or III)} \xrightarrow[\text{light?}]{\text{enzymes}} \text{I} + {}^1/{}_2O_2[6]$$

The net result would be the oxidation of water with the formation of the reducing power required for the reduction of the carbon dioxide.

A way of testing this hypothesis[7] would be to permit photosynthesis in a plant of high carotenoid content with water containing oxygen 18, isolate and purify the various epoxide and furan carotenoid pigments, and determine whether or not these pigments contain a higher percentage of oxygen 18 than an identical sample kept in the dark. An experiment of this type has been attempted on green algae; it is described in detail in the next section.

Experimental

Analysis of H_2O^{18}.—A necessary prerequisite for the experiment is an analysis of the oxygen 18 water to be used in the photosynthesis. The analysis is performed by equilibrating a small sample of CO_2 with a large excess of water, and measuring the O^{18}/O^{16} ratio of the equilibrated CO_2 on a mass spectrograph. (Various difficulties prevent the direct determination of water in the mass spectrograph.) The mass ratio measured for the equilibrated CO_2 is essentially the same as that for the water. The method employed for the equilibration was similar to that of Cohn and Urey.[8]

Photosynthesis of the Algae.—Twenty cc. of fresh packed green algae (*Chlorella pyrenoidosa*) was slurried with 50 cc. of 4% oxygen 18 water. The resulting 70 cc. was divided equally between two large Warburg flasks (150-cc. capacity), each flask containing 0.5 g. of potassium bicarbonate. One of the flasks was wrapped with a metal covered masking tape so as to make it light tight. The two flasks were placed in the Warburg side by side. Photosynthesis was carried out with both top and bottom illumination (a total of 6 G.R. 300 W spot reflectors) until 46.6 cc. of gas had been evolved from the flask exposed to the light. During the same period, the covered flask evolved 14.8 cc. of gas due to CO_2 evolution from the concentrated sodium bicarbonate solution. The total time of light exposure was five hours. Assuming 10 mg. of xanthophylls[9] in 10 g. of algae and 46.6 − 14.8 = 32 cc. of oxygen evolved, the ratio of xanthophylls to water consumed in the experiment was

$$\frac{2.6 \times 10^{-3}\text{ mole of water consumed}}{1.6 \times 10^{-3}\text{ mole of xanthopylls present}} \cong 150$$

Extraction of the Xanthophyll Pigments.—The algae suspensions from the light and dark flasks were treated identically. The algae suspension was centrifuged, and the water decanted off for analysis as above (distillation required first). The algae were then slurried with 400 cc. of absolute methanol, and two or three g. of $CaCO_3$ added. After 18 hours the methanol was filtered off, and the algae re-extracted with 200 cc. of benzene for eight hours, and 200 cc. of methanol for 24 hours. The algae residues were practically colorless after this treatment. The total methanol and benzene extracts were evaporated to 150 cc., and treated with 250 cc. of 12% potassium hydroxide in methanol. After 12 hours this solution was diluted with roughly an equal volume of water, and extracted with peroxide-free ether until the ether extracts were colorless. The ether contained mainly xanthophylls, carotenes, steroids and phytol. The total ether extract was washed five times with water, dried overnight with anhydrous sodium sulfate, and evaporated to dryness at 30–40° under vacuum. (Nitrogen used on the capillary.) The flask containing the pigments was then pumped overnight at high vacuum (10^{-5} mm.). The carotenoid of this solid residue was about 7%.

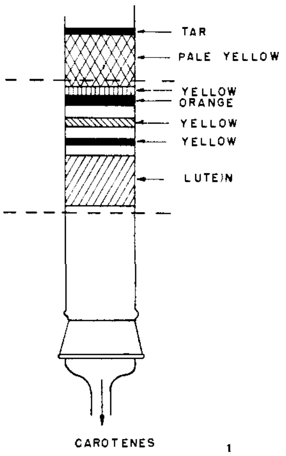


Fig. 1.—Chromatography of plant xanthophylls.

Chromatography of Xanthophylls.—The contents of the flask from the previous operation were dissolved in 10 cc. of benzene. Twenty cc. of petroleum ether (b.p. 35–60°) was added, and the solution placed on a 2 × 10 inch column of tightly packed $CaCO_3$ (Baker and Adams reagent grade) which had previously been wet with a small amount of the same solvent. The vacuum applied to the column for development was about 3″ of mercury. After absorption of the material was

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[4] Karrer and Jucker, "Carotinoide," Verlag Birkhauser, Basel, 1948.

[5] It is known that furan structures (III) when treated with acid yield carotenoids (I), but nothing is known concerning the fate of the oxygen.

[6] It is possible to visualize this as taking place *via* an alternating interconversion of epoxide and furan down the conjugated chain thus bringing the oxygen atoms together.

[7] The germ of a similar idea and some experimental work related to it has recently appeared in a paper by D. I. Sapojnikov and A. B. Lopatkin, *Dokl. Akad. Nauk. SSSR*, **72**, 413 (1950), in which a transformation of carotene into xanthophyll under the influence of light is supposedly demonstrated.

[8] Cohn and Urey, This Journal, **60**, 679 (1938).

[9] The approximate amount found in several trials.

complete, the column was washed with copious quantities of petroleum ether without benzene. The column appeared as shown in Fig. 1.

Elution of the pigments was with methanol. The resulting solution was diluted with an equal volume of dilute aqueous sodium chloride, and extracted with peroxide-free ether. The ether was washed with water, and then evaporated to dryness. This procedure removed methanol soluble impurities in the $CaCO_3$. Direct ether solution of the pigments is not very complete.

The cut shown between the dotted lines in Fig. 1 is free of the carotenes and phytol, but still contains about 60–70% of presumably steroid material. The total xanthophyll content is of the order of 10–12 mg. about half of which is lutein. The other half is composed of four xanthophylls, among which are the furan and epoxide structures.

Rechromatography of this cut raises the purity to about 50–55%. For separation of the various xanthophylls, a developing solvent of petroleum ether and benzene in the ratio of 2:1 works very well.

Pyrolysis of the Xanthophylls.—The oxygen in the xanthophylls must be put in a form suitable for analysis on the mass spectrograph. This was accomplished on a modified combustion train (see Fig. 2) as follows: A stream of helium (200 cc./mm.) is passed through a drying tube (A) to remove water, and over two hot (350°) copper spirals (B) and (C) to remove oxygen.

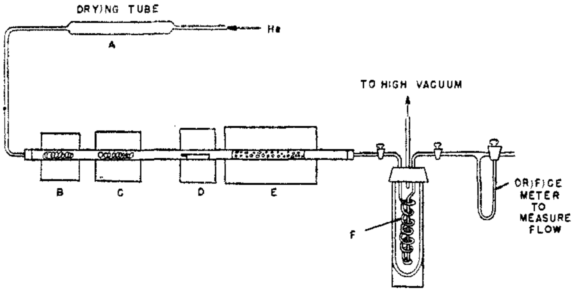

Fig. 2.—Schematic diagram of pyrolysis train.

The sample of carotenoid in a small boat (D) is pyrolyzed at 600° in this stream of dry, oxygen-free helium. The oxygen fragments are all converted to CO by passage over hot carbon (E) at 800°. The CO is then trapped in the spiral (F) which is maintained at about −240° by pumping on a dewar flask containing liquid nitrogen with a high speed vacuum pump (Cenco Megavac). (The nitrogen solidifies.)

The drying agent used was anhydrous magnesium perchlorate (Dehydrite). The copper spirals were used as insurance, the lack of tarnish in the second spiral showing that the helium was oxygen-free. The carbon used was Fisher C.P. sugar charcoal, which had to be pumped on at 800° for 12 hours to complete the decomposition of the sugar. The spiral was packed with coarsely powdered glass to increase the surface area.

It is obvious that before the actual pyrolysis is started, the train must be completely swept out with helium, the various furnaces must be at their correct temperatures and the spiral trap must be at temperature equilibrium (−240°).

Separation of Carbon Monoxide from Helium.—It was originally planned to analyze the CO directly in the helium mixture, but since the ion current in the spectrograph is limited, most of the current was carried by the helium, and the CO peaks were very small. The CO was separated from the helium by absorption of the CO on gas mask charcoal at liquid nitrogen temperatures, and pumping off the helium. The gas mask charcoal was prepared by heating to 120° and pumping at 10^{-6} mm. for four days.

Spectral Analysis of Carotenoids.—A simple approximate analysis of any carotenoid fraction can be carried out on a Beckman or similar spectrophotometer due to the fact that the carotenoid spectra are all similar in shape and extinction coefficient. The shape is a characteristic three-banded system with the highest peak (the middle one) occurring in the region of 4400–4500 Å. in methanol solution. The specific extinction coefficient, α, has a value for most carotenoids around 210–240. α is defined

$$\alpha = \frac{\log_{10} I_0/I}{cl}$$

Where

I_0/I is the reciprocal of the fraction of light transmitted
c is the concentration, g./liter
l is the cell thickness in centimeters

Discussion

A considerable number of difficulties were encountered in the experiment which place certain restrictions on the interpretation of the data. The only sample of oxygen 18 water available was one of 50 cc. containing 4% oxygen 18. This amount of water limited the amount of algae which could be run (about 20 cc. of packed cells for division between the light and dark experiments), and this, in turn, limited the amount of xanthophylls which could be obtained. Now in 10 cc. of packed cells, there are some 4 or 5 mg. of the furan and epoxide carotenoids. This entire amount can be isolated, but it is mixed with roughly an equivalent amount of steroid. To completely free the carotenoid of steroid would mean sacrificing probably three-fourths of the total in chromatography and crystallization. This would leave only about one mg. of carotenoid which would yield one-sixth of the amount of oxygen (as CO) required by the mass spectrography for analysis. If the oxygen 18 content of the water were high, this one mg. of carotenoid could be diluted with a sufficient amount of a normal oxygen source to give the required amount of CO. However, the oxygen 18 content of the water was rather low, and no advantage could be gained by the difficult purification of the furan and epoxide fractions. This is illustrated by Table I (assuming the epoxide and furan oxygens are converted to the same O^{18} content as the water in equilibrium with the algae).

Table I

Dilution		Estimated factor
A. Estimated dilution factor of the original water if the total carotenoid fraction is used for analysis		
Water in algae		0.7
Lutein and other xanthophyll hydroxyl groups		.3
Steroid material		.5
Over-all dilution	=	.1
B. Estimated dilution factor of the original water if furan and epoxide xanthophylls are separated and then analyzed		
Water in algae		0.7
Hydroxyl group dilution		.5
Normal oxygen to be added to give sufficient CO sample		.15
Over-all dilution	=	.05

As a result, it was necessary to pyrolyze the total carotenoid fraction as it existed after two chromatographings. The estimated dilution factor of ten would mean that if the proposed mechanism were operating, the light experiment should yield, after pyrolysis, CO with 0.4%, O^{18}, while the dark run should yield CO which is 0.2% O^{18} (normal oxygen value). The actual results are shown in Table II.

It is seen from this table that both the light and dark samples show an enhancement over normal oxygen. The enhancement can result from O^{18} in the epoxide or furan carotenoids, from the hydroxy groups present in all xanthophyll pigments

TABLE II

Sample	O^{18}, %
1. Original H_2O	4.0
2. Water in equilibrium with algae: light run	3.01
3. Water in equilibrium with algae: dark run	3.06
4. CO sample from xanthophyll fraction of photosynthesized (5 hr.) algae (50% carotenoid)	0.245
5. CO sample from xanthophyll fraction of algae kept in the dark (40% carotenoid)	0.233
6. Normal H_2O	0.204[a]

[a] Thode and Smith, National Research Council of Canada, Atomic Energy Project Report MC-57 (revised), May, 1944.

or from the steroid material. It apparently could not result from exchange with hydroxyl groups already present, however, since such exchanges for ordinary alcohols have been shown to be extremely slow.[8] The mass spectrographic analysis was of sufficient accuracy that the additional enhancement of the light run is real for this one experiment, but the result would have to be obtained several times before the enhancement could be regarded as general. The experiment is thus inconclusive; it suggests only that it would be worthwhile to repeat the work with water of high oxygen 18 content (30–50%) so that the various carotenoid pigments could be isolated in pure form in sufficient quantities for mass analysis, and so that more than one experiment could be run. Such experiments might provide a real clue as to the path of oxygen in photosynthesis.

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The Preparation of 2-Methyl-2,3-dihydrobenzofuran

BY JACOB ENTEL, CLARENCE H. RUOF AND H. C. HOWARD

Alkylated dihydrobenzofurans can be readily produced by refluxing allylic bromides with phenols in the presence of potassium carbonate, rearranging the resulting allyl phenyl ethers to the 2-allylphenols and then closing the ring in the latter with hydrobromic acid.[1] In large scale reactions substitution of the allylic chlorides for the corresponding bromides is economically desirable; such attempts, however, usually result in substantially decreased yields. The use of large quantities of sodium iodide along with the allylic chloride[1] to increase the yields is objectionable due to the cost of the iodide. Reagents cheaper than hydrobromic acid for the ring closure are also desirable.

In the present work a Williamson reaction using allylic chlorides gave yields of allylphenols comparable to those previously obtained with the expensive bromides and a mixture of zinc chloride with hydrochloric acid was successfully substituted for the hydrobromic acid in the ring closure.

Experimental

To 2.5 liters of absolute methanol in a 5-liter 3-necked flask fitted with a reflux condenser and mercury-sealed stirrer was slowly added 235 g. (10.2 moles) of sodium in small pieces. Then 940 g. of phenol (10 moles) in 1 liter of absolute methanol was added slowly and followed by 890 ml. of allyl chloride. After refluxing for 30 hours with stirring, the methanol was distilled off and the organic material was filtered free of precipitated sodium chloride. After washing with 10% potassium hydroxide and drying over anhydrous potassium carbonate, the allyl phenyl ether was fractionated *in vacuo;* yield of the ether 1080 g. (80.6% yield) with b.p. 85 (19 mm.) and n^{25}D 1.5185.[1]

After rearranging the ether by heating as described by Tarbell,[1] 300 g. of the 2-allylphenol was dissolved in 1200 ml. of glacial acetic acid and mixed with 180 g. of anhydrous zinc chloride in 600 ml. of concentrated hydrochloric acid. After refluxing for 30 minutes the mixture was cooled and poured into 2.5 liters of cold water. The organic layer was separated, washed with 10% aqueous potassium hydroxide, extracted with ether and fractionated through a 60-plate column to give 155 g. (51.5% yield) of 2-methyl-2,3-dihydrobenzofuran, b.p. 197° (740 mm.), n^{25}D 1.5284.[1] Triplicate runs gave the same yields.

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RECEIVED OCTOBER 20, 1950

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[1] D. S. Tarbell, "Organic Reaction," edited by R. Adams, Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 22-26.

A New Short-Lived Isotope of Cesium[1]

BY RICHARD W. FINK AND EDWIN O. WIIG

A search has been made for neutron-deficient Cs^{128}, previously expected to be quite short-lived.[2] Spectroscopically pure cesium chloride was bombarded for 2 hours with 96 Mev. protons in the Rochester 130-inch cyclotron in order to prepare 2.4 day Ba^{128}, originally reported[3,4] to decay by emission of 3.0 Mev. positrons through hitherto unknown Cs^{128} to stable Xe^{128}.

Radioactive barium was isolated from the CsCl target by a chemical procedure involving three precipitations of barium chromate from a buffered ammonium acetate–acetic acid solution with extensive washings with 1% ammonium acetate to free it from target CsCl. The $\mathrm{BaCrO_4}$ was next dissolved in 2 *M* hydrochloric acid, and the chromate reduced by boiling with excess hydroxylamine hydrochloride. This was followed by precipitation of chromic hydroxide with ammonium hydroxide. Finally, the barium chloride solution was evaporated and ignited lightly to expel ammonium salts and the barium activities taken up in a drop of HCl. About 200 milligrams of $\mathrm{BaCl_2}$ crystals was then added and periodically the cesium daughters growing in were extracted with concentrated HCl from the solid $\mathrm{BaCl_2}$. Over a period of 9 days after bombardment, a total of 16 such parent–daughter separations were performed.

A typical decay curve of the cesium daughters so isolated is shown in Fig. 1, where the half-lives observed were 10 day Cs^{131}, daughter[5] of 12 day Ba^{131}; 31 hour Cs^{129}, daughter of 2.0 hour Ba^{129}; and new Cs^{128}, daughter of 2.4 day Ba^{128}. The best value for the half-life of Cs^{128} is an average of 11 different determinations and is 3.13 ± 0.2 minutes. The time lapse between cessation of bombardment and completion of chemical separa-

[1] This work was performed under contract with the U. S. Atomic Energy Commission.

[2] R. W. Fink, F. L. Reynolds and D. H. Templeton, *Phys. Rev.*, **77**, 614 (1950).

[3] C. C. Thomas, Jr., and E. O. Wiig, THIS JOURNAL, **72**, 2818 (1950).

[4] R. W. Fink and D. H. Templeton, *ibid.*, **72**, 2818 (1950).

[5] K. Way, *et al.*, *Nuclear Data*, National Bureau of Standards Circular 499 (1950).