procedures formerly employed, namely, repeated chilling, filtering and concentrating in hexane solution and then in ethanol solution are continued. Finally, the concentrate may be fractionated by distillation under very low pressure and the vitamin isolated in the form of the colorless, highly active fraction as already described [*J. Biol. Chem.*, **120,** 635 (1937)].

In Table I are given assay results illustrating the relative vitamin K activities of crude extracts of alfalfa and of the same extracts after the preliminary treatment with phosphotungstic acid. Results with two distillates obtained under very low pressure subsequent to the phosphotungstic acid treatment are also given. The fact that the average blood clotting times of chicks were not greater after the crude extract had been treated with phosphotungstic acid is ample evidence that in this step the losses of vitamin K are very slight, if any. Further advantages of this step are speed and applicability to concentrated solutions. Assays were conducted according to a procedure given elsewhere [*Biochem. J.*, **32,** 1897 (1938)].

TABLE I

AVERAGE CLOTTING TIMES OF CHICKS FED CERTAIN SOURCES OF VITAMIN K

Source of vitamin K	Amount per kilogram of diet[a]	Average blood clotting time, minutes
Hexane extract	10 cc.	3.9
of alfalfa	12 cc.	4.0
Phosphotungstic	≃10 cc.	3.5
acid treated	≃12 cc.	2.4
extract	≃12 cc.	4.1
Molecular	20 mg.	5.4
distillate	20 mg.	4.0

[a] Standard solution representing 1 g. of dried alfalfa per cc.

We wish to acknowledge technical assistance obtained through the WPA under Project A. P. No. 465-03-3-209. We are also indebted to Philip J. Grant, who developed the use of phosphotungstic acid as a precipitant for the green pigment fraction.

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RECEIVED DECEMBER 19, 1938

THE MOLECULAR WEIGHT OF THE DIPHTHERIA TOXIN PROTEIN

Sir:

In connection with an investigation of the biological and chemical aspects of the flocculation reaction involving diphtheria toxin and antitoxin now under way in our respective laboratories, we have had occasion to study the sedimentation, diffusion and electrophoresis behavior of the purified toxin [method of preparation, Pappenheimer, *J. Biol. Chem.*, **120,** 543 (1937)]. By these criteria, the toxin is found to behave as a homogeneous substance.

Sedimentation velocity determinations have been made in the standard oil turbine ultracentrifuge of Svedberg by using the refractive index method to locate the position of the boundaries at finite times during the experiment. For a 1% solution of the diphtheria toxin in $M/15$ phosphate buffer at pH 6.9 and 0.17 M sodium chloride, the scale displacement–distance curves give as sedimentation constant, corrected to the basis of a process taking place in pure water at 20°, $s_{20} = 4.6 \times 10^{-13}$ cm./sec./dyne.

Diffusion experiments with a similar solution, except that the protein concentration was reduced to 0.4%, were made by observing, again by the refractive index method, the blurring of a boundary formed and held stationary in the "U" tube of a Tiselius electrophoresis apparatus. The boundary was moved to the middle of an upper section with the compensation mechanism. The diffusion constant was calculated from the area and maximum height of the usual scale displacement–distance diagram and corrected to a process occurring in water at 20° to give as provisional value $D_{20} = 6.2 \times 10^{-7}$ cm.^{2}/sec.

From the sedimentation constant, s, and diffusion constant, D, the molecular weight is obtained

$$M = \frac{RTs}{D(1 - V\rho)} \cong 72{,}000$$

In the absence of direct determination the toxin protein is assumed to have partial specific volume, $V = 0.75$.

When subjected to a potential gradient in the Tiselius electrophoresis apparatus, containing a 1% solution of the toxin in borate–phosphate buffer at pH = 7 and ionic strength = 0.02, the protein migrates essentially as a single component. However, when the schlieren diaphragm is nearly closed, a trace of faster moving material may be observed. The latter is probably identical with a small amount of inactive protein known to be present.

Electrodialyzed antitoxic pseudoglobulin in 1% solution in $M/15$ phosphate buffer at pH 6.9 and 0.17 M NaCl shows a sedimentation behavior not

unlike that of normal pseudoglobulin. The predominating constituent, about 95%, gives $s_{20} = 7.4 \times 10^{-13}$ cm./sec./dyne and there is present a trace of component with $s_{20} = 18 \times 10^{-13}$ cm./sec./dyne. The antitoxic preparation used was 35% specifically precipitable by diphtheria toxin.

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RECEIVED JANUARY 13, 1939

OXYGEN EXCHANGE BETWEEN CARBON DIOXIDE, BICARBONATE ION, CARBONATE ION AND WATER

Sir:

The rate of oxygen exchange has been used to study the velocity of the reaction between carbon dioxide and water when the *p*H is less than 8. In this range the predominating reaction of carbon dioxide is with the solvent molecules rather than the hydroxide ions.[1] Assuming that the reaction proceeds through the formation of H_2CO_3, and the reversal of this reaction, the equation for the reaction velocity becomes

$$-\frac{d\alpha}{dt} = \frac{k(H_2O)(CO_2)(\alpha - \beta)}{4(CO_2)} \qquad (1)$$

where α is the mole fraction of O^{18} in carbon dioxide, and β is the mole fraction of O^{18} in water, which because of the large quantity of water remains constant, and k is the specific reaction rate constant for the reaction between carbon dioxide and water. The equation is of the first order as is usual in these cases.[2] Moreover, when the water is in excess as, of course, is true in this case, the rate will be independent of the concentration of the reactant.

In our experiments carbon dioxide containing heavy oxygen was dissolved rapidly in water and then samples of this solution were withdrawn from time to time into evacuated vessels in order to separate rapidly the dissolved carbon dioxide from the water. This carbon dioxide was analyzed for its O^{18} content with a mass spectrometer. We find that the velocity constant, $k[H_2O]$, of this reaction is equal to 0.0027 at 0°, the time being in seconds, in good agreement with Stadie and O'Brien.[3] There is no salt effect as shown by making the solution 0.045 molar in sodium chloride. Moreover, there is no hydrogen ion catalysis since 0.02 molar hydrochloric acid does not change the rate.

The velocity of exchange of O^{18} between bicarbonate ion and water has been investigated. It appears that the exchange takes place only through the formation of carbonic acid and carbon dioxide, for the reaction takes place much more slowly under these conditions. In this case the rate depends upon the ratio of the carbon dioxide to the bicarbonate concentrations. The kinetic equation is given by Equation (1) above if $4(CO_2)$ is replaced by $2[3(HCO_3^-) + 2(CO_2)]$.

Pure sodium carbonate containing 0.513% of O^{18} has been dissolved in ordinary water. At 25° the time of half exchange in the case of 0.02 molar solution of sodium carbonate is approximately twenty-eight hours, while no exchange was observed when the solution was 0.02 molar in sodium carbonate and 0.04 molar in sodium hydroxide.[4] The error in our analyses is perhaps less than one per cent. of the percentage of O^{18} present. These results indicate that the reaction proceeds through the formation of the bicarbonate ion.

At 30° we find that the exchange between CO_2 and H_2O is substantially complete in about seven minutes as compared to over an hour at 0°. Such a rapid exchange might account for the decreased O^{18} content found in the experiments by Day and Sheel,[5] on the oxidation process taking place when heavy oxygen is inhaled by rats. The exchange should be very much more rapid in this case because of the high temperature and the possible effect of carbonic anhydrase.

The results of these experiments will be reported in greater detail later.

(1) C. Faurholt, *J. Chim. Phys.*, **21**, 400 (1924).

(2) H. A. C. McKay, *Nature*, **142**, 997 (1938), has shown that this is generally true for exchange reactions.

(3) W. C. Stadie and H. O'Brien, *J. Biol. Chem.*, **103**, 521 (1933).

(4) T. Titani, N. Morita and K. Goto, *Bull. Chem. Soc. Japan*, **13**, 329 (1938). Our greater analytical precision probably accounts for the difference in results reported here.

(5) J. N. E. Day and P. Sheel, *Nature*, **142**, 917 (1938).

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NEW YORK, N. Y. HAROLD C. UREY

RECEIVED JANUARY 23, 1939

CRYSTALLINE β-METHYLMANNOFURANOSIDE AND MANNOSEDIMETHYLACETAL

Sir:

Application of the furanoside synthesis developed in this Laboratory [Pacsu and Green, THIS JOURNAL, **58,** 1823 (1936); Green and Pacsu, *ibid.*, **59,** 1205, 2569 (1937); **60,** 2056, 2288 (1938); Pacsu, *ibid.*, **60,** 2277 (1938)] to *d*-mannosediethylmercaptal resulted in a 60% yield of α-methylmannofuranoside (m. p. 118–119°; $[\alpha]^{20}D$