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 pH 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

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

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carbon dioxide containing heavy oxygen was dissolved rapidly in water and then samples of this solution were withdrawn from time to time into evacuated vessels in order to separate rapidly the dissolved carbon dioxide from the water.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 cataly-

(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).

sis 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.

(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).

COLUMBIA UNIVERSITY   G. ALEXANDER MILLS
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

108°), previously prepared by Haworth and co-workers through the dicarbonate [Haworth and Porter, *J. Chem. Soc.*, 649 (1930)] and direct from mannose and methyl alcoholic hydrogen chloride [Haworth, Hirst and Webb, *ibid.*, 651 (1930)]. When the mother liquor of our preparation was treated with a saturated solution of calcium chloride, a non-reducing, crystalline substance with $[\alpha]^{20}$D $-58°$ in water solution separated. The analytical results and hydrolysis experiments indicated that this substance was a calcium chloride addition compound of β-methylmannofuranoside with the composition of $C_7H_8O_6 \cdot CaCl_2 \cdot 3H_2O$. Further investigations revealed also that the sirupy residue of the preparation of α-methylmannofuranoside from mannose and methyl alcoholic hydrogen chloride would combine with calcium chloride to produce the same addition compound with $[\alpha]^{20}$D $-58°$. Removal of the calcium chloride from the double compound by silver oxalate yielded pure β-methylmannofuranoside, which was secured in crystalline condition from ethyl acetate; m. p. 47°; $[\alpha]^{20}$D $-107°$ in water solution. On the basis of the formula $C_7H_8O_6 \cdot CaCl_2 \cdot 3H_2O$, the rotation of $-106°$ is calculated for the glycoside portion. The two methylmannofuranosides represent the first α,β-pair of methylglycofuranosides that has been obtained in crystalline condition. The half of the rotational difference (107°) of the two isomers is much closer to the value (97°) for the methylglucopyranosides, than to the abnormally low value (74°) for the methylmanno-pyranosides. It is suggested that the non-validity of Hudson's isorotation rules, in the case of the mannopyranosides, is largely due to unequal contents of differently puckered rings in the α- and β-isomers. Since a five-membered ring as it occurs in the furanosides is practically flat, both α- and β-mannofuranosides are considered as being derived from nearly identical rings. Then the small but real deviation (10°) from the "normal" value (97°) for the glucopyranosides can be attributed mainly to an effect produced by the *cis*-hydroxyl groups at carbon atoms 2 and 3 in mannose.

Incidental to this investigation, the crystalline dimethyl acetal of *d*-mannose was prepared from the pentaacetate of mannosediethylmercaptal by the above-mentioned method. After recrystallization from ethyl acetate, the acetal had m. p. 101° and $[\alpha]^{20}$D 0.6° in water solution.

A detailed account of this work will be published shortly.

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THE SULFUR AND PHOSPHORUS CONTENTS OF TOBACCO MOSAIC VIRUS

Sir:

Tobacco mosaic virus protein isolated by chemical means has been reported to contain from 0.0 to 0.59% sulfur and from 0.0 to 0.55% phosphorus.[1] The amounts found vary with the chemical treatment and the manner in which the sample is prepared for analysis. It has now been found that virus protein isolated by the physical method of differential centrifugation and prepared for analysis by drying from the frozen state contains uniformly approximately 0.24% sulfur and 0.60% phosphorus. Neither element is removed by dialysis against water at pH 9.3, a result in accordance with that reported from this Laboratory by Loring.[2] It seems likely that in a previous experiment,[3] in which removal of these two elements was secured, the preparation was more alkaline than pH 9.3. Although the native protein does not give a color reaction with nitroprusside, the denatured protein gives positive tests for sulfhydryl groups. Since sulfur and phosphorus appear to be in organic combination, the nature of their distribution in the protein has been studied.

Sulfur occurs in the form of cysteine or cystine, methionine and sulfate sulfur, and phosphorus, in accordance with previous work, in the form of nucleic acid. Recent unpublished work of Loring indicates that the phosphorus in the virus protein can be accounted for by that isolated in the form of nucleic acid. Baernstein's procedure as modified by Kassell and Brand[4] gives values of 0.04% methionine sulfur, 0.0–0.04% sulfate sulfur, and 0.14% cystine plus cysteine sulfur, thus accounting for practically all of the sulfur. Application of Sullivan's method to hydrochloric-formic acid hydrolysates of the protein indicates that 0.11% sulfur is present as cystine or cysteine, while Lugg's modification[5] of the Folin–Marenzi

(1) Stanley, *Phytopathology*, **26**, 305 (1936); Bawden and Pirie, *Proc. Roy. Soc.* (London), **B123**, 274 (1937); Loring and Stanley, *J. Biol. Chem.*, **117**, 733 (1937).
(2) Loring, *ibid.*, **123**, 126 (1938).
(3) Stanley, *ibid.*, **117**, 325 (1937).
(4) Kassell and Brand, *ibid.*, **125**, 145 (1938).
(5) Lugg, *Biochem. J.*, **26**, 2160 (1932).