complex may be indicated by the equilibrium constant for the equation

$$M^{+2} + P_4O_{12}^{-4} = MP_4O_{12}^{-2}$$

reported by Jones and Monk as being of the order of 7 × 10^{-6}, based on conductivity data at 25°,[6] where $K = [(M^{+2})(P_4O_{12}^{-4})]/MP_4O_{12}^{-2}$.

The non-cross-linked hydrated magnesium polymetaphosphate, when free from alkali metal ions, is only slightly soluble in water. In aqueous suspension it is a very viscous water-immiscible fluid. This is converted to a brittle solid when water is removed by drying in air or by washing with alcohol. It is postulated that when water is removed, the magnesium ions then coördinate with the oxygen of adjacent metaphosphate chains, giving a product which is highly cross-linked through coördination. Magnesium atoms may therefore act as cross-linking units when the metaphosphate is dry, but may not cause cross-linking when the structure is fully hydrated.

Preparation.—One-half gram mole (59 g.) of finely ground non-cross-linked KPO_3 and 413 g. of commercial sodium salt of "Dowex" 50 cation exchange resin were slurried together in 2441 g. of distilled water (always CO_2-free) for 25 minutes, keeping the *p*H of the slurry between 8 and 9 with small additions of dilute NaOH. The viscous solution was separated from the resin by suction through a glass wool pad.

To 2181 g. of this 0.2 *m* solution of Na, K, PO_3 was slowly added, with vigorous agitation, the stoichiometric amount of $MgCl_2·6H_2O$ (44.2 g.) dissolved in 1000 ml. of distilled water, adjusted to *p*H 8.5 with MgO powder. The white rubbery precipitate was collected, squeezed to remove occluded solution, and slightly washed. It was then dissolved in 3000 ml. of distilled water by stirring and macerating the gum. The solution was adjusted to *p*H 8 with MgO powder, and was slowly added to a strongly agitated solution of 177 g. of $MgCl_2·6H_2O$ in 1000 ml. of distilled water adjusted to *p*H 8.5 with MgO powder. The precipitated gum, now very soft and sticky, was transferred to a solution of 44 g. of $MgCl_2·6H_2O$ in 1000 ml. of distilled water, adjusted to *p*H 9 with MgO, and the solution stirred over the precipitate for 16 hours. The viscous, extremely sticky and gummy oil was lightly washed, and then 400 ml. of distilled water (CO_2-free) adjusted to *p*H 8.5 with MgO, was stirred over it for 2 hours; yield of oil, 95 g. A small portion was dried to a film; the remainder was covered with distilled water, adjusted to *p*H 9.6 with MgO, and stored at 10°.

Composition of the air-dried film was as follows: Mg, 9.16, 9.46%; P, 23.04, 23.04%; K, 0.04%; Na, 0.00%; Cl, 0.29, 0.19%. Correcting for the magnesium chloride, Mg/P = 0.511; by difference, the water content was 31.7%. When dried at 110° at 10 mm. pressure, the film was very brittle and the water content was 16%, corresponding to $[Mg(PO_3)_2·2H_2O]_n$. This composition suggests a cross-linked structure in which each Mg ion is coördinated with four oxygen atoms, two from each of two adjacent $(PO_3^-)_n$ chains, plus two molecules of water, to give a total of six oxygens surrounding the metal atom. This white opaque solid reverts to the viscous liquid state after prolonged soaking in water at room temperature. The liquid product stored at 10° underwent some hydrolysis in eight months, the *p*H having dropped from 9.6 to 6.0. Samples containing alkali metal ions seemed to be less stable.

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[6] H. W. Jones and C. B. Monk, *J. Chem. Soc.*, 3475 (1950).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OREGON]

Cysteine Complexes with the Cobalt(III) Ion. III. The Role of Gaseous Oxygen in the Formation of Cysteine Complexes of Cobalt(III) from Cobalt(II) Ion and Cysteine

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The amounts of gaseous oxygen absorbed by cysteine solutions containing various concentrations of cobalt(II) ion have been studied. In dilute solution, with cobalt to cysteine ratios of 1:3 or more, one atom of cobalt was equivalent to 0.75 atom of oxygen; at ratios less than 1:3, one molecule of cysteine was equivalent to 0.25 atom of oxygen. In all experiments varying amounts of cystine were formed, and the oxidation reaction was not stoichiometrically pure. In concentrated solution, the formation of the bis- and red tris-cysteinates of cobalt(III), from cobalt(II) ion and cysteine at appropriate *p*H, proceeds with the absorption of 0.5 atom of oxygen per atom of cobalt. This corresponds exactly with the conversion of cobalt from the (II) to the (III) state, and excludes the formulation of these complexes as disulfide structures.

In entire absence of gaseous oxygen, Michaelis[3] showed that a mixture of cobalt(II) ion and cysteine at *p*H 7–8 gave a cobalt(II) complex "usually slightly olive-green" but pink in the presence of a large excess of cysteine. The cobalt(II) complex was very rapidly oxidized by gaseous oxygen to an intensely dark brown complex which was shown by Schubert[4] to be cobalt(III) bis-cysteinate. Michaelis and co-workers[3,5] and Kendall and Holst[6] reported that during this reaction one atom of oxygen was taken up. The oxidation reaction was not simple, since Kendall and Holst showed that about 23% of cystine was simultaneously formed. This amount of oxygen corresponds to two-thirds the amount necessary to oxidize the cysteine to cystine, or double the amount required to oxidize cobalt(II) to cobalt(III).

In a study of the oxidation of cobalt(II) bis-thioglycolate, Michaelis and Schubert[7] found that one-quarter of a mole of oxygen was absorbed per mole of cobalt. This observation was interpreted as corresponding either to the oxidation of cobalt(II) to cobalt(III), or to the oxidation of sulfhydryl to disulfide. Cryoscopic measurements on the oxidized complex showed it to be dimeric, and a binucleate structure was accordingly assigned. However, the structure of the oxidized complex could not be considered as definitely established, and Michaelis suggested the possibility of a desmotropic relationship between the cobalt(III)-sulfhydryl and the cobalt(II)-disulfide structures.

Since the difficulties of assigning a precise structure in the case of the bis-thioglycolate apply equally to the case of the oxidized cysteine complexes of cobalt, it was considered desirable to in-

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[3] L. Michaelis and E. S. G. Barron, *J. Biol. Chem.*, **83**, 191 (1929).

[4] M. P. Schubert, THIS JOURNAL, **53**, 3851 (1931).

[5] L. Michaelis and S. Yamaguchi, *J. Biol. Chem.*, **83**, 367 (1929).

[6] E. C. Kendall and J. E. Holst, *ibid.*, **91**, 435 (1931)

[7] L. Michaelis and M. P. Schubert, THIS JOURNAL, **52**, 4418 (1930).

vestigate the conditions of formation of the cysteine complexes of cobalt(III). This paper presents the results of a detailed study of the amounts of oxygen absorbed in the formation of each of the cobalt(III) cysteine complexes from cobalt(II) ion and cysteine. A "constant pressure" absorption apparatus was employed, the work of Michaelis was repeated, and the amount of oxygen consumed by a large number of solutions containing different ratios of cobalt to cysteine was determined.

Experimental

Materials.—Cysteine hydrochloride was supplied by Eastman Organic Chemicals, Rochester, New York; and cobalt(II) sulfate of C.P. grade was obtained from the J. T. Baker Chemical Co., Phillipsburg, New Jersey. These materials were assayed for free sulfhydryl and for cobalt by methods described elsewhere.[8] All other chemicals employed were of C.P. grade.

Apparatus.—All *p*H measurements were made using a Beckman *p*H meter. A "constant pressure" oxygen-absorption apparatus was employed which consisted essentially of a 50-ml. conical flask having a bent side-arm of capacity 2.5 ml. connected with the flask *via* a ground-glass joint. The top of the flask was furnished with a capillary T-tube attached to a ground-glass joint which fitted the neck of the flask. One arm of the T-tube led to a gas buret, the other to a stopcock. Both flask and buret were immersed in water-baths the temperature of which was recorded to 0.1°. Solutions in the flask were stirred magnetically.

Absorption Procedure. General.—Cysteine solution was placed in the flask, and cobalt(II) sulfate solution in the side-arm. With the stopcock open to the atmosphere, the solution was stirred rapidly for five minutes to equilibrate with atmospheric oxygen (cysteine solution was always stored under oxygen-free nitrogen). The stopcock was then closed and the air in the apparatus adjusted to atmospheric pressure by means of a reservoir attached to the gas buret. The solution was stirred until no further oxygen-uptake was observed, the pressure was again adjusted to atmospheric, and the volume and temperature were recorded. The side-arm was inverted by rotation at the joint, and the reaction mixture poured in and out of the side-arm to ensure complete mixing. Rapid stirring was then continued. With 10 ml. of cysteine solution over 95% of the reaction was complete within 3 minutes. A constant reading of the buret was obtained within 10 to 15 minutes. Stirring was continued until the volume remained constant over a 15-minute period, when the reaction was assumed to be complete. Gas volumes were corrected to standard conditions, and in cases where slight temperature changes occurred the mean temperature was used in calculations.

Absorption in Dilute Solution in Phosphate Buffer.—Ten-ml. aliquots of 0.04 *M* cysteine in phosphate buffer of *p*H 7.8 were mixed with various amounts of 0.08 *M* cobalt (II) sulfate in distilled water. Mixtures with cobalt to cysteine ratios ranging from 1:0.4 to 1:8.8 were oxygenated. Maximum absorption occurred for those mixtures with a cobalt to cysteine ratio of 1:3, or more. Increasing the ratio to 1:8.8 resulted in no further oxygen absorption. During these experiments appreciable amounts of crystalline cystine were formed.

Absorption in Formation of Cobalt(III) Bis-cysteinate.—The amount of oxygen absorbed in the formation of cobalt (III) bis-cysteinate under Schubert's conditions was determined in the following manner. One ml. of 1 *M* cobalt(II) sulfate was placed in the side-arm of the absorption apparatus. Two ml. of 1 *M* cysteine hydrochloride was titrated to *p*H 8.0, using 7.5 *M* potassium hydroxide, then placed in the flask and the absorption procedure carried out as above. Found: 0.50 and 0.51 atom of O absorbed per Co (2 detn.).

To determine whether any cystine had been formed during the oxidation, 1 g. of the bis-cysteinate formed as above was dissolved in the minimum amount (approx. 700 ml.) of boric acid–borax buffer of *p*H 7.7. No crystalline cystine remained. Sano[9] has determined the solubility of cystine from *p*H 0.83 to *p*H 9.85. At *p*H 7.7 the solubility of cystine is 0.0235 g. per liter ($2.35 \times 10^{-3}\%$). One gram of the bis-cysteinate prepared by Schubert's method contains, therefore, less than 0.002 g. of cystine.

Absorption in Formation of Cobalt(III) Tris-cysteinate (Red Isomer).—The red tris-cysteinate was prepared in the oxygen-absorption apparatus, using Schubert's procedure, as follows. One ml. of 1 *M* cobalt(II) sulfate was placed in the side-arm of the absorption flask. Three ml. of 1 *M* cysteine hydrochloride was titrated to *p*H 5.5, using 7.5 *M* potassium hydroxide, and the oxygen absorbed was determined as above. Found: 0.52 atom of O absorbed per Co (2 detn.).

Results and Discussion

In dilute solution in phosphate buffer with cobalt to cysteine ratios of 1:3 or more, one atom of cobalt was equivalent to 0.75 atom of oxygen. At ratios less than 1:3, one molecule of cysteine was equivalent to 0.25 atom of oxygen. In all experiments varying amounts of cystine were formed, and it appears that the course of the oxidation reaction is dependent upon conditions. The reaction is not stoichiometrically pure and at least two concurrent reactions take place, namely, oxidation of cysteine to cystine and cobalt(II) to cobalt(III). No single chemical equation can be written, therefore, to represent the facts.

In the formation of the bis- and red tris-cysteinates of cobalt(III), from cobalt(II) ion in concentrated solution, exactly 0.5 atom of oxygen was absorbed. This corresponds to the oxidation of cobalt from the (II) to the (III) state. As no cystine was detected in these reactions it may be concluded that, in concentrated systems, the rate of oxidation of the cobalt(II) cysteine complexes greatly exceeds the cysteine–cystine oxidation. The significant fact that emerges from the study of the oxygen-uptake in the formation of the cobalt(II) cysteine complexes is that only enough oxygen is absorbed to oxidize the cobalt to the trivalent state. Any formulations of the cobalt(III) cysteine complexes as disulfide structures are therefore excluded.

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[9] K. Sano, *Biochem. Z.*, **168**, 14 (1926).