THE ELECTROMETRIC TITRATION OF HEMIN AND HEMATIN.

By JAMES B. CONANT, GORDON A. ALLES, and C. O. TONGBERG.

(From the Gates Chemical Laboratory, California Institute of Technology, Pasadena, and the Chemical Laboratory of Harvard University, Cambridge.)

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It was shown 5 years ago (1) that the change from methemoglobin to hemoglobin could be studied by an electrochemical method. The reduction by means of sodium hydrosulfite and the oxidation of the reduced compound (hemoglobin) by ferricyanide were found to involve 1 equivalent. These results have been confirmed and extended in later work (2). An application of the same method to a study of the reduction of alkaline hematin solutions (1) yielded uncertain results which seemed to indicate that 2 equivalents of hydrogen were involved in the reduction of this compound. No definite conclusion could be drawn from the data, however. A continuation of this work has now shown that satisfactory electrochemical titration curves may be obtained *if titanous tartrate is employed as the reducing agent.* The results are very definite and show that the change involves *only 1 equivalent.*

While this work was in progress, two other workers reported results obtained by entirely different methods. These show that the reduction of hematin, like the reduction of methemoglobin, is the change from a ferric to a ferrous compound. The experiments which we now have to report, therefore, serve merely to confirm the recent observations of Haurowitz (3) and those of Hill (4).

Titration of Hemin and Hematin in Borate-Tartrate Buffer Solutions.

In Fig. 1 are shown typical titration curves of hemin solutions with titanous chloride in the presence of tartrate. The crystalline

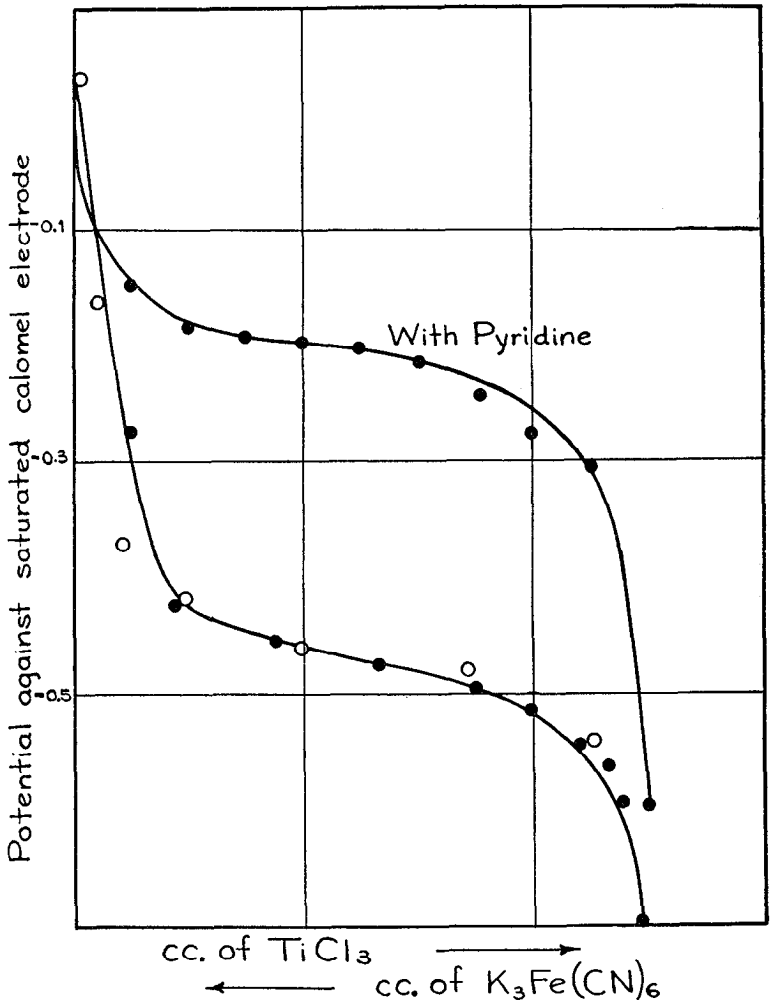

FIG. 1. Electrometric titration of hemin dissolved in borax-tartrate buffer solutions (pH 9.15). Clear circles indicate the points obtained in the back titration with ferricyanide.

hemin[1] was dissolved in 0.1 M borax either at room temperature or by warming, an equal volume of 0.2 M sodium tartrate was added, and the mixture placed in the usual electrochemical cell. After sweeping out all the air with oxygen-free nitrogen, dilute titanous chloride was added from a burette and the potential of two bright platinum electrodes noted. The potential was measured against a saturated calomel electrode. It was found necessary to use special precautions in purifying the nitrogen since the reduced hematin is extraordinarily sensitive to oxygen. Metallic nickel at a red heat or long trains of ammoniacal cuprous solutions were found to be fairly satisfactory.

The potentials are plotted vertically in Fig. 1 and the increments of reducing or oxidizing agent are plotted horizontally. The back titration with potassium ferricyanide is shown by clear circles. It is evident that the end-points are clearly defined. This is particularly true in the case of the titanous chloride titration in the presence of pyridine which causes a change of the position of the curve because the potential of the pyridine hemochromogen system is more positive than that of the reduced hematin system.

The presence of the tartrate ion in the alkaline buffer solution prevents the precipitation of titanous and titanic hydroxides. Thus, an alkaline complex titanous tartrate is in reality the reducing agent employed but, as solutions of it are very sensitive to oxygen, this indirect method of adding it was finally adopted. The addition of 3 per cent of mannite instead of sodium tartrate will also prevent the precipitation of titanous or titanic hydroxide but the titanous mannite complex does not have a sufficiently low potential to give a sharp end-point. The very dilute titanous chloride used was made by diluting the commercial 20 per cent material with boiled water; it was stored in an atmosphere of nitrogen. It slowly changed and was made up fresh every few days. It was standardized just before or after each titration by titrating electrochemically with potassium ferricyanide.

A few of the many results we have obtained are given in Table I

[1] The hemin used in this work was the α-hemin which was prepared by the usual acetic acid method from horse blood. It was purified by solution in chloroform and pyridine and reprecipitation in acetic acid containing sodium chloride. A few experiments with β-hemin prepared by Mörner's procedure showed that its reduction also required only 1 equivalent.

which is self-explanatory. The numbers in the last column show that within the limits of the experimental error there is 1 mol of titanous chloride used in the reduction and 1 mol of ferricyanide in the reoxidation. The result is independent of the method of

TABLE I.

Determination of Number of Equivalents Involved in Reduction of Hemin or Hematin and Reoxidation of the Reduced Compound.

Experiment No.	pH	Iron compound.	Mols × 10^4.	Reagent employed.	Molality of reagent.	Amount of reagent.	Mols of reagent × 10^4.	Ratio of mols of reagent to mols of compound.
		mg.				*cc.*		
1	10.26	61.9	0.94	$TiCl_3$	0.0174	7.00	1.24	1.3
1 a	10.26	61.9	0.94	$K_3Fe(CN)_6$	0.0200	4.50	0.90	0.96
2	9.15	12.4	0.19	$TiCl_3$	0.0189	0.96	0.18	0.95
2 a	9.15	12.4	0.19	$K_3Fe(CN)_6$	0.0200	0.89	0.18	0.95
3	9.15	12.4	0.19	$TiCl_3$	0.0150	1.35	0.20	1.05
4	9.15	12.4	0.19	"	0.0125	1.95	0.24	1.3
5*	9.09	61.9	0.94	"	0.0167	6.00	1.00	1.06
5 a*	9.09	61.9	0.94	$K_3Fe(CN)_6$	0.02	5.00	1.00	1.06
6*	9.1	12.4	0.19	$TiCl_3$	0.0100	2.00	0.20	1.05
7*	9.1	12.4	0.19	"	0.0111	1.80	0.20	1.05
8	9.15	12.4	0.20	"	0.0140	1.40	0.20	1.0
9	9.15	12.4	0.20	"	0.0125	1.80	0.23	1.15

50 cc. of 0.1 M borax and 0.2 M sodium tartrate were used in each experiment; in those marked with an asterisk (*) pyridine was used also; sodium hydroxide was added in Experiment 1. Experiments 1 to 7 were performed with crystalline α-hemin, Experiment 8 with hematin prepared according to Küster (5), and Experiment 9 with dehydrochlorohemin. In Experiments 1, 5, and 7 the hemin was dissolved at room temperature; in the other experiments the iron compound was dissolved by warming it with the borax solution to 70° for 5 minutes, cooling to 20°, and adding the tartrate. The titrations with ferricyanide (Experiments 1 a, 2 a, 5 a) were performed with the solution reduced with $TiCl_3$ in the experiments of the same numbers.

dissolving the hemin and independent of the presence of pyridine. Hematin (Experiment 8) and dehydrochlorohemin (Experiment 9) also require 1 equivalent for the reduction. There can be no doubt from these results that the change in each case involves the reduction or the oxidation of the iron atom.

Aside from the information in regard to the number of equivalents involved in the oxidation or reduction, a satisfactory method of studying the iron-porphyrin compounds is of some interest, since it provides another method of characterizing these substances. This is of particular value in the case of the formation of easily dissociable complexes such as are formed between hemin and pyridine. Although the potentials are not as definite or as reproducible as in the case of the quinones, the method is useful even in extremely dilute solutions. For example, we have been able to obtain fairly satisfactory results in solutions of the pyridine-hemin compound which contained only 3×10^{-7} mols of hemin per liter. An electrochemical titration thus promises to be a useful supplement to the spectroscopic method of detecting and studying those iron-porphyrin derivatives which occur widely distributed in nature. We are now engaged in determining the oxidation-reduction potential of all the various iron-porphyrin compounds which can be obtained, including those formed by the decomposition of hemoglobin (α-hemin, β-hemin, "Verdauung's hematin," *etc.*) and their combinations with such substances as pyridine and the cyanide ion. The results of this work will be the subject of a later paper.

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