

MAGNETOCHEMICAL STRUCTURAL INVESTIGATIONS
WITH PARTICULAR REFERENCE TO HEMOGLOBIN
EQUILIBRIA AND COMPOUND FORMATION

Thesis by

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Introduction

It can be stated, with a fair degree of historical accuracy, that the science of magnetism began with experimental discoveries of the ancient peoples dealing with the attractive power of a mineral ore, lodestone, or magnetite, for iron. An old legend attributes the discovery of magnetite to a Cretan shepherd, who was strongly drawn to the earth by his iron-tacked sandals and iron-tipped crook, while traveling in Magnesia, a region in Asia Minor where magnetite is very plentiful. (1) However, even in the earliest books of the Hindus, the Vedas, a description of the lodestone may be found, and also in the ancient literature of the Chinese, Greeks, and Romans, references may be met bearing on the relationships of magnetic phenomena to earthly directions and celestial motion. It is interesting to note that the pivoted needle, or mariner's compass, was established by the writings of Neckam of St. Albans at about the time of the Crusades in the twelfth century. (2)

The names of many scientists and philosophers immediately present themselves to the research worker in the field of magnetism. Among the ancients, Thales, Socrates, Plato, Aristotle, and many others are all credited with the knowledge of the attraction of lodestone for iron, but like most of the physical science

of the Greeks and Romans it was mostly qualitative and speculative. In the thirteenth century, there is found a man, Peter Peregrinus, of outstanding experimental ability, to whom is given the first credit for the introduction of the term "magnetic pole".(1)

William Gilbert of Colchester (1540-1603) is perhaps the first outstanding worker in magnetism. His famous treatise "De Magnete" reveals him to be a very outstanding personality with a great ability to coordinate experimental facts and to separate them from fiction. His greatest contributions were A. the discovery of the earth's magnetism, and B. the uncovering of the fact that iron ceases to be attracted when red-hot.

The first quantitative magnetic investigations were carried on by John Mitchell (1724-1793) and John Robison (1739-1805). Together they brought forth the fundamental law that "the attraction and repulsion power of magnets decreases as the squares of the distances from the respective poles increase"(1) Mitchell's work was readily repeated and verified by Coulomb (1736-1806) who was the man responsible for the well-known Coulomb law of the mutual action between magnetic poles.

The first application of the mathematical analysis to the clarification of physico-chemical problems in the realm of magnetism and electrostatics was accomplished by Poisson, who used the famous "two-fluid theory" as a

means of deriving Coulomb's law. Poisson's treatment, refined and extended by Kelvin and Green, is the recognized basis of modern statistical theory of magnetism.(1) In the period shortly following, the field became crowded with many investigators. Some of the more important ones were Oersted, Arago, Biot, Savart, and Ampere. Ampere seems to be the most outstanding and is noted for his "molecular-current" theory of magnetism.

Faraday has been called the founder of magnetochemistry by Bhatnagar and Mathur.(1) In 1831, he described his famous laws of electromagnetic induction before the Royal Society. In 1845, he discovered accidentally the diamagnetic qualities of glass, and this, along with the previous discovery by Brugmans of the diamagnetism of bismuth, attracted the attention of chemists to the study of magnetic phenomena. Faraday, in turn, examined large numbers of substances for their magnetic properties in different states and classified them accordingly.

Following Faraday, it is found that the number of research workers in magnetochemistry has undergone another abrupt increase and up until the present day the number has been steadily growing due to the interesting problems presenting themselves in the field. Some of the more important persons in the period following Faraday were Tyndall, Weber, Ewing, and Evershed. The

last three all proposed theories of magnetism. The theories of Ewing and Evershed being pictorial descriptions appealed to those chemists who are not mathematically inclined.

One of the most outstanding magnetic workers was Pierre Curie, who devised in 1895 the most successful magnetic balance. His experimental researches on the magnetic properties of substances in relation to temperature(1895)(1), Honda's work on the elements, and Pascal's work on organic compounds constitute the pillars on which the modern theory of magnetism has been raised.

Some of the later workers in magnetochemistry have made really outstanding contributions to the field. Among them are Langevin, Weiss, Gans, Oxley, Stoner, Van Vleck, and Pauling. Actually, structural magnetochemistry began about 1931 with the independent researches of Bose, Stoner, and Pauling. Pauling's first paper on the nature of the chemical bond* gives many examples of deductions regarding the atomic arrangement, bond angles, and other properties of molecules and complex ions from magnetic data, with the aid of calculations involving bond eigenfunctions.

The magnetochemistry of hemoglobin started with Faraday over ninety years ago, on November 8, 1845.

*L. Pauling- Jour. Amer Chem. Soc., 53, 1367 (1931)

He investigated the magnetic properties of a cake of dried blood and made a note "must try recent fluid blood."^{*} If he had carried out his suggestion and determined the large difference in the susceptibilities of arterial and venous blood, the discovery would have excited a great interest in magneto-hemoglobin-chemistry. The course of blood and hemoglobin research would thus have been very definitely influenced. A. Gamgee^{**} and one or two more recent investigators have reported blood to be about as diamagnetic as water. The difference between arterial and venous blood however was not discovered until later. Felix Haurowitz and Herbert Kittel in 1933 stated^{***} that their magnetic measurements indicated that the N-atoms of the free radical in porphyrins were arranged diagonally opposite. Hisayuki Kudo in 1934 announced^{****} that human and animal bloods were diamagnetic with a susceptibility slightly greater than water.

The first important paper^{*****} was^{written} by Linus Pauling and C. D. Coryell in 1936 at the California Institute. They showed by their magnetic measurements that oxyhemoglobin and carbomoxyhemoglobin possessed no unpaired

- ^{*} L. Pauling and C. D. Coryell - Proc. Nat. Acad. Sci., 22, 210, (1936)
- ^{**} A. Gamgee - Proc. Roy. Soc., London, 68, 503 (1901)
- ^{***} F. Haurowitz and H. Kittel - Ber., 66B, 1046 (1933)
- ^{****} H. Kudo - Acta Med. Scand., 81, 511 (1934)
- ^{*****} L. Pauling and C. D. Coryell - Proc. Nat. Acad. Sci., 22, 210, (1936)

electrons and that ferrohemo-globin contained four un-
paired electrons per heme. This proved that the bonds
from iron to the surrounding atoms were ionic in hemo-
globin, and covalent in oxyhemo-globin and carbonmonoxy-
hemo-globin. Much work has been carried out since this
first original paper. In 1937, Coryell, Stitt, and Paul-
ing* worked out the magnetic properties and structure of
methemo-globin and some of its compounds. The great im-
portance of this work was in explaining the sigmoidal char-
acter of the variation of the magnetic susceptibility of
ferrihemo-globin solutions with pH. In 1938, Taylor and
Coryell made a very intensive study of the magnetic sus-
ceptibility of iron in ferrohemo-globin.** In 1939, Stitt
and Coryell studied magnetically the equilibrium between
ferrohemo-globin, cyanide iron, and cyanide ferrohemo-glo-
bin.***

Much work is still being done in the magnetochemistry
of hemo-globin and it is hoped by the author that the work
done in this dissertation might be a worthy contribution
to the field.

* Coryell, Stitt, and Pauling - Jour. Am. Chem. Soc.,
59, 633, (1937)

** Taylor and Coryell - Jour. Am. Chem. Soc., 60,
1177, (1938)

*** Stitt and Coryell, Jour. Am. Chem. Soc., 61,
1263, (1939)

Theoretical Discussion

In a classical paper in 1905, Langevin(1) gave a new outlook on the facts of para- and diamagnetism. He applied the concepts of the electronic hypothesis to formulate an exact mathematical theory which provided a very satisfactory explanation of both para- and diamagnetism. Since Langevin's treatment was not based on any specific assumption, it could be modified to accord with the principles of the later quantum mechanics.

Diamagnetism --- Substances are said to be diamagnetic when they are repelled by a magnet. There is produced in every substance, upon the application of a magnetic field, a magnetic polarization arising from the "Larmor precession" of the electron orbits, which is an accelerating influence of the field on the electrons.(4) This polarization is negative in direction, thus giving rise to a field which is opposed to the applied field. Since the "Larmor precession" is independent of any thermal variation of the kinetic energy of the atoms, it follows that the diamagnetism should be independent of the temperature. However, this is not universally true, as there have been numerous diamagnetic substances found which are influenced by temperature. Also the diamagnetic polarization of a substance is almost independent of structure; it can be calculated(4)

with reasonable accuracy as the sum of the terms corresponding to the atoms composing the substance, with perhaps some small corrections for the bond-types involved.

Paramagnetism - - A substance is said to be paramagnetic when it is polarized in a direction opposite to its diamagnetic polarization, that is, with the field. In this condition, the substance is attracted by a magnet. This paramagnetic polarization(4), often of large magnitude, results from the presence in the substance of atoms, ions, or molecules with permanent magnetic dipole moments, which in general are due in part to the spin magnetic moments of unpaired electrons and in part to the magnetic moments associated with the orbital motion of the electrons. The orienting tendency of the permanent magnet dipoles in a magnetic field is opposed by the effect of temperature, and, accordingly, paramagnetic polarization is temperature dependent. For ideal systems, having small interaction between the dipoles, it is inversely proportional to the absolute temperature.(4)

Molal susceptibility - - Let χ_{molal} be the molal susceptibility of a substance; N , the Avogadro number; k , the Boltzmann constant; T , the absolute temperature; α , the molecular diamagnetic susceptibility (a negative quantity); and μ , the permanent magnetic dipole moment. It is then possible to relate all these

quantities by the means of the Langevin-Pauli equation.(4)

$$\chi_{\text{molar}} = N\alpha + \frac{N\mu^2}{3kT} = N\alpha + \frac{C}{T} \quad (\text{I})$$

In the extreme right-hand form of the equation, C is called the Curie constant and is equal to

$$\frac{\mu^2}{3kT} \quad (\text{II})$$

The dipole magnetic moment, μ , is usually expressed in Bohr magnetons, with magnitude

$$\frac{eh}{4\pi mc} \quad (\text{III})$$

and in this unit, it is related to C by the equation:(4)

$$\mu = \sqrt{\frac{3kc}{N}} = 2.839 \sqrt{C} \quad (\text{IV})$$

If it is assumed that the orbital contribution to the magnetic dipole moment is zero, or if a correction can be applied, the magnetic dipole moment, μ , is related to the number of unpaired electron spins, n, by the following equation.(4)

$$\mu = \sqrt{n(n+2)} \quad \text{Bohr Magnetons}$$

The following short table shows the relationship between the magnitude of the dipole moment and the number of the unpaired electrons.

No. of Unpaired Electrons	Magnetic Dipole Moment
1	1.73
2	2.83
3	3.88
4	4.90
5	5.92

Calculation of the molal magnetic susceptibility from experimental data --- In the Gouy method(1) for determining magnetic susceptibilities, described in the section of this thesis on "Apparatus and Methods of Measurement", an equation relating the force F_x , or measured change in weight, W , with the field strength, H , and the cross-sectional area of the tube, A , can be written as follows:

$$F_x \quad \Delta W = \frac{1}{2}(K_1 - K_2) AH^2 \quad (VI)$$

where K_1 and K_2 represent the volume susceptibilities of the material in the cylinder and the medium respectively. Then, if three measurements are taken; solution versus water, solvent versus water, and air versus water; it is possible to write down three equations respectively.

$$\begin{aligned} \Delta W_{\text{solution-water}} &= \frac{1}{2}(K_{\text{solution}} - K_{\text{water}})AH^2 \\ \Delta W_{\text{solvent-water}} &= \frac{1}{2}(K_{\text{solvent}} - K_{\text{water}})AH^2 \\ \Delta W_{\text{air-water}} &= \frac{1}{2}(K_{\text{air}} - K_{\text{water}})AH^2 \end{aligned}$$

After combining the above three equations, the following two equations are obtained.

$$K_{\text{solu}} - K_{\text{water}} = \frac{(\Delta W_{\text{solution-water}})(K_{\text{air}} - K_{\text{water}})}{(W_{\text{air-water}})} \quad (\text{VIII-a})$$

$$K_{\text{solv}} - K_{\text{water}} = \frac{(\Delta W_{\text{solvent-water}})(K_{\text{air}} - K_{\text{water}})}{(\Delta W_{\text{air-water}})} \quad (\text{VIII-b})$$

However,

$$K_{\text{solute}} = K_{\text{solution}} - K_{\text{solvent}}$$

Therefore, by subtracting (VIII-b) from (VIII-a), an equation for the volume susceptibility of the solute is obtained.

$$K_{\text{solute}} = \frac{(\Delta W_{\text{solution-water}} - \Delta W_{\text{solvent-water}})(K_{\text{air}} - K_{\text{water}})}{(\Delta W_{\text{air-water}})} \quad (\text{IX})$$

But,

$$K_{\text{solute}} = \frac{\text{susceptibility of solute}}{\text{cm}^3}$$

Hence, equation (IX) must be multiplied by

$$\frac{1000}{C}$$

where C is the molar concentration of the solute, to give the expression for molal susceptibility of the solute.

$$\chi_{\text{molal}} = \frac{(\Delta W_{\text{solu-water}} - \Delta W_{\text{solv-water}})(K_{\text{air}} - K_{\text{water}})(1000)}{(\Delta W_{\text{air-water}})(C)} \quad (\text{X})$$

This expression is used in the thesis to calculate molal magnetic susceptibilities.

Apparatus and Methods of Measurements

All magnetic measurements in this reasearch have been made using the Gouy method(2), which involves measurement of the change in weight, ΔW , of a vertical cylinder of a substance when one end is placed in a field of strength H and the other in zero field. A large half-ring water-cooled magnet was used, the flat surfaces of the pole pieces being 38 mm. in diameter and 22.5 mm. apart. The fields were between 7000 and 9000 oersteds, but it was not necessary to know the absolute field strength since all measurements were made relative to water at a known temperature (20-25°C).

Glass tubes of constant diameter were separated into two compartments by a glass septum, and provided with ground glass caps for the ends and suitable supports for suspension from the arm of a Becker disc-chainomatic balance. The tubes were placed in a thermostated, asbestos-lined compartment, with the septum of the tube between the pole pieces. The substances to be compared occupied the two compartments; solid measurements were made relative to air and solution measurements were made relative to the solvent (water). Solids were introduced in small portions and well tamped in the upper compartment. The solid tube had an internal diameter of 6 mm.,

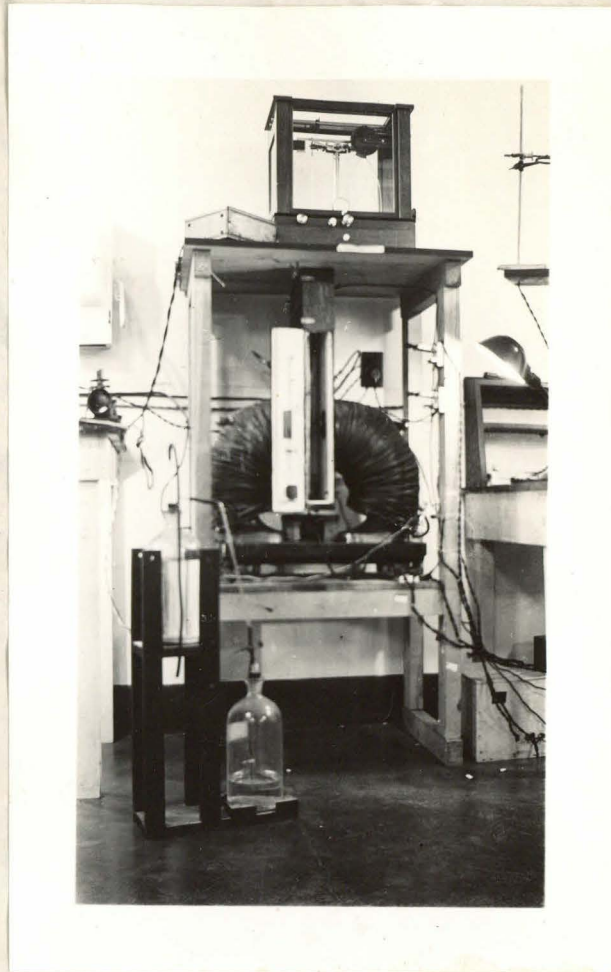
whereas the solution tubes had internal diameters of approximately 18 mm. The compartments were about 15 cm. long.

The tube constants measured were water versus water, to correct for the magnetic properties of the tube, and air versus water, our primary standard in susceptibility.

In all ΔW measurements, four readings were taken; one at zero field, one at an ammeter setting of 9, one at an ammeter setting of 14, and one again at zero. The W 's were obtained by subtracting the average of the zero readings from those at 9 and 14, to give ΔW_9 and ΔW_{14} , respectively. ΔW_{14} was converted to W_9 by dividing it by a factor of 1.347, and it was then averaged with the first ΔW_9 to give the value reported in the tables.

In magnetic titrations, reagents were added by means of a 1cc. glass hypodermic syringe and needle through a rubber stopper placed at the top of the solution compartment. The syringe was graduated into hundredths of a cubic centimeter and could be very easily read.

Measurements of pH values were made with a Beckman glass-electrode pH meter. As a standard, 0.05 M potassium hydrogen phthalate solution, pH 3.98, was used.



The magnet used in this
research at the California
Institute of Technology

Preparation and Standardization
of Hemoglobin Solutions

(a) Preparation of Ferrohemin Solutions:

Bovine blood, defibrinated by rapid stirring, was centrifuged once to remove the serum, and the corpuscles were washed with 0.14 M potassium chloride and centrifuged at least four times. After each period of centrifuging, the upper layer of potassium chloride solution was removed with an aspirator. The blood cells were hemolyzed with peroxide-free ether, the stromata emulsion was centrifuged off, and the dissolved ether was removed by bubbling air through the solutions. The solutions were stored as oxyhemoglobin at approximately 0°C. in an electric refrigerator until used.

(b) Preparation of Ferrihemin Solutions:

1 N Lactic acid was added to the solutions of oxyhemoglobin to bring the pH down to about 4.6-4.9 and the systems were left to auto-oxidize over a period of forty-eight hours at room temperature. Auto-oxidation is complete after this period. The pH was then brought up to 7.0 by adding either NaOH or Na₃PO₄ solutions with rapid stirring. The systems were centrifuged at this pH to remove the small

amount of denatured protein formed during the acidification. The solutions, brought to a desired pH, were then stored as ferrihemoglobin or ferrihemoglobin hydroxide at 0°C. in an electric refrigerator until used.

(c) Standardization of Hemoglobin Solutions:

In the case of oxyhemoglobin, a known volume of solution (20.0-25.0 cc.) was reduced to ferrohemo-
globin with 0.3-0.6 g of $\text{Na}_2\text{S}_2\text{O}_4$ and the ΔW_g was measured. The system was then saturated with CO and another ΔW_g was taken, to correct for the diamagnetism of the protein and the reagents. The change in ΔW_g corresponds to a change in molal susceptibility (per heme) of $12,290 \times 10^{-6}$ c.g.s.u. at 25°C., the effective magnetic moment of ferrohemo-
globin per heme being taken as 5.43 Bohr magnetons.*

In the case of ferrihemoglobin a known volume of solution was made alkaline with NaOH to a pH of about 10.5, at which point there is only methemoglobin hydroxide present, and a ΔW_g was taken. The system was then reduced with 0.3-0.6 g. of $\text{Na}_2\text{S}_2\text{O}_4$, saturated with CO, and another ΔW_g measured. This change in ΔW_g corresponds to a change in molal susceptibility (per heme) of 8350 c.g.s.u. at 25°C., for solutions

*D. S. Taylor and C. D. Coryell, Jour. Am. Chem. Soc., 60, 1177 (1938)

of low ionic strength.**

It is possible then to make an accurate calculation of the concentration, C, in heme-iron, of the hemoglobin solutions by means of the following formula:

$$C = \frac{(\Delta W_9 - \Delta W_9 \text{COHb})(0.748 \times 10^{-6})(10^3)}{(\Delta W_9 \text{ air-water}) \chi \text{ molal}} \quad (\text{XI})$$

where: ΔW_9 is the ΔW_9 for the solution; $\Delta W_9 \text{COHb}$ is the ΔW_9 for the solution saturated with CO; $\Delta W_9 \text{air-water}$ is the ΔW_9 for air versus water; (all ΔW_9 's are corrected for the ΔW_9 of the tube, water versus water)

χ_{molal} is the molal susceptibility of the species in solution, i.e., 12,290 for ferroheme-globin and 8350 for metheme-globin hydroxide; and the factor 0.748×10^{-6} is the value accepted for the volume susceptibility of air versus water at room temperature and can be calculated from values obtained in the International Critical Tables, volume VI.

**C. D. Coryell, F. Stitt, and L. Pauling,
Jour. Am. Chem. Soc., 59, 633 (1937)

Experimental Procedure

Results, Conclusions, and

Remarks

Ferrohemoglobin Ethyl Isocyanide

Warburg, Negelein, and Christian have reported a compound of ferrohemoglobin and methyl isocyanide, having a molar ratio of isocyanide to iron equal to one.* It was hoped by the author that a magnetic study of this type of complex might prove valuable in determining the structure of such compounds. Since ethyl isocyanide is more soluble in water and has a higher boiling point than the methyl derivative, the equilibrium of ferrohemoglobin with ethyl isocyanide was investigated. Preliminary qualitative experiments revealed the compound to be diamagnetic and also to have an absorption spectrum with maxima at approximately 5540 Å and 5250 Å.

20 cc. of cow hemoglobin, the concentration of which was 0.0160 moles per liter in heme-iron, was placed in a differential tube and the ΔW_9 was measured at 20°C. Known volumes of a solution of ethyl isocyanide, of a known concentration, were added successively to the system, the ΔW_9 being measured after each addition. The addition of the reagent was accomplished by the means of a glass syringe, graduated in hundredths of a cubic centimeter.

The titration was repeated once with a new

* O. Warburg, E. Negelein, and W. Christian, *Biochem. Z.*, 214, 26. (1929)

standard solution of ethyl isocyanide. Great care was taken in the second run to eliminate all noticeable sources of error. No apparent change in the ΔW_g with time was noticed.

The results of the two experiments are tabulated in tables I and II. The ΔW_g 's, corrected for the ΔW_g of the tube itself and for the volume changes, are plotted as a function of the volume of the isocyanide solution added. The intersection of the two straight lines in each graphical plot is taken as the end-point of the reaction.

Calculation of the molar ratio of isocyanide to heme-iron:

(a) Titration No. 1:

End-point-- 0.560 cc. of isocyanide solution

$$\frac{(0.0160)(20.0)}{(1000)} = \text{moles of heme-iron}$$

$$\frac{(0.670)(0.560)}{(1000)} = \text{moles of isocyanide}$$

$$\text{molar ratio} = \frac{\text{moles of isocyanide}}{\text{moles of heme-iron}} = 1.17 \text{ Ans.}$$

(b) Titration No. 2:

End-point -- 2.18 cc. of isocyanide solution

$$\frac{(0.0160)(20.00)}{(1000)} = \text{moles of heme-iron}$$

$$\frac{(0.147)(2.18)}{(1000)} = \text{moles of isocyanide}$$

$$\text{molar ratio} = \frac{\text{moles of heme-iron}}{\text{moles of isocyanide}} = 1.00 \text{ Ans.}$$

Conclusions and Remarks:

Spectroscopic and magnetic evidence of the formation of a compound of ferrohemoalbumin with ethyl isocyanide has been obtained. Magnetic data have been presented which demonstrate that within the experimental error the molar ratio of isocyanide to heme-iron is one. It has also been shown that the complex is a very stable one, exhibiting very little dissociation under the conditions of the experiment. If the complex were appreciably dissociated, there should exist a noticeable curvature near the end-point of the graph. No such curvature is apparent.

It is concluded that ferrohemoalbumin ethyl isocyanide is diamagnetic, is very stable with respect to dissociation into ferrohemoalbumin and ethyl isocyanide, and has essentially covalent d^2sp^3 octahedral coordination about the iron. Its coordination bond-type is thus similar to that of oxyhemoalbumin, carbonmonoxyhemoalbumin, and cyanide ferrohemoalbumin.

NOTE: The addition of n-butyl isocyanide to ferrohemoalbumin solutions lowered the susceptibility. However, since denaturation was observed, no attempt was made to interpret the data obtained. The significance of the data was further obscured by the high insolubility of n-butyl isocyanide in aqueous ferrohemoalbumin solutions.

TABLE I -- FERROHEMOGLOBIN - ETHYL ISOCYANIDE TITRATION NO. 1

Total Volume of Isocyanide Soln. Added	Total Volume of System	ΔW_g	ΔW_g for Tube	ΔW_g cor. for Tube	ΔW_g cor. for Tube and Volume change
1. 0.00 cc.	20.00cc.	10.34 mg.	-0.24 mg.	10.58 mg.	10.58 mg.
2. 0.05	20.05	9.05	-0.24	9.29	9.31
3. 0.15	20.15	7.54	-0.24	7.78	7.83
4. 0.25	20.25	5.23	-0.24	5.47	5.54
5. 0.35	20.35	3.02	-0.24	3.26	3.32
6. 0.45	20.45	1.13	-0.24	1.37	1.40
7. 0.55	20.55	-0.76	-0.24	-0.52	-0.53
8. 0.64	20.64	-0.98	-0.24	-0.74	-0.76
9. 0.75	20.75	-1.18	-0.24	-0.94	-0.98
10. 0.85	20.85	-1.12	-0.24	-0.88	-0.92
11. 0.95	20.95	-1.06	-0.24	-0.82	-0.86
12. 1.00	21.00	-1.15	-0.24	-0.91	-0.95

Original conc. hemoglobin soln. = 0.0160 moles Fe / lit.
 Original conc. isocyanide soln. = 0.670 moles / lit.
 Temperature -- 20.0°C.

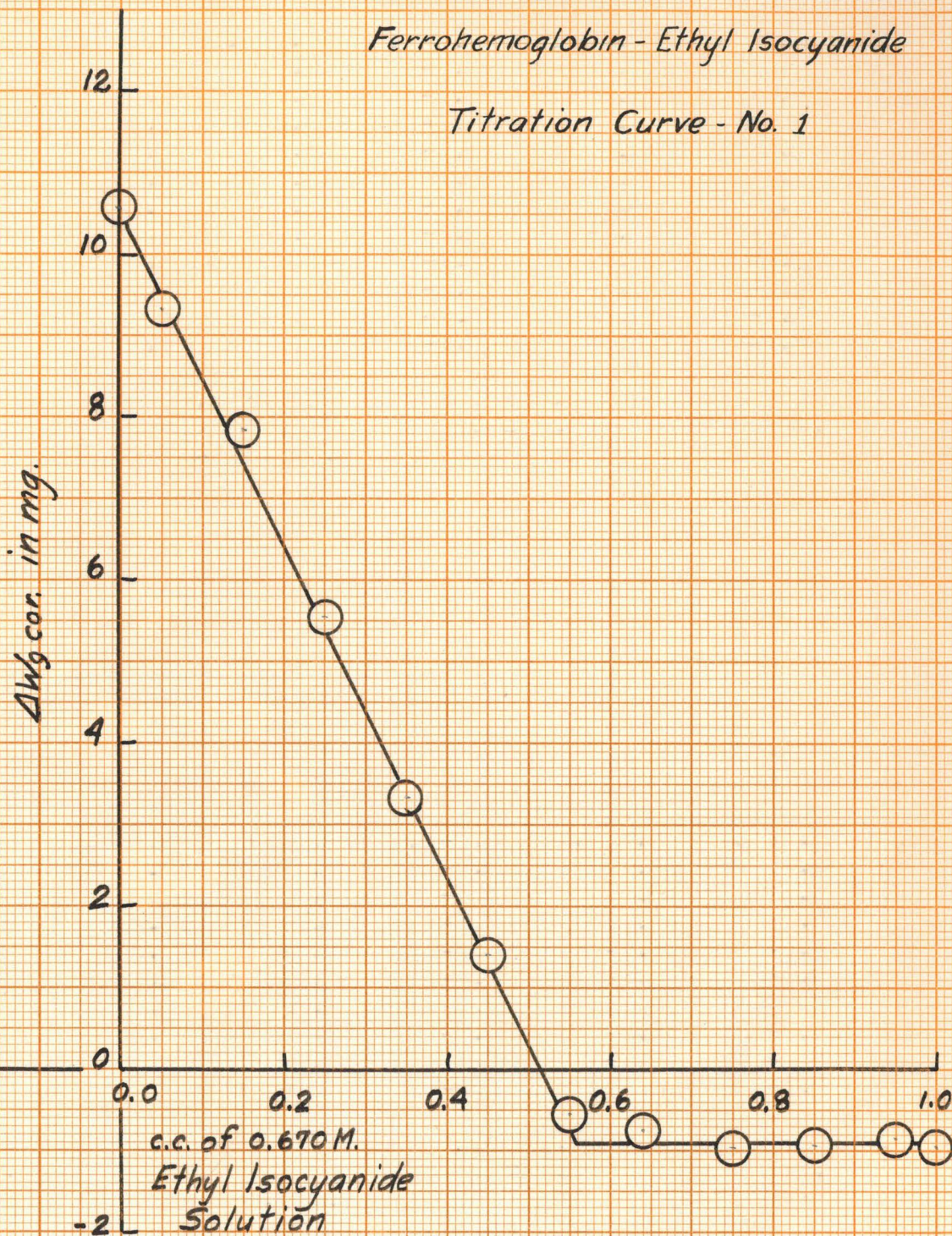
TABLE II - FERROHEMOGLOBIN - ETHYL ISOCYANIDE TITRATION NO. 2

Total Volume of Isocyanide Soln. Added	Total Volume of System	ΔW_g	ΔW_g for Tube	ΔW_g cor. for Tube	ΔW_g cor. for Tube and Volume change
1. 0.00 cc.	20.00 cc.	10.92 mg.	-0.24 mg.	11.16 mg.	11.16 mg.
2. 0.50	20.50	7.98	-0.24	8.22	8.42
3. 1.00	21.00	4.97	-0.24	5.21	5.47
4. 1.50	21.50	1.99	-0.24	2.23	2.40
5. 2.00	22.00	-0.45	-0.24	-0.21	-0.23
6. 2.50	22.50	-1.18	-0.24	-0.94	-1.06
7. 3.00	23.00	-1.18	-0.24	-0.94	-1.08
8. 3.50	23.50	-1.22	-0.24	-0.98	-1.15
9. 4.00	24.00	-1.18	-0.24	-0.94	-1.13
10. 4.50	24.50	-1.33	-0.24	-1.09	-1.33

Original conc. hemoglobin soln. = 0.0160 moles Fe / lit.
 Original conc. isocyanide soln. = 0.147 moles / lit.
 Temperature -- 20.0°C.

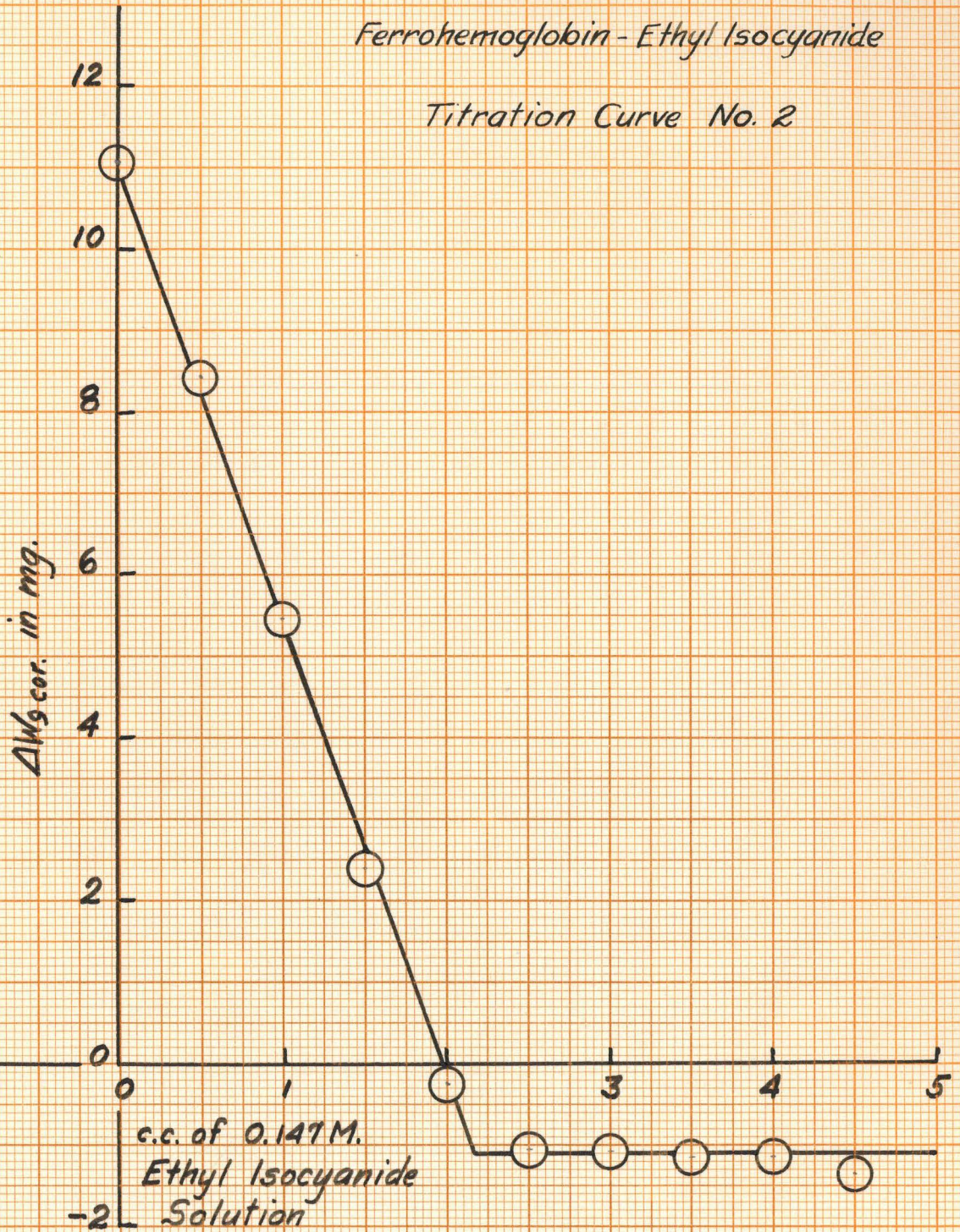
Ferrohemoglobin - Ethyl Isocyanide

Titration Curve - No. 1



Ferrohemoglobin - Ethyl Isocyanide

Titration Curve No. 2



Ferrihemoglobin Imidazole

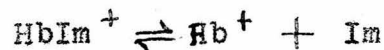
A qualitative experiment indicated a decrease in the susceptibility of a ferrihemoglobin solution upon the addition of imidazole. Therefore it seemed desirable that a series of magnetic titrations should be made at different pH values, namely, 6.86, 8.20, and 10.30, in order to study the equilibrium of this system.

In all titrations, 20 cc. of bovine methemoglobin, at a known pH and a known concentration, was placed in a differential magnetic tube and a ΔW_g measured. Known portions of imidazole solution, made up by weight, were then successively added, the ΔW_g being measured after each addition. In the run at pH 6.86 the imidazole solution was brought to this pH before adding. In all other titrations the imidazole solutions at their original pH values (about 9) were added directly to the ferrihemoglobin systems and the pH of the hemoglobin solutions was measured before and after each titration. The ferrihemoglobin solutions at pH 6.86 and 10.30 contained phosphate buffers; those at pH 8.20 contained borate. Following each experiment the system was reduced with $\text{Na}_2\text{S}_2\text{O}_4$, then saturated with CO , and a ΔW_g measured to correct for the diamagnetism of all reagents.

After several trial experiments five reasonably

satisfactory runs were made. The most precise was at a pH of 6.86. The effect of the addition of imidazole upon ΔW_9 was practically instantaneous. No change in ΔW_9 with time was noticed. The experimental results are tabulated in tables III-V, and the best curves for the three different pH values are plotted in graphs 3-5, in which the fraction of HbIm to the total Hb present is plotted as a function of the "free" imidazole concentration (Im).

Let us assume the following reaction:



The dissociation constant is then

$$\frac{(\text{Hb}^+)(\text{Im})}{(\text{HbIm}^+)} = K$$

This constant has been evaluated at the three different pH values taking into account any large ΔpH during the reaction. (Hb^+) was calculated from the total $(\text{Hb}^+ + \text{HbOH})$ in solution using the pK 8.15 reported by Coryell, Stitt, and Pauling[†] for the ferrihemoglobin:ferrihemoglobin-hydroxide equilibrium. The imidazole was corrected at the pH in question using the acid pK of imidazole, reported by Kirby and Neuberger^{**} to be 6.95.

The method used to calculate the dissociation constant of HbIm is moderately simple. If ΔW_9 is plotted

C. D. Coryell, F. Stitt, and L. Pauling - Jour. Am. Chem. Soc., 59, 633, (1937)

A.H.M. Kirby and A. Neuberger - B.C.J. 32, 1146(1938)

against the volume of imidazole solution added, it is found that the curve approaches a limiting value of ΔW_9 at high concentrations of imidazole. This asymptotic value is taken as the ΔW_9 for the HbIm complex. W_9 for Hb^+ is a function also of pH. Thus, if the pH changes during the course of the reaction by an amount ΔpH , a correction must accordingly be applied to the ΔW_9 of the solution.

Let x be the amount of ferrihemoglobin present, $(1-x)$ the amount of ferrihemoglobin imidazole, ΔW_I the ΔW_9 for ferrihemoglobin at the original pH, $\Delta W_I'$ the ΔW_9 for ferrihemoglobin after pH change with the reaction, ΔW_{II} the ΔW_9 for ferrihemoglobin imidazole (the limiting ΔW_9). Then since ΔW_{9Hb} changes with a change in pH, we have

$$\Delta W_{9Hb} = \Delta W_I \quad \text{for } x=1$$

and

$$\Delta W_{9Hb} = \Delta W_I' \quad \text{for } x=0$$

This gives

$$\Delta W_{9Hb} = \Delta W_I' + (\Delta W_I - \Delta W_I')x \quad (I)$$

Also we have

$$\Delta W_{9HbIm} = \Delta W_{II} \quad \text{for } (1-x)=1 \quad (II)$$

Then, combining (I) and (II), we obtain

$$\Delta W_9 = \Delta W_I' x + (\Delta W_I - \Delta W_I')x + \Delta W_{II}(1-x)$$

This relationship can then be plotted and values read

directly from the graph.

We also have

$$\frac{(\text{Hb}^+)}{(\text{HbOH})(\text{H}^+)} = 10^{8.15}$$

which gives

$$(\text{Hb}^+) = \left\{ (\text{Hb}^+) + (\text{HbOH}) \right\} \frac{10^{8.15}(\text{H}^+)}{\{10^{8.15}(\text{H}^+) + 1\}}$$

or

$$(\text{Hb}^+) = x \frac{\{10^{8.15-\text{pH}}\}}{\{10^{8.15-\text{pH}} + 1\}} \quad (\text{III})$$

If we assume that pH varies linearly with the reaction, we obtain the relation

$$10^{8.15-\text{pH}} = 10^{8.15-\text{pH}-\Delta\text{pH}(1-x)} \quad (\text{IV})$$

Then (III) and (IV) can be substituted into the equilibrium expression along with the determined values of x to give the following:

$$K = \frac{x}{1-x} (\text{Im}) \frac{\{10^{8.15-\text{pH}-\Delta\text{pH}(1-x)}\}}{\{10^{8.15-\text{pH}-\Delta\text{pH}(1-x)} + 1\}} \quad (\text{V})$$

The concentration of "free" imidazole, (Im), is calculated from the experimental data in a straightforward manner.

Great difficulty was experienced in evaluating the limiting value for ΔW_9 because of the wide distribution of points at high concentrations of imidazole, which may have been due to irreversible conditions in this region. The dissociation constants may be in error due to the fact that the asymptotes were chosen so as

to make the least possible variation in the calculated constant.

Conclusions -- There is fair agreement, within experimental error, between the two constants at pH 6.86 and 8.20 but these values are about eight times as great as the constants determined at pH 10.3. The experimental data also fit the theoretical curves better at the lower pH values. Perhaps at pH 10.3 denaturation had occurred to an appreciable extent making it difficult to control experimental conditions. This would give rise to errors in the ΔW_9 measurements.

If the molal susceptibility for HbIm is calculated at three different pH values using the chosen asymptotes, it is noticed that there is a large decrease from 2,940 c.g.s.u. at pH 6.86 to 1,290 c.g.s.u. at pH 10.3. This perhaps may be due to the fact that acid groups on the globin coordinated with the iron atoms of the hemes ionize appreciably at the higher pH values. This conclusion is highly speculative due to the lack of further experimental data.

Experimentally it is noticed that the formation of compounds of ferrihemoglobin hydroxide with F^- , CN^- *, and imidazole there is an increase in pH of the system, indicating a liberation of OH^- , whereas in the addition of NH_3 and C_2H_5OH ** to methemoglobin hydroxide no

* C. D. Coryell, F. Stitt, and L. Pauling - Jour. Am. Chem. Soc., 59, 633 (1937)

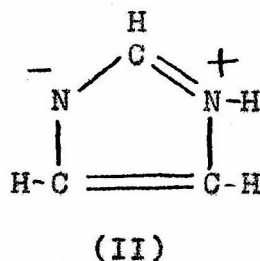
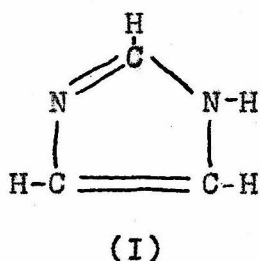
** C. D. Coryell - Unpublished experiments.

increase in pH is observed. Dr. Linus Pauling has offered an explanation of this phenomenon as follows. He assumes that there are groups on the globin coordinated with the iron atoms of the hemes which are acid groups whose energies of dissociation are sensitive to changes in the state of affairs about the coordination center. In the addition of donor molecules to Hb^+ , these molecules occupy the same positions on the iron atoms as do the OH^- ions in HbOH . Replacement of hydroxyl by other anions such as F^- , CN^- , Im^- , has very little effect on the strength of the acid groups on the globin and no increase in ionization occurs. Therefore any addition of one of the above anions would cause only the liberation of OH^- , thus increasing the pH of the system.

When anions coordinated with the iron atoms of the hemes are replaced by neutral molecules an increase in the acid strength of the acid groups occurs, because of the loss of the attraction of the negative charges of the anions for the protons. Increased ionization of the globin acid groups results. Therefore when neutral molecules substitute for the OH^- ions in ferrihemoglobin hydroxide the hydroxyl ions liberated are neutralized by the hydrogen ions from the globin acid groups to form water. This would account for constancy of pH in the experiments with the neutral molecules.

In the case of imidazole there are two resonating

structure which contribute to the valence state of the molecule, namely,



The contribution of (II) may be large enough to give the left nitrogen atom a charge of minus one-half. Since this nitrogen atom is presumably bonded to the iron atom, this would make the imidazole substitution for the hydroxyl ion in ferrihemoglobin hydroxide a case intermediate between the substitution of anions with a charge of minus one and neutral molecules of zero charge.

If the acid group on the globin were very little ionized in acid solution and appreciably ionized in strongly alkaline solution, the apparent dissociation constant of HbIm would be greater at a pH of 6.86 than at a pH of 10.3. From the data relating the experimental, apparent dissociation constant as a function of pH, the dissociation constant of the globin acid group is calculated to be about $10^{-9.5}$. In the absence of further experimental data we may assume the pK of the globin acid group to be about 9.5.

Table III - - Methemoglobin-Imidazole System - pH 6.86

	Volume Im Soln. Added	Exp. ΔW_g Uncorr.	Cor. ΔW_g	($\frac{HbIm}{Total\ Hb}$)	(Im) $\frac{moles}{liter}$	K x 10 ⁵
1.	0.00 cc.	7.33 mg.	8.50 mg.	0.000	0.00000	---
2.	0.40	4.80	6.11	0.357	0.00159	2.7
3.	0.60	3.80	5.16	0.499	0.00253	2.4
4.	0.80	3.20	4.61	0.580	0.00369	2.5
5.	1.00	2.95	4.41	0.610	0.00503	3.1
6.	1.20	2.27	3.74	0.710	0.00611	2.4
7.	1.40	1.93	3.42	0.758	0.00732	2.2
8.	1.60	1.77	3.30	0.776	0.00866	2.4
9.	1.80	1.72	3.29	0.778	0.01010	2.7
10.	2.00	1.44	3.03	0.816	0.01120	2.4
11.	2.50	1.09	2.74	0.860	0.01440	2.2
12.	3.00	0.92	2.64	0.875	0.01750	2.4
13.	4.00	0.58	2.42	0.907	0.02320	2.3
14.	5.00	0.42	2.39	0.911	0.02860	2.7
15.	6.00	0.41	2.54	-----	-----	---
16.	7.00	0.38	2.66	-----	-----	---
17.	8.00	0.15	2.46	-----	-----	---
18.	9.00	0.13	2.61	-----	-----	---
19.	10.00	0.11	2.74	-----	-----	---

Mean K = $2.5 \pm 0.2 \times 10^{-5}$

Conc. Hb Soln. = 0.00974 moles per lit. in heme-iron

Conc. Im Soln. = 0.355 moles per lit.

ΔW_g cor. asymptote = 1.80 mg. χ_{molal} = $2,940 \times 10^{-6}$ c.g.s.u.

Temperature = 25°C.

ΔpH = -0.04 (change neglected)

Table IV-A - - Methemoglobin Imidazole System - pH 8.2

	Volume Im Soln. Added	Exp. ΔW_g	Cor. ΔW_g	$\left(\frac{\text{HbIm}}{\text{Total Hb}} \right)$	$\frac{(\text{Im})}{\text{moles}} \frac{\text{liter}}{\text{liter}}$	$K \times 10^3$
1.	0.00 cc.	7.66 mg	8.83 mg	0.0000	0.00000	
2.	0.30	5.41	6.67	0.287	0.00249	2.9
3.	0.50	4.30	5.59	0.433	0.00466	2.7
4.	0.77	3.12	4.44	0.592	0.00784	2.3
5.	1.00	2.61	3.95	0.662	0.0113	2.4
6.	1.25	2.03	3.37	0.745	0.0147	2.0
7.	1.50	1.79	3.15	0.776	0.0187	2.2
8.	1.75	1.50	2.88	0.813	0.0224	2.0
9.	2.00	1.16	2.53	0.863	0.0261	1.6
10.	2.25	1.14	2.86	0.817	0.0305	2.7
11.	2.50	1.13	2.54	0.862	0.0340	2.1
12.	2.75	1.20	2.64	0.848	0.0378	2.6
13.	3.00	0.72	2.12	0.925	0.0411	1.3
14.	4.00	0.78	2.26	0.904	0.0554	2.3
15.	5.00	0.69	2.24	0.906	0.0685	2.7

Mean $K = 2.3 \pm 0.3 \times 10^{-3}$

Conc. Hb soln. = 0.0118 moles per lit. in heme-iron

Conc. Im soln. = 0.408 moles per liter

ΔW_g cor. asymptote = 1.62 mg. $\chi_{\text{molal}} = 2,180 \times 10^{-6}$ c.g.s.u.

Temperature = 25°C

$\Delta \text{pH} = 0.23$ (assume linear with reaction)

Table IV-B - - Methemoglobin Imidazole System - pH 8.2

	Volume Im soln. Added	Exp. W ₉	Cor. W ₉	HbIm Total Hb	Im moles liter	K x 10 ³
1.	0.00 cc.	7.43 mg	8.60 mg	0.000	0.00000	
2.	0.20	5.73	6.96	0.248	0.00111	1.6
3.	0.40	4.16	5.42	0.457	0.00252	1.3
4.	0.60	3.36	4.64	0.566	0.00505	1.6
5.	0.80	2.86	4.16	0.632	0.00801	1.9
6.	1.00	2.32	3.63	0.707	0.01070	1.8
7.	1.20	1.93	3.24	0.763	0.01370	1.7
8.	1.40	1.69	3.01	0.796	0.01680	1.7
9.	1.60	1.41	2.72	0.838	0.01970	1.5
10.	1.80	1.43	2.77	0.830	0.02310	1.9
11.	2.00	1.38	2.73	0.836	0.02640	2.0
12.	2.50	1.10	2.46	0.874	0.03380	1.9
13.	3.00	0.93	2.31	0.895	0.04150	1.9
14.	4.00	0.77	2.17	0.916	0.05500	1.9
15.	5.00	0.46	1.86	0.963	0.06780	1.0

Mean K = $1.7 \pm 0.2 \times 10^{-3}$

Conc. Hb soln. = 0.0118 moles per liter in heme-iron

Conc. Im soln. = 0.408 moles per liter

ΔW_9 cor. asymptote = 1.62 mg $\chi_{\text{molal}} = 2,180 \times 10^{-6}$ c.g.s.u.

Temperature = 25°C

$\Delta \text{pH} = 0.23$ (assume linear with reaction)

Table V-A - - Methemoglobin-Imidazole System - pH 10.3

	Volume Im Soln. Added	Exp. ΔW_g	Cor. ΔW_g	$\left(\frac{\text{HbIm}}{\text{Total Hb}} \right)$	$\left(\text{Im} \right)$ $\frac{\text{moles}}{\text{liter}}$	$K \times 10^3$
1.	0.00 cc.	5.39 mg	6.53 mg	0.000	0.0000	—
2.	1.00	4.00	5.43	0.199	0.0191	0.48
3.	2.00	2.91	4.41	0.384	0.0366	0.36
4.	3.00	2.23	3.86	0.483	0.0535	0.36
5.	4.00	1.53	3.32	0.581	0.0691	0.31
6.	5.00	1.06	2.90	0.656	0.0836	0.27
7.	6.00	0.87	2.79	0.676	0.0973	0.29
8.	7.00	0.94	3.01	0.637	0.111	0.39
9.	8.00	0.47	2.49	0.731	0.122	0.28
10.	9.00	0.32	2.39	0.749	0.133	0.28
11.	10.00	0.53	2.80	0.674	0.144	0.43

Mean $K = 0.34 \pm 0.06 \times 10^{-3}$

Conc. Hb soln. = 0.0123 moles per liter in heme-iron

Conc. Im soln. = 0.450 moles per liter

ΔW_g cor. asymptote = 1.00 mg. $\chi_{\text{molal}} = 1,290 \times 10^{-6}$ c.g.s.u.

Temperature = 24°C

$\Delta \text{pH} = 0.15$ (change neglected)

Table V-B - - Methemoglobin Imidazole System - pH 10.3

	Volume Im Soln. Added	Exp. ΔW_9	Cor. ΔW_9	$\left(\frac{\text{HbIm}}{\text{Total Hb}} \right)$	$\left(\frac{\text{Im}}{\text{moles}} \right)$ liter	$K \times 10^3$
1.	0.00 cc.	5.36 mg	6.50 mg	0.000	0.00000	
2.	0.50	4.32	5.61	0.162	0.00902	0.29
3.	1.00	3.57	4.96	0.280	0.0181	0.29
4.	1.50	3.00	4.48	0.367	0.0272	0.29
5.	2.00	2.78	4.42	0.378	0.0367	0.37
6.	2.50	2.32	4.04	0.448	0.0453	0.35
7.	3.00	1.90	3.55	0.536	0.0530	0.28
8.	3.50	1.40	3.05	0.627	0.0604	0.22
9.	4.00	1.23	2.92	0.651	0.0683	0.23
10.	4.50	0.95	2.65	0.700	0.0755	0.20
11.	5.00	0.85	2.58	0.713	0.0828	0.21
12.	6.00	0.84	2.69	0.693	0.0974	0.27
13.	7.00	0.43	2.26	0.771	0.110	0.20
14.	8.00	0.48	2.42	0.742	0.122	0.26
15.	9.00	0.43	2.46	0.735	0.133	0.30
16.	10.00	0.45	2.58	0.713	0.144	0.36

Mean $K = 0.27 \pm 0.04 \times 10^{-3}$

Conc. Hb soln. = 0.0123 moles per liter in heme-iron

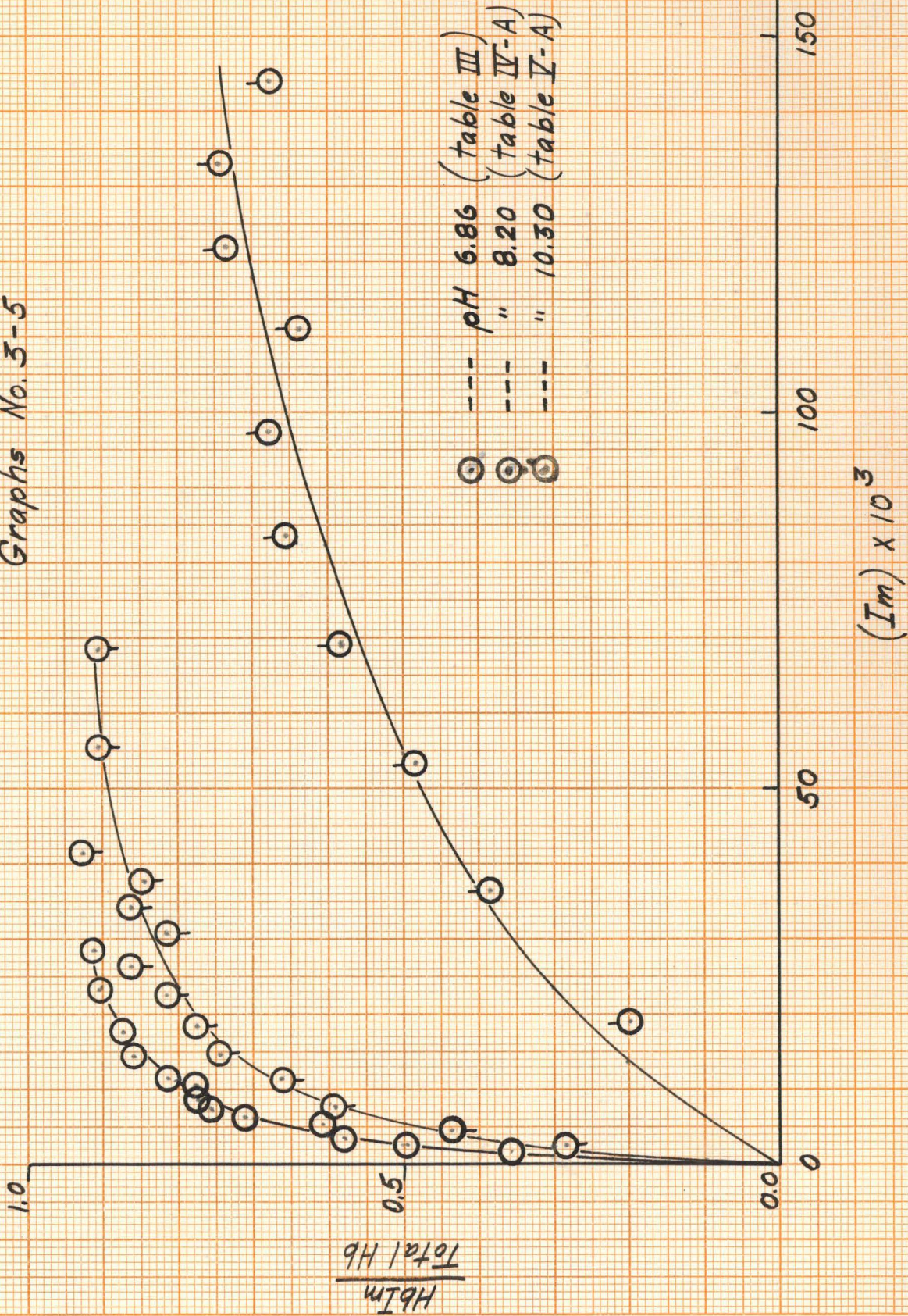
Conc. Im soln. = 0.450 moles per liter

ΔW_9 cor asymptote = 1.00 mg $\chi_{\text{molal}} = 1,290 \times 10^{-6}$ c.g.s.u.

Temperature = 24°C

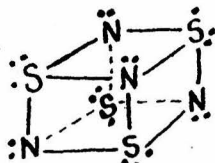
$\Delta \text{pH} = 0.10$ (change neglected)

Graphs No. 3-5



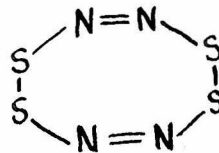
Sulfur Nitride

The following possible structure for N_4S_4 might be suggested.

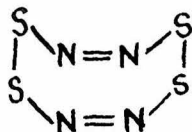


This structure would necessitate a rather large magnetic moment due to the paramagnetic contribution of four unpaired electron spins, one electron on each of the sulfur atoms. This compound was measured magnetically both in crystalline state and in a solution of carbon disulfide and was found to be diamagnetic in each case. On the basis of this evidence, the above structure and any other structure involving unpaired electrons can be ruled out.

A. J. Stosick has suggested that the structure of N_4S_4 might be that of an eight-membered ring.* It can be represented as follows:



This structure would be diamagnetic, very stable, and a cup-wise configuration, such as



would allow the nitrogen bond angles to assume the

* A. J. Stosick - Doctorate Propositions,
California Institute of Technology (1939)

usual value.

Conclusion:

The magnetic data show the compound, N_4S_4 , to be diamagnetic; This supports the structure proposed by Stosick, and in the absence of X-ray and Electron-Diffraction data, it seems to be the best possible structure to write for the present.

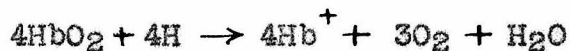
Experimental Notes on
Brief Investigations

Note on the Stability of Oxyhemoglobin
Solutions

- (a) 30 cc. of oxyhemoglobin solution (conc. = 0.0160 moles per liter in heme-iron), prepared by C. D. Russell and C. D. Coryell on November 18, 1938, and kept at 0-4°C. until December 21, 1938, and then kept at room temperature from 11:00 A.M. on, was placed in a stoppered glass magnetic tube and the ΔW_9 was measured as a function of the time. After a period of 1336 hours a spectroscopic measurement was made indicating the presence of methemoglobin. The results are tabulated in table I and the ΔW_9 's are plotted against the time in graph no. 1.
- (b) 25 cc. of oxyhemoglobin solution, same as mentioned above, but kept at 0-4°C. until February 16, 1939, and then kept at room temperature from 9:00 A.M. on, was placed in another stoppered glass magnetic tube and the ΔW_9 was measured as a function of the time. The change in ΔW_9 was not followed as long a time in this run but after a period of about 400 hours, spectroscopic observations showed the presence of ferrohemo-globin. The results are tabulated in table II and the ΔW_9 's are plotted against the time in graph no. 2.

The change in ΔW_0 is almost linear up to approximately 275 hours in experiment (a) and until 215 hours in (b). Both experiments then show a very rapid increase in ΔW_0 with the time, run (b) exhibiting the sharpest increase. After the sharp rise in ΔW_0 , the points in (a) continue to increase linearly, with about the same rate as before, reaching a limiting value, $\Delta W_0 = 11.55$ mg., after 1336 hours; while in (b) the points then follow very closely a horizontal straight line as long as the reaction is followed, $\Delta W_0 = 11.67-11.31$ mg.

It is rather difficult to interpret these results. However, if we assume that the reaction is that of autoxidation of HbO_2 to Hb^+ , as in the following reaction:



the data observed might indicate an auto-catalysis of this reaction. Perhaps the reducing action of bacteria or other living organisms, in using up the oxygen, causes reduction of HbO_2 to Hb , which in turn oxidizes slowly to Hb^+ in the presence of the oxygen left over.

These experimental data furnished very interesting facts concerning the stability of oxyhemoglobin solutions and a study of the kinetics of this reaction at different pH's, temperatures, and other conditions might prove valuable to hemoglobin chemistry.

Table I -- (HbO₂ → Hb⁺) System

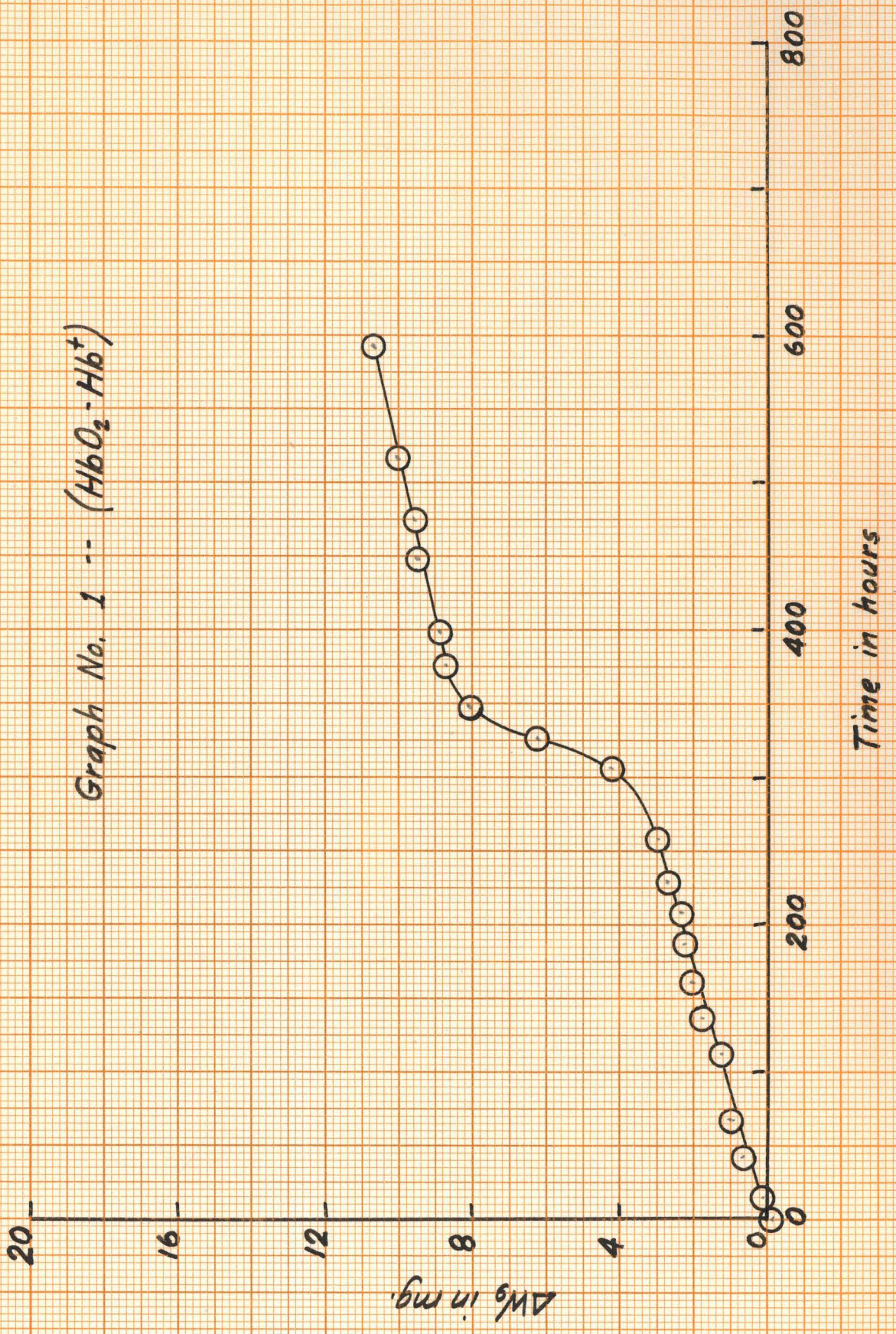
No. of reading	Time in Hours	Exp. ΔW _g in mg.	ΔW _g for the tube in mg.	Exp. ΔW _g corrected for tube in mg.
1.	0.0	1.61	1.70	-0.09
2.	16.0	1.84	1.70	0.14
3.	43.5	2.36	1.70	0.66
4.	68.5	2.72	1.70	1.02
5.	113.5	2.99	1.70	1.29
6.	137.0	3.48	1.70	1.78
7.	162.0	3.71	1.70	2.01
8.	187.5	4.08	1.70	2.38
9.	209.5	4.11	1.70	2.41
10.	232.0	4.52	1.70	2.82
11.	260.0	4.75	1.70	3.05
12.	308.0	5.96	1.70	4.26
13.	327.5	8.00	1.70	6.30
14.	350.0	9.87	1.70	8.17
15.	376.0	10.48	1.70	8.78
16.	399.0	10.66	1.70	8.96
17.	449.0	11.16	1.70	9.46
18.	475.5	11.28	1.70	9.58
19.	519.5	11.71	1.70	10.01
20.	593.0	12.38	1.70	10.68
21.	1336.5	13.25	1.70	11.55

Table II - - (HbO₂ - Hb) System

<u>No. of reading</u>	<u>Time in hours</u>	<u>Exp. ΔW_9 in mg.</u>	<u>ΔW_9 for the tube in mg.</u>	<u>Exp. ΔW_9 corrected for tube</u>
1.	0.0	-0.60	-0.48	-0.12
2.	24.0	-0.28	-0.48	0.20
3.	48.0	0.03	-0.48	0.51
4.	72.0	0.27	-0.48	0.75
5.	96.0	0.44	-0.48	0.92
6.	120.0	0.68	-0.48	1.16
7.	144.0	1.00	-0.48	1.48
8.	168.0	1.03	-0.48	1.51
9.	192.0	1.42	-0.48	1.90
10.	216.0	2.62	-0.48	3.10
11.	240.0	11.19	-0.48	11.67
12.	264.0	11.06	-0.48	11.54
13.	288.0	11.09	-0.48	11.57
14.	312.0	10.83	-0.48	11.31
15.	336.0	10.96	-0.48	11.44

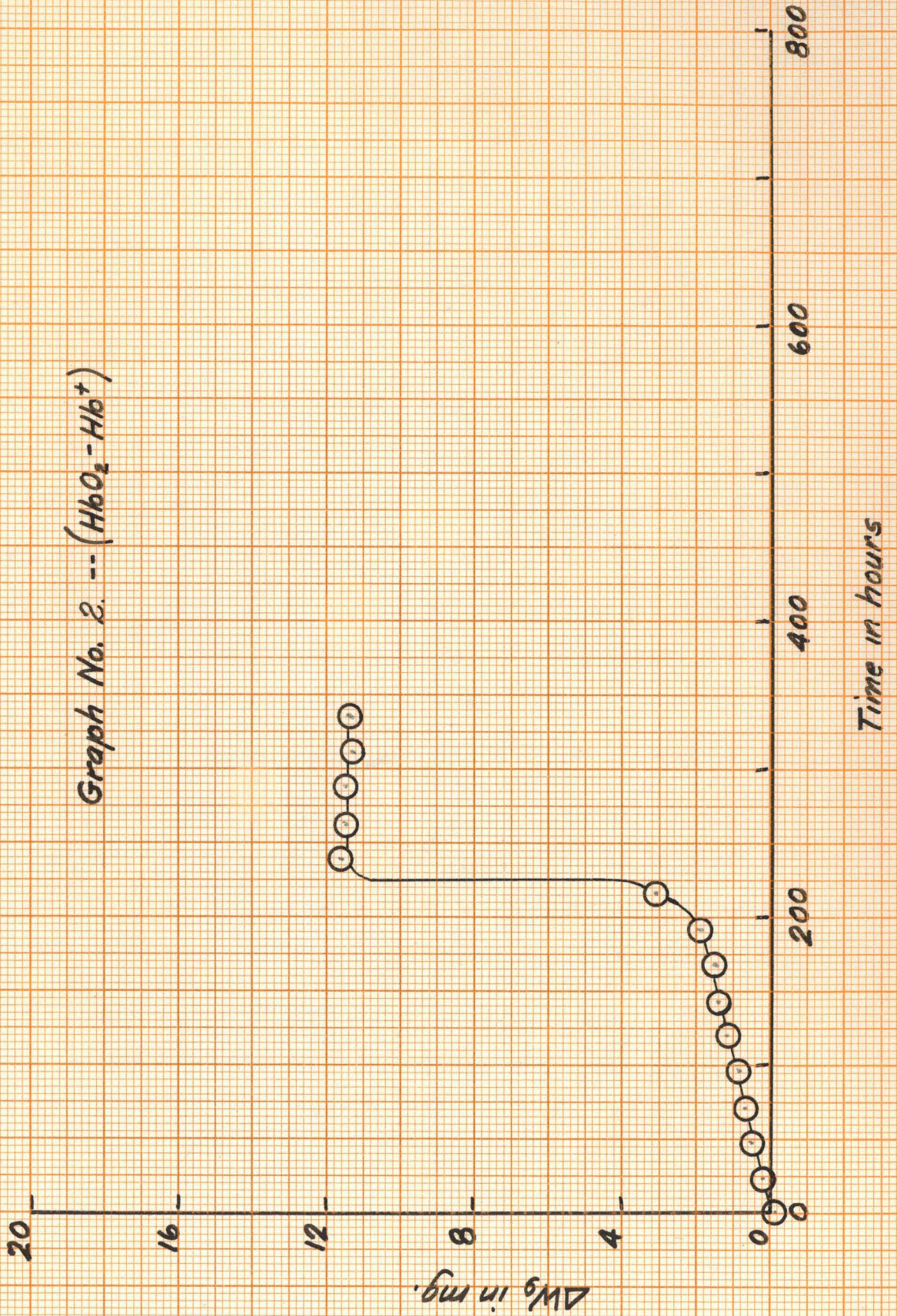
KEUFFEL & ESSER CO., N. Y. NO. 359-11
20 X 20 to the inch, 10th lines heavy.
MADE IN U. S. A.

Graph No. 1 -- (HbO₂ - Hb⁺)



KEUFFEL & ESSER CO., N. Y. NO. 359-11
20 X 20 to the inch, 10th lines heavy.
MADE IN U. S. A.

Graph No. 2. -- (HbO₂ - Hb⁺)



Note on the Methemoglobin Hydroxide-Methyl
Amine Hydrochloride System

Upon the addition of methyl amine hydrochloride to methemoglobin hydroxide at pH=10.15, only a very slight change is noticed in the absorption spectrum of the system. There is, however, a very marked decrease in the susceptibility of the solution. The experimental values are reported in table III in terms of ΔW_9 , uncorrected for the diamagnetism of the reagent added but corrected for the tube. After the addition of nearly 3 g. of $\text{CH}_3\text{NH}_2\text{HCl}$, the pH had fallen to a value of 9.00. Since the weights of $\text{CH}_3\text{NH}_2\text{HCl}$ added were observed only roughly, and since there was such a large change in pH (-1.15 units) during the course of the experiment, no attempt was made to interpret the results obtained.

There is, however, rough qualitative agreement with the unpublished experiments of C. D. Coryell on the $\text{HbOH}-\text{NH}_3$ system. His results show also a decrease in the susceptibility of the solutions with increasing concentration of NH_4OH , but only a very slight change in the absorption spectrum.

(The addition of n-butyl amine to HbOH , at pH 10.2, caused denaturation and no further data were obtained.)

Table III -- HbOH - CH₃NH₂HCl System

<u>System</u>	<u>ΔW_g corrected</u>
1. HbOH soln. at pH 10.15 (25 cc.)	3.41 mg.
2. Addn. of 0.820 g. CH ₃ NH ₂ HCl	1.77 mg.
3. " " 0.793 g. " " "	1.07 mg.
4. " " 0.364 g. " " "	0.27 mg.
5. " " 0.988 g. " " "	0.05 mg.

Note on the Magnetic Susceptibility of
Europium Salts

An attempt was made to determine the magnetic susceptibility of bivalent europium carbonate (EuCO₃) but, no matter how small the cross-section of the glass magnetic tube, the salt was so strongly paramagnetic that the tube was drawn to the pole pieces. If a very low field were used it might be possible to obtain an accurate ΔW.

An attempt was then made to determine the magnetic susceptibility of the bivalent europium ion (Eu⁺⁺) by dissolving the europium carbonate in hydrochloric acid solution and measuring the ΔW_g.

- ΔW_g for solution = 12.28 mg. (uncorrected)
- ΔW_g for air-water = 49.05 " (uncorrected)
- ΔW_g for water-water = 1.35 "
- ΔW_g for HCl soln. = 1.06 " (uncorrected)

$$\chi_{\text{molal Eu}^{++}} = \frac{(11.22)(0.748 \times 10^{-6})(1000)}{(47.70)(0.0186)} = 9420 \times 10^{-6} \text{ c.g.s.u.}$$

Ans.

This value is a little more than a third of the value reported by Selwood.* Undoubtedly the bivalent ion was largely oxidized by the air to Eu⁺⁺⁺.

1 cc. of 4.5 N HClO₄ was then added to the system to oxidize the Eu⁺⁺ and air was passed through to insure

* P. Selwood - Jour. Am. Chem. Soc., 55, 4869 (1933)

complete oxidation. A ΔW_g was then measured.

ΔW_g for solution = 6.60 mg. (uncorrected)
 ΔW_g for air-water = 49.05 mg. (uncorrected)
 ΔW_g for water-water = 1.35 mg.
 ΔW_g for HCl soln. = 1.06 mg. (uncorrected)

$$\chi_{\text{molal Eu}^{+++}} = \frac{(5.54)(0.748 \times 10^{-6})(1000)}{(47.70)(0.0181)} = 4,790 \times 10^{-6} \text{ c.g.s.u.}$$

Ans.

This value if corrected for the diamagnetism of the Eu agrees within experimental error with that reported by Selwood^{*} for the trivalent europium ion.

Summary

1. The reaction between ferrohemoglobin and ethyl isocyanide has been studied magnetically and the stability of the complex toward dissociation has been demonstrated. The molar ratio of isocyanide to heme-iron has been calculated. A covalent d^2sp^3 octahedral configuration for the atomic groups surrounding the iron atom has been postulated.
2. The equilibrium between ferrihemoglobin and imidazole has been investigated at different pH values. Apparent dissociation constants and molal susceptibilities of the complex have been reported as a function of the pH. The pK of the acid groups on the globin coordinated with the iron atoms of the hemes has been evaluated.
3. A structure involving no unpaired electrons has been suggested for sulfur nitride on the basis of magnetic data.
4. The auto-oxidation reaction of oxyhemoglobin solution at room temperature has been briefly investigated.
5. The magnetic properties of the ferrihemoglobin-methylamine hydrochloride system have been qualitatively studied.
6. A value for the molal susceptibility for the trivalent europium ion has been reported.

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