[CONTRIBUTION FROM THE ARMY MEDICAL SERVICE GRADUATE SCHOOL, WALTER REED ARMY MEDICAL CENTER]

Infrared Spectroscopy of Human Hemoglobin. II. The Rate of Exchange of Hydrogen by Deuterium in Solution

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Kinetic studies were carried out on the rate of incorporation of deuterium into the molecule of normal adult human carboxyhemoglobin. Observations were made on hemoglobin in deuterium solutions over a wide range of *p*D values, and changes in intensity with the passage of time were noted in the peaks at 6.35, 6.45 and 6.9 μ. The band at *6.35* μ is associated with ionized carboxyl groups. It was not seen in acid solutions and was intense in alkaline solutions. In solutions near neutrality, the band at 6.35 μ was initially masked by the peak at 6.45 μ; however, with the shift in the peak at 6.45 to 6.9 μ, the band at 6.35 μ emerged as a distinct band. The peak at *6.45* μ is associated with the peptide linkage. It was not seen in strongly alkaline solution and had shifted to the region of 6.55 μ in strongly acid solution. In solutions near neutrality, the 6.45 μ band was initially sharp and intense then rapidly shifted to the region of 6.9 μ. At the end of about 20 minutes, the peak at 6.45 μ was no longer seen. The peak at *6.9* μ is associated with the peptide linkage and the incorporation of deuterium. This band was of greater intensity in acid solution than in alkaline solution. The increase in intensity of this band associated with the shift in the peak from 6.45 μ was less striking in acid and alkaline solution than it was in neutral solution and was completed at the end of about 20 minutes.

While studying the infrared absorption of aqueous solutions of organic acids and their salts, Gore, Barnes and Petersen[1] used deuterium oxide as a solvent to avoid the strong infrared absorption near 3300, 1640 and 800 Kaysers due to water. They measured the rate of exchange of deuterium for hydrogen at the O–H stretching vibration of 3380 Kaysers in *p*-nitrotoluene, and found that the exchange was completed at 80 minutes. There is no information in the literature on the use of this method in the study of proteins. It is the purpose of this paper to report kinetic studies on deuteration of proteins at various *p*D values in the regions of 6 to 7 μ (1655 to 1430 Kaysers).[2]

Methods

Hemoglobin specimens were prepared according to the method of Drabkin,[3] converted to carboxyhemoglobin, dialyzed against distilled water, dried *in vacuo* from the frozen state, and sealed in glass ampoules until used. The carboxyhemoglobin was dissolved in deuterium oxide, deuterium chloride or in sodium deuteroxide, then immediately placed between silver chloride plates with a platinum foil spacer 0.001 inch thick. Deuterium oxide was used as a blank in the reference beam during each run.

A Perkin–Elmer Model 21 recording infrared spectrophotometer was used with a rock salt prism. The correct electrical balance of the instrument was established before each set of studies using the band at 2.0 μ (5000 Kaysers) as a balance point. The patterns were obtained from 5.75 to 7.25 μ (1790 to 1380) at a speed of 4 minutes per micron. Recycling was carried out over the above range until there was no further evidence of shift in the peaks. The time recorded on the figures is the interval between the addition of the deuterium solution and subsequent passage over the 6.5 μ wave length.

Results and Discussion

Wave Length 6.35 μ (1575) (see Figs. 1, 2 and 3). —This band is associated with ionized carboxyl groups[4] and was not visible in strongly acid solutions. At *p*D 5.0, it first became visible and did not change markedly upon standing in deuterium oxide solution. At neutral *p*D the peak was masked by the larger one at 6.45 μ (1550) immediately after placing the hemoglobin in solution. At the end of about 10 minutes, however, the peak at 6.35 μ was unmasked as a distinctly separate band as the peak at 6.45 μ rapidly shifted to 6.9 μ (1450). At the end of about 30 minutes, the 6.35 μ peak stood out clearly with the 6.45 μ band no longer visible or greatly diminished. In samples run at *p*D 9 and above, the 6.35 μ peak became much broader at the base with a tendency toward a U-shape. In strongly alkaline solution, the peak did not change upon standing in deuterium.

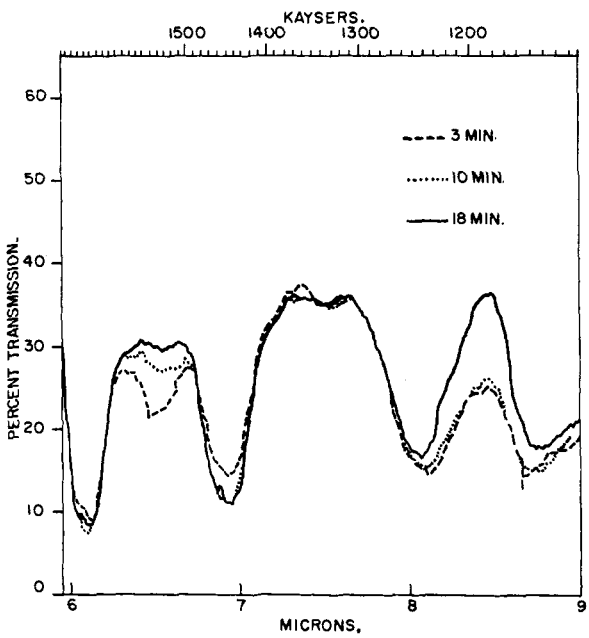

Fig. 1.—Kinetic study of A COHgb in DCl, *p*H 3.0.

Wave Length 6.45 μ (1550) (see Figs. 1, 2 and 3). —This band is probably associated with the deuteration of the peptide linkages.[4b,5] It was not seen in strongly alkaline deuterium solutions, but below the *p*D of 9.0, the peak stood out clearly immediately after placing the hemoglobin in solution. The peak then rapidly decreased in intensity as it shifted to 6.9 μ so as to become greatly diminished or not discernible at the end of 20 to 30 minutes.

(1) R. C. Gore, R. B. Barnes and E. Petersen, *Anal. Chem.*, **21**, 382 (1949).

(2) The term "Kayser" is identical with the term "reciprocal centimeter." See Joint Commission for Spectroscopy, C. J. Bakker, *J. Opt. Soc. Amer.*, **43**, 410 (1953). Throughout the remainder of the paper, the number in parentheses following the wave length refers to Kaysers or reciprocal centimeters.

(3) D. L. Drabkin, *J. Biol. Chem.*, **164**, 703 (1946).

(4) (a) G. Ehrlich and G. B. B. M. Sutherland, *Nature*, **172**, 671 (1953); (b) H. Lenormant and E. R. Blout, *ibid.*, **172**, 770 (1953).

(5) H. Lenormant, *Ann. Chim.*, **383**, 459 (1950).

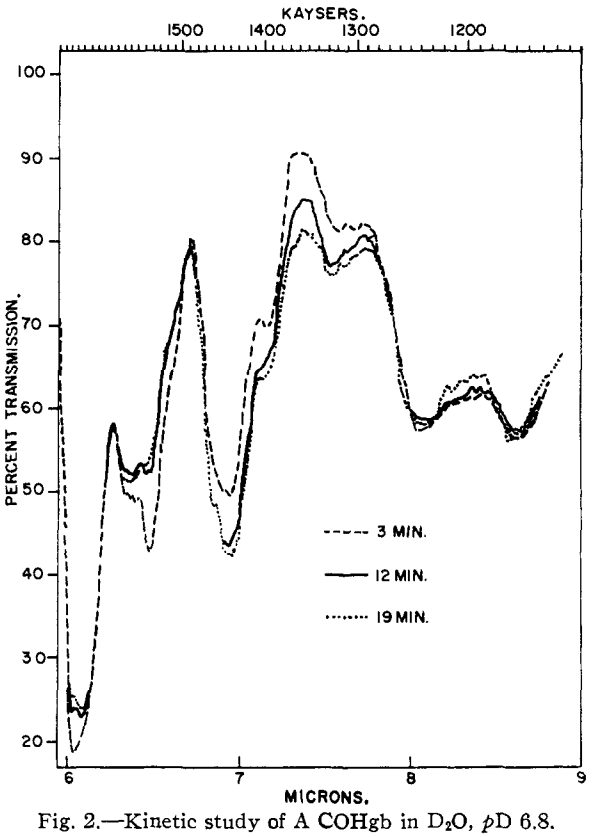

Fig. 2.—Kinetic study of A COHgb in D_2O, *p*D 6.8.

Increasing the acidity of the solution was accompanied by a shift in the peak to the region of 6.55 μ (1525) and a widening of the base of the peak. Upon standing in acid deuterium solution, there is some shift of the peak at 6.55 μ to the region of 6.9 μ, but this is not as noticeable as at a neutral *p*D. This shift was usually completed in about 20 minutes.

Wave Length 6.9 μ (1450) (see Figs. 1, 2 and 3).—This band is probably also associated with the deuteration of the peptide linkages. The peak was visible in all spectra obtained in the liquid phase. The intensity of the peak, however, was greatest in neutral solution, less in acid solution, and least in alkaline solution. In neutral solutions, the peak enlarged rather rapidly on standing. At the end of

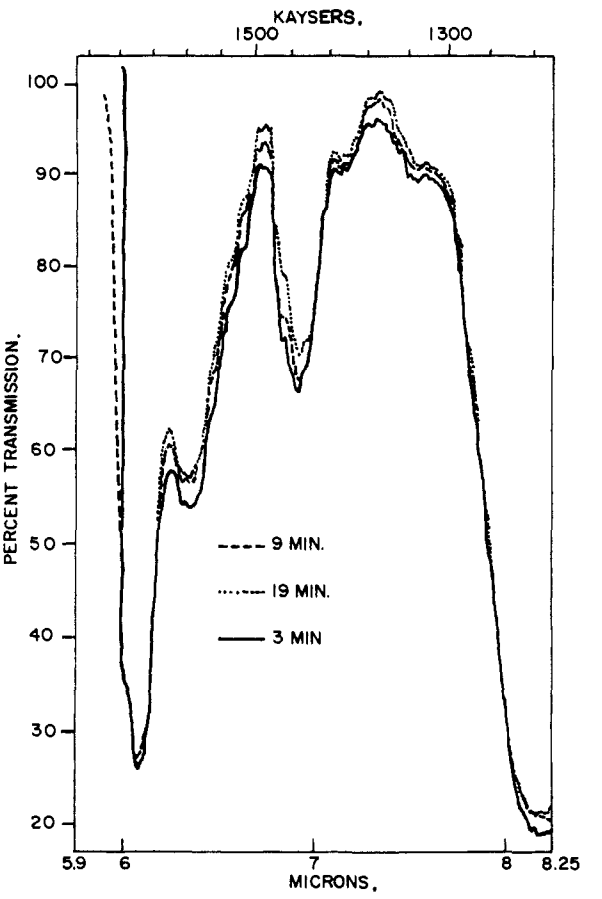

Fig. 3.—Kinetic study of A COHgb in NaOD, *p*D 11.7.

20 to 30 minutes, the shift was completed from the 6.45 μ region to the 6.9 μ region; this resulted in a 6.9 μ band second only to the peak at 6.1 μ in intensity. In strongly acid or alkaline solutions, the increase in intensity of the 6.9 μ peak with time was much less striking, and was usually completed in about 20 minutes.

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