OXYGEN AFFINITY OF CAT HEMOGLOBIN*

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Mammalian hemoglobins provide a group of closely related proteins that are of considerable interest in studies aimed at correlating chemical structure with biological function. The oxygen saturation curves of the blood of various mammals differ widely and it is recognized that these differences may be due to environmental factors, such as pH, concentration, ionic strength, etc., rather than to the primary structures of the hemoglobins per se (1-3). Allen et al. (4), for example, have shown that differences in the oxygen affinities of fetal and maternal blood disappear when hemoglobin solutions prepared from these bloods are dialyzed against the same buffer. In a study of fourteen mammalian species, Bartels et al. (2, 3) found that the oxygen affinity of cat blood was the lowest and that of man was quite high. In order to ascertain whether the relatively low oxygen affinity of cat blood could be attributed to environmental or to structural factors, we have investigated the preparation and properties of cat hemoglobin.

In this work we have found that the relative oxygen affinities of cat and human hemoglobins dialyzed against the same phosphate buffers at pH 6.8 to 7.4 are similar to those reported by Bartels and Harms (2) for the corresponding whole bloods. Our results, therefore, suggest that the relatively low oxygen affinity of cat blood is due to an intrinsic property of cat hemoglobin rather than to environmental factors associated with the blood. We have also found that hemolysates of cat erythrocytes are easily resolved into two

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hemoglobin components by starch gel electrophoresis and by ion-exchange chromatography. The two hemoglobins tentatively identified as major and minor components on the basis of their relative concentration in cat blood, differ from each other in at least one of approximately 31 tryptic peptides. Each of the two hemoglobins exhibits practically the same oxygen affinity as the original hemolysate of the cat erythrocytes.

Methods and Procedure

Blood was collected from laboratory cats by cardiac puncture into NIH formula "A" anticoagulant solution. Human blood was collected by venipuncture into the same anticoagulant. Hemoglobin solutions were prepared by the method of Drabkin (5), except that $AlCl_3$ was omitted from the isotonic saline used to wash the cells. The hemolysates were exhaustively dialyzed against distilled water, centrifuged at 20,000 x g, and stored in the form of CO hemoglobin at 4°C. Hemoglobin concentrations were determined spectrophotometrically as oxyhemoglobin, using molar extinction coefficients 5.6×10^4 at 540 mμ and 5.9×10^4 at 576 mμ (6), or as cyanmethemoglobin (7). The absorption spectra of cat and human hemoglobins were found to be identical. Oxygen affinity at 20°C was determined by the method of Rossi-Fanelli and Antonini (8) using hemoglobin concentrations of 7 ± 2 mg/ml and a light path of 1 mm. A Beckman DBG spectrophotometer, with temperature regulation and linear-log recorder, was used for spectral evaluation of percent saturation at 540, 560 and 576 mμ (9). Hemoglobin solutions were adjusted to the desired pH by dialysis against 0.14 M phosphate buffers. Prior to determining the oxygen saturation curves, carbon monoxide was displaced with oxygen as described by Antonini et al. (10). Starch gel electrophoresis was conducted at 4°C and pH 8.4 in Tris-EDTA-Borate and the hemoglobins were visualized with benzidine (11). The major and minor cat hemoglobins were separated by chromatography on a 3 x 25 cm IRC-50 column using 0.05 M phosphate buffer at pH 6.7.

Results

Figure 1 shows the oxygen saturation curves of hemolysates of human and cat erythrocytes that had been dialyzed against pH 6.75 to 7.35 phosphate buffers. The partial pressures of oxygen required to half-saturate the cat hemolysates ($pO_{1/2}$) are nearly twice those for the human hemolysates. The values obtained for the human hemolysate, 10.3 and 5.4 mm at pH 6.78 and 7.35 are in agreement with values of 11.8 and 5.4 mm respectively calculated from the data of Antonini et al. (12) under the same conditions. The differences between cat and human hemolysates are similar to those reported by Bartel and Harms (2) for the corresponding whole bloods. The difference in oxygen affinity between human and cat blood is therefore inherent in the structures of the respective hemoglobin molecules.

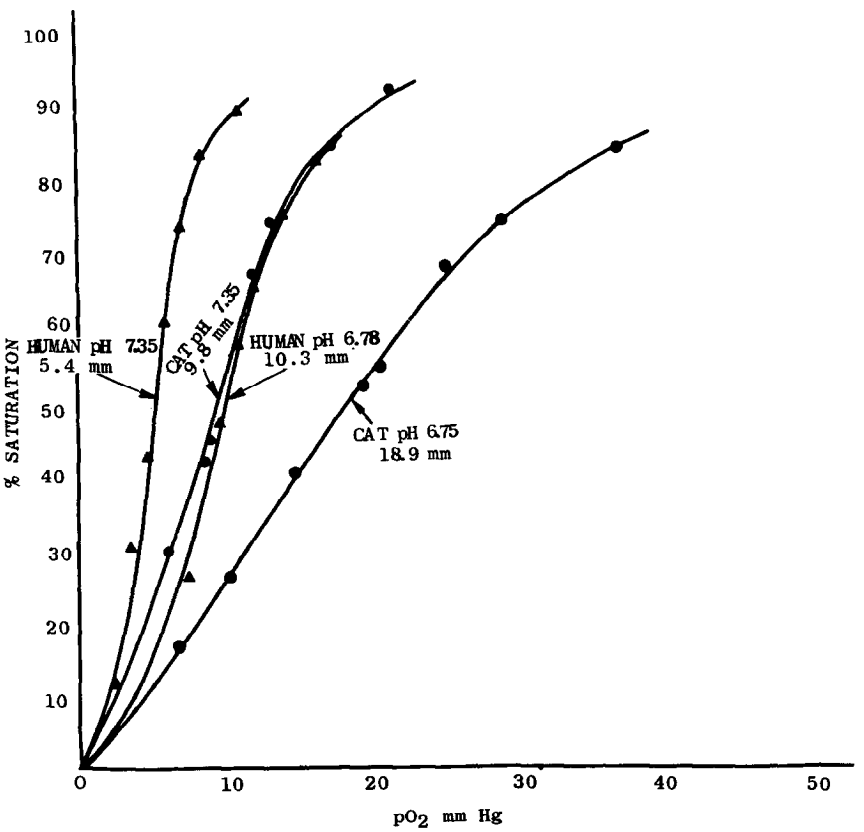


Fig. 1. Oxygen saturation curves of cat and human hemoglobins. The arrows point to the $pO_{1/2}$ values.

Table I shows the magnitude of the Bohr effects and the values of the Hill interaction constant, n, calculated from Fig. 1. It is clear that both cat and human hemoglobins exhibit nearly identical Bohr effects per heme, 0.48-0.49, but show large differences in both "heme-heme" interaction and in oxygen affinity. Smith et al. (13) have recently pointed out that contrary to earlier reports, careful measurements of oxygen equilibria in several mammalian hemoglobins reveal close similarities in Bohr effects. Our data on cat hemoglobin support this observation.

Table I

Bohr Effect and Values of n for Human and Cat Hemoglobins

	Human		Cat	
pH	6.78	7.35	6.75	7.35
$pO_{1/2}$	10.3	5.4	18.9	9.8
Hill Constant, n[1/]	2.8	3.3	1.8	2.0
Bohr Effect[2/]	0.48		0.49	

1/ $n = \log \frac{Y}{1-Y} / \log pO_{1/2}$

2/ Bohr Effect per heme = $\Delta \log pO_{1/2} / \Delta pH$

The electrophoresis of cat hemoglobin is shown in Fig. 2. Contrary to results previously obtained with agar and paper (14, 15), cat hemoglobin is not homogeneous; two distinct components are resolved. Fractionation on IRC-50 gives major and minor components, which migrate toward the anode as single bands. The distribution of the two components varied considerably in the blood of twelve cats, in which the minor fraction comprised from about 10 - 40%. A comparison of the electrophoresis of cat, dog and human hemoglobins A and S is shown in Fig. 3. The more anodic component (minor), exhibits a mobility nearly identical to human hemoglobin A, while the slower moving component (major) is intermediate between human hemoglobin A and dog hemoglobin. The sample of cat hemoglobin shown in the figure 3 was relatively low in the minor component and photographed poorly. Studies on the structures of the two purified cat hemoglobins are in progress. Preliminary results show that they

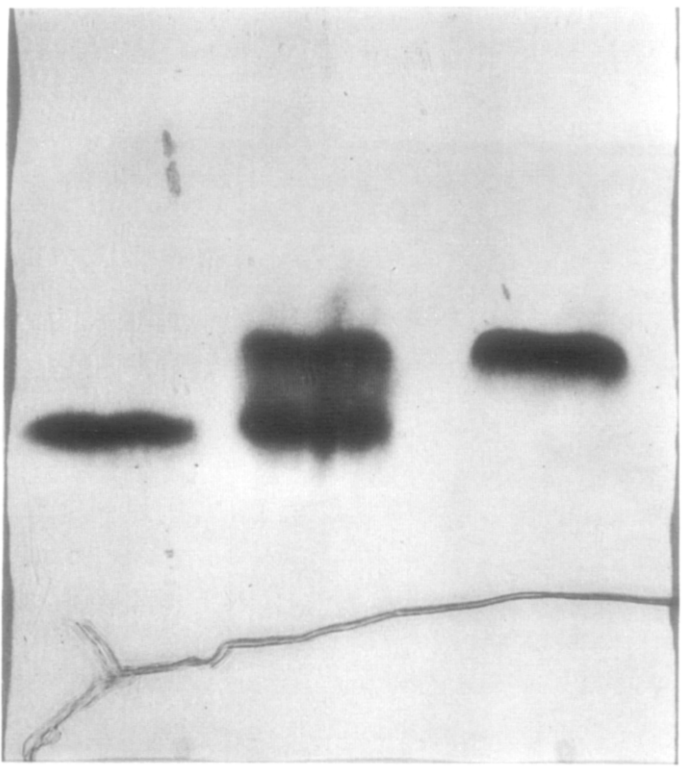
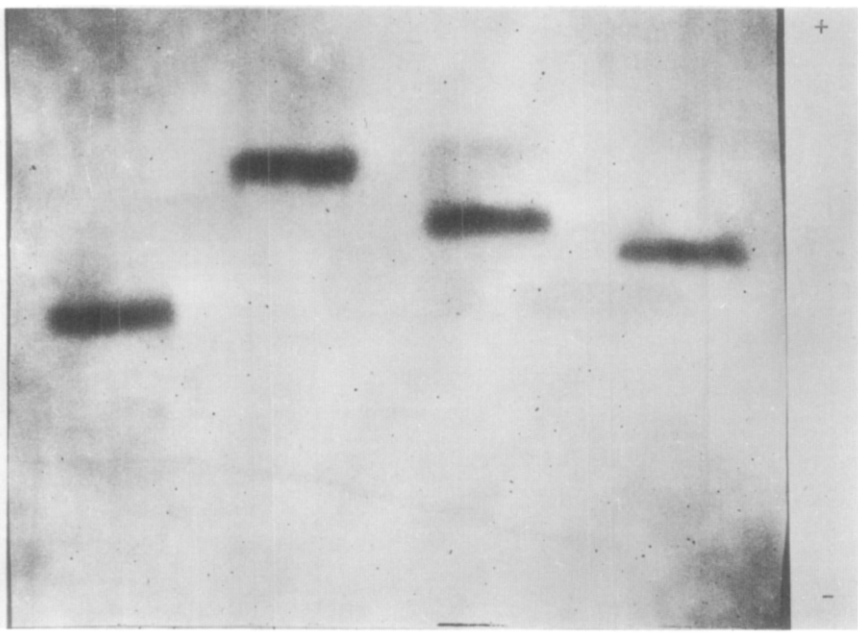


Fig. 2. Starch-gel electrophoresis of cat hemoglobin in Tris-EDTA-Borate at pH 8.4. (The curved lines are a crack in the gel which occurred during development).

differ from each other in at least one of a total of about 31 tryptic peptides. The major component contains one neutral peptide that is absent in the minor hemoglobin, and the latter contains one acidic tryptic peptide absent from the former.[1/] These structural differences however do not affect the oxygen equilibria. The oxygen saturation curves of the isolated components, as

1/ Lessard, J. L. and Taketa, F., unpublished

shown in Fig. 4, are identical at pH 6.8 and 7.2, and practically the same as that of the original hemolysate, $pO_{1/2}$ = 18.9 and 13.3 mm at pH 6.8 and 7.2 respectively.



Hb S Hb A CAT Hb DOG Hb

Fig. 3. Starch-gel electrophoresis of cat, dog, and human hemoglobins A and S; Tris-EDTA-Borate, pH 8.4.

Discussion

The ready availability of cat and human hemoglobins, which differ so widely in their oxygen dissociation curves, provides a basis for investigating relationships between primary structure and the regulation of oxygen binding. We are presently investigating the primary structure of both the major and minor cat hemoglobins and, as mentioned above, they differ from each other by at least one tryptic peptide. In this respect cat blood is similar to sheep blood, in which two components, differing by a single peptide located in the β chain, have been reported (16, 17).

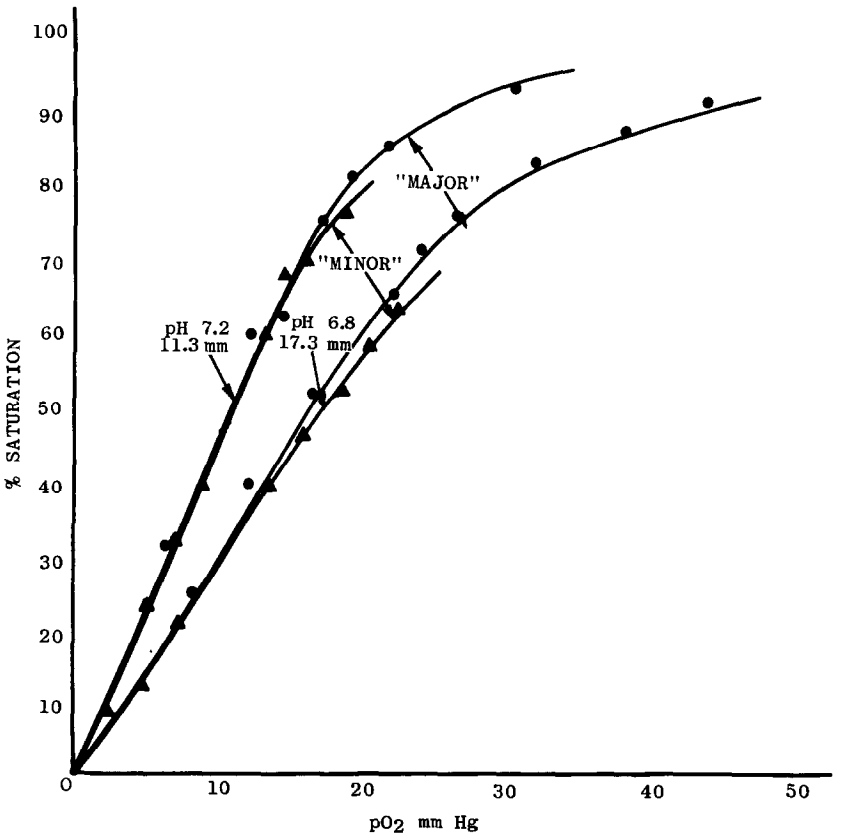


Fig. 4. Oxygen saturation curves of major and minor cat hemoglobins. The $pO_{1/2}$ values are indicated by arrows. Under the same conditions, the dialyzed hemolysates of cat erythrocytes exhibited practically identical $pO_{1/2}$ values, 18.9 and 13.3 mm at pH 6.8 and 7.2 respectively.

Recent data correlating oxygen equilibria with the Bohr effect of mammalian hemoglobins point to a common underlying mechanism which is independent of either the size or habit of the animal or differences in amino acid sequence (13). Although the magnitude of the Bohr effect appears to remain practically constant for different species, oxygen affinity and the values of n seem to vary considerably. The lower oxygen affinity of cat relative to human hemoglobin suggests a stronger interaction of subunits. The relatively lower values of n however, 1.8 to 2.0 for cat and 2.8 to 3.3 for human, are sur-

prising if n actually serves as a measure of subunit interaction. This lack of correlation of n with subunit equilibria is comparable to the relatively high values of n associated with low oxygen affinity of human Hb at high ionic strength (18). Experiments are presently in progress to relate primary structure to the regulation of subunit equilibria. Preliminary results (19) on the oxygen equilibrium of hybrids of cat and human hemoglobin support the conclusions of Antonini et al. (10) that the role played by a given chain in the tetramer is conditioned in a subtle manner by the identity of the other chain.

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