

Hemoglobin Polymorphism in Chimpanzees and Gibbons

Abstract. Hemoglobin polymorphism has been observed in the chimpanzee and two subspecies of gibbons. In chimpanzees, hemoglobins J and B were found in addition to hemoglobin A; J and B differed from A in their alpha and beta chains, respectively. Hemoglobins A and B were observed in different subspecies of gibbons; B differed from A in its beta chain.

Variation in electrophoretic mobility of hemoglobin has been reported for several species of new- and old-world monkeys (1), for orangutans (2), and for gibbons (3). We found that hemoglobin polymorphism exists in the chimpanzee and two subspecies of gibbons.

Hemoglobin samples were obtained from 109 chimpanzees, comprising four subspecies (4)—54 *Pan troglodytes* *schweinfurthi*, 31 *Pan troglodytes* *troglodytes*, 23 *Pan troglodytes* *troglodytes*, and one *Pan troglodytes* *koolo-kamba*—maintained at the 6571st Aeromedical Research Laboratory, Holloman Air Force Base; 14 gibbons (12 *Hylobates lar lar* and 2 *Hylobates lar pileatus*) from the Delta Regional Primate Center of Tulane University; and eight gibbons (*Hylobates lar lar*) from the primate colony of J. Moor-Jankowski, Department of Forensic Medicine of New York University Medical School. Erythrocytes were thoroughly washed with physiologic saline, pH 7.0, and were lysed with two volumes of cold, distilled water. The stroma was removed by centrifugation at 60,000g for 30 minutes, and the clear hemoglobin solution was converted to carbonmonoxyhemoglobin. Horizontal starch-gel electrophoresis (5) was performed in a 13.2 percent starch gel with a tris-EDTA-borate gel buffer, pH 9.1, and a borate buffer, pH 8.6, in the

electrode chambers. A voltage gradient of 15 volt/cm was maintained for 75 minutes. After electrophoresis, hemoglobin zones were stained with a benzidine-peroxide mixture. Column chromatography with Amberlite IRC-50 (CG-50) was used for purification of hemoglobin (6). Aberrant hemoglobin chains were identified by recombination with canine hemoglobin, as suggested by Robinson and Itano (7).

The single major component of chimpanzee and gibbon hemoglobin—chimpanzee hemoglobin A (Hb A^{Ch}) (8) and gibbon hemoglobin A (Hb A^{Gi})—has been shown to be electrophoretically similar to human hemoglobin A. In our study the hemoglobins of 107 of 109 chimpanzees examined electrophoretically had a single major component indistinguishable from human hemoglobin A at pH 9.1. A second minor component having the mobility of hemoglobin A₂ was also present in each case. One chimpanzee showed a second fast major component hemoglobin J (Hb J^{Ch}), similar in electrophoretic mobility to human hemoglobin J (Fig. 1). This component was found by column chromatography to constitute about 30 percent of the total hemoglobin. A second chimpanzee exhibited, in addition to Hb A^{Ch}, a slow major component hemoglobin B (Hb B^{Ch}), similar in electrophoretic mobility to that of human hemoglobin S (Fig. 1). The slow component constituted about 40 percent of the total hemoglobin; Hb A₂^{Ch} was also present.

Recombination of Hb J^{Ch} and Hb B^{Ch} with canine hemoglobin indicated that Hb J^{Ch} differs from Hb A^{Ch} by a substitution in the α -chain, and that Hb B^{Ch} differs from Hb A^{Ch} by a substitution in the β -chain. The two chimpanzees with Hb J^{Ch} and Hb B^{Ch} thus represent heterozygosity for genes of the α -chain and β -chain, respectively (6). In view of the fact that the Holloman colony was not a breeding colony until recently, it is not surprising that additional heterozygotes or Hb J and Hb B homozygotes were not found. The J/A and A/B heterozygotes have been classified as *P. t. schweinfurthi*. At this time, it is premature to speculate on the degree of hemoglobin variation of one subspecies as compared to another. However, the transferrin data of Goodman *et al.* (9) and the blood-group data of Moor-Jankowski *et al.* (4) suggest that *P. t. schweinfurthi* is more heterogeneous than the other three chimpanzee subspecies.

Twenty gibbons (*H. l. lar*) exhibited Hb A^{Gi} (Fig. 1), while the two gibbons of the *H. l. pileatus* subspecies had Hb B^{Gi} (Fig. 1). Recombination of Hb A^{Gi} and Hb B^{Gi} with canine hemoglobin indicates that the two hemoglobins differ in their β -chains. Evidence obtained from peptide-map analysis of the β -chains indicates that the difference is confined to a single peptide (10). Although our sample of the two subspecies is small, the occurrence of only homozygous hemoglobin types is significant in view of the blood-group data of Wiener *et al.* (11). Our data indicate that *H. l. lar* and *H. l. pileatus*, being interfertile, could be allopatric species. An examination of a larger number of hemoglobin phenotypes from the two subspecies, as well as more information concerning their exact geographic habitat, is necessary before the presumed allopatry can be confirmed.

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8. The classification of hemoglobins of non-human primates into five electrophoretic zones is based upon the electrophoretic mobility of human hemoglobin A and the known human hemoglobin variants in starch gel as discussed by H. A. Hoffman and A. J. Gottlieb, in *Holloman Symposium on the Immunology of Chimpanzees and Other Primates*, in press. Hemoglobins having an electrophoretic mobility similar to human hemoglobin I, J, A, S, and C are designated as hemoglobins of the I, J, A, B, and C type, respectively. The species being considered will be denoted by a superscript, for example, Ch for chimpanzee and Gi for gibbon.
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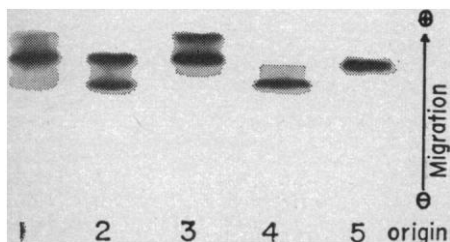


Fig. 1. Starch-gel electrophoresis, pH 9.1, of carbonmonoxyhemoglobin stained with benzidine-peroxide: (1) human hemoglobin (Hb) J/A/S; (2) chimpanzee Hb A/B; (3) chimpanzee Hb J/A; (4) gibbon Hb B; and (5) gibbon Hb A.