Hemoglobin $G_{Coushatta}$: A New Variant in an American Indian Family

Abstract. *A new hemoglobin variant, $G_{Coushatta}$ (α_2^A $\beta_2^{III\ glu \rightarrow ala}$), has been found in three generations of a family from the Alabama Coushatta Indian tribe in east Texas. The propositus suffers from an apparently unrelated, possibly genetic, agranulocytosis. According to the history and the blood-grouping data there is considerable inbreeding in the tribe and remarkably little admixture with other ethnic groups.*

Several surveys have failed to reveal any distinctive hemoglobin variants in American Indians, and the presence of abnormal hemoglobins among them is usually ascribed to admixture with Negroes or other ethnic groups (*1*). The hemoglobin variant described here occurs in four members of a family from the Alabama Coushatta Indian reservation in east Texas—two siblings, their mother, and their maternal grandfather. The hemoglobin of these four persons can be resolved by paper electrophoresis in barbital buffer, *p*H 8.6, into two approximately equal components. These are hemoglobin A and a variant resembling hemoglobin S, but moving slightly faster toward the anode, like hemoglobins P or Stanleyville I (*2*). By zone electrophoresis with alkaline buffers in other media—starch gel, starch block, and cellulose-acetate paper—the variant appears similar to hemoglobin S; in citrate-agar electrophoresis, *p*H 6.2, it does not separate from hemoglobin A, in this respect it resembles hemoglobins D, G, P, and Stanleyville I. However, in chromatography on amberlite resin IRC 50, with citrate buffer at *p*H 6.0, the variant moves between hemoglobins A and S, a mobility shared only by hemoglobin G. In the original sample from the 3-month-old propositus (SKW) there was also an appreciable amount of fetal (F) hemoglobin. Figures 1 and 2 show the mobility of the variant in paper electrophoresis and chromatography.

The variant, for which we suggest the name $G_{Coushatta}$, was separated from hemoglobin A by starch-block electrophoresis. It was then subjected to a combination of electrophoresis and chromatography, the "fingerprinting" procedure of Ingram (*3*). The arginine-containing tryptic peptide III of the β chain of the variant migrated at a slower rate toward the anode than did the corresponding peptide of hemoglobin A. The altered peptide was isolated by electrophoresis and was hydrolyzed and analyzed for amino acids (*4*). A comparison with the corresponding tryptic peptide III of hemoglobin A revealed that one of the two glutamic acid residues of the latter was replaced by an alanine residue in hemoglobin $G_{Coushatta}$. The substitution of glutamic acid for alanine was previously found in a different location, the beta tryptic peptide V, of another hemoglobin variant, $G_{Galveston}$ (α_2^A $\beta_2^{43\ glu \rightarrow ala}$) (*5*). According to the scheme of nomenclature of abnormal hemoglobins (*6*), hemoglobin $G_{Coushatta}$ may be formulated as α_2^A $\beta_2^{III\ glu \rightarrow ala}$.

Because of the rarity of abnormal hemoglobins among Indians of North and South America, the history of the tribe is of interest. The Alabamas and Coushattas (or Koasati) were neighboring groups, both of Muskogean stock of the Creek Confederacy and so closely related that their languages are mutually intelligible. They have lived in east Texas since about 1800, in the vicinity of the present Alabama Coushatta Reservation, although in the latter half of the 19th century one group of Coushattas emigrated to a village in southwest Louisiana. Tribal interdictions against miscegenation are strengthened by an agreement made with the United States government that only full-blooded Indians may live on the reservation. The population, now numbering about 350, has, to a remarkable extent, remained genetically isolated, except from the approximately 200 Louisiana Coushattas living about 150 miles away. The maternal grandfather to whom the hemoglobin variant was traced came originally from this Louisiana group.

Table 1. Frequencies of factors in five blood-group systems in Alabama-Coushatta Indians.

Factors	Percentage	Factors	Percentage
ABO (282 tested)		MNS (108 tested)	
0	98.2	MS	15.7
B	1.8*	Ms	28.7
A	0.0	MSs	25.9
AB	0.0	MNS	1.9
Rh† (173 tested)		MNSs	13.0
CCDee	32.4	MNs	9.3
CcDEe	42.2	NS	1.9
ccDEE	12.1	Ns	2.8
CcDee	5.2	NSs	0.9
ccDEe	1.2		
CCDEe	6.9	Kell (111 tested)	
Duffy (113 tested)		k^bk^b	58.6
Fy(a+b−)	42.5	k^ak^b	33.3
Fy(a+b+)	46.9	K^bk^a	3.6
Fy(a−b+)	10.6	K^bk^b	3.6
Fy(a−b−)	0.0	k^ak^a	0.9

* Four (perhaps all five) persons are closely related. † Genotypes expressed in this form since gene frequencies in this population have not been calculated. Anti-d not tested.

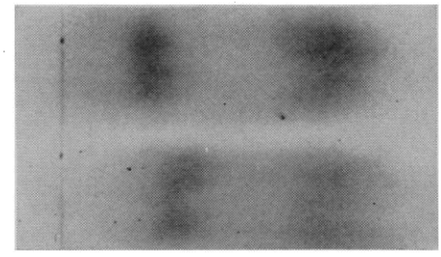

Fig. 1. Paper electrophoresis, barbital buffer, *p*H 8.6, of hemoglobin $G_{Coushatta}$, compared with hemoglobin S. Top, hemoglobin AS; bottom, hemoglobin $AG_{Coushatta}$ (sample from DP, grandfather of propositus).

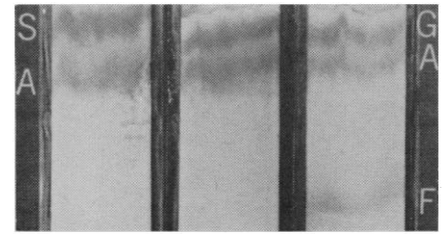

Fig. 2. Chromatography, amberlite resin, IRC 50, citrate buffer, *p*H 6.0, of hemoglobin $AG_{Coushatta}$ compared with hemoglobin AS. Samples from left to right: AS, $AG_{Coushatta}$ (from RW, mother of propositus) and $AG_{Coushatta}$, F (from SKW, propositus, at 3 months of age).

The ethnology of the Alabama Coushattas was described by Dickerson (*7*). Medical and genetic studies by Johnson and McNutt (*8*) revealed that the incidence of diabetes in the tribe is about five times greater than in the general population and that consanguinity is common. The blood group frequencies, as shown in Table 1, are similar to those of other North American Indian populations (*10*), particularly in the marked predominance of type O, Rh+ (D+). Hemoglobin samples from 270 members of the tribe were analyzed by paper electrophoresis, barbital buffer, *p*H 8.6, and only the normal adult type hemoglobin A, was found (*11*). The Louisiana Coushattas, according to all available information, resemble the Texas group in every respect, even in having a similarly high incidence of diabetes.

The Alabama Coushatta girl who is the propositus was first seen here at the age of 3 months. She was suffering from a severe generalized infection

manifested by septicemia, meningitis, and pneumonia caused by *Staphylococcus aureus*. This infection, as well as a reported attack of "thrush" at 3 weeks of age, and a subsequent cutaneous abscess at 6 months, were thought to be related to a striking paucity of granulocytes in her peripheral blood. The initial blood analysis revealed hemoglobin 10 g/100 ml of blood; total leukocytes, 10,000/mm^3; 2 percent neutrophiles, 3 percent stab cells, 54 percent lymphocytes, and 41 percent monocytes. During the illness the hemoglobin value fell to 6.6 g/100 ml, presumably because of infection, since after a blood transfusion and antibiotic therapy the anemia subsided and did not recur. Neutropenia and monocytosis persisted, and in many subsequent blood counts the granulocytes remained few or were completely absent. At the same time the percentage of monocytes and lymphocytes varied reciprocally from about 30 to 65, in a total leukocyte count of 6000 to 15,000/mm.^{3} Examination of the bone marrow revealed that maturation of the granulocytes was arrested at the myelocyte stage. Hematologic studies of both parents failed to disclose any abnormality, and both siblings (one of whom has only hemoglobin A) are healthy. There is no family history of a comparable disorder. Kostmann (*12*), describing a similar type of agranulocytosis in 14 children from a genetically isolated population in Sweden, found that an extreme susceptibility to infection made the condition lethal, and suggested that it was caused by a recessive gene in the homozygous state. Since the three other persons with hemoglobin G$_{\text{Coushatta}}$ are healthy, the grandfather being 66 years old, the agranulocytosis appears unrelated to the presence of the hemoglobin variant.

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Mutarotase Inhibition by 1-Deoxyglucose

Abstract. *The Michaelis-Menten constant for glucose and the inhibitor constant of 1-deoxyglucose for the intestinal mutarotase, glucose, 1-deoxyglucose system are consistent with the properties of the glucose transport system of intestine. The alleged sharing of the intestinal glucose transport system by l-deoxyglucose does not contradict the mutarotase theory.*

1,5-Anhydro-D-glucitol (1-deoxyglucose) has been reported to be transported against a concentration gradient in everted hamster intestinal sacs (*1*) and by hamster intestinal segments (*2*). That 1-deoxyglucose may presumably share a common pathway with glucose in intestinal transport (*1, 3–5*) has been widely used as a central argument against the mutarotase theory (*6*) since 1-deoxyglucose cannot mutarotate.

This report shows that the mutarotase of hamster intestine is inhibited by 1-deoxyglucose and exhibits data from which the Michaelis-Menten constant (K_m) for hamster intestinal mutarotase acting on glucose and the inhibitor constant (K_i) for its inhibition by 1-deoxyglucose may be calculated. These K_m and K_i values serve as a basis for the accurate prediction of the degree of inhibition of transport of glucose in the presence of 1-deoxyglucose, but not for a correct prediction of the degree of inhibition of 1-deoxyglucose transport by glucose. The data of Crane (*3*) on mutual inhibition of transport in studies of glucose and 1-deoxyglucose in hamster intestinal segments are shown to be in accord in these respects with the data for mutarotase.

The entire small intestines of 12 hamsters were removed and 0.025*M* phosphate buffer (*7*) was used to rinse out the lumen. The intestines were weighed, and a tissue extract was made, as described previously for lens (*8*), with 2 ml of buffer per gram of intestine. The hamster intestinal extract was fractionated as follows: For each milliliter of extract, 0.22 g of ammonium sulfate was added and the extract was centrifuged. The precipitate was discarded and 0.18 g of ammonium sulfate was added for each milliliter of supernatant and the mixture was centrifuged. The precipitate was allowed to drain and then it was dissolved in about 5 ml of buffer and dialyzed against water for 1 hour.

Mutarotase was assayed polarimetrically (*8*). The degree of inhibition, the fractional residual activity, K_m and K_i, were also calculated as previously reported (*8*).

The portion of the rate of the mutarotation reaction due to catalysis by the enzyme is $V = (k_e - k_s)S$, where S is the substrate concentration and k_e and k_s are the mutarotation coefficients of the reactions carried out in the presence and absence of the enzyme, respectively. The mutarotation coefficient is the sum of the rate constants of the forward and backward components of the reversible mutarotation reaction and is equal to $0.693/t_{\frac{1}{2}}$; $t_{\frac{1}{2}}$ is equal to the half-time of the reaction.

The average value of several determinations of $t_{\frac{1}{2}}$ for the spontaneous rate of mutarotation was 7.4 minutes (*8*). The value for spontaneous $t_{\frac{1}{2}}$ was independent of the glucose concentration in the range measured.

Table 1 shows the action and Fig. 1 the course of the action of hamster intestinal mutarotase on glucose and the inhibition of this action by 1-deoxyglucose. The course of the spontaneous mutarotation reaction is also shown. Figure 2 shows the determinations of the K_m for hamster intestinal mutarotase by means of a Lineweaver-Burk (*9*) graph. This graph, which