Haptoglobin Binding Capacity of Certain Abnormal Hemoglobins

Abstract. *Hemoglobins H and Bart's failed to bind human haptoglobin. The binding of hemoglobins A_2, F, I, and Lepore was comparable to that of hemoglobin A. These findings suggest that configurational differences or the absence of alpha chain dimers was responsible for the lack of binding of hemoglobins H and Bart's.*

The hemoglobin-haptoglobin complex has been studied extensively during the last 10 years, but the mechanism of its formation is still unclear. According to Jayle and Moretti (*1*), the reaction between haptoglobin (Hp) and hemoglobin (Hb) is stoichiometric. Over a wide *p*H range (4.4 to 10), the hemoglobin-haptoglobin complex is very stable. Haptoglobin apparently binds the globin of hemoglobin (*2*), and human haptoglobin has been found to combine with a variety of animal hemoglobins [monkey, horse, cow, dog, rabbit, and mouse (*1*)]. Haptoglobin has been found to bind fetal hemoglobin and several abnormal human hemoglobins (*3*), notably hemoglobins C, S, and D_{Punjab}.

This study was undertaken to ascertain whether human haptoglobin would bind human hemoglobins which may differ significantly in configuration from Hb A ($\alpha_2^A \beta_2^A$). Hemoglobins H (β_4^A) and Bart's (γ_4^F) are tetramers of a single polypeptide chain; hemoglobin Lepore apparently contains two α^A chains in combination with two chains that contain portions of δ- and β-chains (*4*). Such hemoglobins might be expected to exhibit significant configurational differences from hemoglobin A or from abnormal hemoglobins which contain single amino acid substitutions. An altered configuration has recently been observed on x-ray crystallographic studies of hemoglobin H (*5*).

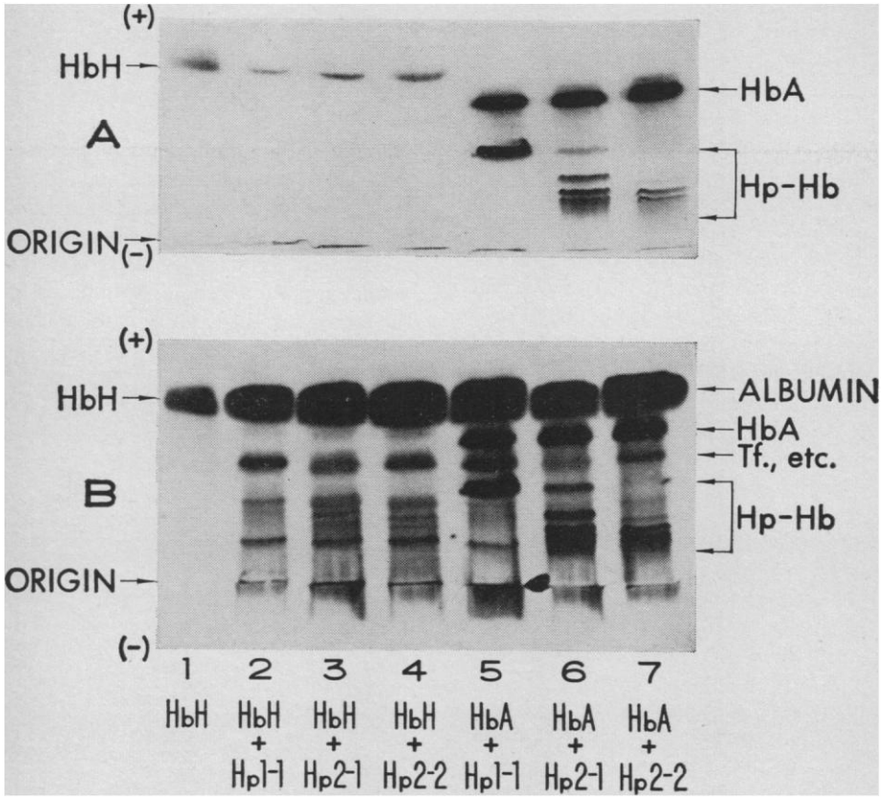

Fig. 1. Horizontal starch electrophoresis of human serum, *p*H 8.6 (*11*), incubated with hemoglobins H and A. *A*, Stained with benzidine. *B*, Counterstain of the same gel with amido black 10-B (Tf, transferrin). *1*, Hb H; *2*, Hb H + serum Hp 1-1; *3*, Hb H + serum Hp 2-1; *4*, Hb H + serum Hp 2-2; *5*, Hb A + serum Hp 1-1; *6*, Hb A + serum Hp 2-1; *7*, Hb A + serum Hp 2-2. Hemoglobin A alone (not shown in the figure) migrates in this system in the same position as unbound Hb A in mixtures *5*, *6*, and *7*. Hemoglobin H concentration is 100 mg per 100 ml of serum. Hemoglobin A concentration is 155 mg per 100 ml of serum. Complex formation between haptoglobin and hemoglobin A is denoted by the haptoglobin-hemoglobin bands in mixtures *5*, *6*, and *7* on the benzidine-stained gel. Such bands are not seen in mixtures *2*, *3*, and *4*, in which hemoglobin H was used.

Hemolysates were prepared by the method of Drabkin (*6*). Hemoglobins A_2, I, and $Lepore_{Boston}$ (*7*) were isolated by starch block electrophoresis (*8*) in 0.05*M* veronal buffer, *p*H 8.6. Hemoglobin F, obtained from cord blood, was isolated on a column of Amberlite IRC-50 according to the method of Allen *et al.* (*9*); developer No. 2 was used. Hemoglobin H was isolated by electrophoresis on starch granules in 0.04*M* phosphate buffer, *p*H 7.5. Hemoglobin Bart's obtained from blood samples from two newborn Negroes was separated by starch block electrophoresis in 0.02*M* phosphate buffer, *p*H 7.5, and was subjected to a second electrophoresis on starch granules in 0.05*M* veronal buffer, *p*H 8.6.

The binding of each hemoglobin (A_2, F, I, Lepore, H, and Bart's) was tested by incubating the serum for 15 minutes with sufficient amounts of hemoglobin to result in saturation or over-saturation of the hemoglobin-binding capacity of the serum. The binding capacity of each serum for hemoglobin A was determined by a modification of Nyman's method (see *10*).

Electrophoretic analyses of the untreated serum and of the serum to which hemoglobin had been added were carried out on horizontal (*11*) and vertical starch gels (*12*). Sliced starch gels were stained with benzidine or with amido black 10-B. The haptoglobin-binding capacity of hemoglobins H and Bart's was further studied by measurement of peroxidase activity (*13*); ethylhydroperoxide was used.

The binding of hemoglobins A_2, F, I, and Lepore to haptoglobin types 1-1, 2-1, and 2-2, as detected by horizontal and vertical gel electrophoresis at *p*H 8.6, did not appear significantly different from the binding of the hemoglobin A control. Of course, small differences in binding would not be appreciated with these methods.

In contrast to other hemoglobins which were apparently equally bound to haptoglobin, hemoglobins H and Bart's were bound little, if at all, to the common haptoglobin types.

When hemoglobin H was added to serum, only a free band of hemoglobin was observed (Fig. 1). This band sometimes split into two components of nearly the same mobility, which were probably the isomeric forms of hemoglobin H (*14*).

Formation of the hemoglobin-haptoglobin complex was not demonstrated when hemoglobin Bart's was used. Some barely discernible benzidine-positive bands observed in the position of the haptoglobin band in a few runs probably represented impurities in the preparations of hemoglobin Bart's. The manipulation of hemoglobins H and Bart's did not appear to account for the absence of binding since hemoglobin A, which had been prepared from the same electrophoretic separations, exhibited an apparently normal haptoglobin-binding capacity. Further evidence of the failure of hemoglobins H and Bart's to form complexes with haptoglobins was derived from the absence of retardation of migration of the haptoglobins on gels stained with amido black. (The haptoglobin-hemoglobin complex migrates faster toward the cathode than the corresponding haptoglobin bands of serum.)

The possibility that globin from the abnormal hemoglobins was blocking the haptoglobin was considered. However, when hemoglobin A was added to serums that had been previously incubated for 15 minutes at 20°C with hemoglobin H or Bart's, the normal hemoglobin-haptoglobin pattern was observed on electrophoresis.

The formation of the hemoglobin-haptoglobin complex was also studied by means of starch gel electrophoresis, with 0.003*M* phosphate buffer (*13*) at *p*H 7.0, where hemoglobin H failed to bind haptoglobin. At *p*H 7.0, hemoglobin Bart's migrated near the haptoglobin band and definite conclusions concerning formation of the complex could not be drawn.

The peroxidase activity of hemoglobins H and Bart's did not increase in the presence of serum, while in hemoglobin A prepared in the same manner a clear increase in peroxidase activity as a consequence of the formation of the haptoglobin-hemoglobin complex was demonstrated.

Neither the structural sites nor the groups involved in the formation of the haptoglobin-hemoglobin complex have been defined. In the studies reported here, two hemoglobins which were tetramers of a single type of polypeptide chain (β_4^A and γ_4^F) failed to bind haptoglobin, although normal hemoglobin A (α_2^A β_2^A), A_2 (α_2^A $\delta_2^{A_2}$), or F (α_2^A γ_2^F) containing β-, δ-, or γ-chains regularly bound at least the common types of human haptoglobins. The failure of β- or γ-tetramers to bind haptoglobin has two obvious alternative explanations: (i) that the presence of α-polypeptide chains is necessary for the binding, or (ii) that the altered configuration demonstrated for hemoglobin H (*5*) and presumed for hemoglobin Bart's results in loss of the haptoglobin binding capacity.

The available evidence does not permit us to decide conclusively which of these possibilities is correct. Studies by others have indicated that electrostatic and van der Waal's forces (*15*) [but probably not SH groups (*16*)] are important in the formation of the complex, but there is little evidence concerning the contribution of the individual hemoglobin polypeptide chains in the complex formation. Single changes in the amino acid sequence of either chain (for example, hemoglobins I^α or S^β) do not interfere with the formation of the haptoglobin-hemoglobin complex. The greater acidic charge of hemoglobin H or hemoglobin Bart's would not account for the failure to bind haptoglobin, since hemoglobin I, which has about the same charge as hemoglobin H, formed the hemoglobin-haptoglobin complex.

At this time there is no method for testing the possibility that α-chains as they occur in the normal hemoglobin-A tetramer are necessary for the formation of the hemoglobin-haptoglobin complex. The α^A-chains that have been isolated (*17*) are monomers and consequently have great changes in configuration. We have, however, been unable to demonstrate complex formation between such α^A-chains prepared in our laboratory and haptoglobin.

The formation of the hemoglobin-haptoglobin complex probably represents a binding between two complementary surfaces as visualized by Pauling (*18*). The failure of hemoglobins H or Bart's to bind haptoglobin is related either to configurational alterations or to the absence of the α^A-chain dimers in these hemoglobins.

RONALD L. NAGEL
HELEN M. RANNEY
Department of Medicine Heredity Unit, Albert Einstein College of Medicine, New York 10061

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Iron Absorption: The Effect of an Iron-Deficient Diet

Abstract. *A diet deficient in iron causes a rapid, marked increase in iron absorption in rats. The increased absorption occurs in the absence of a significant change in iron stores as judged by the effect of an equivalent change in stores produced by phlebotomy, and in the absence of increased erythropoietic activity as judged by the rate of removal of iron-59 from the plasma.*

Many experiments suggest that the intestinal mucosa's absorptive capacity for iron is controlled by erythropoietic activity and by iron stores (*1*). Experiments with rats have suggested to us that the iron in the normal diet is an additional controlling factor.

We have studied iron absorption in male albino rats, using radioactive iron (Fe^{59}) with 250 μg of carrier iron and estimating retention of the isotope by counting the residual radioactivity in the total body (*2*). The investigation reported here began with the chance observation of strikingly increased iron