# MAPPING THE HEMOGLOBIN LOCUS IN MICE TRANSMITTING THE FLECKED TRANSLOCATION[1]

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THIS report describes the chromosomal location of the hemoglobin beta-chain structural locus (*Hbb*) in mice transmitting the flecked (*fd*) translocation (CATTANACH 1961), and the electrophoretic behavior of hemoglobins analyzed by acrylamide gel and cellulose acetate systems. In adult mice, the electrophoretic difference in hemoglobin determined by the locus behaves as a unit character (GLUECKSOHN-WAELSCH, RANNEY and SISKEN 1957). Alleles are $Hbb^s$ (single-type hemoglobin) and $Hbb^d$ (diffuse-type hemoglobin). More recently a third allele, $Hbb^p$, was reported by MORTON (1962). The structural relationship of $Hbb^p$ to $Hbb^s$ and $Hbb^d$, and the nature of its polypeptide chains has not been determined. $Hbb^d$ is probably compound and may represent adjacent structural loci for two kinds of polypeptide chains, beta and delta, whereas $Hbb^s$ represents the structural locus (*i*) for a single kind of polypeptide chain, beta (HUTTON *et al.* 1962a, b). Evidence by RIGGS (1965) and MORTON (1966) suggests that dimerization of beta chains may account at least in part for the single-diffuse electrophoretic phenomenon.

The flecked translocation is an insertion of an autosomal segment carrying the wild-type alleles of albino (*c*) and pink-eyed dilution (*p*), from linkage group I into the X (sex) chromosome (CATTANACH 1961; OHNO and CATTANACH 1962). It is nonreciprocal and no recombination has been observed between the insertion and homologous autosomal segments. The insertion is viable with or without the deletion in females and relatively inviable without the deletion in males. Female mice having the X-autosome insertion and carrying *p* or *c* in homozygous or hemizygous condition in Autosome I exhibit varied degrees of color mosaicism. Males of similar genotype are wild type in color. The location and behavior of *c* and *p* loci in the insertion in the X chromosome (CATTANACH 1961; LYON 1963; OHNO and CATTANACH 1962) are generally consistent with the inactive-X hypothesis (LYON 1961) as modified by RUSSELL (1963). *Hbb* is located close to *c* and still closer to shaker-1 (*sh-1*) in the order *Hbb*, *sh-1*, *c* in linkage group I (POPP and ST. AMAND 1960; POPP 1962). The best estimates of recombination from combined data for the *Hbb* to *c* interval are 7.3 ± 0.75% in females and 3.5 ± .79% in males (POPP and ST. AMAND 1964); for the *Hbb* to *sh-1* interval 1.4 ± .42% in females and 2.0 ± 0.51% in males (POPP 1962). Pink-eyed dilu-

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tion ($p$) is located on the opposite side of $c$ from *Hbb* and recombines with $c$ (in autosomes) with a frequency of 15.7 ± 0.49% for females and 11.9 ± 0.54% for males (GREEN, personal communication).

Since the flecked translocation occurred in an inbred CBA male ($Hbb^d/Hbb^d$) treated with triethylenemelamine (TEM) (CATTANACH 1961), we predicted that either the insertion in X or the deficient autosome would carry the $Hbb^d$ allele. If intact autosomes of linkage group I are fixed for the $Hbb^s$ allele, it becomes possible to test the types of hemoglobin of translocation genotypes and make certain inferences about the location and behavior of the *Hbb* locus, based on our concepts of X-chromosome inactivation. The results of such experiments are reported below.

## MATERIALS AND METHODS

Hemoglobins were electrophoresed in 7.5% standard acrylamide gels in Tris-glycine buffer (stacking pH 8.9; running pH 9.5) (Canalco, Bethesda, Md.), following procedures outlined by ORSTEIN (1964) and DAVIS (1964). Gels were prepared in inverted order, i.e., sucrose layer first, replaced at the last step by hemoglobin mixed in the sample gel. The method of preparing hemoglobin was that described by WOLFE, RUSSELL and PACKER (1963). From 1 to 5 μl of a carbonmonoxyhemoglobin solution was mixed in the sample gel, photopolymerized, and run from 30 to 35 minutes at 5 ma current/gel. Classification of the hemoglobin type of different translocation genotypes was ordinarily made on unstained hemoglobin. When a permanent record was desired, gels were stained with Aniline Blue Black, destained with repeated 7% acetic acid rinses and stored in stoppered tubes in the last acid rinse.

Additionally each hemoglobin type was verified either by electrophoresing the hemoglobin on cellulose acetate strips (Shandon Universial Electrophoresis Apparatus, COLAB, Chicago Heights, Ill.), or by tests of hemoglobin solubility in concentrated potassium phosphate solutions (see WOLFE *et al.* 1963). The cellulose acetate system employed AARONSON and GRÖNWAL buffer, pH 8.9 (Tris(hydroxymethyl)aminomethane, 10 g; disodium ethylenediamine-tetra-acetate, 1.4 g; boric acid, 0.8 g; and distilled water, 1 liter). Samples of a 5 to 10 μl carbonmonoxy-hemoglobin solution were placed on 2.5 × 12 cm Oxoid strips and run for 1 to 2 hr at 0.8 to 1.5 ma current/strip. Strips were stained in 0.2% Ponceau S in 3% trichloroacetic acid, destained, cleared, then mounted in sealing sheets. For determinations of solubility, the simplified procedure of HUTTON *et al.* (1964) was used.

To maintain our flecked translocation stock, light variegated females possessing a deficient Autosome I, intact Autosome I, X-autosome insertion and normal X chromosome (Type I) are mated to normal chinchilla males. Eight regular classes of segregants are expected: Females and males may be normal; they may have deficient Autosome I, intact Autosome I and X-autosome insertion (Type I); they may have intact autosomes and X-autosome insertion (Type II); or they may have a deficient Autosome I, intact Autosome I, and normal X chromosome (presumed lethal). The mutant-type patches in the coats of Type I females are like the coats of $c^{ch}/c$ in color, those of Type II females like $c^{ch}/c^{ch}$. Type I and Type II males are both wild type in color but Type II males are usually small and have a low survival rate.

## RESULTS AND DISCUSSION

Three electropherograms were found in offspring of the flecked female by chinchilla male cross (Figure 1). These correspond to electropherograms found in certain inbred strains and their $F_1$ hybrids. For acrylamide: (1) a single band ($Hbb^s/Hbb^s$) (Figures 1A, C); (2) three bands with the central band more concentrated, called intermediate ($Hbb^s/Hbb^d$) (Figure 1D); and (3) three

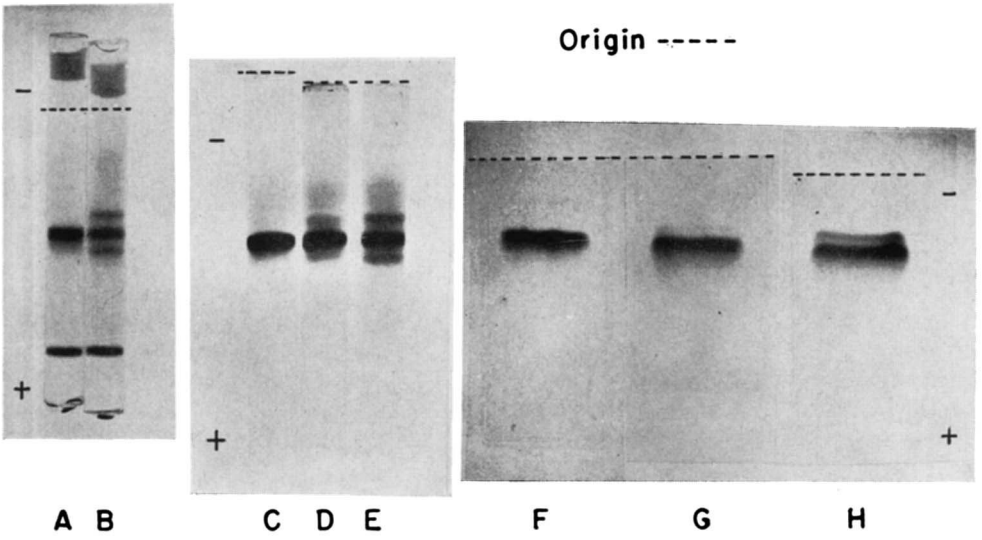


FIGURE 1.—Electrophoretic patterns of adult mouse hemoglobin. A-E acrylamide; F-H cellulose acetate. A, B, unstained single- and diffuse-type patterns, respectively. Both were photographed within the glass column in which they were run. The dark region at the top of each tube is residual hemoglobin in the sample gel, while the band at the bottom is bromphenol blue, a tracking dye. C, D, and E are single-, intermediate- and diffuse-type patterns respectively, stained with Aniline Blue Black. F, G, and H are single-, intermediate- and diffuse-type patterns respectively, stained with Ponseau S.

bands with anodal and cathodal bands more prominent and central band less prominent than in D, called diffuse ($Hbb^d/Hbb^d$) (Figures 1B, E). For cellulose acetate: (1) a single band ($Hbb^s/Hbb^s$) (Figure 1F); (2) two bands but cathodal band faint ($Hbb^s/Hbb^d$) (Figure 1G); and (3) two bands with cathodal band more prominent than in G ($Hbb^d/Hbb^d$) (Figure 1H).

In preliminary electrophoretic tests of hemoglobin of mated pairs, light variegated (Type I) females were invariably intermediate whereas normal chinchilla males were invariably single. Further tests extended to their offspring established that intact autosomes in our stock were fixed both for $Hbb^s$ and chinchilla ($c^{ch}$). Coat color and hemoglobin types of parents and offspring, together with their proposed genotypes, are shown in Figure 2.

Light variegated (Type I) females and wild-type (Type I) males have intermediate-type hemoglobin patterns. Dark variegated (Type II) females and wild-type (Type II) males have single-type hemoglobin patterns (both are trisomic for the length of the insertion). *Lack of a sex difference in electrophoretic pattern between translocation genotypes that possess the X-insertion suggests that* $Hbb^d$, *unlike* $c^+$, *is absent from the X-insertion.* The evidence is critical in the case of Type II females and males, both of which are homozygous for $Hbb^s$ in their two intact Autosomes I. Type II males presumably have $c^+$ in their single X active in all melanocytes. If $Hbb^d$ were included in the translocation rather than in the deficient autosome the locus theoretically would be active

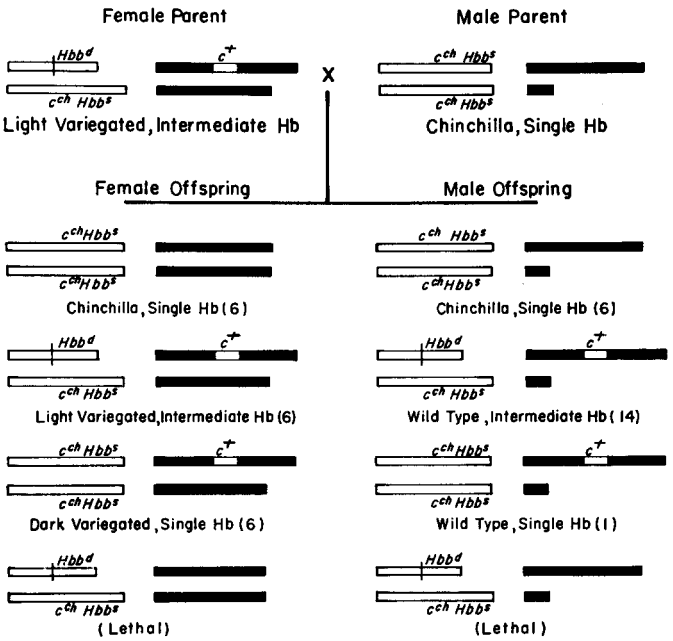


FIGURE 2.—Coat-color and hemoglobin type of segregants from a cross of flecked females, heterozygous for *T(X;1)Ct*, and of chinchilla males not carrying the translocation. Chromosomes are: short, clear rod—deficient Autosome I; long, clear rod—intact Autosome I; solid rod with segment transposed from Autosome I—X-inserted (sex) chromosome; long, solid rod—normal X chromosome; short, solid rod—Y chromosome. Gene symbols are: $Hbb^s$—single-type hemoglobin; $Hbb^d$—diffuse-type hemoglobin; $c^{ch}$—chinchilla; $c^+$—wild-type color. Numbers of offspring tested for hemoglobin pattern are indicated in parentheses following phenotype. An excessive number of wild-type, intermediate-hemoglobin males (Type I, second row) is probably a result of sampling error. Nine presumed Type I males were trial mated and confirmed to be sterile. Males trisomic for the insertion (Type II, third row) have reduced viability and are smaller than normal. The one very small male presumed to be Type II had single-type hemoglobin. It did not survive to mating. Two unbalanced types (bottom row) are presumed early prenatal lethals. Results suggest that $Hbb^d$ is located outside the break, in deficient Autosome I.

in all erythropoietic cells of Type II males, with diffuse hemoglobin present in circulating erythrocytes in amounts detectable by our methods. Assuming that synthetic rate of beta-chain polypeptide bears a direct relationship to beta-chain loci present, then Type II males should synthesize hemoglobin in the ratio 2 single : 1 diffuse. Type II females should synthesize hemoglobin in the ratio 4 single : 1 diffuse, if we assume that that part of the insertion in the X chromosome containing $Hbb^d$ was active in one half of erythropoietic cells. *No diffuse-type hemoglobin was detected* in either Type II females or males, though we could demonstrate presence of diffuse-type hemoglobin in corresponding mixtures made in vitro.

Repression of $Hbb^d$ or an inability to function (e.g., break in a part of the operon) in an X-inserted position is also an untenable explanation of the data, unless repression occurs selectively in 3× dose combinations. Type I females and

males would have single rather than intermediate hemoglobin, since only one *Hbb* gene would be active, namely $Hbb^s$ in the intact autosome. Neither can the results be adequately explained by gradients of X-inactivation with $Hbb^d$ nearer to a center or region of inactivation, or by selective proliferation or death in the young embryo of erythroid stem cells having particular functional states. The single-type hemoglobin pattern in the Type II male argues against the latter possibility.

Light variegated (Type I) females of the flecked stock were outcrossed to males of inbred strain 129/Re. Mice of strain 129/Re are homozygous chinchilla and electrophoretically diffuse. Two electropherograms were apparent among the offspring. Type I females (2 examined) and Type I males (2 examined) were diffuse; Type II females (4 examined) and Type II males (3 examined) were intermediate and could not be distinguished from Type I females and males of the previous cross. Results reinforce interpretations made for the previous cross.

Genetic evidence presented in this paper and that presented by EICHER (1967) supports the conclusion that one of two breakpoints postulated to have occurred in a chromosome comprising linkage group I in the flecked translocation lies between *Hbb* and *sh-1*. The transposed segment apparently includes the *sh-1* locus but not the *Hbb* locus. The data permit certain inferences about genetic length of the translocation. If one conservatively begins from *p* and includes *sh-1* but not *Hbb*, the best estimate of the genetic length of the translocation is 15 map units (based on recombination data, males) or 23 map units (based on recombination data, females), and this compares favorably to an estimated 20% increase in length of the X-inserted chromosome obtained by microscopic measurement (OHNO and CATTANACH 1962).

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## SUMMARY

Mice of our flecked translocation stock *T(X;1)Ct* possessing a deficient Autosome I, intact Autosome I, and the X-autosome insertion, of either sex (Type I), have electrophoretically intermediate hemoglobin, whereas mice possessing two intact Autosomes I and the X-autosome insertion, of either sex (Type II), have electrophoretically single hemoglobin. These results are interpreted to mean that the beta-chain structural locus (*Hbb*) is located in the deficient autosome adjacent to the missing (transposed) segment, and that function of the locus is unimpaired.

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