Hemoglobin F_{Texas}: Gamma-Chain Variant

Abstract. *An abnormal fetal hemoglobin, designated hemoglobin F_{Texas}, was found in the cord blood of five Negro infants (all related and three of them siblings) and of one Caucasian infant. Studies on the chemical structure of this variant indicate that there is a substitution of a lysyl residue for either the fifth or sixth glutamyl residue of the gamma-chain.*

Many genetically determined variants of adult hemoglobin are known, which carry a single amino acid substitution at a specific residue of either the α-polypeptide or the β-polypeptide chain. Because the α-chains of adult hemoglobin A ($\alpha_2^A \beta_2^A$) and of fetal hemoglobin F ($\alpha_2^A \gamma_2^F$) are under the same genetic control, an abnormality of the α-chain results in abnormal hemoglobins of both the adult and fetal types. When the amino acid substitution occurs at one of the residues of the β-chain, only the adult hemoglobin is abnormal. A similar alteration of the amino acid sequence of the γ-chain of fetal hemoglobin has not been demonstrated. We now report on an analysis of a γ-chain variant, designated hemoglobin F_{Texas}.

This variant, found in samples of cord blood from two Negro siblings (from the W family) and two other infants, Negro and Caucasian respectively, made up about 12 percent or less of the total cord blood hemoglobin (*1*). In blood samples taken at intervals after birth, the amount was less in each succeeding sample, and the variant was barely detectable when the infants were 6 months old. In samples drawn thereafter, the variant was not detectable, nor was it detectable in the blood of the parents. This same variant has now been found in two other samples of cord blood. one from a third sibling of the W family and the other from their paternal cousin. More complete questioning has revealed that the presumably unrelated Negro infant of the earlier report (*1*) is actually a paternal cousin, several times removed, of the W siblings (*2*).

In zone electrophoresis in alkaline buffers, hemoglobin F_{Texas} moves more slowly towards the anode than does hemoglobin C, and it appears somewhat inhomogeneous (Fig. 1*a*). The variant hemoglobin from the third sibling (WIII) and that from the cousin (LFR) were isolated by chromatography on diethylaminoethyl (DEAE) cellulose in a phosphate buffer, *p*H 8.6, after prior separation by starch-grain electrophoresis in veronal buffer, *p*H 8.6. The electrophoretic inhomogeneity was still apparent in these isolated samples, but in the ultraviolet absorption spectrum there was only one tryptophan "fine-structure" band, at about 289 mμ, as is characteristic of fetal hemoglobin. In citrate-agar electrophoresis, *p*H 6.2, the isolated variant moved between hemoglobins F and A, as it did in chromatography on amberlite resin, IRC 50, citrate buffer, *p*H 6.0.

In order to determine which polypeptide chain of hemoglobin F_{Texas} is abnormal, the isolated variant was subjected to the hybridization procedure of Itano and Singer (*3*), in which the unknown hemoglobin is added to one known to be abnormal in either the α- or the β-chains. Both hemoglobins are dissociated (by appropriate acidification) into their component polypeptide chains, and these chains (upon neutralization) recombine at random. A similar hybridization may be achieved with hemoglobin from another species, such as the dog (*4*).

When this procedure was applied to a mixture of hemoglobin F_{Texas} and the variant hemoglobin C (known to be aberrant in the β-chain), no new products were formed (Fig. 1*d*). This result would be expected if the alteration in F_{Texas} was also not in the α-, but rather in the β- or γ-polypeptide chains. When hemoglobin F_{Texas} was hybridized with the α-chain aberrant variant, hemoglobin Stanleyville I (*5*)—identical with G_{Phila} (*6*)—two new species of hemoglobin appeared. One of these corresponded in mobility to hemoglobin A, or to hemoglobin F when the fetal form of G_{Phila} was used for hybridization. The mobility of the other was that which would be expected from the doubly abnormal hybrid molecule $\alpha_2^{G_{Phila}} \gamma_2^{F_{Texas}}$ (Fig. 1, *b* and *d*). Hybridization of F_{Texas} with canine hemoglobin produced two new species of hemoglobin compatible with the structure: $\alpha_2^{canine} \gamma_2^{F_{Texas}}$ and $\alpha_2^A \beta_2^{canine}$, respectively (Fig. 1*c*).

Hemoglobin F_{Texas} was studied in immunodiffusion tests (*7*) with rabbit antiserum prepared by injection of hemoglobin antigens which had been purified by both electrophoretic and chromatographic methods. These antiserums enabled us to differentiate α-, β-, γ-, and δ-polypeptide chains, but not to detect small amino acid differences within these chains. In tests such as that with hemoglobin F antiserum in Fig. 2, hemoglobin F_{Texas} appears to contain α- and γ-chains. It is thus indistinguishable from hemoglobin F, but different from all hemoglobins which do not contain both α- and γ-chains (hemoglobins A, S, C, A_2, and Bart's) (*8*). Similar results were obtained with antiserums which had been appropriately absorbed so that each contained antibody against only one

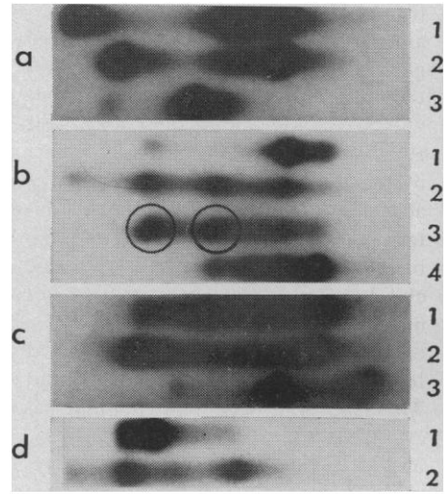

Fig. 1. Starch gel electrophoresis, tris (hydroxymethyl) aminomethane ethylenediaminetetraacetic acid borate buffer, *p*H 8.6, benzidine stain. Anode to right. Separated hemoglobins listed from right to left. *a*, F_{Texas} compared with other types of hemoglobin. Pattern 1, cord blood hemolyzate of WIII. Hemoglobins: A, F, and F_{Texas}. Pattern 2, hemolyzate from infant with hemoglobin C trait. Hemoglobins: A, F, and C. Pattern 3, hemolyzate from patient with sickle cell anemia. Hemoglobins: F, S, and small amount of A_2. *b*, Hybridization of F_{Texas} with $F_{G_{Phila}}$. Pattern 1, cord blood hemolyzate of WIII. Hemoglobins: A, F, and F_{Texas}. Pattern 2, F_{Texas} and $F_{G_{Phila}}$ dissociated and recombined. Hemoglobins: F, $F_{G_{Phila}}$, F_{Texas}, and doubly abnormal hybrid $\alpha_2^{G_{Phila}} \gamma_2^{F_{Texas}}$. Pattern 3, cord blood hemolyzate of M.L. (with G_{Phila} trait), F_{Texas} added. Circled fractions used for hybridization. Hemoglobins: A, F, $F_{G_{Phila}}$, and added F_{Texas}. Pattern 4, hemolyzate of M.L. at 10 weeks. Hemoglobins: A, F, G_{Phila}, and $F_{G_{Phila}}$. *c*, Hybridization of F_{Texas} and canine hemoglobin. Pattern 1, F_{Texas} and canine hemoglobin, dissociated and recombined. Hemoglobins: Canine $\alpha_2^{canine} \gamma_2^{F_{Texas}}$, $\alpha_2^A \beta_2^{canine}$, and F_{Texas}. Pattern 2, mixture of F_{Texas} and canine hemoglobin, control. Hemoglobin: Canine and F_{Texas}. Pattern 3, hemolyzate of patient with sickle cell trait. Hemoglobins: A, S, and small amount of A_2. *d*, Comparison of hybridizations of hemoglobin F_{Texas} with C and F_{Texas} with $F_{G_{Phila}}$. Pattern 1, F_{Texas} and C, dissociated and recombined. Hemoglobins: C and F_{Texas}. Pattern 2, F_{Texas} and $F_{G_{Phila}}$, dissociated and recombined. Hemoglobins: F, $F_{G_{Phila}}$, F_{Texas}, and doubly abnormal hybrid $\alpha_2^{G_{Phila}} \gamma_2^{F_{Texas}}$.

type of polypeptide chain. Hemoglobins F and F_{Texas} reacted strongly (and identically) with antibodies to α- or to γ-chains, and both failed to react with antibodies to β- or δ-chains.

Approximately 20 mg of the abnormal hemoglobin component, isolated as before from the cord blood of WIII, were available for detailed analysis. Of this, 5 mg were hydrolyzed with trypsin, essentially as described by Ingram (*9*). For peptide analysis, the tryptic hydrolyzate (1 mg) of the variant hemoglobin and of normal hemoglobin F were each chromatographed (0.6 × 13 cm column; Spinco type 15A resin, pyridine acetic acid gradient), and the components were detected by the ninhydrin system of an amino acid analyzer (*10*). The peptide pattern of the variant hemoglobin resembled the pattern of hemoglobin F more closely than it did the patterns of hemoglobins A or A_2 (Fig. 3). At least two consistent differences were detected and are indicated by arrows. The first difference is a significant increase in the size of a peak eluted at about 75 ml. The second difference is a new peak eluted at about 168 ml.

The remaining 15 mg of variant hemoglobin were converted to globin, and the cysteinyl residues were reacted with ethylenimine (*11*) to form the *S*-aminoethyl derivative (*10*). The aminoethylated α-chains (AEα) and γ-chains (AEγ) were separated by countercurrent distribution according to the procedure (*12*) for adult hemoglobin. The AEγ-chain was hydrolyzed with trypsin and the resultant peptides were chromatographed on a preparative column (0.9 × 20 cm, Spinco type 15A resin) (*10*). Fractions corresponding to each peak in the chromatogram were collected. Amino acid analyses of about one-half of the material in the peaks observed were made after hydrolysis with 6*N* HCl at 110°C for 22 hours. Because of the small amount of material available for analysis, the amino acid analyzer (Spinco Model 120) was modified to include a long-path photometer which gave a tenfold increase in sensitivity (*13*). Analysis of the zone corresponding to the peak designated by the arrow at 75 ml volume in Fig. 3 indicated that the zone was a mixture of two or more peptides. The exact composition of these peptides could not be determined because of lack of material. The peak designated by the arrow (Fig. 3) at 168 ml volume contained a single peptide. The amino acid composition of this new peptide

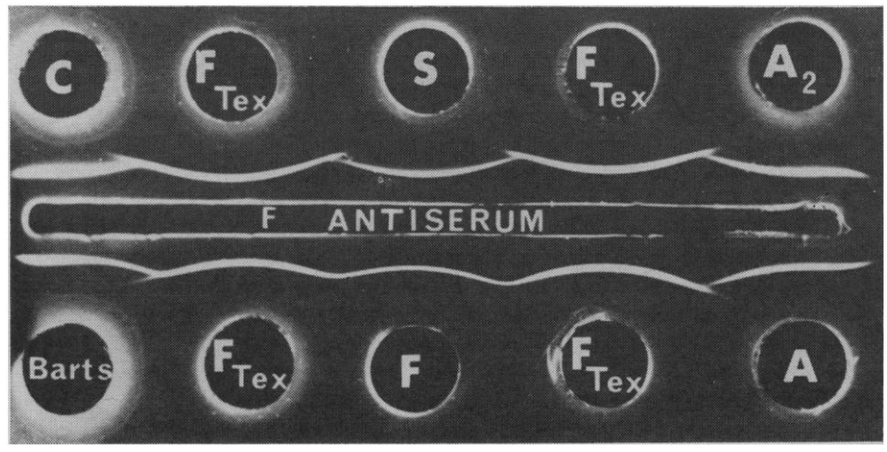

Fig. 2. Immunodiffusion reactions of F_{Texas}, compared with other types of hemoglobin. F antiserum in center trough, various hemoglobins in surrounding wells, as indicated.

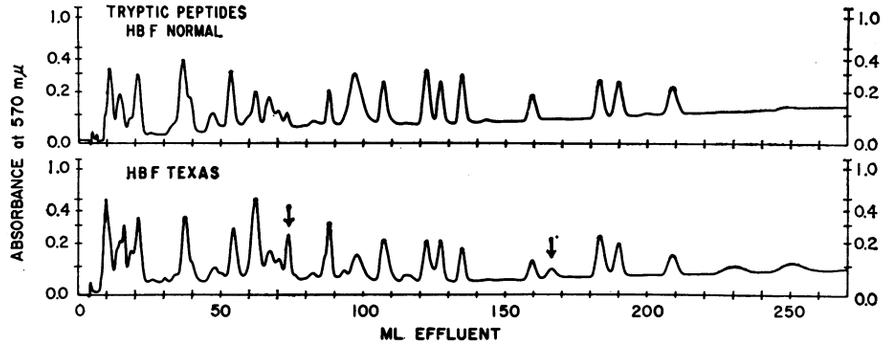

Fig. 3. Automatic peptide patterns of tryptic hydrolyzates of normal hemoglobin F and hemoglobin F_{Texas}.

was similar to that of the normal γ-T-1 peptide except for the presence of one glutamyl residue, rather than two, and two lysyl residues, rather than one. The sequence of the normal γ-T-1 peptide (*14*) is: Gly-His-Phe-Thr-Glu-Glu-Asp-Lys. The sequence postulated for the new abnormal peptide on the basis of the amino acid analysis is: Gly-His-Phe-Thr-(Glu,Lys)Asp-Lys. Apparently, this abnormal peptide resulted from an incomplete tryptic hydrolysis of the abnormal lysine residue. The abnormal peak eluted at about 75 ml (Fig. 3) probably contains one of the extra tryptic peptides which result from complete tryptic hydrolysis of the abnormal γ-T-1 peptide. Because of the limited amount of abnormal hemoglobin available, it has not yet been possible to determine whether it is the fifth or the sixth glutamyl residue which has been substituted by the lysyl residue.

These studies on the structure of hemoglobin F_{Texas} indicate that it is composed of normal α-chains and abnormal γ-chains. At least one amino acid substitution, a lysyl residue for either the fifth or sixth glutamyl residue, appears in this abnormal γ-chain. This amino acid substitution—a lysyl for a glutamyl—is known to occur in genetic changes of the β-chain of hemoglobin A, such as in hemoglobin C and hemoglobin E (*15*). Such a substitution is consistent with the relative electrophoretic mobilities, since hemoglobin F_{Texas} is slower than hemoglobin C by about as much as hemoglobin F is slower than hemoglobin A.

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References and Notes

1. R. G. Schneider, F. Arat, M. E. Haggard, *Nature* **202**, 1364 (1964).
2. We thank H. Moreland and J. C. Sharp, Jr., for blood samples and genetic data.
3. H. A. Itano and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.* **44**, 522 (1958).
4. E. R. Huehns, E. M. Shooter, G. H. Beaven, *J. Mol. Biol.* **4**, 323 (1962).
5. P. Dherte, J. Vandepitte, J. A. M. Ager, H. Lehmann, *Brit. Med. J.* **2**, 142 (1959); R. G. Schneider and M. E. Haggard, *ibid.*, p. 285.

6. C. Baglioni and V. M. Ingram, *Biochim. Biophys. Acta* **48**, 253 (1961); B. H. Bowman and R. G. Schneider, in preparation.
7. R. G. Schneider and F. Arat, *Brit. J. Haematol.* **10**, 15 (1964).
8. We thank K. Suzuki for the immunodiffusion tests and photography.
9. V. M. Ingram, *Biochim. Biophys. Acta* **28**, 546 (1958).
10. R. T. Jones, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 297 (1964).
11. M. A. Raftery and R. D. Cole, *Biochem. Biophys. Res. Commun.* **10**, 467 (1963).
12. R. J. Hill, W. Konigsberg, G. Guidotti, L. C. Craig, *J. Biol. Chem.* **237**, 1549 (1962).
13. R. T. Jones and G. Weiss, *Anal. Biochem.* **9**, 377 (1964).
14. W. A. Schroeder, J. R. Shelton, J. B. Shelton, J. Cormick, R. T. Jones, *Biochemistry* **2**, 992 (1963).
15. J. A. Hunt and V. M. Ingram, in *Biochemistry of Human Genetics* (Little, Brown, Boston, 1959), pp. 114–143.
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Histone Regulation of Lactic Dehydrogenase in Embryonic Chick Brain Tissue

Abstract. *Histone, when added at low concentrations to cultures of embryonic chick brain tissue, causes an inductive response in lactic dehydrogenase activity, whereas at higher concentrations of histone the response is repressive. This control is shown to operate by altering protein synthesis. Thus, histones exercise a primary regulatory function in the chick. Unlike lactic dehydrogenase, acetylcholine esterase is stable in this system and is not affected by histones or inhibitors of protein synthesis.*

Evidence of a cytological and biochemical nature suggests that histones take part in the regulation of genetic activity (*1*). The biochemical evidence shows that histones can inhibit the priming activity of DNA in directing RNA synthesis and that this inhibition is reversed when the histones are removed (*2*, *3*). Since polycationic molecules, such as polylysine, have a similar effect (*3*), the question arises whether histones have any specificity in inhibiting different regions of the DNA in a chromosome. Evidence for such specificity of histones on the chromatin from different tissues of the pea embryo has been presented by Bonner *et al.* (*4*). Since developmental processes involve both inductive and repressive responses, the position of histones as primary genetic regulators would be strengthened if it could be shown that under certain conditions they can bring about an induction or derepression of particular genes, while repressing these same genes under different conditions. We now present evidence that histones can indeed perform such an antithetic control function in the case of lactic dehydrogenase synthesis in embryonic chick brain tissue.

Small pieces (about 1 mm^3) of 14-day embryonic chick brain tissue (cerebral hemispheres) were cultured in Charity-Waymouth medium at 37°C in a CO_2-incubator on a gyratory shaker. Homogenates of washed tissue were assayed for lactic dehydrogenase (LDH) activity (*5*), and for acetylcholinesterase (AChE) (*6*). Protein was determined by the bromosulfalein method (*7*), and specific activities are expressed as micromoles of product (diphosphopyridine nucleotide or thiocholine, respectively) produced per hour per milligram of protein. Histone was prepared from the liver of 1-year-old White Leghorn chickens (*8*). The amino acid analysis showed a typical histone composition with a molar ratio of lysine to arginine of 1.97. For the measurement of amino acid–incorporating activity in the tissue ^{14}C-leucine was used. Radioactivity incorporated into protein, precipitated by 5 percent trichloroacetic acid from a 1*N* NaOH digest of the tissue, was measured on a Nuclear-Chicago counter.

The amount of lactic dehydrogenase in the control cultures was kept relatively constant by continuous synthesis and degradation (Fig. 1). Each point in this and subsequent figures represents an average value for three or more duplicate experiments. Actinomycin D (60 μg/ml) and puromycin (100 μg/ml) caused a decrease in enzyme activity which indicates a half-life for the enzyme of about 50 to 60 hours. The decrease of LDH activity in response to 100 μg of poly-L-lysine per milliliter (Fig. 1) is about the same as that caused by actinomycin. This repression by polylysine was not due to a direct inhibition of the enzyme, for no inhibitory effects were observed at the concentrations used.

In contrast to the effect (Fig. 1) of antibiotics and polylysine, which caused a decrease in the enzyme activity at all concentrations used (10 to 200 μg/ml), increasing the concentration of histone caused a stimulation of LDH synthesis which was maximum at 100 μg of histone per milliliter, but at a concentration of 400 μg/ml became

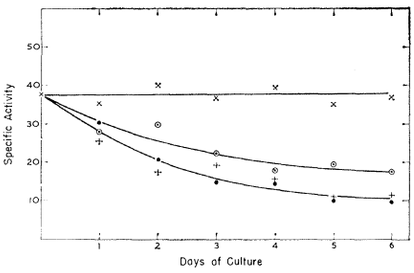

Fig. 1. Lactic dehydrogenase activity in cultures exposed to 60 μg/ml of actinomycin D (● ——— ●), 100 μg/ml of puromycin (⊙ ——— ⊙), and 100 μg/ml of poly-L-lysine (+ ——— +), compared with the control (× ——— ×).

a repression comparable to that caused by antibiotics or polylysine. Commercial calf thymus histone caused the same pattern of response, although the magnitude was slightly different (Fig. 2, upper curve). As with polylysine, the effect of 400 μg of histone per milliliter was not due to a direct inhibition of the enzyme, since there was no such inhibition in incubated homogenates until relatively high concentrations of histone were reached (5 to 10 mg/ml). A neutral protein such as bovine serum albumin at concentrations from 10 to 1000 μg/ml had no effect upon LDH activity in the cultures.

Evidence that the observed increases and decreases of LDH activity were not due to the accumulation of dissociable activators or inhibitors was provided by the fact that mixing and dilution experiments on homogenates

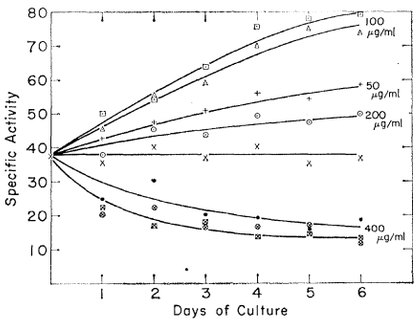

Fig. 2. Lactic dehydrogenase activity in cultures exposed to the concentrations of adult chicken liver histone shown in curves △ ——— △, + ——— +, ⊙ ——— ⊙, and ● ——— ● compared with the control (× ——— ×). Upper curve (⊡ ——— ⊡) is the response to 100 μg/ml of commercial calf thymus histone. Lower curve: 100 μg/ml of chicken liver histone plus 60 μg/ml of actinomycin D (⊠ ——— ⊠) and 100 μg/ml of chicken liver histone plus 100 μg/ml of puromycin (⊗ ——— ⊗).