tinate guinea pig erythrocytes do not agglutinate washed blood platelets from the same animals. Similarly, fluorescein-conjugated antiserums react strongly with intact erythrocytes and their ghosts but do not show any labeling with platelets or leukocytes.

Our studies show that approximately 20 percent of the protein bound to washed, erythrocyte ghost membranes can be readily solubilized and obtained free of other membrane elements simply by dialysis against ATP and 2-mercaptoethanol. This protein is different antigenically from the serum proteins and cytoplasmic proteins released from erythrocytes during hemolysis. This protein also has the remarkable property of polymerizing in the presence of divalent cations to form coiled filaments similar in the ultrastructure to the actin filaments of muscle. However, the physical, chemical, and antigenic properties of the erythrocyte membrane protein are quite different from those of muscle actin extracted from the same animals (*6*).

It is not yet clear where and in what form this protein is bound to the intact erythrocyte membrane. In that filamentous material is lost from the inner surface of the ghost membrane as a result of the extraction procedure used, this is probably a source of the soluble protein. However, the agglutination of intact red blood cells by antiserums prepared against the extracted protein suggests that antigenic material is also accessible on the outer surface of the membrane. Whether the same antigenic material is present on both the inner and outer surfaces of the membrane is unknown; it is possible that the antiserums contain antibodies to a minor protein component which are not detectable by the double-diffusion assay.

Antibodies directed against the extracted protein react only with red blood cells and their ghost membranes; other blood cells from the same animals are nonreactive. This specific reaction against erythrocyte membranes suggests either that they have a unique membrane protein or that this type of protein—if more widespread—is antigenically specific for different cell types. The distribution and cross reactivity of membrane proteins of other cell types remain to be determined.

The functional role of the protein in the intact erythrocyte membrane cannot yet be determined. Because the ghosts break up into small fragments under the conditions in which the protein is solubilized, it seems likely that this protein is somehow involved in maintaining the structure of the ghost membrane. Because this protein appears to be a new molecular species and is extractable from erythrocyte ghost membranes, we suggest that it be called Spectrin (Latin: derived from ghosts).

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References and Notes

1. V. T. Marchesi and G. E. Palade, *Proc. Nat. Acad. Sci. U.S.* **58**, 991 (1967).
2. ———, *J. Cell Biol.* **35**, 385 (1967).
3. Samples of washed membranes ranging from 0.5 to 1.0 g dry weight were dialyzed against 5 liters of a solution of ATP and mercaptoethanol for 24 hours at 4°C. The most recent preparations were subjected to a second 5-liter dialysis under the same conditions. This method was devised with the idea that this loosely bound protein component had solubility properties similar to those of muscle actin. This protein can also be solubilized from membranes treated with ethylenediaminetetraacetate in other media of low ionic strength, such as distilled water. It is not clear whether ATP and 2-mercaptoethanol stabilize the protein once it is free of the membrane.
4. Protein was measured by the Lowry procedure [O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin as the standard.
5. Samples of extracted protein were analyzed for sialic acid by the thiobarbituric acid method [L. Warren, *J. Biol. Chem.* **234**, 1971 (1959)]; neutral sugars, by paper chromatography; amino sugars, by amino acid analyzer; and sterols and phospholipids, by gas chromatography. We thank Drs. V. Ginsburg and E. Korn for help in these analyses.
6. E. Steers, Jr., and V. T. Marchesi, in preparation.
7. We thank Dr. G. E. Palade for criticism of the manuscript.

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Hemoglobin Hijiyama: A New Fast-Moving Hemoglobin in a Japanese Family

Abstract. *A variant of hemoglobin A, named Hb Hijiyama, found in two generations of a Japanese family living in Hiroshima, Japan, has a higher anodal electrophoretic mobility than hemoglobin A; a gain of two negative charges per molecule is indicated. Fingerprinting and amino acid analysis showed the biochemical anomaly to be in the beta chain at residue 120, where lysine is replaced by glutamic acid. In the heterozygote carriers of the abnormal hemoglobin there is no apparent association with clinical or hematologic abnormalities.*

During a systematic survey for hemoglobinopathies among visitors to the outpatient clinic of the Atomic Bomb Casualty Commission (*1*) in Hiroshima, Japan, between June 1963 and November 1965, 9262 blood samples were screened for abnormal hemoglobins by agar-gel electrophoresis (*2*). Two abnormal hemoglobins, both β-chain anomalies, were discovered. We now describe identification of the anomaly of one of these hemoglobins, which has been named Hb Hijiyama.

The index case was a 53-year-old Japanese housewife in good health; her red-blood-cell indices, morphology, and fragility were within normal limits. Of four children, only her eldest son, a 23-year-old university student in excellent health, had the abnormal hemoglobin; his red-cell indices and morphology were normal. The proband's parents were deceased. Blood samples from three paternal uncles and an aunt living in Hawaii were normal (*3*).

Agar-gel electrophoresis at *p*H 8.6 of hemolysates from the proband or her son produced two bands: one was in the position of Hb A; the other migrated more rapidly toward the anode. For estimation of the magnitude of the negative charge, Hb A and Hb Hofu ($\alpha_2\beta_2^{126\ Glu}$) (*4*) were compared with Hb Hijiyama on starch-gel electrophoresis (*5*). The distance between Hb A and Hb Hijiyama was approximately

Table 1. Comparison of amino acid contents of aminoethylated peptides from the abnormal spot of Hb Hijiyama with those of βTp-12B and βTp-13 of Hb A. The values found for Hb Hijiyama were obtained by automatic amino acid analysis (see text); those expected for Hb A are given by Jones (*10*) and Braunitzer *et al.* (*14*). Abbreviations: Lys, lysine; His, histidine; Thr, threonine; Glu, glutamine or glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine.

Amino acid	Residues (No.)		
	Hb Hijiyama abnormal peptide		Hb A βTp-12B + βTp-13, expected
	Found	Integral	
Lys	0.87	1	2
His	2.03	2	2
Thr	1.04	1	1
Glu	3.58	4	3
Pro	1.95	2	2
Gly	1.19	1	1
Ala	2.80	3	3
Val	1.77	2	2
Leu	0.96	1	1
Tyr	.86	1	1
Phe	2.27	2	2

twice that between Hb A and Hb Hofu, the latter also being a fast-moving hemoglobin (Fig. 1A).

The abnormal hemoglobin was readily separated from Hb A by starch-block electrophoresis (*6*). Hemoglobin Hijiyama constituted 58.4 percent of the proband's hemolysate; the proportion of Hb A_2 was normal, as were alkaline resistance and solubility (*7*).

The α- and β-chains were separated from one another by carboxymethylcellulose column chromatography of hemolysates treated with *p*-chlormercuribenzoate, or by urea-dissociation electrophoresis (*8*). From the column, β-Hijiyama was eluted more rapidly than β-A. On urea dissociation, β-chain migration toward the anode was in the following order: Hijiyama, Hofu, A (Fig. 1B).

In addition to the evidence from urea-dissociation electrophoresis, hybridization tests (*9*) of Hb Hijiyama with canine hemoglobin demonstrated clearly that the anomaly of Hb Hijiyama was in the β-chain. Thus ($\alpha_2^{can}\beta_2^{Hijiyama}$) migrated more rapidly toward the anode than ($\alpha_2^{can}\beta_2^{A}$), whereas the migration rates of ($\alpha_2^{Hijiyama}\beta_2^{can}$) and ($\alpha_2^{A}\beta_2^{can}$) were identical.

To further characterize the anomaly, β-chain globin and the aminoethylated preparation were subjected to fingerprinting (*10*). Hemoglobin Hijiyama globin fingerprints appeared to be almost identical with those of Hb A; no peptide spots seemed to be missing, nor were any abnormal spots apparent. However, specific staining of the fingerprints for tyrosine (*11*) showed that the spot normally formed by an overlap of βTp-1 and βTp-13 (*12*), although positive in Hb A, was negative in Hb Hijiyama; specific staining for histidine, on the other hand, showed no differences between Hb A and Hb Hijiyama. The βTp-1 contains histidine but no tyrosine, while βTp-13 has tyrosine but no histidine; here is presumptive evidence that, in the globin print of Hb Hijiyama, βTp-13 is either abnormal or absent.

In fingerprints of the aminoethylated β-chain globin of Hb Hijiyama, βTp-12B and βTp-13, normally present in Hb A, were absent, and a new spot appeared directly over Tp-10,11 (Fig. 2). Amino acid analysis (*13*) of this unusual spot proved it to have the same number of amino acid residues as has βTp-12B + βTp-13 of Hb A, but it had one less lysine and one more glutamic acid or glutamine residue (Table 1). Thus the abnormal peptide of β-Hijiyama corresponds to the amino acid residues from β113 through β132 in β-A (*14*).

The substitution could be at either β120 or β132, the two positions occupied by lysine in the normal β-chain

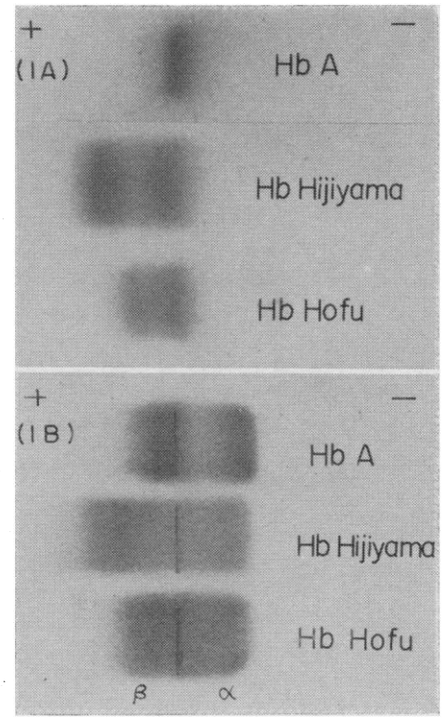

Fig. 1. (A) Starch-gel electrophoresis of Hb A, Hb Hijiyama, and Hb Hofu; *p*H 8.6. The distance between Hb A and Hb Hijiyama is approximately twice that between Hb A and Hb Hofu. (B) Urea-dissociation electrophoreses of the α- and β-chains are clearly evident and correspond to the pattern exhibited by the parent hemoglobins.

in this region; the evidence indicates that the substitution is at β120. The C-terminal lysine at β120 is normally subject to tryptic hydrolysis, but in the abnormal chain, in the absence of lysine, hydrolysis at this point, resulting in a combined abnormal peptide corresponding to βTp-12B and βTp-13, would not occur. A further consequence of this substitution would be that the acid-insoluble core of β-Hijiyama would extend from β83 to β132, in contrast to the core of β-A, which includes residues from β83 to β120. Such an assumption is borne out by the fact that, in ordinary fingerprints of non-aminoethylated Hb Hijiyama, although βTp-13 was missing, an abnormal spot did not appear, presumably because it was combined with the insoluble core, which does not appear in this method of fingerprinting.

Evidence against substitution at β132, on the other hand, is the fact that βTp-14, the tryptic peptide adjacent to β132 (and βTp-13), was in its expected position in the fingerprint of β-Hijiyama, which fact may be taken to indicate that tryptic digestion had hydrolyzed the C-terminal of lysine at β132.

The replacement for lysine at β120 may be either glutamine or glutamic acid, since methods used by us do not distinguish between them. Hemoglobin Hofu, with a known substitution of a negative glutamic acid for a neutral valine residue, has two more negative charges per molecule than has Hb A.

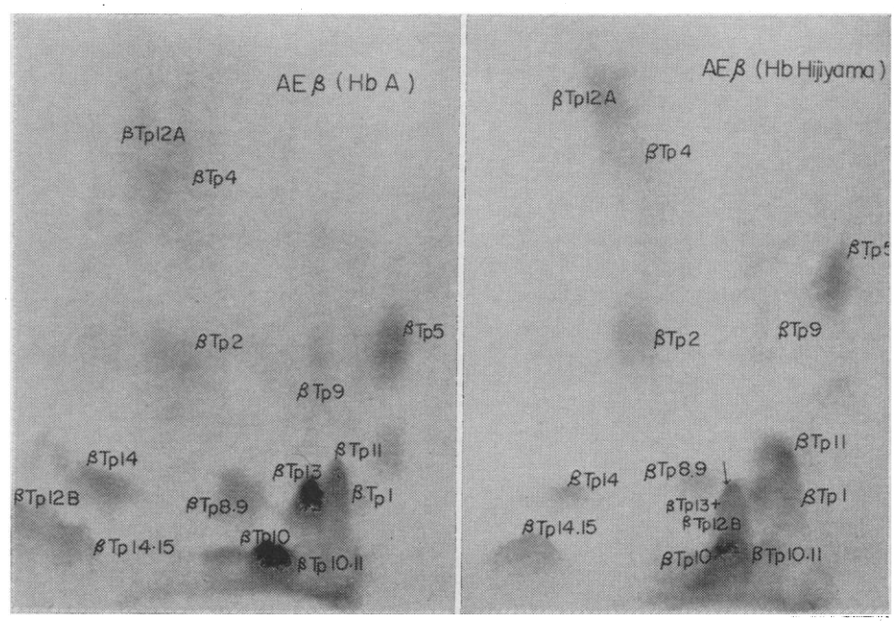

Fig. 2. Fingerprints of the aminoethylated chains of Hb Hijiyama and Hb A. (Left) Normal β A; (right) β Hijiyama. In the latter, an abnormal spot (arrow) appears immediately above βTp-10, 11, and βTp-12B and βTp-13 are absent.

If the substitution in Hb Hijiyama were a neutral glutamine for a positive lysine residue, resulting also in two more negative charges per molecule, one would expect the two abnormal hemoglobins to have approximately the same electrophoretic mobility; in fact, Hb Hijiyama moves at about twice the speed of Hb Hofu. Thus the enhanced electrophoretic mobility of Hb Hijiyama over both Hb A and Hb Hofu can be explained on the basis of a substituent that contributes two negative charges per chain or four per molecule. This condition is fulfilled only if a negative glutamic acid replaces a positive lysine residue (*15*).

At least two other abnormal hemoglobins have been reported in which glutamic acid is substituted for lysine, and in which anodal migration at *p*H 8.6 is relatively rapid (*15*). In Hb I, the substitution is in the α-chain at the 16th residue; in Hb Nβ, in the β-chain at the 95th residue. Therefore Hb Hijiyama is yet another example to be added to the expanding list of abnormal hemoglobins. According to present convention, Hb Hijiyama is designated $\alpha_2\beta_2^{120\ \mathrm{Glu}}$.

The abnormality in our two cases seems to have no clinical effect, but individuals homozygous for the trait would offer convincing evidence in this respect. In view of the rarity of the gene, it is extremely unlikely that such individuals will be found, even in Japan with its somewhat elevated frequency of consanguinity.

The hemolysate of the proband contains 58 percent of the abnormal hemoglobins and stands at the top of the list of β-chain variants with respect to the relative proportion in the heterozygote's blood (*4*). The significance of this observation is obscure; it may indicate that Hb Hijiyama has a slightly greater affinity for heme than has Hb A, or that the protein-synthesis mechanisms for Hb Hijiyama are slightly more efficient than those for Hb A (*16*). We cannot yet choose between these and alternative explanations.

The trait is apparently mediated by a gene at the β-chain locus, allelic to the normal gene. Beale and Lehmann (*15*) noted that, according to the generally accepted concepts of the genetic code (*17*), single-base mutations in the triplet codons for amino acids in proteins account for all currently known hemoglobin variants. Hemoglobin Hijiyama is no exception. Thus, in messenger RNA, in which the code for lysine is either AAA or AAG, substitution of G in the first position produces GAA or GAG, either of which specifies glutamic acid (A, adenine; G, guanine).

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References and Notes

1. The Commission is a cooperative research agency of the U.S. NAS–NRC and the Japanese National Institute of Health, Ministry of Health and Welfare; funds are provided by the U.S. AEC, the Japanese National Institute of Health, and the U.S. PHS. Individuals from whom blood was drawn are voluntary participants in an adult health study and an *in utero* study of the long-term aftereffects of exposure to ionizing radiation. Methodology of these studies is described by: J. W. Hollingsworth, G. W. Beebe, M. Ishida, A. B. Brill, in *The Use of Vital and Health Statistics for Genetic and Radiation Studies* (United Nations, New York, 1962), pp. 77–100; J. W. Wood, R. J. Keehn, S. Kawamoto, K. G. Johnson, *Amer. J. Public Health*, **57**, 1374 (1967).
2. S. Shibata and I. Iuchi, *Acta Haematol. Japon.* **24**, 51 (1961).
3. We thank Grant Stemmermann, Kuakini Hospital, Honolulu, Hawaii, for obtaining these four blood samples.
4. S. Shibata, I. Iuchi, T. Miyaji, *Acta Haematol. Japon.* **29**, 115 (1966).
5. O. Smithies, *Biochem. J.* **61**, 629 (1955).
6. S. Ueda, *Japan. J. Clin. Hematol.* **3**, 26 (1962).
7. K. Singer, A. I. Chernoff, L. Singer, *Blood* **6**, 413 (1951); C. A. J. Goldberg, *Clin. Chem.* **4**, 146 (1958).
8. E. Bucci and C. Fronticelli, *J. Biol. Chem.* **240**, 551 (1962); T. Take, *J. Biochem.* **49**, 206 (1961).
9. S. Shibata, I. Iuchi, S. Ueda, T. Miyaji, I. Takeda, *Acta Haematol. Japon.* **25**, 675 (1962).
10. M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.* **13**, 469 (1930); V. M. Ingram, *Biochim. Biophys. Acta* **28**, 539 (1958); C. Baglioni, *ibid.* **48**, 392 (1961); R. T. Jones, *Symp. Quant. Biol.* **29**, 297 (1964).
11. R. Archer and C. Crocker, in *Techniques in Protein Chemistry*, J. L. Bailey, Ed. (Elsevier, Amsterdam, 1962), pp. 21–2.
12. βTp, β tryptic peptide.
13. Fingerprints were lightly stained with 0.02 percent ninhydrin, the spots were cut out, and the peptides were eluted with 6*N* HCl; 22-hour hydrolysis at 105°C. Amino acid analysis was performed automatically with a Yanagimoto Automatic Amino Acid Analyzer, model LC-5, by the methods of S. Moore, D. H. Spackman, W. H. Stein, *Anal. Chem.* **30**, 1185 (1958); D. H. Spackman, W. H. Stein, S. Moore, *ibid.*, p. 1190.
14. G. Braunitzer, K. Hilse, V. Rudloff, N. Hilschmann, *Advan. Protein Chem.* **19**, 1 (1964).
15. D. Beale and H. Lehmann, *Nature* **207**, 259 (1965); R. B. Schneider, J. B. Alpern, D. Beale, H. Lehmann, *J. Lab. Clin. Med.* **68**, 940 (1966). Hb Nβ is also known as Hb N Baltimore or Hb N Memphis.
16. H. Lehmann and R. G. Huntsman, *Man's Hemoglobins* (North-Holland, Amsterdam, 1966), pp. 144–7.
17. M. Nirenberg, P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, C. O'Neal, *Proc. Nat. Acad. Sci. U. S.* **53**, 1161 (1965).
18. Aided by PHS research grant GM 09469-05. This report was presented in part before the 10th Annual Meeting of the Japanese Society of Human Genetics, Kumamoto City, Japan, 9–10 October 1965.

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Crystal and Molecular Structure of Adenosine 3′,5′-Cyclic Phosphate

Abstract. *The structure of adenosine 3′,5′-cyclic phosphate has been determined by single-crystal x-ray diffraction. The two molecules in the asymmetric unit show different conformation about the glycosidic bond, while other structural details are essentially the same. The furanose rings are puckered with the C(4′) atom out of the best four-atom plane. The bond lengths and angles appear to be normal.*

Adenosine 3′,5′-cyclic phosphate (cyclic 3′,5′-AMP) has a role in many metabolic processes and can influence the activity of certain enzymes. For example, in tissues it is a factor in the conversion of inactive glycogen phosphorylase to the active form. Furthermore, cyclic 3′,5′-AMP stimulates the production or secretion of steroids and other hormones in some tissues.

Inasmuch as molecular configuration is probably related to biological activity, we have studied the configuration of the molecule and compared its conformational aspects with those of the related compounds, 3′-AMP (*1*) and 5′-AMP (*2*).

Crystals of cyclic 3′,5′-AMP were grown as fine needles by diffusion of xylene into an aqueous solution at *p*H 2. A small crystal of dimensions 0.3 by 0.1 by 0.03 mm was mounted for collecting photographic data. The orthorhombic unit cell has the following approximate dimensions: $a = 10.63$ Å, $b = 35.75$ Å, $c = 7.80$ Å. The only systematically absent reflections were $h00$, $0k0$ and $00l$ for odd h, k and l respectively. The space group was assumed, therefore, to be $P2_12_12_1$. Density of the crystals was measured by flotation in a solution of dichloromethane and tetrabromoethane and found to be 1.75 g cm^{-3}. This suggests that there are two molecules of cyclic 3′,5′-AMP per asymmetric unit, along with the equiv-