A Case of a Newborn Infant with Hb M Iwate

Reiko Kumagai, Hisae Niitsu, Ikuyo Katsura, Nori Nakayashiki and Syusaku Katsura

Department of Legal Medicine, School of Medicine, Iwate Medical University, Morioka 020

Kumagai, R., Niitsu, H., Katsura, I., Nakayashiki, N. and Katsura, S. A Case of a Newborn Infant with Hb M Iwate. Tohoku J. exp. Med., 1986, 150 (3), 337-343 — Hemoglobin of a newborn infant who was suspected to have Hb M Iwate was examined. The infant hemolysate was separated into five fractions by column chromatography on Amberlite CG-50, and two of these fractions showed absorption spectra corresponding with that of Hb M Iwate. Five bands were found after the isoelectric focusing of the hemolysate, and two of these bands were brown. The two Hb M fractions obtained by column chromatography was focused to the positions of the brown bands. One of these Hbs M corresponded with Hb M Iwate (α^2_B) from an adult carrier of this trait, but the other was not found in adult hemolysates. The latter species of Hb M was shown to be composed of the abnormal α chain and the normal γ chain (α^γ y_2), and was assumed to be specific for infants. A quantitative estimation of the hemoglobins in the infant hemolysate showed that there was no difference between the relative quantities of the fetal and adult forms of Hb M Iwate.

The patients with Hb M Iwate (α^27His-Tyr^β_2) are locally found among peoples in some region in Iwate Prefecture, and show a cyanotic face. Previously, we reported a case of blood transfusion to a patient with Hb M Iwate and cervical cancer (Katsura et al. 1983). A great grandson of the patient showed a cyanotic face at the birth and was suspected of methemoglobinemia.

Hb M is divided into two types, one of which consists of abnormal α chains and normal β chains (Hb M Iwate and Hb M Boston), and the other has normal α chains and abnormal β chains (Hb M Saskatoon, Hb M Hyde Park and Hb M Milwaukee-1). Newborns with the α-chain M hemoglobins show a cyanotic face at the birth. In newborns with the β-chain M hemoglobins, on the other hand, cyanosis does not appears until the γ chain has been replaced by β chain several months after delivery.

Nakatsuji et al. (1983) studied a number of abnormal hemoglobins of more than 50 newborn infants, all carrying hemoglobin variants, from structural and genetic aspects. However, quantitative analyses of Hb M in newborns have

Received March 18, 1986; accepted for publication October 27, 1986.
scarcely been reported. We present here our analysis of the hemoglobins of a newborn infant carrying Hb M Iwate.

**MATERIALS AND METHODS**

*Family history.* A full-term infant weighing 2,280 g was born and seen to be cyanotic at the birth. Skin of his mother seems cyanotic and brown colored. His family history is shown in Fig. 1, and Hb M Iwate was detected in the blood of his great grandmother (Katsura et al. 1983).

*Hemolysate.* Heparinized blood obtained from the newborn infant was washed with normal saline and hemolyzed by adding distilled water. Hemolysate without membrane was obtained by centrifugation at 12,000 rpm for 30 min, and stored at -80°C till used.

*Column chromatography.* Column chromatography of the hemolysate was carried out according to the method of Hayashi et al. (1967). A half ml of the hemolysate dialysed against 0.05 M potassium phosphate buffer (pH 7.0) was applied on a column (1 × 60 cm) of Amberlite CG-50 equilibrated with the same potassium phosphate buffer. The column was first eluted with the equilibration buffer and then with 0.5 M NaCl.

*Thin-layer isoelectric focusing on polyacrylamide gel.* Polyacrylamide gel (PAG) was made as follows: For the gel of 11 × 13 × 0.1 cm in size, 2.5 ml of 29.1% acrylamide, 2.5 ml of 0.9% N,N'-methylenebisacrylamide, 1.75 ml of 87% glycerol and 0.75 ml of carrier ampholite (a mixture of equal quantities of Ampholines of pH 6-8 and pH 7-9) were mixed and made up to 15 ml with distilled water. The solution was degassed for 5 min and 0.4 ml of 1% ammonium persulphate was then added. As electrode solution, 1.0 M phosphoric acid was used at the anode and 1.0 M sodium hydroxide at the cathode. Hemolysate containing 0.05% potassium cyanide was applied to the cathodal edge of the PAG plate. Electrophoresis was performed for 3 hr with a maximum voltage of 1,250 V and a maximum ampere of 20 mA. The plate was stained with Coomassie Brilliant Blue R-250 after fixation with sulfosalicylic acid and trichloroacetic acid.

*Hemoglobin quantitation.* Hemoglobin was quantitatively measured by the methods of de Duve (1948). Each hemoglobin band fractionated by the thin-layer isoelectric focusing was cut out and completely extracted with 1 ml of distilled water in a test tube. The extraction was carried out overnight with adequate shaking. To the hemoglobin band extract, 1 ml of pyridine-alkali reagent (100 ml of pyridine and 30 ml of 1 N sodium hydroxide were mixed and made up to 300 ml with distilled water) was added. After several minutes, sodium hydrosulfite was added and then absorbance at 556 nm of the

![Fig. 1. The genealogical table of the infant baby. Individuals indicated by closed symbols showed methemoglobinemic faces. →, propositus.](image-url)
hemoglobin band extract was immediately determined. Relative quantity of each of the hemoglobin bands was estimated.

**Analysis of hemoglobin chains.** Hemoglobin chains were separated by the PAG electrophoresis of Alter et al. (1980). Hemoglobin solutions at about 1 mg/ml were prepared by adding sample buffer (a mixture of 5 ml of 8 M urea, 0.5 ml of acetic acid and 0.5 ml of 2-mercaptoethanol) to hemolysates and isolated hemoglobin preparations. Ten μl each of the hemoglobin solutions was applied in sample wells of a PAG plate (13.7 × 11.0 × 0.2 cm in size) and electrophoresed at 8.5 mA for 20 hr.

**RESULTS**

**Chromatogram and absorption spectrum of the infant hemolysate**

The infant hemolysate was separated on Amberlite CG-50 column into three fractions (fractions a, b and c) by eluting with 0.05 M potassium phosphate buffer, and two fractions (fractions d and e) were developed with 0.5 M NaCl (Fig. 2). Fraction a was red, and fractions b and e were chocolate brown. Absorption spectra of fractions a and c were the same as that of normal oxyhemoglobin. The absorption maxima of fractions b and e were found at 540 and 575 nm (Fig. 3), and those of methemoglobin converted from these two fractions were 485 nm and 585 nm (Fig. 4). These spectra agreed with those of Hb M Iwate (Shibata et al. 1967). Absorption spectrum of fraction d corresponded with that of normal methemoglobin.

**Patterns of isoelectric focusing and quantitation of hemoglobin bands**

Five bands, three anodic red bands and two cathodc brown bands, were clearly found in the infant hemolysate (Fig. 5). The five bands were called bands

![Diagram](image)

Fig. 2. Chromatogram of the infant hemolysate on Amberlite CG-50. Three fractions (a, b and c) were eluted with 0.05 M potassium phosphate buffer (pH 7.0) and two fractions (d and e) with 0.5 M NaCl.
1, 2, 3, 4 and 5 in the order from anode to cathode. Bands 2, 3 and 4 correspondingly migrated with Hb A0, Hb Fδ and adult Hb M Iwate used as controls, respectively. No hemoglobin with the same migration as band 5 was found in normal cord, normal adult or Hb M Iwate adult hemolysates.
Fractions $a$, $b$, $c$ and $e$ obtained by the column chromatography of the infant hemolysate migrated to the positions of bands 3, 5, 2 and 4 of the isoelectric focusing, respectively. Fraction $d$ showed the same pattern as methemoglobin $A_o$ in the isoelectric focusing without potassium cyanide.

Band 1, which was assumed to be Hb $F_1$ (Drysdale et al. 1971), composed 5.5% of the total hemoglobin of the infant. Bands 2, 3, 4 and 5 constituted 18.5, 53.8, 6.6 and 15.5% of the total hemoglobin, respectively.

**Hemoglobin chains**

Electrophoretic analysis for hemoglobin chain revealed that fraction $b$ of the
Amberlite CG-50 column chromatography consisted of three bands, one of which moved close behind the normal α chain and the others comigrated with normal γ chains (Fig. 6). The band following the normal α chain showed the same migration as did the abnormal α chain of Hb M Iwate obtained from the hemolysate of an adult woman carrying this trait.

**DISCUSSION**

Hb M Iwate is eluted subsequently to application of 0.5 M NaCl when analyzed by Amberlite CG-50 column chromatography of Hayashi et al. (1967). In the present study, 0.5 M NaCl elutes fraction e of the hemolysate from the infant with Hb M Iwate corresponded to Hb M Iwate both chromatographically and spectrophotometrically. Fraction b, which was eluted before the application of 0.5 M NaCl, also showed an absorption spectrum very similar to that reported for Hb M Iwate but clearly different from that for Hb M Boston (Shibata et al. 1967) (Figs. 3 and 4). Fraction b migrated to the position corresponding to band 5 in the isoelectric focusing; band 5 was found only in the hemolysate from the infant with Hb M Iwate. It is thus suggested that the hemoglobin of fraction b may be a type of Hb M Iwate specific for infants.

Chain analysis of fraction b indicated that the fraction was composed of normal γ chains and abnormal α chains which had the same migration with that of the abnormal α chain of Hb M Iwate. The electrophoretic pattern of chains of Hb M Iwate in this report was consistent with that of urea-dissociation paper electrophoresis reported by Shibata et al. (1961). Therefore, the hemoglobin of fraction b could be regarded as fetal Hb M Iwate (αβγγ).

Nakatsuji et al. (1983) reported that the levels of αX containing hemoglobin types in newborns heterozygous for α chain variants varied between 16 and 29% with some exceptions. Katsura et al. (1983) reported that a relative amount of Hb M Iwate was 24% in the blood of a patient with cervical cancer. In the present study, the relative amount of fetal Hb M Iwate (αβγγ) was estimated to be 22.4% of the total fetal hemoglobin (Hb Fα and fetal Hb M), and that of adult Hb M Iwate (αββ) to be 26.3% of the total adult hemoglobin (Hb Aα and adult Hb M). Although no discrete band corresponding to the αM-containing counterpart (αβγγN-acetylγγN-acetyl) of Hb Fα (αβγγN-acetyl) was separated from band 4 after the isoelectric focusing, it is probable that a small amount of this species of fetal Hb M Iwate coexisted with adult Hb M Iwate in band 4 (Drysdale et al. 1971). If the ratio of αβγγN-acetylγγN-acetyl to adult Hb M is assumed to the same as that of αβγγN-acetylγγN-acetyl to Hb Aα, the relative quantity of adult Hb M Iwate can be reduced from 26.3% to 21.6%.

The relative quantities of fetal and adult forms of the abnormal hemoglobin of the infant with Hb M Iwate corresponded with those of the newborns with αX variants reported by Nakatsuji et al. (1983). In addition, the values presented here were similar to the quantity of Hb M Iwate of the adult patient described by
Katsura et al. (1983). It could be concluded that there is no difference between the relative quantities of the fetal and adult forms Hb M Iwate of individuals heterozygous for this abnormal hemoglobin.

References


