Two types of embryonic hemoglobins (Hb) containing \( \alpha \) chains have been identified in the blood of several neonates of Chinese origin with homozygous \( \alpha \)-thalassemia. In addition to Hb Portland I (\( \alpha_2\beta_2 \)) which was previously reported, another embryonic hemoglobin has been detected and found to contain \( \gamma \) chains and \( \beta \) chains. It is being designated Hb Portland II and has the formula \( \alpha_2\beta_2\gamma_2 \). It has a mobility slightly slower than that of Hb A on starch gel electrophoresis at pH 8.6 and has been found in the hemolysates of blood of some but not all hydropic infants. Another component with a mobility faster than that of Hb A on starch gel has been isolated from the blood of some hydropic neonates. This latter component is postulated to be \( \alpha_2\beta_2 \). The occurrence of Hb Portland I and Hb Portland II in these hydropic neonates is consistent with the hypothesis that, in the absence of normal \( \alpha \) chain production, \( \gamma \) chains are continued to be produced at later states of development than normal and form tetramers with each of the \( \beta \)-like globin chains. Because Hb Portland II has not been found in blood from all hydropic neonates, we postulate that the presence of this hemoglobin in these fetuses may be correlated with the gestational age of the fetus at the time of birth.

The existence of embryonic hemoglobins in human embryos was first described by Huehns et al. (1). Two embryonic hemoglobin, Gower I (first thought to be \( \alpha \), but later identified as \( \gamma\delta \)) and Gower II (\( \alpha\delta\varepsilon \)) predominate only during the first 10 to 12 weeks of fetal development (1-4). It is believed that these hemoglobins are synthesized by erythroid cells derived from the yolk sac (5). They are also found in trace amounts in normal cord blood and in some K562 cell line cultures which have been induced with hemin (6). Hb Portland I, another embryonic hemoglobin, is composed of two \( \alpha \)-like chains (embryonic \( \gamma \) chains) and two \( \gamma \) chains. Hb Portland I (\( \gamma_2\alpha_2 \)) was discovered in a female Chinese infant having multiple congenital anomalies and complex autosomal mosaicism (7, 8) and later reported in infants with hydrops fetalis due to homozygous \( \alpha \)-thalassemia (9, 10), a disease condition first described by Lie-Injo and Jo (11). Capp et al. (8) provided the structural evidence that demonstrated a new hemoglobin chain which they designated the \( \gamma \) chain. Their findings suggested not only another hemoglobin chain but at least one more globin gene locus. The similarity in the structure of human \( \alpha \) and \( \gamma \) globin chains led to the conclusion that this \( \gamma \) chain was probably an \( \alpha \)-like embryonic chain (12). Based on the findings of Kamvoura and Lehmann (13) and Huehns and Farooqui (14), it can be postulated that \( \gamma \) chain should combine with all \( \beta \)-like chains (i.e., \( \alpha \), \( \gamma \), \( \beta \), and \( \delta \)). We have looked into this possibility by attempting to study all the hemoglobin components in the hemolysates of blood from several neonates with homozygous \( \alpha \)-thalassemia.

MATERIALS AND METHODS

Blood Samples—Blood from stillborn neonates with hydrops fetalis due to homozygous \( \alpha \)-thalassemia was obtained from the delivery room of the General Hospital, Kuala Lumpur, Malaysia. The washed red blood cells were packed in Dry Ice and shipped by air to the United States. Hemolysates were prepared from these samples by a standard method using deionized distilled water and tolutene (15).

Starch Gel Electrophoresis—Hemoglobin Portland I, Hb Bart's, and other hemoglobin components were separated by starch gel electrophoresis (16). Starch gel electrophoresis was carried out in Tris-EDTA-boric acid buffer, pH 8.8. The hemoglobin components were eluted from the starch according to the method described by Lie-Injo (17).

Polyacrylamide Gel Electrophoresis—Hemoglobin Portland I, Hb Bart's, and other components were separated by isoelectric focusing using both analytical and preparative techniques. The analytical isoelectric focusing was performed in a 2-mm thick slab of polyacrylamide gel containing 2.4% Amphotole carrier amphotole in the pH range 6-8 without glycerol. After a 30-min prerun, 10-20 µl of 1 mg/ml of Hb sample predialyzed against 1% Amphotole solution were applied to the gel surface by means of a paper wick (14 × 8 mm) and the hemoglobin separation was completed after 12 h at 4°C with an LKB 2117 Multiphor Cell, using 10 watts from a constant wattage power supply. Measurement of the pH of the gel was performed using an isoelectric focusing combination surface electrode (Ingold) before staining the polyacrylamide gel. The hemoglobin staining was performed according to LKB Notes 138 and 250 using bromphenol blue.

Separation of Globin Chains by Reverse Phase HPLC—The HPLC system was assembled from the modular components: Altex Beckman model 332 gradient liquid chromatograph, an Altex-Hitachi model 100-30 UV-VIS variable wavelength detector with 5-µl analytical flow cell, and a Hewlett-Packard recorder of a HP5830A gas chromatograph system which was programmed to compute the per cent of area of the peaks and retention times. HPLC grade acetonitrile was obtained from Burdick and Jackson and methanol from J. T. Baker Chemical Co. All other chemicals were reagent grade.

Globin was prepared by acid acetone precipitation (18) from the whole hemolysate or from the purified hemoglobin components. For globin separation both analytical (3.9 mm × 30 cm) and preparative (7.8 mm × 30 cm) C-18 Bondapak columns (Waters) were used. The

Received for publication, September 6, 1983

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Printed in U.S.A.
mobile phase solvents "A" and "B" were those described by Shelton and co-workers (18). The gradient programs for separating globin chains by the short duration (50 min) and long duration (180 min) procedures using analytical and preparative columns are described in detail elsewhere (19) but are presented in summary with the results.

**Polyacrylamide Gel Electrophoresis**—Globin chains were separated by the method of Alter et al. (20) using electrophoresis on 12% polyacrylamide gels containing 6 M urea and 2% Triton X-100 in 5% acetic acid.

Slab gels (12 × 16 cm × 1.5 mm) with 6 or 12 spaces were employed for analytical electrophoresis using 5% acetic acid as electrophoresis buffer. The sample buffer consisted of 5 ml of deionized 8 M urea, 0.5 ml of glacial acetic acid, 0.5 ml of 2-mercaptoethanol, and 2 mg of pyronin Y. Five to ten μg of globin were mixed with 20 μl of sample buffer. Electrophoresis was performed for 17 h at a constant current of 8.5 mA at room temperature.

The gels were stained for 30 min with 0.5% Coomassie brilliant blue in 7% acetic acid, 30% methanol and destained with the acetic acid-methanol by diffusion. The gels were dried by placing in a Bio-Rad slab dryer (model 224) for 1 h at 45 °C.

**Polyacrylamide Isoelectric Focusing in NP40, 8 M Urea**—Lyophilized globin samples of 50–200 μg were dissolved in 8 M urea, 3% NP40, 10% 2-mercaptoethanol, at a concentration of 5 mg/ml, focused in 12.5 × 2.6 cm × 1.5 mm containing 8 M deionized urea, 2% pH 6-8 and 0.2% pH 3.5–10 Ampholine (LKB), 3% NP40 and stained with Coomassie brilliant blue G-250. The gels were prefocused for 2 h at 200 V and finally focused for 2 h, 30 min after the application of globin samples. Although these conditions are very similar to those recently described by Gianni et al. (21), they were developed independently.

**RESULTS**

The results described here were obtained on samples from several neonates with homozygous α-thalassemia hydrops fetalis syndrome. All these infants were of Malaysian-Chinese origin. The results obtained from the separation of various hemoglobin components and their constituent globin chains (Figs. 1–6 and Tables I and II) indicate that there was no detectable α chain in blood from any of these infants.

Starch gel electrophoresis of the whole hemolysate of a neonate with homozygous α-thalassemia at pH 8.8 produced four bands. These bands are designated components I (major band), II, III, and IV (a faint slow moving band) as shown in Fig. 1.

Component I had a mobility identical to Hb Bart’s on starch gel electrophoresis. Its identity as Hb Bart’s was confirmed (19, 22) by (a) separating the G, and A, chains by HPLC procedures, (b) isoelectric focusing of G, and A, globin chains, (c) globin chain electrophoresis on polyacrylamide gel of globin samples from Hb Bart’s (A), (d) examinations of tryptic peptides of globin samples using HPLC procedures and Amicon A5 cation exchange chromatography, and (e) amino acid composition of the G, zone separated by HPLC, which agreed well with published data.

Component II had a mobility on starch gel electrophoresis at pH 8.6 faster (more anodic) than Hb A and component III had a mobility slightly slower than Hb A. The hemolysate of blood from one hydropic stillborn produced only two visible bands corresponding to components I and III whereas other stillborns produced all of the four bands. That the starch gel component II is identical to Hb Portland I (ε2ε2) was confirmed by (a) separating the G, A, and ε chains by HPLC procedures, (b) separating the γ tryptic peptides by HPLC, and (c) amino acid composition of the γ and ε chains (19, 22).

The tryptic digests of the globin from components II and III when separated by A-5 chromatography were found to contain a tryptic peptide unique to the ε chain as identified by Capp et al. (zone XIX of Fig. 1c in Ref. 9). The amino acid composition of the ε-like chain was that of ε ε from Hb Portland I (22). In addition, several other differences were found for the tryptic digests of components II and III compared to the γ chain tryptic digest. Hence it was concluded that these components were hemoglobin chains which contained ε chains. A hemoglobin with the composition of Hb Portland I was also purified from two other hydropic neonates as component II by starch gel electrophoresis.

Examination of component III from two hydropic stillborn fetuses by using a short duration HPLC method (Fig. 3) also confirmed the presence of ε chains by the characteristic retention time of 37 min. The evidence that the HPLC zone with a retention time of 37 min is ε chain has been given in a previous report (19, 22). In addition to very small amounts of G, and A, (which are considered to be contaminants) what appeared to be a unique globin chain with a retention time of about 15 min was also present in component III. The elution position of the chain with retention time of about 15 min is identical to that of the β globin chain of Hb A. That this chain is the same as the β of normal Hb A is based upon the following results: (a) separation of β and ε chains from the purified hemoglobin component by polyacrylamide isoelectric focusing (Fig. 4B); (b) globin chain electrophoresis on polyacrylamide gel of material present in the zone with retention time of 15 min; it had a mobility identical to that of β A chain (Fig. 5B); (c) examination of its tryptic peptides which had a pattern identical to those of the β chain (Fig. 6).

The presence of β chain was further confirmed from the amino acid compositions of two tryptic peptides which corre-
Hemoglobins Containing $\xi$ Chains

**Fig. 2. Analytical polyacrylamide gel electrofocusing (pH range 6–8) of hemoglobins from (A) Hb A; (B) cord blood; (C) neonate with hydrops fetalis due to homozygous $\alpha$-thalassemia.** Isoelectric points were determined prior to hemoglobin staining which was performed by using bromphenol blue. Bands I and III were identified as Hb Portland I and Hb Bart’s. Band II is postulated as Hb Portland II whereas Band IV is a $\gamma$ chain-containing hemoglobin component (see text for details).

A zone from the HPLC separation of chains of Hb Portland II (Fig. 3) with a retention time of 33 min was observed ahead of the main $\xi$ chain zone (RT of 37 min). This was also observed in component III from two other hydropic neonates. After aminoethylation and tryptic hydrolysis, both of these zones gave identical peptide patterns by HPLC. No differences in amino acid composition could be detected between RT 33 and RT 37 (22). Whether or not these two zones from the $\xi$ chain are due to chemical modifications or sequence heterogeneity remains to be shown.

The analytical isoelectric focusing pattern (Fig. 2) of the hemolysate from the suspected hydropic neonate confirms the absence of Hb A, Hb F, Hb A$,\gamma$, or any other $\alpha$ chain containing hemoglobin. Lane C of Fig. 2 also shows separation of four bands (Bands I, II, III, and IV) in the whole hemolysate from a hydropic infant. Bands I and III were identified as Hb Portland I and Hb Bart’s. This was established (a) by examining by isoelectric focusing the behavior of components I and II separated and purified by starch gel electrophoresis and (b) by separating tryptic peptides using A-5 chromatography. Band I and II were found to contain the unique peptide $\xi$ TI by ion exchange chromatography and hence are considered $\xi$ chain-containing hemoglobins. Since Band I has been shown to be Hb Portland I with an isoelectric point of 6.37, it is postulated that Band II is Hb Portland II. Band IV which was not observed in all hydropic neonates contained only $\gamma$ chains and is postulated to be some modified form of Hb Bart’s.

We found that the blood hemolysates from hydropic neonates which were sent from Malaysia frozen in Dry Ice did not give satisfactory separations by starch gel electrophoresis.

**Fig. 3. Preparative separation of Hb Portland II ($\xi_2\beta_2$) globin on Waters $\mu$Bondapak C-18 preparative column (7.8 mm x 30 cm).** The hemoglobin was obtained from the CKL hydrops as component III on starch gel electrophoresis. The reverse phase column was attached with a guard column (containing C-18 Porasil packing), and the separation was made at a flow rate of 3.5 ml/min using a 55-min program. The gradient program and the column cleaning procedure are given in Ref. 19. About 6 mg of the globin was dissolved in 12 ml of phosphate solution and stirred for 1½ h at room temperature prior to loading sample after centrifugation. The loading procedure is also given in Ref. 19. Similar results were obtained from component III of the CTV hydrops (22).

**Fig. 4. Analytical isoelectric focusing of hydrops globin chains.** The globin was made by acid-acetone precipitation method of the following CKL hydrops samples: A, whole hemolysate; B, Hb Portland II separated by starch gel electrophoresis; and C, Hb Bart’s separated by starch gel electrophoresis. About 50–100 $\mu$g of the globin were dissolved in sample solution and focused in pH 6–8 gel containing 8 M urea and NP40. The gel was stained with Coomassie brilliant blue, destained, and dried.
or isoelectric focusing on polyacrylamide gels if stored for several weeks. For this reason we could not purify various hemoglobin components from all of the blood samples. However, the whole globin from this stored material could be used to separate the chains by HPLC procedures as shown in Table II. The tryptic peptides of the globin chains of Hb Bart’s (component I), Hb Portland I (component II), and Hb Portland II (component III) were investigated by converting pure hemoglobin components to globin.

DISCUSSION

The results obtained by electrophoresis and analysis of the constituent globin chains of the hemoglobin components from hemolysates of blood from infants with hydrops fetalis indicate a total absence of $\alpha$-chain production. The results presented here also provide clear evidence that in addition to Hb Portland I ($\xi_{2y2}$) identified earlier by Capp et al. (7) most of these neonates with homozygous $\alpha$-thalassemia produce another hemoglobin-containing $\xi$ chain with the composition of $\xi_2\beta_2$. Preliminary studies on the globin chain which elutes at a retention time of 15 min in an HPLC chain separation procedure have identified it as $\beta$ chain by the presence of tryptic peptides $\beta$T-3 and $\beta$T-5 and the similarity of its tryptic peptide pattern to that of the $\beta$ chain of Hb A. These findings rule out the possibility that this chain is an $\epsilon$ chain as found in Hb Gower I, $\xi_{2y2}$.

All 10 infants with homozygous $\alpha$-thalassemia that were studied had detectable to significant amounts of $\xi$ chains. We also demonstrated that most also had $\beta$-globin chains in their cord blood. The conclusion that all these neonates were producing Hb Portland I ($\xi_{2y2}$), Hb Portland II ($\xi_2\beta_2$), or both of these hemoglobinogens in detectable amounts in addition to predominant amounts of Hb Bart’s is based upon starch gel electrophoresis and HPLC separations. The presence of Hb Portland II in some hydropic neonates has also been confirmed by separating this hemoglobin as component III on starch gel followed by subsequent analysis of the globin chains from three different hydropic fetuses. One hydropic fetus (CKL) which did not have any significant amount of Hb Portland I contained about 15% of Hb Portland II. The cord blood of another hydropic neonate (GTT) did not have any detectable amounts of $\beta$ chain in the whole globin preparation or in any of its hemoglobin components but rather contained only Hb Portland I in addition to the predominant amount of Hb Bart’s. Similar results were also obtained from hydrops CFM.

Hemoglobin Portland I ($\xi_{2y2}$) was separated as component II by starch gel electrophoresis. It moves faster than Hb A but slower than Hb Bart’s (Fig. 1). A number of authors have detected Hb Portland I in samples from early human embryos, hydropic neonates, and human leukemic cell lines K562 (6, 7, 23–25).

Hemoglobin X separated by Todd et al. (10) by starch gel electrophoresis using Tris-EDTA-borate buffer at pH 8.65 may have been a mixture of Hb Portland I and Hb Portland II. The electrophoretic mobility of their Hb X appears to be identical to Hb Portland II (i.e. component III as shown in Fig. 1). This conclusion was strengthened further by the compositional analysis of their Hb X which Todd et al. found consisted of $\gamma$ chains, $\xi$ chains, and $\beta$ chains. In view of the data presented here, it seems very likely that the hemoglobin designated Hb X by Todd et al. (10) contained Hb Portland II.

Hemoglobin components with the mobility of Hb Portland I (starch gel component II) and Hb Portland II (starch gel component III) were also separated by Pootrakul et al. in a survey of hemoglobinogens (26) from a neonate with Hb Bart’s hydrops in Thailand. In that survey a hemoglobin component separated by starch gel electrophoresis with mobility identical to that of Hb Portland II was assumed to be Hb F ($\alpha_2\gamma_2$). The presence of Hb F as any fraction in hydropic fetuses must be incorrect because hydrops fetalis results from complete deletion of $\alpha$ genes (27, 28) and, therefore, a complete lack of production of $\alpha$ chains. Thus we propose that the hemoglobin component identified by Pootrakul et al. (26) as Hb F was in fact Hb Portland II.

Hemoglobin Portland I is now recognized as a normal...
Embryonic human hemoglobin. Its presence in trace amounts in normal newborn babies and significant proportions during early embryonic life has been reported by several investigators (5, 29). We postulate that Hb Portland II (\(\xi_2\beta_2\)) may not be detectable in normal newborn neonates because the normal infant produces significant amounts of \(\alpha\)-chain and the affinity of \(\alpha\)-chain for adult \(\beta\)-chain may be greater than \(\xi\)-chain for \(\beta\). In addition, because the \(\xi\)-chain in normal newborn neonates is produced in very small amounts, it would be difficult to detect in trace amounts even if Hb Portland II is produced. Therefore, it can be argued that Hb Portland II can only be detected in special conditions where the \(\xi\) and \(\beta\) globin chain synthesis is active, and synthesis of the \(\alpha\)-chain is either absent or markedly reduced.

Because \(\alpha\)-thalassemic hydroptic neonates cannot make \(\epsilon\)-chains but continue to synthesize \(\xi\)-chain, we postulate that during early embryonic life these fetuses may produce Hb Gower I (\(\xi_2\epsilon_2\)) followed by Hb Portland I (\(\xi_2\gamma_2\)). From the fact that some hydroptic infants are found to have significant amounts of Hb Portland II (\(\xi_2\beta_2\)) in addition to large amounts of Hb Bart’s (\(\gamma_4\)), it is concluded that these fetuses were producing \(\beta\) chain before birth. The fact that there was no Hb Portland II found in these hydroptic fetuses in detectable amounts leads to the suggestion that \(\beta\) chains may have greater affinity for \(\xi\) chain than \(\gamma\) chains have for \(\xi\). Based on this assumption it is also speculated that those hydroptic neonates which are born closer to full term have more active synthesis of adult \(\beta\)-chains and thus produce Hb Portland II. Furthermore, we propose that hydroptic infants which are born prematurely produce mainly Hb Portland I because little or no \(\beta\) chain is synthesized prior to 30 weeks of intrauterine life. In short, when no \(\alpha\) chains are available to form tetramers with \(\beta\), \(\gamma\), and \(\delta\) chains then \(\xi\) chains may form these tetramers. The proportion of the various tetramers depends upon the relative abundance of \(\beta\), \(\gamma\), and \(\delta\) chains and their relative affinity for the \(\xi\) chains.

Starch gel component IV has not been investigated further as yet. It is unlikely to be Hb Gower I (\(\xi_2\epsilon_2\)) because \(\epsilon\)-globin synthesis is active only during early embryonic life and most of the hydroptic neonates included in this study were born close to full term. Moreover, based on the electrophoretic mobility of component IV on starch gel, it is unlikely to be Gower I because Gower I moves slower than Hb Portland I1 because Gower I moves slower than Hb Portland I because Gower I moves slower than Hb Portland I because Gower I moves slower than Hb Portland I. It is postulated that component IV may have the composition \(\xi_2\beta_2\). The existence of component IV as a \(\xi\)- and \(\beta\)-containing hemoglobin component would be more consistent with adult \(\delta\) chain production closer to full term after adult \(\beta\) chain synthesis is fully activated. A hemoglobin designated X with a composition \(\gamma_2\beta_2\) has recently been reported in some embryonic neonates and in human K562 cell lines when induced by hemin (6). Components III or IV are unlikely to be Hb X (\(\gamma_2\epsilon_2\)) because no \(\epsilon\) chain could be detected in samples from these hydroptic neonates.

The presence of Hb Portland I and Hb Portland II suggests that \(\xi\) is \(\alpha\)-like in its association with non-\(\alpha\) chains. The \(\alpha\) chain forms similar products with \(\gamma\) and \(\beta\) chains during fetal development as fetal type Hb F (\(\alpha_2\gamma_2\)) and adult Hb A (\(\alpha_2\beta_2\)).

### REFERENCES


### TABLE I

Amino acid composition of two tryptic peptides of \(\beta\)-globin chain of Hb Portland II (\(\xi_2\beta_2\)) from CTY and CKL hydroptic infant

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>(\xi)-like peptide</th>
<th>(\beta)-like peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT 55.7</td>
<td>RT 66.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.3 (1)</td>
<td>4.5 (1)</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.8 (1)</td>
<td>1.1 (1)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>13.3 (2)</td>
<td>11.7 (3)</td>
</tr>
<tr>
<td>Threonine</td>
<td>0</td>
<td>4.9 (1)</td>
</tr>
<tr>
<td>Serine</td>
<td>0</td>
<td>2.5 (3)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.9 (2)</td>
<td>4.3 (2)</td>
</tr>
<tr>
<td>Proline</td>
<td>9.6 (2)</td>
<td>7.8 (2)</td>
</tr>
<tr>
<td>Glycine</td>
<td>19.5 (3)</td>
<td>10.4 (2)</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.6 (1)</td>
<td>5.0 (1)</td>
</tr>
<tr>
<td>Valine</td>
<td>16.5 (3)</td>
<td>5.5 (1)</td>
</tr>
<tr>
<td>Methionine</td>
<td>0</td>
<td>3.1 (1)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.5 (1)</td>
<td>5.1 (1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.0 (1)</td>
<td>11.5 (3)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.6 (1)</td>
<td>4.1 (19)</td>
</tr>
</tbody>
</table>

### TABLE II

Per cent area of the globin chains from the HPLC separation of 1 mg of whole globin from hydroptic neonates

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Hydronic neonate</th>
<th>RT* 15 min</th>
<th>RT* 19 min</th>
<th>RT* 21 min</th>
<th>RT* 23 min</th>
<th>RT* 37 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CKL</td>
<td>6.5</td>
<td>50</td>
<td>37</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CTT</td>
<td>1.0</td>
<td>48</td>
<td>25</td>
<td>17.9</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>WM</td>
<td>6.6</td>
<td>49</td>
<td>37</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>YLL</td>
<td>10</td>
<td>52</td>
<td>34</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TAM</td>
<td>9.7</td>
<td>62</td>
<td>18</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CH</td>
<td>7.4</td>
<td>67</td>
<td>18</td>
<td>7.6</td>
<td></td>
</tr>
</tbody>
</table>

*These values represent percentage of the total area due to the absorption at 220 nm and not the actual amount of the globin chain (HPLC).
Hemoglobins Containing \(\xi\) Chains