Immunochromical Quantitation of Proteins in Single Cells
I. Carbon Anhydrase B, \( \beta \)-Chain Hemoglobin and \( \gamma \)-Chain Hemoglobin in Some Normal and Abnormal Erythrocytes

By David Gitlin, Teruo Sasaki and Pekka Vuopio

The nature of the transition from hemoglobin \( F \) synthesis to hemoglobin \( A \) synthesis in the fetus and newborn remains unknown. Although it is evident from the elution method developed by Kleihauer, Braun and Betke\(^1\) that hemoglobin \( F \) and hemoglobin \( A \) may coexist in single erythrocytes,\(^2\) quantitative data regarding the amounts of these hemoglobins in individual cells are lacking. An attempt to quantitate specific hemoglobins in single erythrocytes by combining microspectrophotometry with the elution technic has not proven very successful, the method being insensitive to levels of hemoglobin \( F \) which were less than 10 per cent or more than 80 per cent of the total.\(^5\) Excellent methods for the quantitation of total hemoglobin in single cells have been developed using photometry\(^6\) or electron microscopy,\(^7\) but the methods do not distinguish between specific hemoglobins. Immunochemical methods have been used successfully to differentiate between cells which do or do not contain hemoglobin \( F \),\(^8,10\) but the methods are qualitative, not quantitative.

In the present study, specific antisera against \( \gamma \)-chain hemoglobin, \( \beta \)-chain hemoglobin and carbonic anhydrase \( B \) were used to determine the quantities of these proteins in single erythrocytes in normal fetal, newborn and adult blood. In addition, cells from patients with sickle cell hemoglobin or with thalassemia were examined for \( \gamma \)-chain hemoglobin and carbonic anhydrase \( B \).

Materials and Methods

Antigens and Antisera

A 3S \( \gamma_1 \)-globulin which has been shown\(^1\) to be immunologically and structurally identical to erythrocyte carbonic anhydrase \( B \) was isolated from pooled normal adult plasma by DEAE-cellulose chromatography and Sephadex filtration as described elsewhere,\(^12\) and it was used in this study as the antigen for the preparation of antisera specific for carbonic anhydrase \( B \). Hemoglobin \( A \) was crystallized from concentrated lysates of adult erythrocytes by means of ammonium sulfate fractionation.\(^13\) The antigen used for the preparation of antisera against hemoglobin \( F \) was alkali-resistant hemoglobin prepared by a modification of the method of Singer, Chernoff and Singer\(^14\) from washed erythrocytes obtained from umbilical cord blood of a full term normal infant as described elsewhere.\(^8\)

All of the antisera used in this study were prepared in rabbits with the antigen in

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From the Department of Pediatrics, University of Pittsburgh School of Medicine and the Children's Hospital, Pittsburgh, Pennsylvania.

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Freund’s complete adjuvant. The antiserum against carbonic anhydrase B (anti-CAB) gave only a single line with erythrocyte lysates on immunoelectrophoresis\textsuperscript{15} and in agar gel diffusion\textsuperscript{16} which was identical to that obtained between the antiserum and a purified preparation of erythrocyte carbonic anhydrase B generously contributed by Dr. P. O. Nyman of the University of Göteborg. The antisera against hemoglobin A contained antibodies against carbonic anhydrase B and reacted with hemoglobin F as well as with hemoglobin A: the antisera were adsorbed before use with purified hemoglobin F obtained by DEAE-cellulose fractionation of neonatal erythrocytes\textsuperscript{17} and with a carbonic anhydrase non-heme protein fraction from carboxymethylcellulose chromatography of adult erythrocyte lysates.\textsuperscript{18} The adsorbed antisera vs hemoglobin A also reacted with hemoglobin S but not with hemoglobin F, and hence were specific for β-chains, whether normal or substituted as in hemoglobin S. The crude antisera against hemoglobin F were carefully adsorbed with hemoglobin A obtained by carboxymethylcellulose chromatography and with purified carbonic anhydrase B; the resulting antisera reacted with hemoglobin F but not with A or S, and hence were specific for γ-chains.

Quantitation of Proteins in Lysates

The concentrations of hemoglobins F and A and carbonic anhydrase B in lysates of washed erythrocytes were determined immunochemically by the method of Mancini and her colleagues\textsuperscript{19} using the specific antisera described above. Total hemoglobin, however, was determined by Drabkin’s method.\textsuperscript{20}

Immunochernical Quantitation in Single Erythrocytes

The quantitation of carbonic anhydrase B and β-chain and γ-chain hemoglobins in single erythrocytes was performed using given dilutions of a specific antiserum in agar gel: the given antiserum was diluted at 56 C. with 0.1 M pH 8.6 borate buffer containing 0.05 M NaCl and with appropriate amounts of 3 per cent agar in the same buffer-saline so that the final agar concentration was 1.5 per cent; in this manner, final dilutions of 1:2, 1:4, or 1:8 of the antiserum in agar were prepared. The antiserum-agar solutions were poured into square areas on 25 × 75 mm. glass microscope slides (Fig. 1), the areas being bordered on 2 sides by 10 to 15 mm. × 25 mm. pieces of glass cut from other microscope slides and on the other 2 sides only by the edges of the slide; surface tension kept the agar from overflowing the edges of the slide and the agar could be poured to a depth of approximately 2 mm. After gelation, the glass retainers were removed and a thin layer of a washed suspension of erythrocytes in 0.15 M NaCl was spread over the surface of the agar; the suspension of erythrocytes was adjusted prior to spreading such that the cells on the agar surface were well separated from each other (Fig. 2, A and C). The positions of the erythrocytes

Fig. 1.—(A) Microscope slide showing position of glass retainers. (B) Antiserum in agar in situ before removal of glass retainers.
Fig. 2.—(A and C) Erythrocytes, obtained at delivery from infant of 23 weeks' gestation, immediately after spreading on agar-antiserum gel; antiserum was specific for γ-chains and diluted 1:4 in the agar. Arrow in A indicates 2 erythrocytes close together. Magnification 56 x. (B and D) Same fields as in A and C respectively taken with darkfield illumination showing precipitation rings which were found after 7 days; agar was dried prior to examination of slide. Arrow in B indicates single ring obtained from the 2 erythrocytes shown in A; sharp white dots in these and subsequent figures are artifacts, not precipitin reactions. Magnification 56 x.

on the slide were quickly photographed at 100X or 200X using a light microscope equipped with a graduated mechanical stage, the positions photographed being marked by the vernier scales of the stage. The slides were then placed in a closed atmosphere of toluene vapor for 40 minutes to ensure lysis of all erythrocytes, and then transferred to a small humidity chamber consisting of a petri dish at the bottom of which had been placed a layer of absorbent paper soaked in water. A few drops of toluene were placed on the paper and the dishes were sealed in Saran Wrap® (Dow Chemical Co., Midland, Mich.). The slides were left undisturbed at approximately 23 C. for 7 days. At the end of this time, the slides were removed from the petri dishes and 25 × 25 mm. squares cut from cellulose acetate electrophoresis membranes (Beckman Instruments, Palo Alto, Calif.) were placed on top of the agar. The slides were then dried at 37 C., a process which took about 4 hours, and the cellulose acetate membranes were removed from the dried agar by placing the slide vertically in 0.05 M NaCl until the cellulose acetate fell away, a process which took but a few minutes. The slides were examined with the same microscope used to photograph the erythrocytes earlier but now adapted as a dark field microscope by simply changing the condenser; in this way the erythrocyte areas originally photographed could be identified by the same vernier stage readings. Areas toward the center of the agar showing immunological precipitation were photographed (Fig. 2, B and D) using 35 mm. Kodak Tri-X film; areas along the edges of the agar were not taken for measurement, due to the fact that premature dehydration along the edges tended to take place even in the humidity chamber, leading to smaller precipitation rings than when dehydration did not occur. To measure the diameters of the precipitation rings, the negative images were enlarged by projection to a final magnification of 450 diameters, and all measurements were made from the projected images.
Fig. 3.—(A, B and C) Immunochemical precipitation obtained with adult erythrocytes on agar containing antiserum specific for β-chains showing increased diameter of radial precipitation with increased dilution of antiserum. (A) Antiserum diluted 1:2. (B) Antiserum diluted 1:4. (C) Antiserum diluted 1:8. Magnifications all 150×. (D, E and F) Precipitation of carbonic anhydrase B (arrows) in adult erythrocytes. (D) Antiserum diluted 1:2. (E) Antiserum diluted 1:4. (F) Antiserum diluted 1:8. The immunochemical precipitates were easier to distinguish from other reflecting objects on the slide than in the photographs. Magnifications all 150×.

To determine the absolute content of specific protein in individual erythrocytes, it was necessary to relate the diameter of the precipitation rings obtained with a specific antiserum to the amount of specific protein in the cell. For this purpose it was necessary to determine:
1) the average content of that protein per cell, C, in a given erythrocyte suspension, and this was estimated as \( [C] V / N \), where \([C]\) was the concentration of the protein in a lysate of the erythrocytes, \(V\) was the volume of the lysate, and \(N\) was the number of erythrocytes in the lysate;
2) the fraction, \(f\), of the total number of erythrocytes in the same cell suspension which contained the specific protein, and this was determined by comparing the number of erythrocytes, \(N_r\), found in a given photographed area with the number of precipitation rings, \(N_r\), in the same area (Fig. 2), or \(N_r/N_r = f\); and
3) the diameters of at least 200
precipitation rings from the same erythrocyte suspension at each of 2 or 3 different dilutions of antiserum in agar (Fig. 3), and from these values the average ring diameter, $\bar{R}$ and the median ring diameter, $R_m$, for each antiserum dilution were determined. The diameters of the precipitation rings under the conditions of this study were proportional to the logarithm of the amounts of specific protein released from the cell.\textsuperscript{21} The proportionality constant for this relation was determined from the values of $R_m$ obtained at the different dilutions of antiserum; dilution of antiserum by a factor of 2 reduced the amount of antigen required to yield a given ring size by the same factor,\textsuperscript{22} or stated another way, the same ring diameter as that formed by a given amount of antigen per cell with antiserum diluted 1:2 was formed at half that amount of antigen per cell when the antiserum was diluted 1:4. In practice then, the proportionality was established by first plotting the median diameters of the precipitation rings, $R_m$, vs the logarithms of the corresponding relative amounts of antigen per cell; the $R_m$ value with antiserum diluted 1:4 was temporarily and arbitrarily set as equivalent to the logarithm of 1.00 units of antigen; the $R_m$ value obtained with antiserum diluted 1:2 was then equivalent to the logarithm of 0.5 units of antigen and that obtained with antiserum diluted 1:8 was equivalent to the logarithm of 2.00 units of antigen. Thus, a standard curve for the antiserum was constructed relating the diameter of the precipitation ring obtained with antiserum diluted 1:4 to the relative content of antigen per erythrocyte. The relative content of specific protein per cell was then converted to absolute amounts per cell using the fact that the average ring diameter, $\bar{R}$, was equivalent to the logarithm of $C/f$, or the logarithm of the average amount of specific protein per cell in those cells which contained the antigen; the conversion of the standard curve to absolute terms, then, was accomplished simply by adding log $C/fC_{\alpha}$ to the logarithms of the relative content, where $C_{\alpha}$ was the relative content per cell at $\bar{R}$.

To obtain high values of $C$ and $f$, and thus keep any error in these factors to a minimum in standardizing the antisera, adult erythrocytes were used for standardization of the anti-$\beta$ chain antisera in terms of hemoglobin A and for the standardization of the anti-CAB antisera; umbilical cord blood in which over 90 per cent of the hemoglobin was present as F was used to standardize the anti-$\gamma$ chain antisera in terms of hemoglobin F. Under the conditions of this study, the limit of sensitivity of the method was less than $2 \times 10^{-14}$ g. for carbonic anhydrase B and less than $10^{-13}$ g. for $\beta$-chains or $\gamma$-chains as hemoglobin.

Rather than plot the exact diameter of each precipitation ring to obtain the frequency distribution curve for specific protein content in erythrocytes of a given blood sample, the
ring diameters were segregated into groups with increments of 2.25 to 4.50 μ. Only those precipitation rings which were separated from all other precipitation rings were taken for measurement, assuring that the antigen content of one cell did not affect the size of the precipitation ring formed by another; it should be noted that unless 2 cells were separated by half the diameter of the smaller of 2 overlapping precipitation rings, it was possible to obtain a single ring from the 2 cells (arrows in Fig. 2, A and B, and in Fig. 8, D) emphasizing the necessity of spreading the cells well apart from each other.

RESULTS

All of the erythrocytes in 3 blood samples from normal male adults contained β-chains, the range being from 18.8 to 62.2 μg per cell. The cumulative distribution of β-chains as hemoglobin in the erythrocytes of one of these bloods as a percentage of those cells containing β-chains is shown in Figure 4 and the frequency distribution is shown in Figure 5: the range was 18.8 to 45.4 μg per cell with a median of 28.5 μg; erythrocytes from the same individual 6 months earlier gave similar results with a range of 18.8 to 52.6 μg per cell and a median of 26.5 μg. Approximately 0.5 to 0.9 per cent of the erythrocytes contained γ-chains (Fig. 6), the content per cell in terms of hemoglobin ranging from 2.5 to 12.5 μg. (Figs. 4 and 5), the median being 5.1 μg. Carbonic anhydrase B was found in approximately 86 per cent of the erythrocytes, the content per cell ranging from 0.07 to 2.12 μg, with a median of 0.50 μg.

In cords bloods from 3 normal newborns of 38 weeks' gestation, 95 to 98
per cent of the erythrocytes contained \( \gamma \)-chains (Fig. 7), the content per cell as hemoglobin ranging from 8.6 to 94.0 \( \mu \text{g} \). In the blood represented in Figures 4 and 5, 96 per cent of the erythrocytes had detectable \( \gamma \)-chains which, as hemoglobin, ranged from 15.0 to 82.5 \( \mu \text{g} \) per cell, the median being 35.6 \( \mu \text{g} \). Of the erythrocytes in the latter blood, 15 per cent contained detectable \( \beta \)-chains (Fig. 7), the content per cell as hemoglobin being 14.8 to 43.3 \( \mu \text{g} \); (Figs. 4 and 5) with a median at 26.8 \( \mu \text{g} \); the cumulative \( \beta \)-chain distributions in the erythrocytes of the 3 cord bloods were quite similar. Although the ranges and the medians for \( \beta \)-chain content were similar in both adult and cord blood erythrocytes, the \( \beta \)-chain content of the erythrocyte below the median was lower in cord cells than in adult cells, while above the median the \( \beta \)-chain content per cell tended to be lower in the adult erythrocyte. Approximately 6 to 12 per cent of cord blood erythrocytes were found to contain carbonic anhydrase B; in the blood represented in Figures 4 and 5, 12 per cent of the cells contained the enzyme with a range per cell of 0.04 to 2.18 \( \mu \text{g} \) and a median of 0.33 \( \mu \text{g} \).

When individual cord erythrocytes were studied for the simultaneous presence of carbonic anhydrase B and \( \gamma \)-chains or carbonic anhydrase B and \( \beta \)-chains (Fig. 8), it could be calculated from the percentage of cells containing each protein that a single cell might contain any combination of the three proteins assuming, of course, that no erythrocyte totally lacked both
Fig. 7.—Immunochemical precipitation obtained with cord erythrocytes of 38 weeks' gestation on antiserum specific for \( \gamma \)-chains (A) and antiserum specific for \( \beta \)-chains (B, C and D). Magnification \( 132 \times \).

\( \gamma \) and \( \beta \) chains. For example, 8 per cent of the cord erythrocytes of Figures 4 and 5 were found to have both \( \gamma \)-chains and carbonic anhydrase B, while 4 per cent were found to have carbonic anhydrase B but no \( \gamma \)-chains. On the other hand, 5.2 per cent of the erythrocytes examined contained carbonic anhydrase B but no \( \beta \)-chains. On the basis of these figures, since 12 per cent of the cord erythrocytes had carbonic anhydrase B, 2.8 per cent, or 12 per cent—(4 per cent + 5.2 per cent), possessed carbonic anhydrase B plus \( \gamma \)-chains and \( \beta \)-chains (Table 1). It had been noted that 15 per cent of the erythrocytes contained \( \beta \)-chains and 96 per cent contained \( \gamma \)-chains; therefore, 11 per cent, or 15 per cent—4 per cent, contained both \( \beta \)-chains and \( \gamma \)-chains, and since 2.8 per cent contained all 3 proteins, 8.2 per cent, or 11 per cent—2.8 per cent, possessed \( \beta \)-chains and \( \gamma \)-chains but no carbonic anhydrase B. In addition, it can be calculated that approximately 80 per cent of the cord erythrocytes, or 96 per cent—(2.8 per cent + 8.2 per cent + 5.2 per cent), contained only \( \gamma \)-chains and neither \( \beta \)-chains nor carbonic anhydrase B.

From these calculations, it would appear that few if any of the cells contained \( \beta \)-chain hemoglobin without also containing either \( \gamma \)-chains or carbonic anhydrase B or both.

In an infant of 23 weeks' gestation born prematurely, at least 99.5 per cent of the cells were found to contain \( \gamma \)-chains and 0.52 per cent contained both \( \gamma \)-chains and carbonic anhydrase B. Very small amounts of \( \beta \)-chains were
Fig. 8.—Immunochemical precipitation. (A) Erythrocytes from infant of 23 weeks' gestation on agar with antisera specific for γ-chains and carbonic anhydrase B; arrow indicates a cell with both proteins. (B and C) Erythrocytes from infant of 38 weeks' gestation on agar with antisera specific for γ-chains and carbonic anhydrase B. (D) Adult erythrocytes on agar with antisera specific for β-chains and carbonic anhydrase B; at site indicated by arrow 2 precipitation centers for carbonic anhydrase B indicated the presence of 2 erythrocytes so close to each other that only a single precipitation ring for hemoglobin was obtained. Magnifications all 100 ×.

found in approximately 0.1 per cent of the cells, but the areas of immunochemical precipitation were similar in size to those obtained for carbonic anhydrase B; therefore, the number of cells containing both carbonic anhydrase B and β-chains, or the number of cells containing carbonic anhydrase B without β-chains, could not be determined, due to overlapping of the precipitation areas of the two proteins. No cells contained carbonic anhydrase B without γ-chains. The range and distribution of the γ-chain content was re-

Table 1.—The Percentage of Circulating Erythrocytes Containing γ-chains, β-chains and/or Carbonic Anhydrase B as Calculated from the Numbers of Cells Containing Either One or Two of the Three Proteins and Assuming That no Cells Lacks Both γ-chains and β-chains.

<table>
<thead>
<tr>
<th>γ-chains</th>
<th>β-chains</th>
<th>Carbonic anhydrase B</th>
<th>Circulating erythrocytes at 38 weeks' gestation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>+</td>
<td>+</td>
<td>4.0</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>5.2</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2.8</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>8.2</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>79.8</td>
</tr>
</tbody>
</table>
Fig. 9.—Cumulative distributions of carbonic anhydrase B and γ-chains as a function of those cells containing the specific protein rather than as a function of the total erythrocytes. Dotted lines = normal adult erythrocytes. Upper graphs = individuals heterozygous for hemoglobins S and A (hollow symbols) and individuals homozygous for hemoglobin S (solid symbols). Lower graphs = parents heterozygous for β-chain thalassemia (hollow symbols) and their 2 children with thalassemia major (solid symbols).

The range for carbonic anhydrase B content was from 0.07 to 0.57 μg. The range for carbonic anhydrase B content was from 0.07 to 0.57 μg.

In 2 individuals with hemoglobins S and A, the γ-chain content of the erythrocytes in terms of hemoglobin was similar to that in normal adult erythrocytes (Figs. 9 and 10), but in 4 patients homozygous for hemoglobin S a shift in the distribution curve to increased γ-chain content was evident as well as an increase in the percentage of erythrocytes containing γ-chains (Figs. 9 and 10). As can be seen from Figure 9, the erythrocyte carbonic anhydrase B content in those cells which contained carbonic anhydrase B did not deviate from the normal in the same manner as did γ-chain content.

In 2 siblings with thalassemia major and in their parents, from 2.4 to 4.8 per cent of the erythrocytes (Fig. 10) possessed γ-chains. The increase in γ-chain hemoglobin noted in the parents of the thalassemic children, as with the patients homozygous for hemoglobin S, was attributable to increased cell content of γ-chains as well as to an increase in the number of cells con-
Fig. 10.—Frequency distribution of γ-chain content as a function of the total erythrocytes in blood sample. From left to right: upper series = normal adult, individual with both hemoglobins S and A, an individual homozygous for hemoglobin S; middle series = an individual homozygous for hemoglobin S, another individual homozygous for hemoglobin S, parent heterozygous for β-chain thalassemia; lower series = the other parent heterozygous for thalassemia and the 2 siblings with thalassemia major. All of these individuals are those also represented in Figure 9.

It would appear that γ-chains and β-chains as determined immunochemically may coexist in the same erythrocyte: approximately 11 per cent of the erythrocytes in the cord blood of infants delivered at 38 weeks' gestation contained both γ-chains and β-chains, a value similar to that found for hemoglobins F and A by the elution method, and approximately 1 per cent of the erythrocytes from normal male adults contained γ-chains as well as β-chains. As Kleihauer and Betke have indicated, therefore, the change from the predominantly hemoglobin F cell of the fetus to the predominantly hemoglobin A cell of the adult is not attributable to the sudden appearance of
adult type cells with simultaneous disappearance of fetal cells, but is a transitional phenomenon accompanied by the appearance of cells containing both hemoglobins. However, as has been noted in the present study, in those cord erythrocytes in which β-chains were found the range and median of the β-chain content was remarkably similar to those found for the β-chain content of adult erythrocytes, suggesting that once the cistron for β-chain synthesis is activated, or the operon for β-chain synthesis is derepressed, β-chain synthesis must rapidly approach adult rates even in the presence of γ-chain synthesis.

Although 95 to 98 per cent of the cord erythrocytes contained γ-chains, approximately 2 to 5 per cent of the erythrocytes contained β-chains but no detectable γ-chains. Thus, complete repression of γ-chain synthesis did occur in a significant number of erythrocytes by 38 weeks' gestation. On the other hand, approximately 0.5 to 0.9 per cent of the normal adult erythrocyte population contained definite amounts of γ-chains, indicating that repression of γ-chain synthesis was not necessarily complete even in the adult cell. Although normal adult cells may appear to be devoid of hemoglobin F using the elution technic and other methods, small amounts of hemoglobin F have been demonstrated in normal adult blood by others using specific antisera or column chromatography in accord with the results of the present study. Why repression of γ-chain synthesis should be complete in some cells but not in others is not apparent, but it is interesting to note that in most genetic deficiencies of protein synthesis, particularly those of the plasma proteins, the defect is not complete, and small amounts of specific protein are synthesized. Whatever the normal repression mechanism for γ-chain synthesis, it appears to be imperfect in some cells at least.

Derepression of carbonic anhydrase B synthesis, at least in part, appeared to be independent of derepression of β-chain synthesis, despite the superficially parallel increase in synthesis of the two: at 23 weeks' gestation, 0.5 per cent of the erythrocytes contained small amounts of carbonic anhydrase and all of these cells contained γ-chains; at 38 weeks' gestation, all or almost all of the cells which contained β-chains but no detectable γ-chains, amounting to approximately 4 per cent of the erythrocytes, did indeed have carbonic anhydrase B, but approximately 5 per cent of the total erythrocytes had γ-chains and carbonic anhydrase B but no β-chains, and about 8 per cent had β-chains and γ-chains but no carbonic anhydrase B. Since the developmental sequences of individual proteins in cells other than the erythrocyte can be independent events, it is not surprising that the development of synthesis of specific non-heme proteins in the erythrocyte can be independent of the development of specific hemoglobin synthesis.

The frequency distribution for β-chain content, as hemoglobin, in adult erythrocytes and that for β-chains plus γ-chains in cord erythrocytes is remarkably similar to those reported for total hemoglobin in individual cells using photodensitometry with light microscopy or electron microscopy. The presence of γ-chain hemoglobin in individual cells in patients with sickle-cell anemia or thalassemia has been well demonstrated by others using...
the elution technique. In the present study, the frequency distribution curve for γ-chains in individuals homozygous for hemoglobin S and in two persons heterozygous for thalassemia assumed the familiar sigmoid curve with a shift to increased cell γ-chain content. In 2 patients homozygous for thalassemia, however, the γ-chain content of the erythrocytes did not even approach a single Gaussian distribution curve, but instead suggested the presence of perhaps 2 abnormal cell populations. Unfortunately, β-chain distributions for the latter erythrocytes were not done, so that the data are insufficient for informative speculation as to the underlying mechanisms involved.

SUMMARY

An immunochemical method for the quantitative determination of specific soluble proteins in individual erythrocytes has been described. Application of the method revealed:

1. From 0.5 to 0.9 per cent of the normal adult erythrocytes studied contained small amounts of γ-chains, from 2.5 to 12.5 μg, as hemoglobin per cell.
2. In cord blood of normal infants of 38 weeks’ gestation, significant numbers of erythrocytes were found which contained either γ-chains or β-chains or both, independently of carbonic anhydrase B.
3. Once initiated within a given erythrocyte, β-chain synthesis in that cell rapidly approached adult rates. Derepression of carbonic anhydrase B synthesis was independent of derepression of hemoglobin synthesis, and in fetal cells which contained carbonic anhydrase B the amount found was well below that of most adult erythrocytes.
4. In 4 patients homozygous for hemoglobin S and in 2 persons heterozygous for thalassemia, an increase in F hemoglobin was associated with an increase in γ-chain content of individual erythrocytes as well as an increase in the number of erythrocytes containing γ-chains; in 2 patients homozygous for thalassemia, an unusual distribution of cells with increased γ-chain content was observed.
catenas γ in le erythrocyto individual e etiam con un augmento del numero de erythrocytos continente catenas γ. In 2 homozygoticos pro thalassemia, un distribution inusual del cellulas con augmentate contentos de catena γ esseva observe.

ACKNOWLEDGMENT

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REFERENCES


DAVID GITLIN, TERUO SASAKI and PEKKA VUOPIO