MEMBRANE-BOUND REDOX PROTEINS OF THE MURINE FRIEDEL VIRUS-INDUCED ERYTHROLEUKEMIA CELL

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ABSTRACT

We have obtained and studied a 105,000-g pellet from T-3-C1-2 cells, a cloned line of Friend virus-induced erythroleukemia cells. By difference spectrophotometry, the pellet was shown to contain cytochrome $b_5$ and cytochrome P-450, hemeproteins that have been shown to participate in electron-transport reactions of endoplasmic reticulum and other membranous fractions of various tissues. The pellet also possesses NADH-cytochrome $c$ reductase activity which is inhibited by anti-cytochrome $b_5$ $\gamma$-globulin, indicating the presence of cytochrome $b_5$ reductase. This is the first demonstration of membrane-bound forms of these redox proteins in erythroid cells. Dimethyl sulfoxide-treated T-3-C1-2 cells were also shown to possess membrane-bound cytochrome $b_5$ and NADH-cytochrome $c$ reductase activity. We failed to detect soluble cytochrome $b_5$ in the 105,000-g supernatant fraction from homogenates of untreated or dimethyl sulfoxide-treated T-3-C1-2 cells. In contrast, erythrocytes obtained from mouse blood were shown to possess soluble cytochrome $b_5$ but no membrane-bound form of this protein. These findings are supportive of our hypothesis that soluble cytochrome $b_5$ of erythrocytes is derived from endoplasmic reticulum or some other membrane structure of immature erythroid cells during cell maturation.

KEY WORDS: erythroleukemia - cytochrome $b_5$ - NADH-cytochrome $c$ reductase - cytochrome P-450 - erythrocytes

The murine Friend virus-induced erythroleukemia cell line has been used as a model to study erythroid differentiation (10). The T-3-C1-2 cloned line of these erythroleukemia cells has a very low level of spontaneous differentiation (18, 34). Upon treatment with dimethyl sulfoxide (DMSO), however, this cell line (like other erythroleukemia lines) undergoes changes similar to those associated with erythroid differentiation. The changes include the appearance of erythroid membrane antigen (17), accumulation of mRNA for globin synthesis (34), and synthesis of globin chains and hemoglobin (18).

In the present study, we have used the Friend virus-induced erythroleukemia T-3-C1-2 line as a model for early erythroid cells to study cytochrome $b_5$, a protein found in reticulocytes and mature erythrocytes. In non-nucleated erythroid cells, cytochrome $b_5$ (16, 31) and cytochrome $b_5$ reductase (16, 20, 30, 39) exist in the cytoplasm as soluble molecules. Erythrocyte cytochrome $b_5$ is similar to cytochrome $b_5$ solubilized from microsomes of the liver. The amino acid composition of bovine erythrocyte cytochrome $b_5$ I is in very good agreement with a segment (residues 1-97) of bovine liver microsomal cytochrome $b_5$ (6). Tryptic digestions...
of bovine erythrocyte cytochrome $b_5$ and bovine liver microsomal cytochrome $b_6$ yield core hemeproteins which are indistinguishable on the basis of electrophoretic migration and amino acid composition (6, 15).

It is our contention that, in the immature erythroid cell, cytochrome $b_6$ is associated with membranes, and that sometime during the maturation process this hydrophobic membrane-bound protein is converted to a water-soluble molecule. In this paper we will present evidence that cytochrome $b_6$ in an immature erythroid cell system exists as a membrane-bound protein. Some of these results have been presented previously in abstract form (36).

**MATERIALS AND METHODS**

**Growth and Preparation of Friend Erythroleukemia Cells**

Friend virus-induced erythroleukemia cells, clonal line T-3-C1-2, were obtained from Dr. S. Orkin of the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md. The cells had been maintained in culture in the laboratory of Dr. R. Ruddon, Department of Pharmacology, The University of Michigan. This clonal line has been described as a "genetically homogeneous population of erythroleukemic cells" (27). These cells were assayed periodically by Dr. Allen Lau, using the uridine phosphorylase assay (22), and were found to be free of mycoplasma contamination (21).

The cells were grown in suspension with RPMI-1640 powdered tissue culture medium (9.4 g/liter) to which NaHCO$_3$ (1.8 g/liter), penicillin (0.07 g/liter), streptomycin (0.1 g/liter), and 1 N HCl (3.4 ml/liter) had been added. The medium was also supplemented with 10% fetal calf serum (KC Biological, Inc., Lenexa, Kans.). The growth medium was sterilized by filtration through a 0.22-$\mu$m Millipore filter (Millipore Corp., Bedford, Mass.). All cultures were initiated in culture in the cold until it was postfixed in 2% osmium tetroxide-0.1 M cacodylate buffer, pH 7.2, and then dehydrated in a graded ethanol series. The dehydrated pellet was washed in propylene oxide, soaked in propylene oxide and Epon, and then embedded in Epon. Sections were prepared, post-stained with uranyl acetate (9) and lead citrate (33), and examined with an AEI Corinith 275 electron microscope.

**Preparation of a 105,000-g Pellet**

A 105,000-g pellet was prepared from untreated and DMSO-treated cells by a modification of the method of Omura and Sato (26). The cells were homogenized with 3 vol of cold 0.25 M sucrose containing 0.001 M EDTA, pH 7.6, in a Potter-Elvehjem homogenizer equipped with a Teflon pestle (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.). The homogenate was centrifuged at 12,100 g for 25 min in a Sorvall RC-2B centrifuge at 0°C (DuPont Instruments-Sorvall, Du Pont Co., Newton, Conn.). The precipitate was discarded and the supernatant fraction was centrifuged at 105,000 g for 1 h in a Beckman L5-65 preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The resulting pellet was then resuspended in 1.15% KCl with a Dounce homogenizer (Kontes Co., Vineland, N.J.) and recentrifuged at 105,000 g for 1 h. The washed, 105,000-g pellet was resuspended in 0.1 M potassium phosphate, pH 7.0. Protein determinations were made by the method of Lowry et al. using bovine serum albumin as standard (23).

**Difference Spectra of the 105,000-g Pellet**

Difference spectra of the 105,000-g pellet preparations were measured in an Aminco Chance DW-2 spectrophotometer (American Instrument Co., Traverse Laboratories Inc., Silver Spring, Md.) using cuvettes of 1-cm optical path. The pellets from either untreated or DMSO-treated cells were placed in both the sample and reference compartments. After recording the baseline, NADH was added to the sample cuvette and the difference spectrum was recorded. The amount of cytochrome $b_6$ present was calculated from the reduced minus oxidized difference spectrum using $\Delta\varepsilon_{max}(424-409 \text{ nm}) = 185$ according to

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1 This procedure suggested by Dr. S. Orkin.
NADH Cytochrome c Reductase Assay

The NADH cytochrome c reductase activity of the 105,000-g pellet was assayed by measuring the rate of reduction of cytochrome c (horse heart type III; Sigma Chemical Co., St. Louis, Mo.) using NADH (24). A value of 21.0 for the change in the millimolar extinction coefficient at 550 nm for ferrocytochrome c was used in the calculation of specific activity (25). The reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM KCN (to inhibit contaminating mitochondria), 0.1 mM cytochrome c, microsomes (10 ml of 1 mg/ml solution), and water to a total vol of 1 ml. The reaction was started by the addition of NADH and the absorbance changes at 550 nm were recorded at 30°C. In experiments where anti-cytochrome bs- y-globulin was added, all components (minus NADH) were pre-incubated for 20 min at 30°C with the y-globulin and the reaction was then started with the addition of NADH. The rabbit anti-cytochrome bs- y-globulin (a generous gift from Dr. T. Omura and Dr. G. Mannering) had been prepared against trypsin-solubilized rat liver microsomes cytochrome b, which showed a single band on acrylamide gel electrophoresis.

Isolation of Soluble Cytochrome bs from Mouse Erythrocytes

Soluble cytochrome bs was isolated from mouse erythrocytes by a modification of the procedure used to isolate soluble cytochrome bs from the supernatant fraction of bovine erythrocytes (6). The isolation procedure was carried out at 4°C using deionized-distilled water throughout. Mouse whole blood (Type I, fresh, in citrate) was obtained from Pel Freez Biologicals Inc., Rogers, Ariz. The blood was centrifuged at 3,000 g for 10 min. The supernatant fraction and leukocytes (along with some erythrocytes) were removed by aspiration. The packed cells were washed in 0.9% NaCl and centrifuged at 3,000 g for 10 min. The resulting packed erythrocytes (4.5 ml) were then lysed with 3 vol of water. The hemolysate was stored at -70°C. The hemolysate was thawed, the pH was adjusted to 6.0, and the stromal fraction was removed by centrifugation at 12,100 g for 30 min. The supernatant fraction was diluted with 2 vol of water and applied to a 0.5 x 5-cm DEAE-cellulose column which had been previously equilibrated with 0.003 M potassium phosphate buffer, pH 7.2. After a wash with the same buffer, the column was eluted sequentially with 0.01 M KH2PO4, 0.05 M KH2PO4, and finally 0.2 M KH2PO4. Absolute and difference spectra of the isolated cytochrome bs were recorded with a Cary 14 spectrophotometer (Cary Instruments, Fairfield, N.J.).

Attempted Isolation of Soluble Cytochrome bs from the 105,000-g Supernatant Fraction of T-3-CI-2 Cell Homogenate

The 105,000-g supernatant fraction of the erythroleukemia cell homogenate was diluted with 3 vol of water and then subjected to the same column chromatographic procedure that was used for the isolation of soluble cytochrome bs from mouse erythrocytes. Absolute and difference spectra of the eluted fractions were recorded with a Cary 14 spectrophotometer.

RESULTS

Morphology of Untreated and DMSO-Treated Murine Friend Erythroleukemia Cells, Clonal Line T-3-CI-2

Fig. 1 shows electron micrographs of both untreated and DMSO-treated T-3-CI-2 cells harvested after 96 h of growth. The untreated T-3-CI-2 cell (Fig. 1A) shows a subcellular pattern similar to that of very early erythroid cells (proerythroblast to basophilic erythroblast), as has been previously reported (35). The cell has a high nuclear-to-cytoplasmic ratio, numerous mitochondria, and a very electron-dense cytoplasm due to the presence of large numbers of ribosomes. Rough endoplasmic reticulum is present in small amounts.

The DMSO-treated cell (Fig. 1B) has a subcellular pattern similar to that of the late erythroblast cells (polychromatophilic to orthochromatophilic erythroblast). The cell still possesses ribosomes, mitochondria, and small amounts of rough endoplasmic reticulum, but it has a lower nuclear-to-cytoplasmic ratio. One prominent difference between the untreated and the DMSO-treated cell is that the latter cell exhibits the presence of complex vacuolar structures containing numerous virus particles. Also, an increase in the numbers of budding viruses is seen. These changes are characteristic of erythroleukemia cells induced to differentiate by DMSO (11, 12, 35). Fig. 1C shows a portion of a DMSO-treated cell which shows several budding viruses.

Detection of Membrane-bound Cytochrome bs and Cytochrome P-450

We obtained a 105,000-g pellet from both untreated and DMSO-treated erythroleukemia cells.
FIGURE 1  Electron micrographs of T-3-C1-2 erythroleukemia cells. (A) An untreated cell after 96 h of growth. The cell has a large nucleus (N), numerous mitochondria (M), and traces of rough endoplasmic reticulum (arrow head). Bar, 0.5 μm. × 30,000. (B) A T-3-C1-2 cell after 96 h of DMSO treatment. The cell retains a nucleus (N), mitochondria (M), and low levels of rough endoplasmic reticulum (arrow head). Complex vacuolar structures (CVS) containing numerous virus particles are seen frequently. An increase in the number of budding viruses is also seen. Bar, 0.5 μm. × 30,000. (C) A portion of a T-3-C1-2 cell after 96 h of DMSO treatment which shows several budding viruses. Bar, 0.5 μm. × 45,000.

The yield of protein in the 105,000-g pellet was 8.5 mg/10⁹ cells for untreated cells and 5.0 mg/10⁹ cells for DMSO-treated cells. The visible spectral properties of the 105,000-g pellet from the erythroleukemia cells are summarized in Table I. The NADH reduced minus oxidized difference spectrum (Fig. 2) shows absorbance maxima at 426, 526, and 559 nm. The spectrum corresponds to that of mouse liver microsomal cytochrome b₅ observed in this study and to the spectrum of microsomal cytochrome b₅ of other tissues. A similar NADH reduced minus oxidized spectrum was
Comparison of Visible Spectral Properties of the 105,000-g Pellet from Erythroleukemia Cell with Liver Microsomal Cytochrome b5, Liver Microsomal Cytochrome P-450, and Erythrocyte Soluble Cytochrome b5

<table>
<thead>
<tr>
<th></th>
<th>Reduced minus oxidized</th>
<th>Reduced CO-complex minus reduced*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet (105,000 g) from untreated T-3-C1-2 cells</td>
<td>426, 526, 559$</td>
<td>451</td>
</tr>
<tr>
<td>Pellet (105,000 g) from DMSO-treated T-3-C1-2 cells</td>
<td>427, 526, 559$</td>
<td>419, 535, 571</td>
</tr>
<tr>
<td>Mouse liver microsomal cytochrome b5</td>
<td>426, 528, 559$</td>
<td>none</td>
</tr>
<tr>
<td>Mouse liver microsomal cytochrome P-450</td>
<td>—</td>
<td>451</td>
</tr>
<tr>
<td>Mouse erythrocyte soluble cytochrome b5</td>
<td>424, 525, 558*</td>
<td>—</td>
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</tbody>
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* Reduced with sodium dithionite.
‡ Reduced with NADH.

Figure 2. The NADH reduced minus oxidized difference spectrum of the 105,000-g pellet obtained from T-3-C1-2 erythroleukemia cells. The sample and reference cuvettes contained pellet (7 mg protein/ml) suspended in 0.1 M phosphate buffer, pH 7.0. NADH was added to the sample cuvette and the spectral difference measured. Curve 1 was recorded with a full scale absorbance of 0.05 (scale on the right). Curve 2 was recorded with a full scale absorbance of 0.1 (scale on the left). Curve 3 represents the recorded oxidized vs. oxidized baseline. Absorbance maxima observed with the 105,000-g pellet from the DMSO-treated cells.

The reduced CO-complex minus reduced difference spectrum of the 105,000-g pellet from untreated T-3-C1-2 cells (Fig. 3) shows an absorbance maximum at 451 nm which is typical of the spectrum of cytochrome P-450, the CO-inhibited hydroxylase of microsomes. The reduced CO-complex minus reduced difference spectrum of the pellet from the DMSO-treated cells corresponded to that of carbonmonoxymeglobin minus deoxymeglobin. This indicates that the pellet had not been washed completely free of the hemoglobin synthesized by T-3-C1-2 cells as a response to the DMSO treatment. No peak or shoulder at 450 nm could be seen. However, we cannot determine from this spectrum whether cytochrome P-450 was absent in the 105,000-g pellet of DMSO-treated cells or whether the spectrum of cytochrome P-450 was masked by the contaminating hemoglobin.

The quantitation of cytochrome b5 and cytochrome P-450 in these cells is summarized in Table II. The amounts of cytochrome b5 in the untreated and DMSO-treated cells are similar (0.014 and 0.016 nmol/mg protein, respectively). These amounts (expressed either on the basis of number of cells or milligrams of pelleted protein) are small compared to the amount of microsomal cytochrome b5 in mouse liver. The amount of membrane-bound cytochrome P-450 in the erythroleukemia cell is likewise small relative to the amount of microsomal cytochrome P-450 in the liver cell. The ratio of cytochrome b5 to cytochrome P-450 in erythroleukemia cells is comparable to that of mouse liver microsomes.

Figure 3. The reduced CO-complex minus reduced difference spectrum of the 105,000-g pellet obtained from untreated T-3-C1-2 erythroleukemia cells. The sample and reference cuvettes contained pellet (7 mg protein/ml) suspended in 0.1 M phosphate buffer, pH 7.0. Carbon monoxide was bubbled through the sample cuvette and then sodium dithionite was added to both sample and reference cuvettes.
TABLE II

Amounts of Membrane-Bound Redox Proteins in Mouse Erythroleukemia Cells

<table>
<thead>
<tr>
<th>Source</th>
<th>Pellet from untreated T-3-CI-2 cells</th>
<th>Pellet from DMSO-treated T-3-CI-2 cells</th>
<th>Mouse liver microsomes*</th>
<th>Mouse erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/10^9 cells)</td>
<td>8.5</td>
<td>5.0</td>
<td>93.5</td>
<td>none</td>
</tr>
<tr>
<td>Cytochrome b&lt;sub&gt;5&lt;/sub&gt; (nmol/10^9 cells)</td>
<td>0.12</td>
<td>0.08</td>
<td>50.5</td>
<td>0.04</td>
</tr>
<tr>
<td>(nmol/mg protein)</td>
<td>0.014</td>
<td>0.016</td>
<td>0.54</td>
<td>-</td>
</tr>
<tr>
<td>Cytochrome P-450 (nmol/10^9 cells)</td>
<td>0.22</td>
<td>-</td>
<td>150</td>
<td>none</td>
</tr>
<tr>
<td>(nmol/mg protein)</td>
<td>0.025</td>
<td>-</td>
<td>1.60</td>
<td>none</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase activity (µmol/min/10^9 cells)</td>
<td>0.70</td>
<td>0.75</td>
<td>97</td>
<td>-</td>
</tr>
<tr>
<td>(µmol/min/mg protein)</td>
<td>0.082</td>
<td>0.15</td>
<td>1.04</td>
<td>-</td>
</tr>
<tr>
<td>(µmol/min/10^9 cells in the presence of anti-cytochrome b&lt;sub&gt;5&lt;/sub&gt; γ-globulin)</td>
<td>0.16</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(µmol/min/mg protein in the presence of anti-cytochrome b&lt;sub&gt;5&lt;/sub&gt; γ-globulin)</td>
<td>0.019</td>
<td>0.029</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* From the unpublished data of Dr. Kostas Vatsis, Department of Biochemistry, The University of Michigan. The values per 10^9 cells were calculated using the value of 15.9 mg microsomal protein/g of liver and the reported value of 1.7 × 10^8 hepatocytes/g of liver (40).

Evidence for Membrane-bound Cytochrome b<sub>5</sub> Reductase

The 105,000-g pellets from both untreated and DMSO-treated cells were shown to possess NADH-cytochrome c reductase activity (Table II). Strittmatter and Velick (38) have shown that in liver microsomes this activity is dependent upon both cytochrome b<sub>5</sub> reductase and cytochrome b<sub>5</sub>, and thus can be used as evidence that both proteins are present. Further evidence for the presence of cytochrome b<sub>5</sub> in the pellets from untreated and DMSO-treated erythroleukemia cells was provided by demonstration of inhibition of the NADH-cytochrome c reductase activities with anti-cytochrome b<sub>5</sub> γ-globulin (Table II). Approx. 80% inhibition of the reductase activity of these pellets was observed when 40 mg of anti-cytochrome b<sub>5</sub> immunoglobulin were used/mg of protein. At this concentration the same immunoglobulin preparation showed 80% inhibition of the reductase activity using solubilized liver microsomal proteins. 3

Cytosolic Cytochrome b<sub>5</sub>: Its Presence in Mouse Erythrocytes and Its Absence in Mouse Erythroleukemia Cells

Soluble cytochrome b<sub>5</sub> was detected and isolated for the first time from mouse erythrocytes. Fig. 4 shows the DEAE-cellulose elution profile for a preparation of soluble cytochrome b<sub>5</sub> derived from 4.5 ml of packed erythrocytes from mouse blood. The oxidized, reduced, and reduced minus oxidized spectra are similar to the spectra of soluble cytochrome b<sub>5</sub> from human, bovine, and rabbit erythrocytes (6, 31). The reduced minus oxidized difference spectrum (Fig. 5) shows absorbance maxima at 424, 525, and 558 nm. The amount of soluble cytochrome b<sub>5</sub> calculated from this spectrum was 0.87 nmol/ml of packed erythrocytes or 0.04 nmol/10^9 erythrocytes.

When this procedure was carried out on the 105,000-g supernatant fraction from homogenates of untreated and DMSO-treated erythroleukemia cells, no soluble cytochrome b<sub>5</sub> was detected. This finding indicates an absence or very low levels of cytochrome b<sub>5</sub> in the cytosol of these cells even after DMSO treatment. Oxyhemoglobin was spectrally identified in the initial fractions from

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3 Personal communication from Dr. S. Kuwahara and Dr. R. Schwen of the laboratory of Dr. G. Mannering.
FIGURE 4 Partial purification of mouse erythrocyte cytochrome \( b_5 \) by chromatography on DEAE-cellulose. The diluted, stroma-free mouse erythrocyte hemolysate was applied to a 0.5 × 5-cm DEAE-cellulose column, and the column was then eluted sequentially with 10 mM KH₂PO₄, 10 mM KCl, 50 mM KH₂PO₄, and 0.2 M KH₂PO₄ as described in the text. The arrow on the left denotes the beginning of the 50 mM KH₂PO₄ wash and the arrow on the right the beginning of the 0.2 KH₂PO₄ wash.

FIGURE 5 Reduced minus oxidized difference spectrum of mouse erythrocyte cytochrome \( b_5 \). Fractions 8, 9, and 10 from the DEAE-cellulose column (Fig. 4) were pooled and added to the sample and reference cuvettes. The material in the sample cuvette was reduced by the addition of sodium dithionite.

DEAE-cellulose chromatography of the 105,000-g supernatant fraction of homogenates from DMSO-treated T-3-C1-2 cells. This finding suggests that, in the erythroleukemia cell, hemoglobin is present in its ferrous form.

DISCUSSION

We have isolated a 105,000-g pellet from Friend virus-induced erythroleukemia cells and have demonstrated that the pellet contains cytochrome \( b_5 \), cytochrome P-450, and NADH-cytochrome \( b_5 \) reductase activity. This is the first report of the presence of membrane-bound forms of these proteins in erythroid cells. The levels of these proteins in erythroleukemia cells are low. Nonetheless, there is no doubt that they arose from these cells, because there were no suggestions that contaminating cells were present in this culture.

Cytochrome \( b_5 \), cytochrome P-450, and cytochrome \( b_5 \) reductase have been shown to be major constituents of microsomes from kidney, lung, and other tissues (13). Our detection of these proteins in the 105,000-g pellet might suggest that we have isolated microsomes from erythroleukemia cells. The small amounts of these membrane-bound redox proteins in erythroleukemia cells, relative to hepatocytes, would be in keeping with the relative amounts of endoplasmic reticulum which can be observed by electron microscopy within these cells.

However, our data do not allow us to establish whether these redox proteins are derived from endoplasmic reticulum rather than from contaminating mitochondrial, nuclear, or other membrane fractions. Both erythroleukemia cells and normal erythroid cells, at various stages of maturation, have been shown by electron microscopy to possess large nuclei and numerous mitochondria, in addition to small amounts of endoplasmic reticulum (1, 28, 35). Peroxisomal and outer mitochondrial membranes (but not inner mitochondrial membranes) of rat liver have been shown to possess cytochrome \( b_5 \) (7, 32, 37), and mitochondria from adrenal glands contain substantial amounts of cytochrome P-450 (4). Moreover, cytochrome P-450, NADPH-cytochrome \( c \) reductase activity, cytochrome \( b_5 \), NADH-cytochrome \( c \) reductase activity, or combinations of these redox proteins have been detected in nuclear membrane preparations from the livers of a variety of species (2, 8, 19, 41).

In this paper, we also report the isolation of soluble cytochrome \( b_5 \) from the cytoplasmic fraction of mouse erythrocytes. Soluble cytochrome \( b_5 \) had been isolated previously from beef (6), human (14, 16, 31), rabbit (31), and pork (5) erythrocytes. In contrast to the detection of soluble cytochrome \( b_5 \) in the mature erythrocytes of the mouse, no soluble cytochrome \( b_5 \) could be detected in the cytoplasmic fraction of mouse erythroleukemia cells.
The Friend virus-induced erythroleukemia cell serves as a model for early immature cells of the erythroid series. Morphologically, this cell has many of the characteristics of immature erythroblasts. Approx. 1% of the erythroleukemia cells spontaneously differentiate to the level of polychromatophilic and orthochromatophilic erythroblasts and synthesize hemoglobin (11, 12). Upon treatment with DMSO, Friend virus-transformed cells exhibit changes that are analogous to those seen in the differentiation of normal erythrocyte precursors. The polychromatophilic- and orthochromatophilic-like cells that result from DMSO stimulation (35) develop erythrocyte membrane antigen (17), accumulate mRNA for globin synthesis (34), and synthesize globin chains indistinguishable from those of the adult mouse (3, 29).

It is our contention that the soluble cytochrome b₅ and cytochrome b₅ reductase present in the cytoplasm of mature erythrocytes are derived from membraneous structures (endoplasmic reticulum, mitochondria, or nuclei) of immature erythrocytes by proteolysis during the maturation process. The endoplasmic reticulum and nuclei, along with most of the other subcellular organelles (excluding mitochondria), have disappeared from the cell by the late orthochromatophilic erythroblast stage. The finding that the amount of cytochrome b₅ is present in normal, early immature, erythroleukemia cell suggests that only particulate cytochrome b₅ is present in normal, early immature, erythrocyte membrane-bound cytochrome b₅ and cytochrome b₅ reductase were detected in the 105,000-g supernatant fraction. Apparently, DMSO-induced differentiation of erythroleukemia cells to the polychromatophilic or orthochromatophilic erythroblast stage does not result in the solubilization of cytochrome b₅. The exact stage at which solubilization of membrane-bound cytochrome b₅ occurs during normal erythroid maturation remains to be elucidated.

The authors would like to thank Dr. Raymond Ruddon for making his laboratory available for the growth and maintenance of the erythroleukemia cell line, Dr. Allen Lau for showing us how to grow and maintain these cells, Dr. Robert Gray for performing the electron microscopy, and Dr. Kostas Vatsis for his interest and input into this work.

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