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

SHELLEY R. SLAUGHTER and DONALD E. HULTQUIST

From the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

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 b₅ and cytochrome P-450, heme proteins 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 b₅ γ-globulin, indicating the presence of cytochrome b₅ 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 b₅ and NADH-cytochrome c reductase activity. We failed to detect soluble cytochrome b₅ 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 b₅ but no membrane-bound form of this protein. These findings are supportive of our hypothesis that soluble cytochrome b₅ of erythrocytes is derived from endoplasmic reticulum or some other membrane structure of immature erythroid cells during cell maturation.

KEY WORDS  erythroleukemia  -  cytochrome b₅  -  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 b₅, a protein found in reticulocytes and mature erythrocytes. In non-nucleated erythroid cells, cytochrome b₅ (16, 31) and cytochrome b₅ reductase (16, 20, 30, 39) exist in the cytoplasm as soluble molecules. Erythrocyte cytochrome b₅ is similar to cytochrome b₅ solubilized from microsomes of the liver. The amino acid composition of bovine erythrocyte cytochrome b₅ I is in very good agreement with a segment (residues 1-97) of bovine liver microsomal cytochrome b₅ (6). Tryptic digests
of bovine erythrocyte cytochrome $b_5$ and bovine liver microsomal cytochrome $b_6$ yield core heme-peptides 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 $b_5$ 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 $b_5$ 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.). Inocula containing 10$^4$ cells/ml of medium were placed in one 35-mm plastic flask containing 3 ml of 0.22-$\mu$m Millipore filter (Millipore Corp., Bedford, Mass.). All cultures were initiated at a concentration of 3 $\times$ 10$^5$ cells/ml of medium in a total vol of 200 ml. The cultures were maintained in one 1 Corning plastic flasks (Corning Glass Works, Science Products Div., Corning, N.Y.). The flasks were briefly gassed with 5% CO$_2$−95% air and incubated at 37°C. When cells were treated with DMSO, the cultures were initiated in the same way except for the addition of DMSO to a final concentration of 3%. All cells were incubated for 96 h. Cells were harvested by centrifugation at 600 g for 10 min. Pellets were washed with 0.9% NaCl and frozen at −70°C.

Cell counts were performed in hemocytometers with 0.9% NaCl as diluent. Cell viability was determined by Erythrosin B dye exclusion. Hemoglobin was stained as follows: $^1$ Immediately before use, 5.0 ml of H$_2$O and 0.5 ml of 30% H$_2$O$_2$ were combined with 1.0 ml stock benzidine solution (3% benzidine in 90% acetic acid). Two drops of stain were added to 0.2 ml of cell suspension. After 15 min, the number of benzidine-positive cells were counted in a hemocytometer.

**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., Travenol 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 $b_5$ present was calculated from the reduced minus oxidized difference spectrum using $\Delta\varepsilon_{434-409}$ = 185 according to

$^1$ This procedure suggested by Dr. S. Orkin.
Omura and Sato (26). To obtain the reduced CO-complex minus reduced difference spectrum, CO was bubbled through the sample cuvette and then solid sodium dithionite was added to both sample and reference cuvettes. The content of cytochrome P-450 was determined from the reduced CO-complex minus reduced difference spectrum using $\Delta$E$_{nm}$ (450–490 nm) = 91 (26).

**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 μl 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 b$_5$-γ-globulin was added, all components (minus NADH) were pre-incubated for 20 min at 30°C with the γ-globulin and the reaction was then started with the addition of NADH. The rabbit anti-cytochrome b$_5$-γ-globulin (a generous gift from Dr. T. Omura and Dr. G. Mannering) had been prepared against trypsin-solubilized rat liver microsomes, which showed a single band on acrylamide gel electrophoresis.

**Isolation of Soluble Cytochrome b$_5$ from Mouse Erythrocytes**

Soluble cytochrome b$_5$ was isolated from mouse erythrocytes by a modification of the procedure used to isolate soluble cytochrome b$_5$ 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 × 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 KH$_2$PO$_4$, 0.05 M KH$_2$PO$_4$, and finally 0.2 M KH$_2$PO$_4$. Absolute and difference spectra of the isolated cytochrome b$_5$ were recorded with a Cary 14 spectrophotometer (Cary Instruments, Fairfield, N.J.).

**Attempted Isolation of Soluble Cytochrome b$_5$ 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 b$_5$ 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 b$_5$ and Cytochrome P-450**

We obtained a 105,000-g pellet from both untreated and DMSO-treated erythroleukemia cells.
The yield of protein in the 105,000-g pellet was 8.5 mg/10^9 cells for untreated cells and 5.0 mg/10^9 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

| Component                                      | Reduced minus oxidized | Reduced CO-complex minus oxidized |
|------------------------------------------------|------------------------|-----------------------------------|
| Pellet (105,000 g) from untreated T-3-C₁-2 cells | 426, 526, 559*         | 451                               |
| Pellet (105,000 g) from DMSO-treated T-3-C₁-2 cells | 427, 526, 559*         | 419, 535, 571                     |
| Mouse liver microsomal cytochrome b₅           | 426, 528, 559*         | none                              |
| Mouse liver microsomal cytochrome P-450        | —                      | 451                               |
| Mouse erythrocyte soluble cytochrome b₅        | 424, 525, 558*         | —                                 |

* 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.

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 deoxymoglobin. 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

| Source                  | Pellet from untreated T-3CI-2 cells | Pellet from DMSO-treated T-3CI-2 cells | Mouse liver microsomes* | Mouse erythrocytes |
|-------------------------|-------------------------------------|----------------------------------------|-------------------------|---------------------|
| Total protein (mg/10⁹ cells) | 8.5                                 | 5.0                                    | 93.5                    | none                |
| Cytochrome b₅ (nmol/10⁹ cells) | 0.12                                | 0.08                                   | 50.5                    | 0.04                |
| (nmol/mg protein)       | 0.016                               | 0.016                                  | 0.54                    | —                   |
| Cytochrome P-450 (nmol/10⁹ cells) | 0.22                                | —                                      | 150                     | none                |
| (nmol/mg protein)       | 0.025                               | —                                      | 1.60                    | none                |
| NADH-cytochrome c reductase activity (µmol/min/10⁹ cells) | 0.70                                 | 0.75                                   | 97                      | —                   |
| (µmol/min/mg protein)   | 0.082                               | 0.15                                   | 1.04                    | —                   |
| (µmol/min/10⁹ cells in the presence of anti-cytochrome b₅ γ-globulin) | 0.16                                 | 0.15                                   | —                       | —                   |
| (µmol/min/mg protein in the presence of anti-cytochrome b₅ γ-globulin) | 0.019                               | 0.029                                  | —                       | —                   |

* From the unpublished data of Dr. Kostas Vatsis, Department of Biochemistry, The University of Michigan. The values per 10⁹ cells were calculated using the value of 15.9 mg microsomal protein/g of liver and the reported value of 1.7 × 10⁹ hepatocytes/g of liver (40).

Evidence for Membrane-bound Cytochrome b₅ 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 b₅ reductase and cytochrome b₅, and thus can be used as evidence that both proteins are present. Further evidence for the presence of cytochrome b₅ 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 b₅ γ-globulin (Table II). Approx. 80% inhibition of the reductase activity of these pellets was observed when 40 mg of anti-cytochrome b₅ 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.²

Cytosolic Cytochrome b₅: Its Presence in Mouse Erythrocytes and Its Absence in Mouse Erythroleukemia Cells

Soluble cytochrome b₅ 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 b₅ 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 b₅ 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 b₅ calculated from this spectrum was 0.87 nmol/ml of packed erythrocytes or 0.04 nmol/10⁹ 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 b₅ was detected. This finding indicates an absence or very low levels of cytochrome b₅ in the cytosol of these cells even after DMSO treatment. Oxyhemoglobin was spectrally identified in the initial fractions from
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 x 5-cm DEAE-cellulose column, and the column was then eluted sequentially with 10 mM KH$_2$PO$_4$, 10 mM KCl, 50 mM KH$_2$PO$_4$, and 0.2 M KH$_2$PO$_4$ as described in the text. The arrow on the left denotes the beginning of the 50 mM KH$_2$PO$_4$ wash and the arrow on the right the beginning of the 0.2 KH$_2$PO$_4$ wash.

**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.

S. R. SLAUGHTER AND D. E. HULTQUIST  *Redox Proteins of Erythroleukemia Cells* 237
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 \( b_5 \) and cytochrome \( b_5 \) reductase present in the cytoplasm of mature erythrocytes are derived from membraneous structures (endoplasmic reticulum, mitochondria, or nuclei) of immature erythroid cells by proteolysis during the maturation process. The endoplasmic reticulum and nuclei, along with many of the other subcellular organelles (excluding mitochondria), have disappeared from the cell by the late orthochromatophilic erythroblast or reticulocyte stage. Because the erythroleukemia cell is a model for a normal immature erythroid cell, our demonstration of membrane-bound cytochrome \( b_5 \) but no soluble cytochrome \( b_5 \) in the erythroleukemia cell suggests that only particulate cytochrome \( b_5 \) is present in normal, early immature, erythroid cells. The finding that the amount of membrane-bound cytochrome \( b_5 \) present in mouse erythroleukemia cells is greater than the amount of soluble cytochrome \( b_5 \) present in the cytoplasm of mouse erythrocytes is compatible with membraneous structures being the origin of the soluble cytochrome \( b_5 \).

As has been reported by other workers, we found that DMSO caused the erythroleukemia cells to differentiate, as evidenced by positive benzidine staining of the cells, the red color of the cell pellet, the spectral demonstration of hemoglobin, and the orthochromatophilic-like morphological features of the cells. Electron microscopy of these DMSO-treated cells revealed small amounts of endoplasmic reticulum to be still present. Cytochrome \( b_5 \) and cytochrome \( b_5 \) 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 \( b_5 \). The exact stage at which solubilization of membrane-bound cytochrome \( b_5 \) 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.

This work was supported by American Cancer Society Institutional Grants, an Institutional Equal Opportunity Award, United States Public Health Service Research Grant AM-09250, and United States Public Health Service Training Grant GM-00187.

Received for publication 4 May 1978, and in revised form 4 June 1979.

REFERENCES

1. ACKERMAN, G. A., J. A. GRASSO, and R. A. KNIGHT. 1963. Erythropoiesis in the mammalian embryonic liver as revealed by electron microscopy. Lab. Invest. 10:787-796.

2. BERZENTY, R., L. K. MACILLAY, and F. L. CRANI. 1972. The purification and biochemical characterization of bovine liver nuclear membranes. J. Biol. Chem. 247:5549-5561.

3. BOYER, S. H., K. O. WU, A. N. NOYES, W. SCHER, C. FRIEND, H. D. PREISLER, and A. BANK. 1972. Hemoglobin biosynthesis in murine virus-induced leukemic cells in vitro: Structure and amounts of globin chains produced. Blood 40:823-835.

4. BROWNIE, A. C., E. R. SIMPSON, F. R. SKEELTON, W. B. ELLIOTT, and R. W. ETTABBOUR. 1970. Interaction of androgens with the adrenal mitochondrial cytochrome system. Arch. Biochem. Biophys. 141:18-25.

5. CAMPUS, S. 1977. The erythrocyte cytochrome \( b_5 \). Physiol. (Bucharest) 19:85-87.

6. DOUGLAS, R. H., and D. E. HULQUIST. 1978. Evidence that the two forms of bovine erythrocyte cytochrome \( b_5 \) are identical to segments of microsomal cytochrome \( b_5 \). Proc. Natl. Acad. Sci. U.S.A. 75:3118-3122.

7. FOWLER, D. J., R. BAUMANN, P. TRAGT, H. BEAUFAY, F. J. BERTHET, M. WIBO, and P. HAUSER. 1976. Analytical study of microsomes and isolated subcellular membranes from rat liver. V. Immunological localization of cytochrome \( b_5 \) by electron microscopy. Methodology and application to various subcellular fractions. J. Cell Biol. 71:353-359.

8. FRANZ, W. R., D. H. DEVLIN, B. ESMEN, E. D. JANUSCH, and H. KLINGO. 1976. Nuclear membranes from mammalian liver: Isolation procedure and general characterization. J. Cell Biol. 71:379-395.

9. FRASER, J. M., and Y. R. PARKS. 1965. A routine technique for double staining ultrathin sections using uranyl and lead salts. J. Cell Biol. 28:157-161.

10. FRIEND, C., C. M. PULS, and E. DEHAVAN. 1966. Erythrocyte maturation in vivo of murine (Friend) virus-induced leukemic cells. Natl. Cancer Inst. Monogr. 22:505-520.

11. FRIEND, C., H. D. PREISLER, and W. SCHER. 1974. Studies on the control of differentiation of murine virus-induced erythroleukemic cells. Curr. Top. Dev. Biol. 8:101-111.

12. FRIEND, C., and W. SCHER. 1975. Cytosol b5: formation and intracellular transport in hemoglobin synthesis in murine virus-induced leukemia. J. Cell Biol. 24:153-163.

13. GARTENBACH, D. 1963. Determination of hemoglobin in human microsomes. Comp. Biochem. Physiol. 5:367-379.

14. HULQUIST, D. E., R. T. DEAN, and R. H. DOUGLAS. 1974. Hostoge-
neous cytochrome b; from human erythrocytes. Biochim. Biophys. Res. Commun. 60:28-34.

15. HULTQUIST, D. E., R. H. DOUGLAS, and R. T. DEAN. 1975. The methemoglobin reduction system of erythrocytes. In Proceedings of the Third International Conference on Red Cell Metabolism and Function. G. Brewer, editor. Alan R. Liss, Inc., New York. 297-300.

16. HULTQUIST, D. E., D. W. REED, and P. G. PASSENGER. 1969. Isolation, characterization, and enzymatic reduction of cytochrome b(556) from human erythrocytes. Fed. Proc. 28:682.

17. IZAKA, Y., M. FURUSAWA, and H. SUGANO. 1973. Erythrocyte membrane-specific antigens in Friend virus-induced leukemia cells. Bibl. Haematol. 39:955-967.

18. IZAWA, Y., J. ROSS, P. LEDER, J. GIELEN, S. PACKMAN, P. EBERT, K. HAYASHI, and H. SUGANO. 1974. Erythroid differentiation of cultured Friend leukemia cells. In Differentiation and Control of Malignancy of Tumor Cells. W. Nakahara, T. ORO, T. SUGIMURA, and H. SUGANO, editors. University Park Press, Baltimore, Md., 515-546.

19. KASPER, C. B. 1971. Biochemical distinctions between the nuclear and microsomal membranes from rat hepatocytes. The effect of phenobarbital administration. J. Biol. Chem. 246:577-581.

20. KUMA, F., and H. INOMATA. 1972. Studies on methemoglobin reductase. J. BioL Chem. 247:556-560.

21. LAU, A. F., and R. W. RUDDON. 1977. Proteins of transcriptionally active and inactive chromatin from Friend erythroleukemic cells. Exp. Cell Res. 107:35-6.

22. LEVINE, E. M. 1972. Mycoplasma contamination of animal cell cultures: A simple, rapid detection method. Exp. Cell Res. 74:99-109.

23. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

24. MACKLER, B. 1967. Microsomal DPNH-cytochrome c reductase. Methods Enzymol. 10:551-553.

25. MASSEY, V. 1959. The microestimation of succinate and the extinction coefficient of cytochrome c. Biochim. Biophys. Acta. 34:255-256.

26. OMURA, T., and R. SATO. 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its heme protein nature. J. Biol. Chem. 239:270-279.

27. ORLEN, S. H., F. J. HAKOH, and P. LEDER. 1975. Differentiation in erythroleukemic cells and their somatic hybrids. Proc. Natl. Acad. Sci. U.S.A. 72:109-112.

28. ORLEF. D. 1970. Ultrastructural analysis of erythropoiesis. In Regulation of Hematopoiesis. A. Gordon, editor. Appleton-Century-Crofts, New York. 271-296.

29. OSTERBERG, W.-H. MELDERS, G. SEITZ, N. KLEGEN, and S. DURE. 1972. Synthesis of mouse hemoglobin and globin mRNA in leukemic cell cultures. Nature New Biol. 239:531-534.

30. PAXON, P. G., and D. E. HULTQUIST. 1972. Soluble cytochrome b; reductase from human erythrocytes. Biochim. Biophys. Acta. 275:62-73.

31. PAXON, P. G., D. W. REED, and D. E. HULTQUIST. 1972. Soluble cytochrome b; from human erythrocytes. Biochim. Biophys. Acta. 275:51-61.

32. REMACI, J. 1978. Binding of cytochrome b; to membranes of isolated subcellular organelles from rat liver. J. Cell Biol. 79:291-313.

33. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

34. ROSS, J., Y. IZAWA, and P. LEDER. 1972. Globin messenger-RNA induction during erythroid differentiation of cultured leukemia cells. Proc. Natl. Acad. Sci. U.S.A. 69:3620-3623.

35. SATO, T., C. FRIEDEN, and E. DEHAVEN. 1971. Ultrastructural changes in Friend erythroleukemia cells treated with dimethylsulfoxide. Cancer Res. 31:1402-1417.

36. SLAUGHTER, S. R., and D. E. HULTQUIST. 1977. Demonstration of microsomal cytochrome b; cytochrome b; reductase, and cytochrome P-450 in Friend erythroleukemic cells. Fed. Proc. 36:928.

37. SOTTOCAVA, G., L. B. KYLENHEIMAN, L. ERFER, and A. BERG-STRAND. 1967. An electron-transport system associated with the outer membrane of liver mitochondria. A biochemical and morphological study. J. Cell Biol. 32:415-438.

38. STRITTMATTER, P., and S. VELICK. 1956. A microsomal cytochrome reductase specific for diphosphopyridine nucleotide. J. Biol. Chem. 221:277-286.

39. SUGITA, Y., S. NOMURA, AND Y. YONEYAMA. 1971. Purification of reduced pyridine nucleotide dehydrogenase from human erythrocytes and methemoglobin reduction by the enzyme. J. Biol. Chem. 246:6072-6078.

40. WEIBEL, E. R., W. STAUBLI, H. R. GNAGI, and F. A. HESS. 1969. Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereologic methods, and normal morphometric data for rat liver. J. Cell Biol. 42:68-91.

41. ZBANSKY, D. B., K. A. BERNOCHAIK, L. N. KLECKOVA, AND V. V. DELEKOVSKAYA, AND V. V. DELEKOVSKAYA. 1969. Isolation and biochemical characterization of the nuclear envelope. Nature (Land.). 228:257-259.