Myeloid Differentiation of Human Blood Mononuclear Cells in Liquid Culture

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Human blood mononuclear cells were prepared from peripheral blood or single donor platelethpheresis residues by depletion of adherent cells and T lymphocytes. In double-layer soft agar culture, 5 x 10^6 such cells yielded from 33 to 165 colonies. For liquid culture, these cells were suspended in McCoy's 5A medium with 15% fetal bovine serum and 0%, 20%, or 40% conditioned medium (CM) and incubated for up to 14 days at 37°C in a humidified 5% CO₂-95% air atmosphere. The number of cells in cultures with CM decreased about 0%-10%, while cell counts from cultures without CM decreased about 45%-65%. In cultures with CM, 5%-20% of the cells were classified as blasts after 3-5 days. After 7-11 days, blasts and promyelocytes comprised up to 53% of all cells. After 9-11 days, cells with specific granules and maturing nuclei comprised up to 56% of all cells. At 11 days, up to 66% of the cells contained peroxidase-positive granules. Cultures without CM contained no more than 5% blasts and promyelocytes and less than 5% maturing granulocytic cells. ³H-thymidine and Na₂³⁵SO₄ incorporation reached a peak at 3-5 days and at 5-11 days, respectively, in cells from cultures containing CM.

HEMATOPOIETIC progenitor cells can be identified by their capacity to produce clones of differentiated progeny in culture.¹² Those progenitor cells committed to granulocytic and macrophage differentiation (GM-CFC) will form eosinophil, neutrophil, and/or macrophage colonies in semisolid agar if provided with appropriate nutrient media, and a source of colony-stimulating factor.³ Such cultures contain cellular aggregates of greater than 40 maturing eosinophils, granulocytes, and/or macrophages following 10-14 days of incubation.¹⁴ This culture technique has proven extremely useful in the study of murine and human granulopoietic cells.³ However, technical problems with harvesting developing colonies in the semisolid agar system have made sequential biochemical analysis of the events of proliferation and differentiation very difficult.

We have therefore attempted to develop a system that permits proliferation and differentiation of hematopoietic progenitor cells in liquid culture. Murine and human granulopoietic progenitor cells recovered from bone marrow have been studied in a liquid culture system but the presence of immature granulocytic cells has made myeloid differentiation difficult to evaluate.⁸⁹

We have found that human peripheral blood mononuclear cells depleted of adherent cells and T lymphocytes proliferate and differentiate into granulocytic cells in liquid culture in nutrient medium containing conditioned medium obtained from adherent blood mononuclear cells incubated with 2-mercaptoethanol.

Proliferation and differentiation were assessed by sequential enumeration and morphological and cytochemical examination of aliquots removed from the cultures, and estimation of cellular incorporation of radioactively labeled thymidine and sulfate.

MATERIALS AND METHODS

Source of Stem Cells

Platelethpheresis residues were used to prepare concentrates of progenitor cells as previously reported.¹⁰ Peripheral blood (75-120 ml) was collected from normal donors in syringes rinsed with preservative-free heparin. Informed consent was obtained from each donor, and the research was carried out according to the Declaration of Helsinki. The Human Experimentation Committee of the Upstate Medical Center has approved this study.

Progenitor Cell Purification

Cells were processed using sterile techniques at room temperature unless otherwise noted. Cells obtained from platelethpheresis residues were sedimented on Ficoll-diatrizoate (Lymphocyte Separation Medium, LSM, Litton Bionetics, Inc.) as previously described.¹⁰ Peripheral blood collected from individual donors was mixed with equal volumes of Hanks balanced salt solution (HBSS), divided into 40-ml aliquots in 50-ml conical graduated centrifuge tubes, underlayered with 10 ml of LSM, and centrifuged at 400 g for 30 min.¹⁰¹¹ The cells at the interface were collected, combined in a 50-ml conical graduated centrifuge tube, and washed thrice with 50 ml HBSS.

Adherent Cell Depletion

The mononuclear cells recovered from platelethpheresis residue were depleted of adherent cells as previously reported.¹⁰ Mononuclear cells recovered from normal peripheral blood were resuspended in 15 ml of RPMI 1640 containing 20% fetal bovine serum. The cell suspension was depleted of adherent cells by adding aliquots(s) of 15 ml to each 150 x 15 mm plastic Petri dish and incubating for 1 hr at 37°C in a humidified atmosphere of 5% CO₂-95% air.¹² The nonadherent cells were decanted and read-

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herited. The nonadherent cells were again decanted, and hematopoietic precursor cells were further concentrated by depletion of T cells. The adherent cells were used to prepare conditioned media as outlined below.

**T-Cell Depletion**

A T-cell-depleted fraction was prepared from the nonadherent cells by a nonimmune rosette sedimentation technique. The nonadherent cells were collected by centrifugation at 150 g for 15 min and resuspended at a concentration of 15 x 10^6 cells/ml in Eagle's minimum essential medium (MEM) containing 3% of 1 M Hepes and 1% bovine serum albumin (BSA). Aliquots of this cell suspension were mixed with 15% v/v sheep red blood cells (SRBC). The nonadherent cells were again decanted, and hematoxylin-stained preparations were mixed with 15% v/v sheep red blood cells (SRBC). The nonadherent cells were collected by centrifugation at 150 g for 15 min and resuspended at a concentration of 10^6 cells/ml in McCoy's 5A medium with 15% fetal bovine serum and 0%, 20%, or 40% CM. Volumes of medium containing 10^6 cells were removed every 48–72 hr. The cell suspension was centrifuged at 1000 rpm for 1 min in a Sorvall CW-1 centrifuge at room temperature. The supernate was decanted and placed in 10-ml plastic tubes at 4°C. The pellet was resuspended in 3 ml of 0.05 M Ficoll-Hypaque and washed with 10 successive 3-ml volumes of 0.05 M NaHCO_3 in 0.15 M NaCl. The filters were allowed to dry overnight at room temperature. Each was then placed in a 20-ml glass vial with 10 ml of SintiVerse (Fisher Scientific) and the radioactivity measured on a Searle Mark III model 6880 liquid scintillation spectrometer (Searle Analytic Inc.).

**Conditioned Media (CM)**

The adherent cells were recovered by adding 5 ml of calcium- and magnesium-free HBSS to each Petri dish and gently removing the cells from the bottom of the dish using a rubber policeman. The adherent cells were combined and washed twice with 50 ml of HBSS. The final preparation was resuspended at 10^6 cells/ml in McCoy's 5A medium containing 15% fetal bovine serum and 0.5 mM 2-mercaptoethanol. Two-milliliter aliquots (2 x 10^6 cells) were placed in 35-mm Petri dishes and were incubated at 37°C in a 5% CO_2-95% humidified air atmosphere. After 7 days, the supernates were collected by decanting into a 50-ml conical graduated centrifuge tube. The tubes were centrifuged at 400 g for 30 min, and the supernates were decanted, filtered through a 0.45-μm Millipore filter, and stored at –20°C until use.

**Liquid Culture**

Mononuclear cells recovered following sedimentation on LSM, depletion of adherent cells, and depletion of T lymphocytes were employed in the liquid and double-layer agar culture assays. For liquid culture, 10 x 10^6 cells were suspended in 10 ml of McCoy's 5A medium containing 15% fetal bovine serum, 100 U/ml of penicillin, 50 μg/ml of streptomycin, and 0%, 20%, or 40% CM and placed in a loosely capped 25-ml plastic centrifuge tube. The tubes were centrifuged at 400 g for 30 min, and the supernates were decanted, filtered through a 0.45-μm Millipore filter, and stored at –20°C until use.

**Gel Filtration of Cell Extracts and Media**

The procedure was performed as described previously, using 9 x 10^6 cells and 9 ml of media recovered from cultures containing 40% CM and Na_2^35SO_4. Cell extracts and media were gel filtered on Sephadex G-200 before and after papain digestion.

**Incorporation of Radioactive Sulfate**

Na_2^35SO_4 was added at a concentration of 10 μCi/ml to 30-ml tissue culture flasks containing 10 x 10^6 mononuclear cells suspended in 10 ml McCoy's 5A medium with 15% fetal bovine serum and 0%, 20%, or 40% CM. Volumes of medium containing 10^6 cells were removed every 48–72 hr. The cell suspension was centrifuged at 1000 rpm for 1 min in a Sorvall CW-1 centrifuge at room temperature. The supernate was decanted and placed in 10-ml plastic tubes at 4°C. The pellet was resuspended in 3 ml of 0.05 M NH_4SO_4 in 0.15 M NaCl. The resultant cell suspension was placed on a Whatman GF/C Millipore filter and washed with 10 successive 3-ml volumes of 0.05 M NH_4SO_4 in 0.15 M NaCl. The filters were allowed to dry overnight at room temperature. Each was then placed in a 20-ml glass vial with 10 ml of SintiVerse (Fisher Scientific) and the radioactivity measured on a Searle Mark III model 6880 liquid scintillation spectrometer (Searle Analytic Inc.).

**Incorporation of Radioactive Thymidine**

Aliquots of 10^6 cells from liquid cultures with 0%, 20%, or 40% CM were removed every 48–72 hr. 3H-thymidine at a concentration of 1 μCi/ml (specific activity 18–25 μCi/mmole) was added, and the cell suspension was placed in a loosely capped 5-ml glass centrifuge tube and reincubated for 24 hr at 37°C in a humidified 5% CO_2-95% air atmosphere. Following incubation, the suspension was centrifuged at 1000 rpm for 1 min in a Sorvall CW-1 centrifuge at room temperature. The supernate was discarded and the pellet resuspended in 3 ml of 0.9% NaCl. The resultant cell suspension was collected on a Whatman GF/C Millipore filter and washed 10 times with 3-ml volumes of 0.9% NaCl. The filters were allowed to dry overnight, then placed in 20-ml glass vials with 10 ml of SintiVerse and the radioactivity measured on a Searle Mark III model 6880 liquid scintillation spectrometer for 10 min.

** Autoradiography**

Aliquots of medium containing 0.5–1 x 10^6 cells from cultures incubated with Na_2^35SO_4 or 3H-thymidine were centrifuged at 150 g for 15 min, the supernates were discarded, and the pellets resuspended in 0.25 ml of McCoy's 5A medium containing 15% fetal bovine serum. These cell suspensions were centrifuged onto glass slides in a Shandon cytocentrifuge, the resultant preparations were stained with Wright-Giemsa, or for peroxidase or α-naphthyl acetate esterase according to methods outlined below. A 500-cell differential count was performed on each of the various stained preparations utilizing a light microscope and 1000 x magnification.

**Staining Techniques**

**Peroxidase.** This enzyme was detected utilizing 3-amino-9-ethyl-carbazole. Peroxidase activity produces an intense red-brown granular deposit in the cytoplasm.

**α-Naphthyl acetate esterase.** The α-naphthyl acetate esterase staining reaction was performed according to the method of Yam et al. Enzymatic activity is detected by intense red-brown granular deposits in the cytoplasm.
fixed in absolute methanol for 10 min, dipped in Kodak NTB,
emulsion, and then placed in light-tight black boxes. After storage
for 14–21 days at 4°C, the slides were developed, stained with
Wright-Giemsa, and examined by light microscopy.

**GM-CFC Assay**

The double-layer agar culture technique of Pike and Robinson
was employed. The underlayer contained 10^6 blood leukocytes
recovered from a normal donor in 1 ml of McCoy's SA medium with
15% fetal bovine serum and 0.5% agar, or a mixture of 0.4 ml CM
and 0.6 ml of McCoy's SA medium with 15% fetal bovine serum
containing agar at a final concentration of 0.5%. The overlayer
contained the various mononuclear cell fractions suspended at a
concentration of 5 x 10^5 cells in 1 ml of McCoy's SA medium
containing 15% fetal bovine serum and 0.3% agar. The cultures were
incubated for 14 days at 37°C in a humidified atmosphere of 5%
CO₂-95% air. Colonies were defined as aggregates of greater than
40 cells and were counted using an inverted microscope.

**Liquid Culture Assay**

Starting preparations contained 90%–97% cells
resembling lymphocytes, 3%–6% basophils, and 0%–
4% monocytes as determined from Wright-Giemsa
stained preparations.

**Cell Counts**

The data reported in Figs. 1 and 2 were obtained
from two representative experiments. Total cell counts
decreased about 50% (range 10%–75%) in 5 cultures
prepared without CM (Fig. 1) but were unchanged
over the first 5 days of incubation in 9 cultures
prepared with CM (Fig. 2). Following 9–11 days of
incubation, cell counts obtained from 9 cultures
containing 20%–40% CM decreased about 10% (range
0%–30%), while cell counts from 5 cultures without
CM decreased about 65% (range 50%–75%). The cell
counts of cultures incubated with 40% CM for up to
11 days were the same whether the mononuclear cells
depleted of adherent cells, and T lymphocytes were
obtained from plateletpheresis residues or from
normal peripheral blood.

**RESULTS**

**Differential Counts**

Cells from cultures of 10⁶/ml of mononuclear cells
depleted of adherent cells and T lymphocytes obtained
from 5 plateletpheresis residues incubated without
CM were examined cytologically at intervals of 2–3
days (Fig. 1). After 3–5 days in culture, no blasts were
identified. After 7–9 days, blasts and promyelocytes
comprised <1% of the cells, and <1% of the cells were
classified as maturing neutrophils. Maturing basophils
eosinophils were also rarely identified. Monocytes
were not detected until 9 days in culture. Up to 5% of
the cells (0.5 x 10^5) were monocytes beginning at 9
days (Fig. 1).

Cells from 9 individual cultures grown with 40%
CM were similarly examined (Fig. 2). After 3–5 days,
5%–20% of the cells (0.5–2.0 x 10^5) had developed
large nuclei with fine chromatin and prominent
nucleoli, and were classified as blasts (Fig. 3). After
7–11 days of culture, the number of blasts increased
and azurophilic granules appeared in many of the cells
(Fig. 3), which were then classified as promyelocytes.
The blasts and promyelocytes comprised 23%–53% of
the cells (2.3–5.3 x 10^5). After 9–11 days of culture,
Fig. 3. Wright-Giemsa-stained cytocentrifuge preparations recovered from liquid cultures containing nonadherent, T-cell-depleted mononuclear cells examined at 1000x with a light microscope. (A) Starting preparation containing 90%-97% cells resembling lymphocytes. (B) Immature mononuclear cells observed following 5 days in culture with 40% CM. (C) A promyelocyte observed following 7 days in culture with 40% CM. (D) Polymorphonuclear leukocyte observed following 11 days in culture with 40% CM. (E) Autoradiographs from cultures containing 40% CM and Na₂SO₄ prepared following 11 days in culture. Mononuclear cells with silver grains localized in cytoplasmic granules.
26%–56% of the cells (2.6–5.6 × 10^3) contained mature nuclei and specific granules, predominantly neutrophilic. A smaller proportion (20%–30%) of the cells contained eosinophilic- and basophilic-specific granules. Polymorphonuclear leukocytes were observed after 11 days in culture (Fig. 3). Similar results were obtained when mononuclear cells depleted of adherent cells and T lymphocytes prepared from peripheral blood of 5 normal donors rather than plateletpheresis residues were planted in liquid cultures containing CM (Fig. 4). The total and differential cell counts were identical in cultures containing 20% or 40% CM, but the cellular uptake of Na_2^{35}SO_4 was greater in cultures containing 40% CM (Fig. 5). Thus, 40% CM was employed in the experiments outlined below.

**Cytochemistry**

Peroxidase-positive granules were first detected after 7 days in culture in cells containing nonspecific granules. However, less than 1% of the cells were peroxidase-positive at 7–9 days. At 9–11 days, 25%–66% (2.5–6.6 × 10^3) of the cells examined contained peroxidase-positive granules. Up to 11 days of culture, only rare cells containing α-naphthyl acetate esterase positive granules were observed. After 11 days of incubation, less than 10% of the cells contained α-naphthyl acetate esterase positive granules. No difference was noted in the cytochemical profiles of cells recovered from cultures containing 20% or 40% CM.

**Na_2^{35}SO_4 Uptake**

**Cells from plateletpheresis residues.** Prior to culture, 10^6 mononuclear cells obtained from 5 plateletpheresis residues yielded 30–160 cpm when incubated with 10 μCi Na_2^{35}SO_4 for 2 hr in the liquid culture system herein described. Figure 5 illustrates the Na_2^{35}SO_4 uptake/10^6 cells in cultures containing 0%, 20%, and 40% CM. Cellular uptake of Na_2^{35}SO_4 was markedly stimulated by CM at the 40% level. The peak uptake was observed after 7–11 days in cultures with 40% CM and significant cellular uptake of Na_2^{35}SO_4 continued for up to 18 days.

**Cells from peripheral blood samples.** The incorporation of Na_2^{35}SO_4 into mononuclear cells recovered from peripheral blood of 5 normal donors and incubated with 40% CM is illustrated in Fig. 4. After 5 days of culture, Na_2^{35}SO_4 uptake was 19,000 cpm and remained between 12,000 and 18,000 cpm during the 7–11 days in culture. The peak uptake appeared to occur earlier (5–7 days) with the mononuclear cells obtained from normal peripheral blood than with similar cells recovered from plateletpheresis residues (7–11 days).

**Gel Filtration**

Figures 6 and 7 illustrate the gel filtration patterns obtained from cell extracts and medium of experiments in which Na_2^{35}SO_4 was incorporated in the medium. The principal radioactive component was eluted from the Sephadex G200 column near the void volume, indicating it was of high-molecular weight. An aliquot was removed from the 3–10-ml eluent...
volume, lyophilized, and digested with papain for 48 hr. Recovery of radioactivity following papain digestion was 90% for samples derived from both cell extract and medium. The elution of the radioisotope from these preparations was significantly retarded, suggesting the original labeled components are proteoglycans.

**3H-Thymidine Uptake**

Radioactivity was measured on aliquots of 10^6 cells incubated with 3H-thymidine. Prior to culture, 10^6 mononuclear cells yielded 100–200 cpm when incubated with 1 μCi 3H-thymidine for 2 hr in the liquid culture system herein described. The 3H-thymidine uptake of cells from cultures containing CM was 1100 cpm after 1 day, 2000 cpm after 2 days, 9000 cpm after 3 days, 5000 cpm after 5 days, and 800–2500 cpm after 7–11 days (Fig. 8). The 3H-thymidine uptake of cells removed at intervals for 11 days from cultures without CM remained between 50 and 100 cpm.

**Autoradiographs**

 Autoradiographs were prepared from cells obtained from the liquid cultures in which Na_2^{35}SO_4 and 3H-thymidine incorporation were determined. After 3–5 days, cytocentrifuged preparations from cultures incubated with 40% CM and labeled with 3H-thymidine had silver grains localized over the nuclei of 25% of the cells. After 5–11 days, less than 5% of the cells had silver grains localized over the nuclei in cultures incubated with 40% CM and labeled with 3H-thymidine.

The cells containing the silver grains appeared to be both mature and immature mononuclear cells as determined by light microscopy. No silver grains were detected on radioautographs of cells recovered at intervals from cultures incubated without CM for up to 11 days before labeling with 3H-thymidine.

Radioautographs of cells incubated 9–11 days with 40% CM and Na_2^{35}SO_4 demonstrated silver grains...
localized over nonspecific granules in 25% of the cells. The cells containing radioactivity appeared to be immature myeloid cells (promyelocytes and myelocytes) (Fig. 3). No silver grains were detected over cells recovered at intervals of 0–5 days from cultures with CM. Silver grains were detected over less than 5% of the cells recovered at intervals of 5–9 days from cultures with CM. No silver grains were detected over cells recovered at intervals from cultures grown without CM but with Na$_2^{35}$SO$_4$ for up to 11 days.

**GM-CFC**

The colony-forming capacities of mononuclear cells depleted of adherent cells and T lymphocytes were evaluated in double-layer agar cultures. Using a peripheral leucocyte feeder, 33–192 colonies (mean 118 ± 47) were formed from 5 × 10$^5$ cells recovered from 9 normal donors (5 plateletpheresis residues and 4 peripheral blood samples). Similar numbers of colonies were formed by cells recovered from these two sources. Using a feeder layer containing 40% CM, 20–165 colonies (mean 99 ± 12) were formed after 14 days in cultures containing cells recovered from 4 normal donors.

In 3 experiments employing peripheral blood mononuclear cells, the number of colonies formed by 5 × 10$^3$ cells in double-layer agar cultures was compared with Na$_2^{35}$SO$_4$ uptake/10$^6$ cells in simultaneously performed liquid cultures. After 14 days of incubation, 33, 81, and 192 colonies were formed in double-layer agar cultures, and 2000, 6000 and 11,900 cpm, respectively, were detected in cells recovered after 7 days of incubation in liquid cultures containing 40% CM.

**DISCUSSION**

Interest in the study of hematopoiesis has been greatly enhanced by the development of in vitro culture techniques to assess progenitor cells committed to erythroid,$^{18}$ megakaryocytic,$^{19}$ and granulocyte-macrophage$^{1-3}$ differentiation. These committed progenitor cells are identified by their capacity to produce colonies (cellular aggregates) of maturing progeny in cultures containing semisolid media. The present studies are based on the observation that peripheral blood mononuclear cells will differentiate into granulocytes and macrophages in liquid culture. Conditioned medium prepared from adherent human peripheral blood cells was found to be effective in the standard double-layer culture system and was subsequently employed as a source of stimulating factor in our liquid system. We have previously demonstrated that GM-CFC could be enriched up to six-fold from human peripheral blood by removing mature granulocytes, erythrocytes, adherent cells, and T lymphocytes.$^{20}$ Since this enriched preparation was devoid of immature granulocytic cells, differentiation could be readily detected by examination of stained cells by light microscopy. Further information was obtained utilizing $^3$H-thymidine to study proliferation and Na$_2^{35}$SO$_4$ to study differentiation, and by evaluation of the cytochemical reactivity of the cultured cells.

Our starting cell preparation (nonadherent, T-lymphocyte-depleted mononuclear cells) contained 90%–97% lymphocytes, 3%–6% mature basophils, and 0%–4% monocytes and yielded 33–165 colonies in the standard double-layer agar culture. When planted in the liquid culture system incorporating conditioned media, sequential morphological and cytochemical studies revealed the appearance of maturing granulocytic cells. Cellular proliferation was indicated by $^3$H-thymidine uptake (9000–5000 cpm after 3–5 days) and $^3$H-thymidine localization in nuclei in cultures containing CM. The total cell count remained relatively constant during 11 days of culture, suggesting a significant turnover or renewal of cell populations in cultures containing CM. Fifteen to 53% of cells recovered after 7–9 days of culture with CM were blasts and promyelocytes, but only about 1% of the promyelocytes had granules that contained peroxidase. After 9–11 days of culture, 20% of the cells were promyelocytes and up to 56% of the cells were maturing granulocytic cells (predominantly myelocytes and metamyelocytes). The peroxidase-reactive granulated cells were 25%–66% of the total.

We also employed Na$_2^{35}$SO$_4$ incorporation to identify developing myeloid cells. Previous studies employing short-term suspension cultures of bone marrow cells have demonstrated virtually all of $^{35}$SO$_4$ incorporated in immature myeloid cells,$^{17}$ primarily in chondroitin-4-sulfate as identified by paper chromatography of extracts of cells or media.$^{17}$ In 5 single donor plateletpheresis residues, Na$_2^{35}$SO$_4$ uptake was maximum at 11,900 cpm/10$^6$ cells after 11 days in cultures containing 40% CM. Nonadherent, T-lymphocyte-depleted mononuclear cells recovered from the peripheral blood of 5 normal donors yielded 20,000 cpm/10$^6$ cells after 5 days in cultures containing 40% CM and Na$_2^{35}$SO$_4$. Autoradiographs revealed accumulation of radioactivity in the primary granules of the promyelocytes and myelocytes. Gel filtration on Sephadex G-200 of culture medium and cell extracts obtained after 11 days of culture with 40% CM revealed a sharp peak of radioactivity just behind the void volume of the column. Following papain digestion of the cell extracts and media, the peak of radioactivity was eluted later. These findings are in agreement with previous reports employing short-term cultures of bone marrow cells.$^{17}$
Cells recovered from liquid cultures incubated without CM incorporated very little Na$_{235}$SO$_4$ (100–800 cpm/10$^6$ cells) and $^3$H-thymidine (maximum 200 cpm/10$^6$ cells). Less than 5% of the cells were blasts and promyelocytes after 11 days of culture. Thus, cells cultured without CM fail to proliferate or differentiate into granulocytes.

We have demonstrated that human peripheral blood mononuclear cells can be stimulated to produce maturing granulocytic progeny in a liquid culture. In cultures containing CM, there is significant $^3$H-thymidine incorporation. The proliferating cell appears to be a mononuclear cell as detected by autoradiographs. The granulocytic nature of this progenitor cell was identified by development of maturing progeny in culture characterized as promyelocytes, myelocytes, metamyelocytes, band and polymorphonuclear forms containing granules reactive with peroxidase stains and incorporating Na$_{235}$SO$_4$. The results from 3 experiments suggest that the amount of Na$_{235}$SO$_4$ incorporated in cells recovered from liquid cultures correlates with the number of colonies formed by these cells in double-layer agar culture.

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