

## Long-Term Culture of Normal and Leukemic Human Bone Marrow

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### A. Introduction

Clonal culture systems using semisolid media for the detection of granulocyte-macrophage (Bradley and Metcalf 1966; Pike and Robinson 1970; Pluznik and Sachs 1966), erythroid (Stephenson et al. 1971), lymphoid (Fibach et al. 1976; Metcalf et al. 1975a; Sredni et al. 1976) and megakaryocytic (Metcalf et al. 1975b) progenitors have contributed greatly to investigations of hematopoiesis. Unfortunately, such systems possess two major limitations: (1) observations are restricted to relatively short time intervals and (2) the interaction between different kinds of cells cannot easily be studied.

More recently, liquid culture systems have also been investigated. Golde and Cline (1973) described the short-term liquid culture of human marrow using an *in vitro* diffusion chamber in which cells grew both in suspension and on a dialysis membrane. Proliferation and maturation of granulocytes and macrophages in these chambers persisted for 4 weeks. Dexter et al. (1973) reported the development of a liquid system for the cocultivation of mouse thymus and bone marrow in which CFU<sub>c</sub>s (granulocyte-macrophage progenitor cells) were generated for at least 10 weeks and CFU<sub>s</sub>s (pluripotent stem cells) were present for 14 days. The same group (Dexter and Lajtha 1974; Dexter and Testa 1976; Dexter et al. 1977) later developed a method for the long-term culture of mouse bone marrow cells alone in liquid medium. Such cultures produce both CFU<sub>c</sub>s and CFU<sub>s</sub>s for several months. Hematopoiesis in this system is dependent upon the presence of a marrow-derived adherent population consisting of three cell types: phagocytic mononuclear cells, endothelial

cells, and giant lipid-laden adipocytes. Initially, only certain lots of horse serum had the ability to stimulate the growth of these essential adipocytes. Greenberger (1978) reported that "deficient" lots of horse serum could be reconstituted with corticosteroids.

Long-term liquid culture systems for the cultivation of human bone marrow cells are urgently needed. Moore and Sheridan (1979) reported the establishment of human marrow cultures using conditions similar to those described by Dexter et al., but CFU<sub>c</sub> production was limited to 6–8 weeks. More recently, Moore et al. (1979) reported sustained long-term hematopoiesis in liquid cultures of marrow from a subhuman primate, the tree shrew (*Tupaia glis*). We now present additional information concerning a recently described method (Gartner and Kaplan 1980) for the long-term culture of human marrow cell populations based on modifications of the murine system.

### B. Materials and Methods

#### I. Media

Fischer's complete growth medium consisted of Fischer's medium (Gibco #320–1735) supplemented with 10<sup>-7</sup> M hydrocortisone sodium succinate (Upjohn) and either 25% horse serum (HoS; Flow Labs), 25% fetal bovine serum (FBS; Microbiological Associates), or 12.5% each of HoS and FBS. McCoy's complete growth medium consisted of the following: McCoy's 5a medium, modified, (Gibco #430–1500), 10<sup>-7</sup> M hydrocortisone, 1% sodium bicarbonate solution (Gibco #670–5080), 1% MEM sodium pyruvate solution (Gibco 320–1360), 1% MEM vitamin solution (Gibco #320–1120), 0.8% MEM amino acids solution (Gibco

#320-1135), 0.4% MEM nonessential amino acids solution (Gibco #320-1140) 1% L-glutamine, 200mM solution (Gibco 320-5030), 1% penicillin-streptomycin solution, 10,000 u pen/ml, 10,000 mcg strep/ml (Gibco #600-5140), and either 25% HoS, 25% FBS, or 12.5% of each. Only freshly prepared medium was used for the initiation and maintenance of cultures. All serum was heat inactivated at 56°C for 1 h and stored frozen prior to use. In the presence of  $10^{-7}$  M hydrocortisone, all lots of HoS tested were able to support the development of a good stromal layer. Only those lots of FBS which sustained the growth of our fastidious human lymphoma cells (Epstein and Kaplan 1974; Kaplan et al. 1979) were used for these cultures.

## II. Initiation and Maintenance of Cultures

Normal marrow specimens were obtained from resected ribs of patients undergoing thoracotomy. Immediately after removal from the patient, the rib was cut into segments 2–3 cm in length and immersed in cold, Ca++-free Dulbecco's phosphate-buffered saline (PBS; Gibco) supplemented with 1% pen-strep solution and beef lung sodium heparin (Upjohn), 10 µ/ml final concentration. All extraneous connective tissue was cut away from the segments and they were gently rinsed with fresh Ca++-free Dulbecco's PBS. Care was taken not to flush the marrow from the segments at this step. Using two 6" blunt-ended forceps, the rib segments were pried apart longitudinally. With the tip of a forcep, the marrow was scraped into a 100 mm glass or plastic petri dish containing 20 ml cold complete growth medium (two dishes were used for the marrow from each resected rib.) The medium containing the marrow clumps was then transferred to a 50 ml centrifuge tube (Corning) and pipetted moderately vigorously to break apart the cell clumps and create a single cell suspension. (In our hands, cultures initiated from single cell suspensions have consistently proven superior to those in which cell clumps were seeded). Viable cell counts were performed using 0.04% trypan blue; viability was consistently 98%–100%. Wright-Giemsa-stained slides of the fresh specimens were also prepared to insure that the marrow was normal. Between  $1-2 \times 10^9$  nucleated cells were usually recovered from a rib. Two  $\times 10^7$  viable nucleated cells in 10 ml of complete growth medium were seeded into plastic T-25 flasks (Corning) or  $1 \times 10^7$  nucleated cells were seeded into Cluster<sup>6</sup> 35 mm wells (Costar) containing glass coverslips (Corning.) Cultures were incubated at 33°C in 5% CO<sub>2</sub> in air. (Our early experiments demonstrated the superiority of 33°C over 37°C in terms of supporting long-term hematopoiesis, although 37°C accelerated the development of the stromal layer. In contrast, Moore and Sheridan (1979) reported that 37°C was superior for their human marrow cultures.) Weekly feedings consisted of removal of 5 ml spent medium containing

nonadherent cells and addition of 5 ml fresh medium. The nonadherent cells were counted, used for morphologic and cytochemical studies, and assayed for CFU<sub>c</sub>.

Aspirates of normal sternal marrow were obtained from patients undergoing cardiovascular surgery. Aspirates were also obtained from the iliac crests of patients with acute myeloid leukemia (AML) before treatment, while in remission, and during relapse. These specimens were drawn into heparinized syringes and immediately transferred to tubes containing 2 ml of the previously described complete McCoy's growth medium. Wright's Giemsa-stained preparations of the leukemic aspirates were examined to estimate the seeding density required for the development of the stromal microenvironment in culture. Specimens comprised almost exclusively of myeloid blasts required greater seeding densities for development of the stromal microenvironment. Cell counts were also performed. With the leukemic specimens, between  $5 \times 10^6$  and  $2 \times 10^7$  nucleated cells were seeded per T-25 flask, depending on the total number of nucleated cells available. From the normal marrow aspirates,  $1-2 \times 10^7$  nucleated cells were seeded per T-25 flask. Two to five days after the initial seeding of the aspirate suspensions, the nonadherent cells were removed from each flask independently and separated on individual "mini" Ficoll-Hypaque gradients (Boyum 1968) using 8 ml cell suspension in PBS plus 3 ml Ficoll-Hypaque solution. The cells at the gradient interface were washed three times in Dulbecco's PBS and returned to the original flasks which contained some adherent cells. Aspirate cultures were fed weekly as described for rib-derived cultures.

## III. CFU<sub>c</sub> Assay

One million human peripheral blood mononuclear cells were suspended in 1 ml volumes of 0.5% Noble agar (Difco) in McCoy's 5a medium supplemented with 15% FBS as described by Pike and Robinson (1970) and seeded into 35 mm wells of cluster<sup>6</sup> plates (Costar).  $1 \times 10^5$  viable bone marrow cells in 1 ml volumes of 0.3% Noble agar in McCoy's 15% FBS medium were overlayed on the 0.5% agar layer. Only 0–1-day-old underlays were used. Clusters and colonies were scored at day 12–14. Clusters contained 20–50 cells and colonies contained more than 50 cells. CFU<sub>c</sub> values represent the average of at least three cultures, and three wells per culture were assayed.

## IV. Cytochemical stains

For morphologic characterization nonadherent cells and coverslip cultures were stained with Wright's-Giemsa; cytochemical tests were also performed for the presence of nonspecific esterase and myeloperoxidase activity (Yam et al. 1971).

**Table 1.** Characteristics of the adherent population

Culture medium <sup>a</sup>	Serum		Length of time to confluence (days)	Longevity (months)	Presence of adipocytes <sup>b</sup>
	FBS %	HoS %			
Fischer's		25	14-21	>12	3+ - 4+
Fischer's	25		12-14	> 6	0 - 1+
Fischer's	12.5	12.5	12-14	> 6	3+ - 4+
McCoy's		25	14-21	> 6	3+ - 4+
McCoy's	25		7-12	> 6	0 - ±
McCoy's	12.5	12.5	7-10	> 6	3+ - 4+

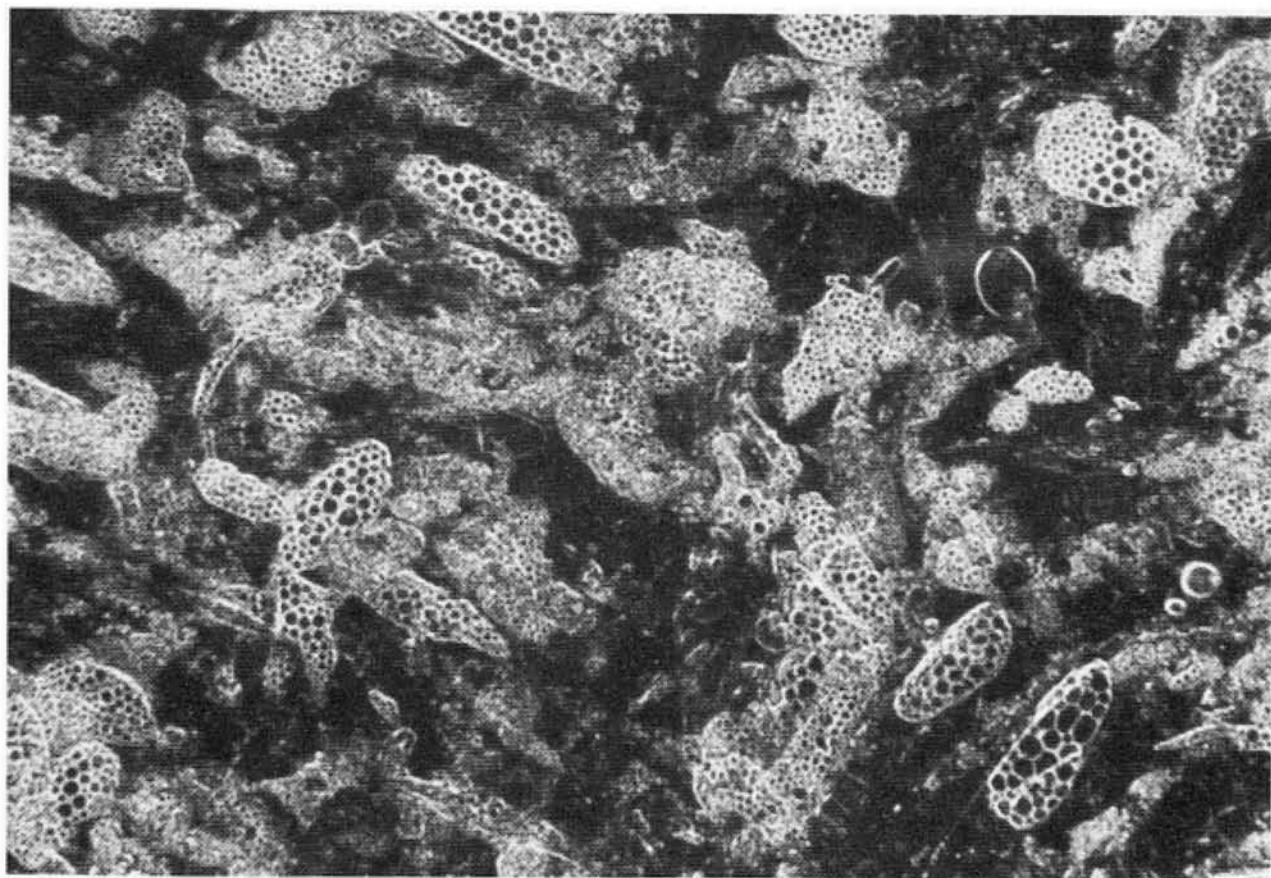
<sup>a</sup> All cultures were supplemented with  $10^{-7}$ M hydrocortisone

<sup>b</sup> Stromal layers of very old cultures were composed almost exclusively of adipocytes

### C. Results

Table 1 demonstrates that adipocyte-containing confluent stromal layers could be initiated and maintained for periods of at least 5 months in either Fischer's or McCoy's medium when supplemented with both horse serum and hydrocortisone. The stromal layers of very old cultures (greater than 6 months), especially those grown in Fischer's medium, were comprised almost exclusively of adipocytes (Fig.

1). Greenberger (1979) also reported the induction by corticosteroids of lipogenesis in adipocytes in human marrow cultures. In the absence of horse serum, either medium, despite supplementation with hydrocortisone and FBS, failed to support the development of significant numbers of lipid-laden adipocytes. In such cultures almost no nonadherent cells were recovered after 6-8 weeks. Cultures grown in McCoy's medium supplemented with FBS with or without HoS developed confluent



**Fig. 1.** A human marrow culture at 6 months illustrating abundant adipocytes.  $\times 20$

stromal layers earlier than those supplemented with HoS alone or grown in Fischer's medium.

Fischer's growth medium supplemented with hydrocortisone and 25% HoS or 12.5% HoS and 12.5% FBS was able to support the generation of CFU<sub>c</sub>-forming cells for up to 12 weeks in some cases. However, the number of colony-forming cells recovered from such cultures was usually less than 50 per  $10^5$  cells assayed. In contrast, McCoy's complete growth medium supplemented with both FBS and HoS not only provided adequate nutrition for the early development and maintenance of stromal layers containing abundant adipocytes but also contributed either directly or indirectly (through the stromal microenvironment) to longterm hematopoiesis and significantly greater yields of CFU<sub>c</sub>.

Figure 2 illustrates the appearance of a typical culture at day 14. A confluent stromal layer can be seen underneath the nonadherent cells. A most important feature of these long-term cultures is the presence of "cobblestone"-like areas, presumably regions of hematopoiesis from which the nonadherent cells arise. Figures 3 and 4 illustrate such cobblestone-like areas in cultures at 6 weeks and 16 weeks,

respectively. At higher magnification (Fig. 5), the flattened polygonal cells in such areas are seen to be so tightly packed together that their boundaries may be difficult to discern. With time some of these cells become more rounded granules can be seen to appear within their cytoplasms and ultimately they become non-adherent.

When coverslip cultures were gently washed extensively to remove nonadherent cells and stained for myeloperoxidase activity, it was observed that cobblestone areas did indeed contain both myeloid and monocyteoid cells. Coverslip cultures stained for nonspecific esterase activity revealed monocyteoid cells scattered throughout the stromal layer, in islands of hematopoiesis, and sometimes in large, tight clusters within the stromal layer. In general, nonspecific esterase-positive cells were much more intensively stained outside of the cobblestone areas.

The kinetics of the generation of cells with colony-forming ability in these long-term cultures appeared to follow two different patterns, designated the "hyperproliferative" and "homeostatic" patterns. The hyperproliferative pattern describes cultures in which the

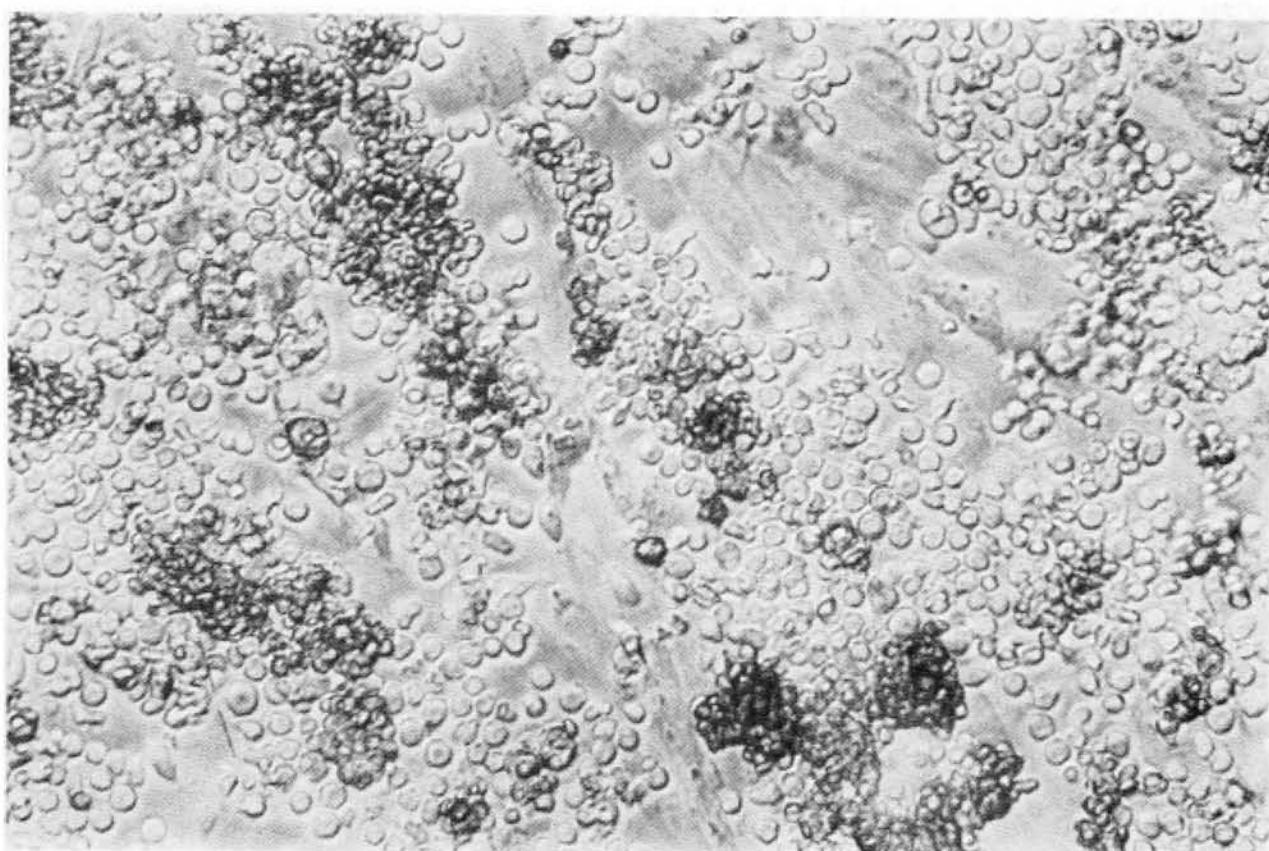
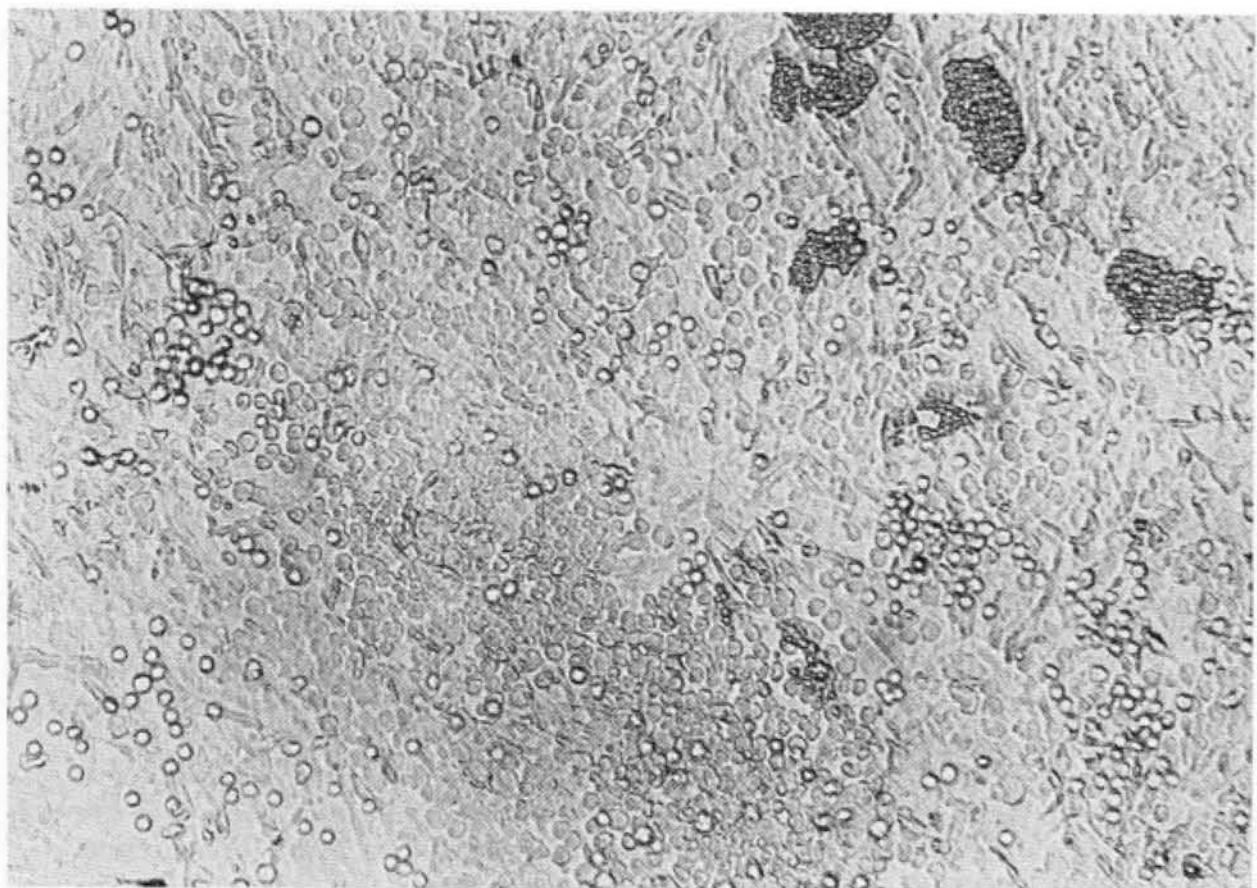
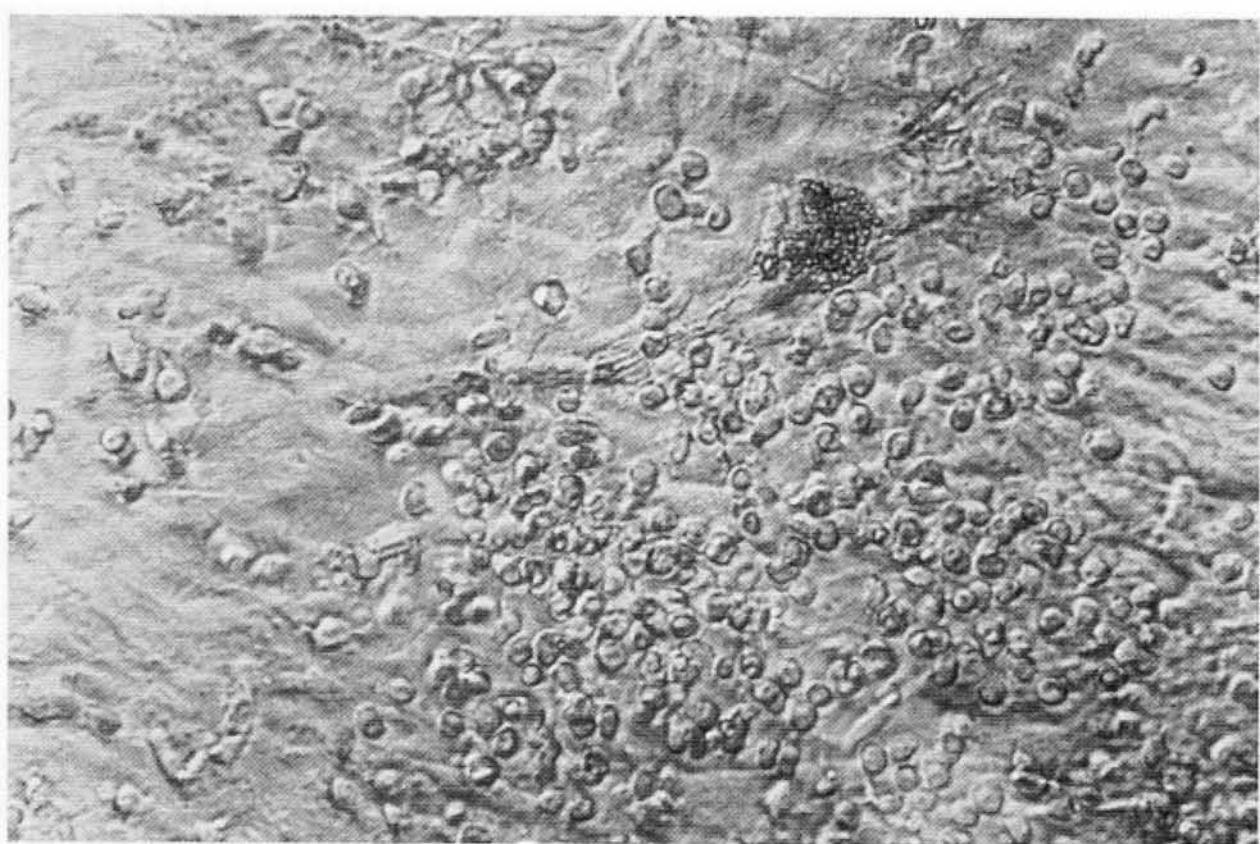


Fig. 2. A human marrow culture at 14 days.  $\times 81$



**Fig. 3.** A human marrow culture at 6 weeks illustrating adipocytes and cobblestone areas of active hematopoiesis.  $\times 50$



**Fig. 4.** A human marrow culture at 16 weeks illustrating a cobblestone area.  $\times 77$

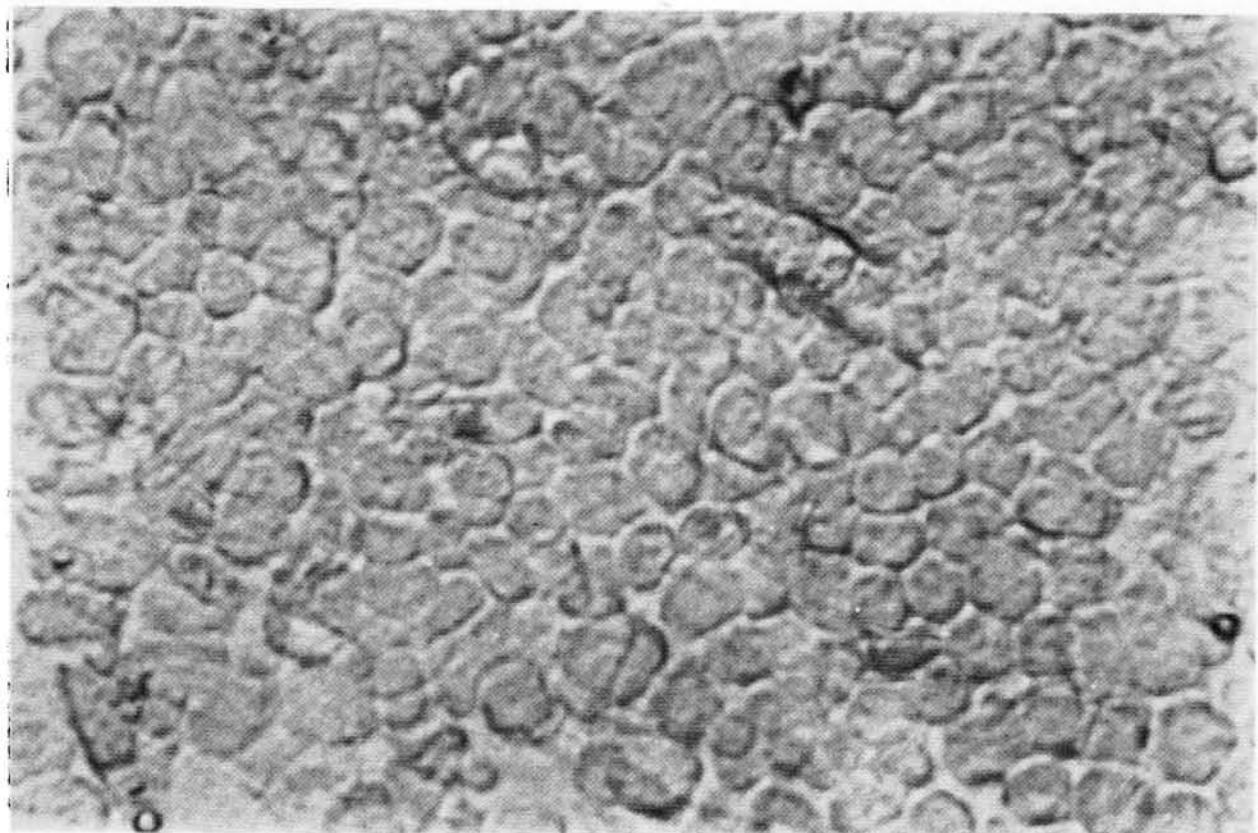
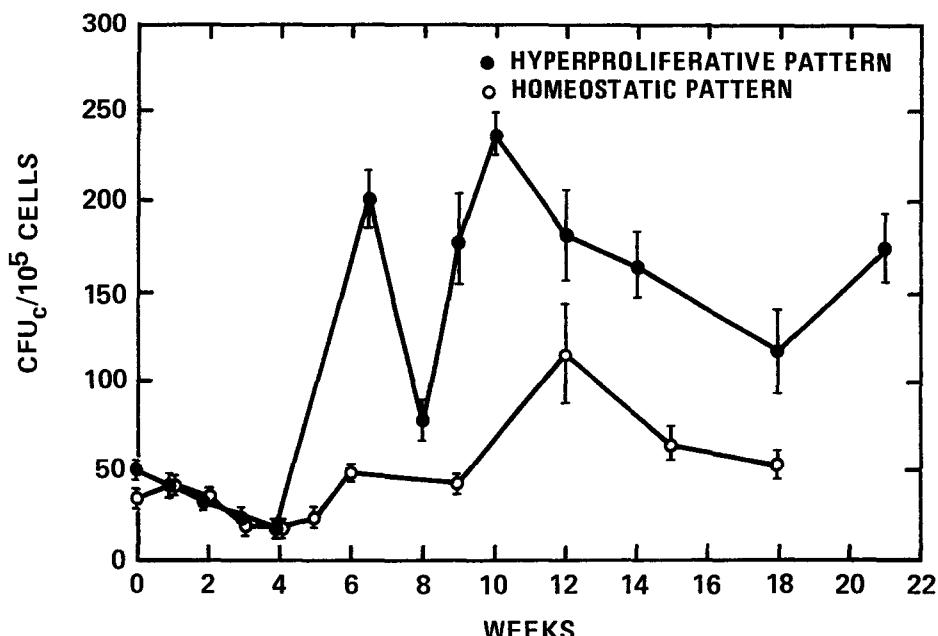


Fig. 5. A human marrow culture at 14 weeks illustrating a very early cobblestone area.  $\times 324$

number of CFU<sub>c</sub>s generated exceeds 100 per  $10^5$  cells in serial assays over several weeks. In contrast, the homeostatic pattern describes a steady-state situation in which a lower level of CFU<sub>c</sub>s, usually between 25–75 per  $10^5$  cells, is continuously present in the cultures. An example of each pattern is shown in Fig. 6. Of nine different rib specimens incubated in McCoy's complete growth medium supplemented with both FBS and HoS, four showed the hyperproliferative pattern and five the homeostatic pattern. With the other growth media only the homeostatic pattern was observed, and only in rare instances were CFU<sub>c</sub> detected beyond 12 weeks. With all growth media and at all time intervals most colonies were very large, containing more than 500 cells. An example is shown in Fig. 7. Adipocyte colonies were also occasionally seen in the agar cultures. After approximately 8 weeks the numbers of nonadherent cells recovered weekly usually varied between  $2 \times 10^5$  and  $2 \times 10^6$  per culture. Before 7–8 weeks, between  $1 \times 10^6$  and  $1.5 \times 10^7$  cells were routinely recovered. Such recovery frequencies were common to both the hyperproliferative and homeostatic patterns.

The numbers of CFU<sub>c</sub>s did not increase during the first 4 weeks of culture (Fig. 6). In the murine system Dexter et al. (1977) described the "recharging" of cultures at 4 weeks by the addition of fresh marrow. Addition of fresh cells was found to be unnecessary for prolonged hematopoiesis in *Tupaia glis* marrow cultures (Moore et al. 1979; Moore and Sheridan 1979). We have found that recharging did not enhance the production of CFU<sub>c</sub>s in our human marrow cultures and in some cases proved deleterious either by destroying the stromal microenvironment or by decreasing the number of CFU<sub>c</sub>s recovered. In the absence of recharging, the numbers of CFU<sub>c</sub>s began to increase between 4–6 weeks. In most normal marrow cultures, CFU<sub>c</sub> production continued for at least 20 weeks.

Table 2 indicates the relative abundance of different morphologic cell types in the nonadherent populations. Figs. 8 and 9 illustrate the nonadherent population from cultures at 9 weeks and 12 weeks, respectively. In older (greater than 8 weeks) cultures grown in Fischer's or McCoy's medium with FBS only, relatively small numbers of nonadherent cells were recovered, the vast majority of which



**Fig. 6.** Serial CFU<sub>c</sub> production in long-term human marrow liquid cultures. The results represent the means  $\pm$  SEM of three wells for each of three cultures

were mature macrophages. This finding is in agreement with that of Moore and Sheridan (Moore and Sheridan 1979; Moore et al. 1979) in long-term human marrow cultures grown in Fischer's medium with HoS. With Fischer's medium plus 25% HoS or 12.5% HoS and 12.5% FBS, some immature monocyteoid and both immature and mature myeloid cells were also present but in small numbers. In contrast, cultures grown in McCoy's FBS plus HoS growth medium contained many more immature monocyteoid and myeloid cells as well as mature myeloid cells. Spontaneous mitotic figures were also seen in stained preparations of these nonadherent cells. The numbers of myeloid cells often exceeded the

numbers of mature macrophages in the McCoy's FBS plus HoS cultures, unlike the situation in Fischer's medium. In younger cultures (less than 6–8 weeks) monocyteoid and myeloid cells at various stages of differentiation were generally observed, and fewer differences were detected between the different growth media.

Aspirates of normal sternal marrow taken from patients during cardiovascular surgery and of leukemic marrow from the iliac crests of AML patients were also cultured. The probability of successful establishment of cultures from such specimens appeared dependent upon the quality of the aspirate and, in the case of the leukemic specimens, the previous medi-

**Table 2.** Characteristics of the nonadherent population

Culture medium <sup>a</sup>	Serum		Monocyteoid <sup>b</sup>		Myeloid <sup>b</sup>		CFU <sup>c</sup> -forming Capacity
	FBS %	HoS %	Immature	Mature	Immature	Mature	
Fischer's		25	1+	4+	1+-2+	1+-2+	1+
Fischer's	25		0-±	4+	0-±	0-±	0-±
Fischer's	12.5	12.5	1+	4+	1+	1+-2+	0-1+
McCoy's		25	1+	3+	1+	1+-2+	1+
McCoy's	25		0-±	2+	0-±	0-±	NT <sup>c</sup>
McCoy's	12.5	12.5	1+-2+	3+	2+-4+	2+-4+	2+-4+

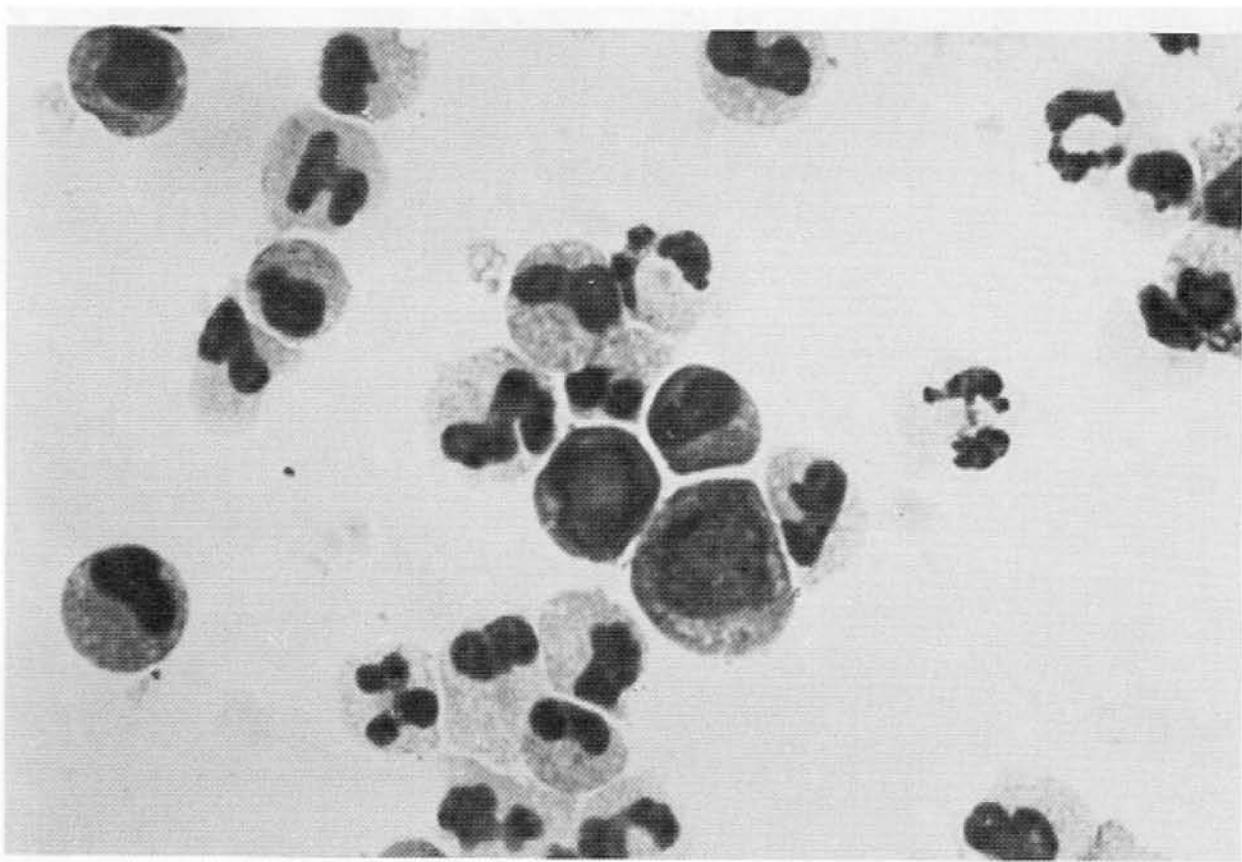
<sup>a</sup> All cultures supplemented with 10<sup>-7</sup>M hydrocortisone

<sup>b</sup> Based on Wright's-Giemsa, nonspecific esterase, and myeloperoxidase staining characteristics

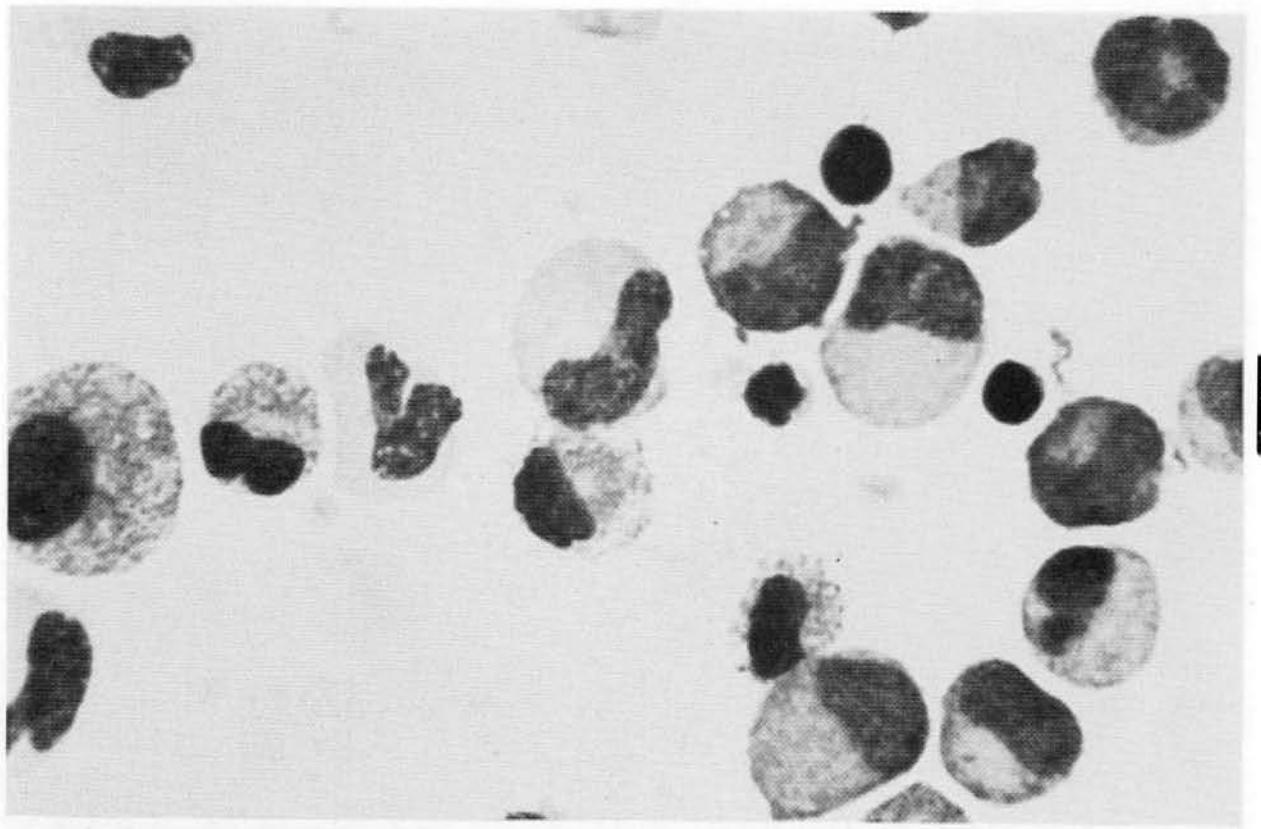
<sup>c</sup> NT=not tested; too few nonadherent cells were recovered after 8 weeks



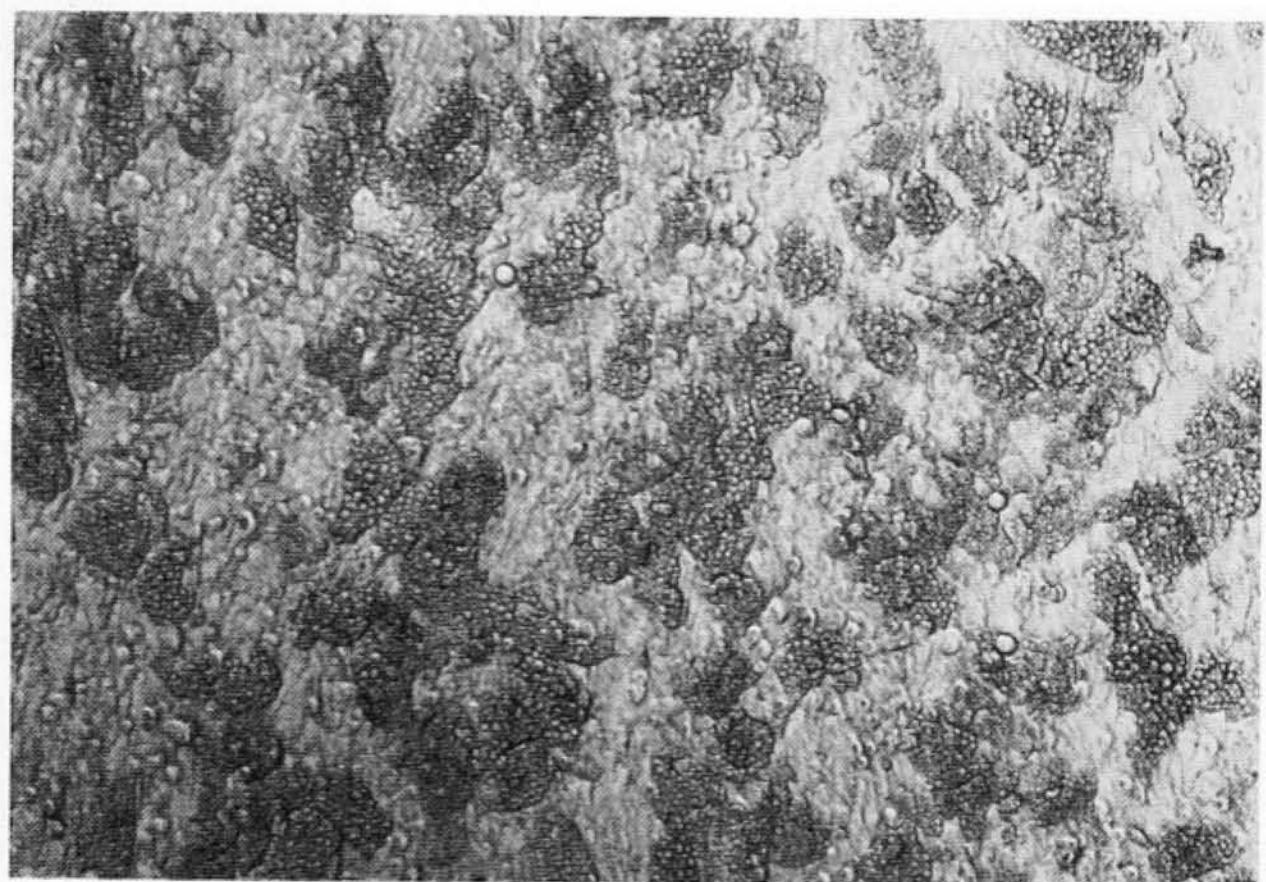
**Fig. 7.** Agar culture colony arising from a CFU<sub>c</sub> harvested from a 14-week-old human marrow culture in liquid medium.  $\times 19$



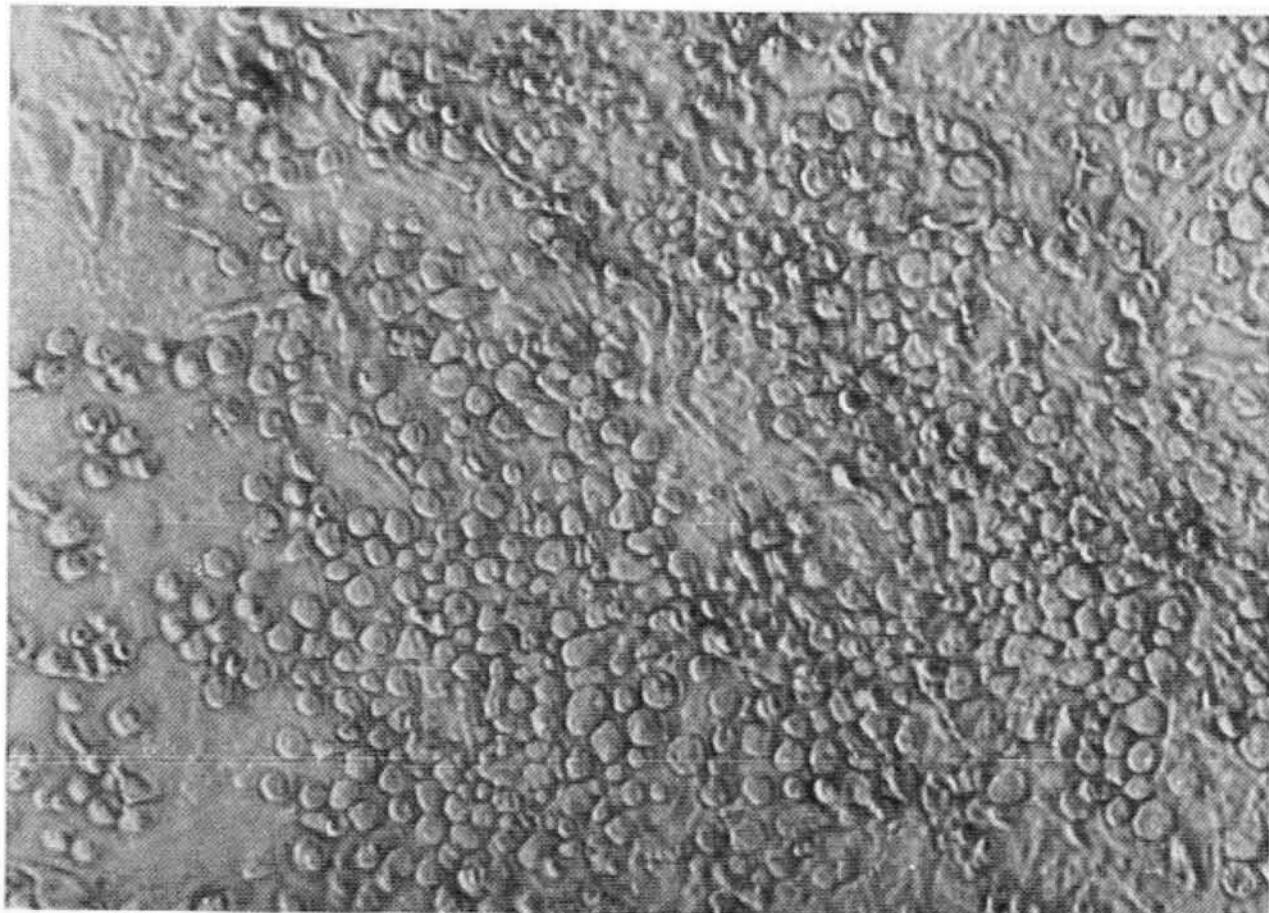
**Fig. 8.** Nonadherent population from a 9-week-old human marrow culture.  $\times 310$



**Fig. 9.** Nonadherent population from a 12-week-old human marrow culture.  $\times 324$



**Fig. 10.** A 1-month-old human marrow culture from an aspirate from AML patient T. H.  $\times 50$



**Fig. 11.** A 1-month-old human marrow culture from a second aspirate from AML patient T. H.  $\times 80$

cal treatment of the patient. Cultures in which cobblestone areas were observed were established from both normal and leukemic aspirates. Figure 10 illustrates such a culture at 4 weeks from an AML patient in remission. Faint cobblestone-like areas can be seen sandwiched between the abundant adipocytes. Figure 11 illustrates a 4 week culture from the same patient established from an additional aspirate taken 1 month later. Although we have been able to recover colony-forming cells from cultures of normal aspirate specimens for at least 8 weeks, the duration of granulopoiesis has been much more variable than that observed in cultures from rib specimens.

#### D. Discussion

It has been demonstrated that hematopoiesis in long-term liquid cultures of murine (Allen and Dexter 1976) and *Tupaia glis* (Moore et al. 1979) marrow is dependent upon the presence of lipid-laden adipocytes. Our results suggest that this is also the case for human marrow. Horse serum in conjunction with hydrocortiso-

ne appears to be essential for the initial growth and/or differentiation of the adipocytes. In limited studies cultures grown in the presence of freshly collected pooled human serum and  $10^{-7}M$  hydrocortisone, with or without the addition of FBS, failed to develop mature adipocytes.

The numbers of lipid-containing adipocytes in cultures from different specimens varied tremendously, especially in younger cultures (less than 8 weeks). Interestingly, some cultures established from AML marrow aspirates often possessed extremely large numbers of adipocytes within 2 weeks after initiation of the cultures. In contrast, occasionally other AML marrow cultures completely failed to develop any adipocytes. In those cases where more than 1 aspirate was obtained at different time intervals from the same AML patient considerable variation was also seen between specimens with regard to the numbers of adipocytes observed in the cultures, without any evident correlation with the patient's clinical condition or the status of the disease. The culture of additional leukemic specimens may elucidate this problem.

The cobblestone areas were often not seen immediately adjacent to the adipocytes, especially in younger cultures. This suggests that direct cell-to-cell contact between granulocyte-macrophage committed progenitors and well-differentiated adipocytes may not be required for the maturation of the progenitors.

It seems clear that the successful maintenance of long-term hematopoiesis in these cultures is crucially dependent upon the development and preservation of an intact, competent stromal layer. Factors which affect the integrity of the adherent layer, such as increased acidity of the culture medium or sometimes the addition of an extraneous cell population to the culture, resulted in a decrease in hematopoiesis as measured by CFU<sub>c</sub> production. The regeneration of the stromal population in the original culture vessel has not occurred to any significant degree in our cultures. (This is unlike the situation reported for cultures of tree shrew marrow where hematopoiesis was restored along with the regeneration of the stromal layer [Moore et al. 1979]). Thus, great care must be taken to preserve the integrity of the stromal micro-environment.

Our recent efforts to subculture stromal populations have met with limited success. When adherent cells trypsinized from old cultures of normal marrow were reseeded into new culture vessels, we have again observed the growth of an adherent population to confluence. Lipid-laden adipocytes are lost during trypsinization, so that subcultured layers are initially comprised only of large, polygonal or fibroblastoid cells and macrophages. In some cases, lipid-containing cells have appeared some time later in these cultures, suggesting that adipocyte precursors are trypsinizable or that the lipid droplets are lost during trypsinization and require considerable time to be regenerated. The restoration of long-sustained hematopoiesis in such trypsinized cultures has been generally unsuccessful, though cobblestone areas have occasionally been observed.

Trypsinization has proven valuable in generating confluent stromal layers in cultures from AML patients. With some such specimens, very few adherent cells (other than macrophages) were present in the cultures and confluence of an adherent population was never reached. Such cultures were later trypsinized and the recovered cells seeded at higher cell densities.

Confluence of an adherent layer was thus ultimately obtained in many cases.

An additional problem in the culture of leukemic specimens was the lack of an appropriate assay system to monitor granulopoiesis in these cultures. In the case of some of the specimens from patients in remission, the nonadherent cells recovered from the cultures did not form colonies in soft agar, although such nonadherent cell populations morphologically resembled those recovered from cultures of normal marrow.

We believe that both the hyperproliferative and homeostatic patterns described for CFU<sub>c</sub> production reflect the actual de novo generation of granulocyte-macrophage progenitor cells in the cultures. It appears that the numbers of CFU<sub>c</sub> detected over time in such cultures exceed the numbers of granulocyte-macrophage committed progenitor cells initially seeded. Moreover, serial CFU<sub>c</sub> assays would have been expected to reveal a rapid depletion of CFU<sub>c</sub> as weekly samples were withdrawn, if CFU<sub>c</sub> were merely persisting rather than proliferating in the cultures. This interpretation is further supported by the observation of mitotic figures and by the fact that production of CFU<sub>c</sub> did not begin to increase in the hyperproliferative pattern until about 4 weeks. Myeloperoxidase-stained preparations have demonstrated that cobblestone areas do contain myeloid cells. The alternative possibility that granulocyte-macrophage committed progenitors remain dormant and undetectable within the stromal matrix for long periods of time until they receive appropriate signals for proliferation and maturation or until they are sloughed off as the culture ages seems less likely in the face of these observations. The differences between the two growth patterns may be attributable to age and other constitutional factors affecting individual hematopoietic activity; they may have been more readily apparent because most of our rib specimens came from older patients. Greenberger et al. (to be published) have demonstrated that the capacity for long-term hematopoiesis in murine marrow cultures differs considerably from strain to strain. Studies of the stromal microenvironment of these cultures are currently underway which may provide a better understanding of the source of CFU<sub>c</sub>s.

Sampling problems have been encountered during attempted recovery of nonadherent cells from these cultures. The nonadherent

cells tend to "hover" closely over the stromal layers. Phase contrast observations immediately following feeding of the cultures reveal that even with gentle agitation of the culture vessel, large numbers of nonadherent cells remain close to the stromal layer. Possibly the viscosity of the growth medium enhances this effect. Therefore, we believe that the numbers of nonadherent cells harvested in the aliquots of spent medium may not accurately reflect the numbers of nonadherent cells present in the cultures. More vigorous agitation of the culture vessel results in some destruction of the delicate stromal microenvironment and cessation of hematopoiesis. We are presently attempting to improve our methods of quantitation.

Erythroid (BFU-E) (Testa and Dexter 1977) and megakaryocytic (CFU-M) (Williams et al. 1978) progenitors are also produced in long-term murine cultures. The persistence for at least 16 weeks of pre-T cells in murine cultures has also been recently reported (Jones-Villeneuve et al. 1980). To date we have not attempted to assay for such populations in our cultures of human marrow. However, we have established three permanent, polyclonal cell lines of Epstein-Barr virus transformed B-lymphoblastoid cells from marrow cultures of different nonleukemic specimens. All three arose from what appeared to be hematopoietically exhausted stromal layers, one developing at 3 months, one at 4 months, and one at 6 months after initiation of the cultures. All were derived from cultures in Fischer's medium with 25% HoS and hydrocortisone. In the case of the 6-month-old stromal layer, no cobblestone areas remained, and no nonadherent cells had been recovered for 2 months prior to the appearance of the B cells. Moore and Sheridan (1979) reported the conversion of 10% of their human cultures to a lymphoblastoid morphology by the 6th week of culture. We are at present uncertain as to whether our observations represent the generation of B cells from immature precursors in these old cultures or the delayed outgrowth of long-lived EBV-transformed mature B cells. Given the complexity of the stromal microenvironment, it is quite possible that small numbers of mature B cells could have survived undetected.

Methods for the culture of human bone marrow are essential for furthering our understanding of normal hematopoiesis and of

hematopoietic disease states as well as the effects of therapeutic regimens on marrow subpopulations. Although there are still many improvements to be made in the application of the liquid phase murine culture system to human marrow, we believe the modifications we have described allow for the routine establishment of such cultures in a reproducibly successful way.

### Acknowledgments

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