Diversity of Human Granulopoietic Precursor Cells: Separation of Cells That Form Colonies in Diffusion Chambers (CFU-d) From Populations of Colony-forming Cells in vitro (CFU-c) by Velocity Sedimentation


Normal human bone marrow contains cells that form granulocytic colonies in fibrin clot diffusion chambers implanted intraperitoneally in sublethally irradiated mice (CFU-d). A series of experiments was performed to determine the relationship between CFU-d and the cells that form colonies in agar culture in vitro (CFU-c). Low-density bone marrow cells (<1.070 g/cm^3) were separated by velocity sedimentation and colony-forming cells in each fraction assessed by culture in diffusion chambers and in soft agar. CFU-d sedimented more slowly than the vast majority of CFU-c, with a peak sedimentation velocity of 5.0 ± 0.4 mm/hr. Two different CFU-c populations could be distinguished. One was a rapidly sedimenting cell population (7.3 ± 0.9 mm/hr.) that formed colonies after 1 wk incubation in vitro. On day 14, however, colonies were derived from more slowly sedimenting cells that had a peak sedimentation velocity of 6.0 ± 0.5 mm/hr. Clusters (3-50 cells), scored on day 7 in agar culture, were a heterogeneous population, possibly derived from both day 7 and day 14 CFU-c. Mixing experiments did not show evidence of cell interaction that could explain the observed velocity differences. The velocity sedimentation profiles of colony-forming cells in DNA synthesis (S phase) and non-S phase were determined by assessment of the proportion of separated cells sensitive to pulse treatment with high specific activity ^3H-thymidine (^3H-TdR) prior to culture. The results excluded the possibility that the assays simply detected identical cells in various stages of the mitotic cycle. Rather, three different populations of precursor cells were distinguishable by the culture techniques employed. In a series of five unseparated normal bone marrow samples, neutrophilic CFU-d had a small proportion in S phase (7% ± 10%) as measured by ^3H-TdR suicide experiments. The average proportion of CFU-c in S phase was 21% ± 7% and 48% ± 4% for day 14 and day 7 CFU-c, respectively.

Families of committed granulocyte-macrophage progenitor cells can be recognized by their capacity to form colonies in vitro in response to macromolecules, termed colony-stimulating activity (CSA). In the mouse, such colony forming units in culture (CFU-c) derive from multipotent hematopoietic stem cells, CFU-s, that give rise to colonies in the spleen when injected into lethally irradiated recipient mice. Application of physical separation techniques, such as velocity sedimentation, density gradient separation, and cell adherence procedures, has confirmed that CFU-s and CFU-c are different cells and has further shown that each compartment comprises several sub-
populations that differ in their capacity of self-replication or responses to
growth regulators in vitro.9-13
There is no assay system available for the detection of multipotent stem cells
in human bone marrow. However, clonal techniques have now been developed
based on the observation that human marrow cells can proliferate and differ-
entiate to granulocytes in diffusion chambers implanted intraperitoneally (i.p.)
in irradiated mice.14,15 The work reported here shows that cells forming colonies
in fibrin clot diffusion chambers (CFU-d) can be separated from the vast ma-
jority of CFU-c and that two different CFU-c populations can be distinguished
by means of velocity sedimentation. Part of these results have been briefly pre-
sented elsewhere.16

MATERIALS AND METHODS
Normal human bone marrow was obtained from healthy volunteers by aspiration from the
superior posterior iliac spine. Heparin was added as an anticoagulant. Buffy coats were collected
after centrifugation of the aspires at 200 g for 10 min and washed twice in modified McCoy’s
5A medium17 supplemented with 15% fetal calf serum (FCS).

Density cut separation. Buffy coat cells were separated into two fractions according to density
by a density “cut” at 1.070 g/cm³ in bovine serum albumin (BSA) as described previously.17

Velocity sedimentation. The low-density cells (<1.070 g/cm³) were separated according to
sedimentation velocity at 1 g using the procedure described by Miller and Phillips.18,19 Nu-
cleated cells (5-10 x 10⁶) were suspended in 40 ml 0.2% BSA (fraction V, Armour) in isotonic
phosphate-buffered saline (PBS), loaded on top of a gradient of 0.4%-2% BSA in PBS, and al-
lowed to sediment at 4°C in a 17-cm Stadut sedimentation chamber. After 4-5 hr 30-ml fractions
were collected and the cells were counted and assayed for colony-forming units (CFU) as de-
scribed below. For practical reasons the fractions containing the most rapidly sedimenting cells
were pooled prior to culture.

Unseparated control samples of low-density cells were stored in test tubes at 4°C in 0.2% BSA-
PBS solution during the separation procedure and assayed for CFU in parallel with the separated
cell fractions.

Assays for CFU

CFU-c. Cells were suspended in modified McCoy’s 5A medium supplemented with 10% FCS
and 0.3% bactoagar (Difco) and cultured in 35-mm Petri dishes with feeder layers containing
10⁶ peripheral blood leukocytes in 0.5% agar medium as a source of CSA.3 The cultures were
incubated at 37°C in a humidified atmosphere of 7.5% CO₂ in air and scored for clusters (3-50
cells/aggregate) and colonies (>50 cells) after 7 and 14 days of incubation. Three to five plates
were scored per point. Colonies and clusters were aspirated by means of fine Pasteur pipettes,
smeared on glass slides, fixed, and stained with May-Grunwald Giemsa stain for individual typing
according to morphology.

CFU-d. Fibrin clot diffusion chambers (Millipore; pore size 0.22 μm) containing cells in modi-
ified McCoy’s 5A medium and 20% FCS were prepared as previously described.14,20,21 The chambers were sealed and implanted i.p. into 5-7 wk-old conventionally outbred female Swiss
mice (CD-1; Charles River, Wilmington, Mass.) that received 600 R total body irradiation from a
137Cs source 2-4 hr before implantation. After 7 days the chambers were removed, cleaned on
the outer surfaces, and reimplanted into newly irradiated mice. Two chambers were implanted into
each mouse. After a total of 14 days the cultures were harvested,14 stained with May-Grünwald
Giemsa stain, and scored for neutrophilic and eosinophilic colonies (>30 cells). The number of
eosinophil colonies was sometimes too low to give valid information. In that case only results with
neutrophilic CFU-d were presented. An average of 12 chambers (range 6-18) were scored per
point.
A few modifications of the diffusion chamber technique were introduced for the present series
of investigations compared with previously published experiments. Apart from a slightly increased
number of colonies in diffusion chambers, essentially the same results were obtained with the
present technique.
The number of cells inoculated per culture varied with the particular experiment, depending on the number of cells available. Unsedimented low-density cells were plated in agar at 1-2 x 10^5 cells/culture or inoculated into diffusion chambers at 1.5 x 10^5 cells/chamber unless otherwise stated. Fractions of cells obtained after velocity sedimentation were plated in agar cultures at 2-10 x 10^4 cells and in diffusion chambers at 4-10 x 10^4 cells/culture. In each velocity sedimentation experiment a constant number of cells from each fraction (or pool of fractions) were plated per culture. The number of colonies was found to be proportional to the cell dose in the range used.

Tritiated thymidine suicide technique. To assess the proportion of colony-forming cells in DNA synthesis (S phase), tritiated thymidine (³H-TdR) with high specific activity was used as an S-phase specific cytotoxic agent.22,23 Samples of low-density buffy coat cells or cell fractions obtained after velocity sedimentation were plated in agar cultures at 2-10 x 10^4 cells and in diffusion chambers at 4-10 x 10^4 cells/culture. The reduction in the number of colonies after pulse incubation with ³H-TdR was expressed as a percentage of the number in control cultures.

Statistical methods. The standard error of the ratio between ³H-TdR-sensitive and total CFU was calculated as described by Blackett.24

RESULTS

Density cut separation of normal human bone marrow buffy coat cells. Separation of normal human bone marrow cells according to density has been used as a method to remove inhibitory polymorphonuclear granulocytes from the low-density fraction (< 1.070 g/cm^3), which contains the majority of CFU-c.17,25,26 To see if CFU-d, like CFU-c, were low-density cells, buffy coat of bone marrow was separated by density cut at 1.070 g/cm^3 into two fractions and the recovery of CFU in each fraction was measured. Parallel cultures of unseparated buffy coat cells were plated to assess the number of CFU before separation. As shown in Table 1, all colonies were found in cultures of low-density cells. Density separation resulted in an absolute enhancement of colony formation by low-density cells in agar culture by approximately 50%. This enhancement was abolished when low- and high-density cells were mixed in the original proportion and cocultured in agar, confirming that dense leukocytes inhibited colony formation in agar.26

<table>
<thead>
<tr>
<th>Table 1. Density Cut Separation of Normal Bone Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/Culture</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Nucleated Cells/Culture*</td>
</tr>
<tr>
<td>Unseparated</td>
</tr>
<tr>
<td>Low-density fraction (&lt; 1.070 g/cm^3)</td>
</tr>
<tr>
<td>High-density fraction (&gt; 1.070 g/cm^3)</td>
</tr>
<tr>
<td>Coculture, low-and high-density fractions</td>
</tr>
</tbody>
</table>

*3 x 10^5 unseparated cells yielded 2.6 x 10^5 low-density and 0.4 x 10^5 high-density cells. The table shows the number of colonies formed by 3 x 10^5 unseparated cells, 2.6 x 10^5 low-density cells, and 0.4 x 10^5 high-density cells.
†10-14 chamber/group.
‡Agar colonies counted on day 7.
Since both CFU-c and CFU-d were found in the low-density fraction, the density cut procedure was used in further experiments. In a series of 16 normal bone marrows the average numbers of CFU per 10^5 low-density cells were 5.7 neutrophilic CFU-d, 1.6 eosinophilic CFU-d, 55 CFU-c counted day 14, and 85 CFU-c counted on day 7 of incubation. However, large variations in their relative numbers were found.

Separation of CFU-d and CFU-c populations by velocity sedimentation. Normal low-density bone marrow cells were separated by velocity sedimentation, and fractions of cells were collected and assayed for CFU-d and CFU-c. The number of colony-forming cells in each fraction was calculated and normalized to the percentage of the number of CFU in the peak fractions. The velocity sedimentation profiles of precursor cells seen in Fig. 1 show the mean of three experiments. CFU-d that formed neutrophilic colonies had a peak sedimentation rate of 5.2–5.4 mm/hr. Diffusion chamber eosinophilic colonies had a sedimentation profile very similar to that of neutrophilic colonies. By scoring agar cultures on both day 7 and day 14 of incubation, two different CFU-c populations could be distinguished. One was a rapidly sedimenting cell population (7.0–7.2 mm/hr) that formed colonies after 1 wk incubation in vitro. On day 14, however, colonies were derived from more slowly sedimenting cells, which had a peak sedimentation velocity of approximately 6.0 mm/hr.

In four subsequent experiments similar differences in the three sedimentation profiles were observed. The sedimentation rates of granulocytic CFU-d were found to vary between 4.7 and 5.4 mm/hr. There was a parallel variation of CFU-c forming colonies on day 14 of 5.5–6.4 mm/hr and of CFU-c that
formed colonies after 7 days of 6.4–8.2 mm/hr. Such changes may have been due to minor technical variations between the experiments.

Colonies and clusters in agar cultures were examined for cellular morphology. On day 7, most aggregates contained neutrophilic granulocytes, but in one experiment mononuclear cells predominated. Most colonies on day 14 were composed of neutrophil granulocytes and/or mononuclear cells and macrophages; 10%–20% were eosinophil colonies. There was no significant differences in the proportion of the various colony types in slowly and rapidly sedimenting fractions.

Relationship between clusters and colonies in agar cultures, day 7. When agar cultures were scored for clusters and colonies after 7 days of incubation, the cluster-to-colony ratio decreased with increasing sedimentation rate. Cultures of rapidly sedimenting cells had an average cluster-to-colony ratio of 1:1, whereas slowly sedimenting cells formed about 20–100 clusters to each colony. This is shown semilogarithmically in Fig. 2, which depicts distributions of colonies (counted on days 7 and 14) and clusters (day 7). There was no parallel relationship between the logarithmic profiles of day-7 clusters and colonies indicating a change in their ratio with increasing sedimentation velocity. Cells forming clusters after 7 days of culture were markedly heterogeneous with respect to velocity and formed a profile intermediate between the two colony-forming cell populations. In contrast, no difference in the sedimentation profiles of clusters and colonies was apparent when the cultures were scored on day 14. Qualitatively similar results were found in nine other experiments. No significant differences in the cellular composition of clusters in different fractions were found.

Fig. 2. Velocity sedimentation profiles of clusters (3–50 cells) and colonies (>50 cells) on day 7 and colonies on day 14 in agar cultures. Absolute numbers depicted semilogarithmically to show that cluster-to-colony ratio on day 7 decreased with increasing sedimentation rate of precursor cells. Clusters were assumed to derive from heterogeneous cell populations, one of which was identical with day-7 colony-forming CFU-c. Maximum estimate of the number of clusters in this compartment was obtained by multiplying the number of day-7 colonies in each fraction with the cluster-to-colony ratio observed in cultures of the most rapidly sedimenting cells. By subtracting their number from total observed clusters, a profile of "excess clusters" was calculated. Note that this profile was parallel with the profile of colonies on day 14.
One possible explanation for the heterogeneity in cluster-to-colony ratio was that there were intrinsic differences between the cells that gave rise to clusters and colonies day 7. Since the majority of colonies scored on day 7 were only slightly larger (50-80 cells) than the arbitrary size limit (50 cells) that distinguished colonies from clusters, it was likely that some of the clusters were derived from the same population of cells that gave rise to the colonies day 7. A maximum estimate of the number of clusters formed in each fraction by day-7 CFU-c was then obtained by multiplying colony numbers with the low cluster-to-colony ratio observed in the most rapidly sedimenting fractions. The “excess clusters” that according to this estimate did not derive from day-7 CFU-c formed a profile parallel with that of day 14 CFU-c (Fig. 2), suggesting that some of the colonies scored on day 14 were detectable as clusters on day 7.

This calculation was based upon the assumption that the proportion of clusters and colonies formed by day 7 CFU-c was constant within all fractions and not influenced by cellular interactions. Mixing experiments, described below, failed to disclose such interacting cells.

**Mixing experiments.** Various cell fractions were mixed and cocultured in agar and in diffusion chambers to exclude the possibility that the observed profiles of CFU-d and CFU-c resulted from interactions with other cells that stimulated or inhibited colony formation in some of the fractions. The observed number of colonies in the cocultures was compared with the number expected by culture of each fraction alone. No consistent differences between expected and observed values were found (data not shown). Similarly, there was no evidence that the heterogeneity in the cluster-to-colony ratio was due to inhibitory or stimulatory cells affecting the proliferation rate or growth potential of aggregate forming cells in certain velocity fractions.

By comparing the number of colonies in agar or diffusion chamber cultures of unseparated bone marrow to the number obtained in all fractions, the recovery of colony-forming cells after separation was found to average 70%, for all types of CFU (range 50%-80%), and to equal the recovery of total nucleated cells. Thus no net enhancement or inhibition of colony formation resulted from the cell separation per se. The cluster-to-colony ratio in agar cultures of unseparated marrow was intermediate between the highest and lowest ratios obtained in separated fractions.

**Distribution of S-phase and non-S-phase CFU.** Since the velocity sedimentation procedure separates cells primarily according to size (larger cells having faster sedimentation rates) and cells double their size during the mitotic cycle, the reason for the observed velocity differences could be that the assays detected identical cells in various stages of the mitotic cycle. To exclude this possibility, normal bone marrow cells were separated by velocity sedimentation, and half of each fraction was subjected to high specific activity ³H-TdR suicide to estimate the number of CFU in S phase. Control and ³H-TdR-exposed samples were cultured in agar and in diffusion chambers to assess the number of CFU. Results from one of three consistent experiments are shown in Fig. 3. The distributions of non-S-phase CFU were determined from cultures of ³H-TdR-treated samples. Profiles of S-phase CFU (±1 SEM) were obtained by subtracting the number of non-S-phase CFU from total CFU as measured
by culture of the control samples. The profiles were normalized to percentage of the number of total CFU in the peak fractions. Note that the most rapidly sedimenting fractions were pooled into one sample and that the average sedimentation velocity of these cells was higher than it appears from Fig. 3.

As seen in Fig. 3, three populations of S-phase cells with different sedimentation velocity ranges could be distinguished. Similarly, the non-S-phase components of the three populations clearly differed in sedimentation rate. The majority of the non-S-phase cells sedimented more slowly, i.e., were smaller than their corresponding S-phase compartments, and were therefore G1 or G0 cells. Cells that double their size during the mitotic cycle increase their sedimentation rate by a factor 1.59. The S-phase component of a cell population will therefore sediment between the peak of G1-cells and 1.59 times their rate. The three S-phase profiles shown in Fig. 3 are consistent with this prediction.

Recovery of total and $^3$H-TdR-resistant CFU after separation was calculated by serial addition of the number of CFU in all fractions, and the ratio of $^3$H-TdR-sensitive CFU to total CFU was subsequently calculated. For CFU-c this ratio was similar to the proportion of $^3$H-TdR-sensitive CFU assessed in parallel cultures of unseparated marrow, as indicated in Fig. 3. Since only a
Table 2. CFU Sensitivity to $^3$H-TdR in Normal Low-Density Bone Marrow
(Percentage Sensitive CFU ± 1 SEm)

<table>
<thead>
<tr>
<th>CFU-d Colonies</th>
<th>CFU-c Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophilic</td>
<td>Day-14 Colonies</td>
</tr>
<tr>
<td>8 ± 16</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>26 ± 34</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>10 ± 20</td>
<td>2 ± 20</td>
</tr>
<tr>
<td>22 ± 24</td>
<td>3 ± 20</td>
</tr>
<tr>
<td>31 ± 30*</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>Average</td>
<td>7 ± 10</td>
</tr>
</tbody>
</table>

*The number of colonies formed by $^3$H-TdR-treated cells was 131% of the number in the control cultures. The results obtained with unseparated bone marrow in the experiment presented in Fig. 3 are included in the table.

Proliferative state of CFU populations. The results indicated the existence of three different cell types that could be distinguished with the culture techniques employed. To obtain information about the proliferative activity of these populations during normal conditions, the proportion of $^3$H-TdR-sensitive CFU in low-density bone marrow was measured (Table 2). Approximately 50% of CFU-c that formed colonies on day 7 were sensitive to $^3$H-TdR. On an average, significantly smaller fractions of day-14 CFU-c (21%) and of neutrophilic CFU-d (7%) were sterilized after pulse exposure to $^3$H-TdR ($p < 0.001$). Provided that the duration of the S phase was similar for each cell type, this suggested that day-7 CFU-c were rapidly proliferating cells whereas day-14 CFU-c and neutrophilic CFU-d either proceeded more slowly through the mitotic cycle or had a significant proportion in a noncycling (G0) state.

The standard errors of the ratio between $^3$H-TdR-sensitive and total CFU were in general agreement with the values predicted by Blackett. As discussed by that author, the degree of statistical confidence is low when the percentage $^3$H-TdR-sensitive CFU is close to zero. Due to the large standard error of each observation it is mainly the average $^3$H-TdR sensitivity of CFU in the entire series of experiments that is of interest. However, the data presented in Table 2 did not exclude the possibility of interexperimental variation in the proliferative state of CFU. The number of eosinophilic colonies found in diffusion chambers was too low to give information about the cycling state of eosinophilic CFU-d.

DISCUSSION

The velocity sedimentation profiles of CFU-d that formed neutrophilic or eosinophilic colonies after 14 days in diffusion chamber culture were different from the profiles of CFU-c. Furthermore, two different CFU-c profiles were obtained by counting agar colonies on day 7 and on day 14. This suggested that at least three different populations were detected in the assays. A similar difference in the profiles of CFU-c that formed colonies on day 7 and day 14 was recently described by Johnson et al. The velocity profiles of the three CFU...
populations overlapped each other. However, by comparing the absolute numbers of CFU in each fraction rather than comparing the normalized profiles, evidence was obtained that one class of CFU (i.e., CFU-d) was not identical with a subpopulation of one of the other CFU populations (i.e., day-14 CFU-c). Similar evidence also excluded the possibility that day-14 CFU-c were simply a mixture of CFU-d and day-7 CFU-c.

Mixing experiments and recovery studies suggested that the profiles were not significantly influenced by stimulatory or inhibitory cells. To minimize the significance of potential interacting cell populations, the present study utilized low-density bone marrow cells depleted of polymorphonuclear granulocytes. These cells inhibit colony formation in agar by inhibiting monocyte CSA production but have no significant effect on diffusion chamber colony formation, which is not absolutely dependent upon CSA-producing monocytes in the chamber inoculum. In this context it is also of interest that in one experiment, where nonadherent (monocyte depleted) low-density bone marrow cells were separated by velocity sedimentation, the same differences in CFU-d and CFU-c were observed (Jacobsen N, Broxmeyer HE: Unpublished data).

Cell cycle analysis of separated cells showed that each CFU population had its own S-phase and non-S-phase components, suggesting that they represented independent cell populations rather than different components of the mitotic cycle of the same cell type.

As shown in Fig. 2, the cells that gave rise to clusters and colonies in day-7 agar cultures could be partially segregated. Metcalf and MacDonald described a similar segregation of cluster- and colony-forming cells in cultures of mouse bone marrow, except that in the mouse cluster-forming cells sedimented more rapidly than colony-forming cells. There was no evidence that the heterogeneity in cluster-to-colony ratio was due to interacting cells that stimulated or inhibited proliferation in some fractions.

In contrast to CFU-d and CFU-c that formed colonies, the profile of day-7 cluster-forming cells dispersed over considerably more fractions than expected for homogeneous cells in mitotic cycle. This suggested that the clusters were derived from at least two different cell populations. The minimum size limit of 50 cells employed to distinguish colonies from clusters on day 7 was arbitrary. It was therefore likely that one cluster-forming cell population was identical with the day-7 colony-forming cells, which may form aggregates of varying size as a result of stochastic variations in the number of divisions in culture. By extrapolating from the cluster-to-colony ratio in cultures of the most rapidly sedimenting cells, a second compartment of clusters could be delineated. These “excess clusters” were derived from cells that had the same sedimentation velocity as CFU-c that formed the colonies on day 14. This may suggest that some of the day-14 colonies were represented by clusters on day 7. However, when the peak number of excess clusters and day-14 colonies were compared, it was clear that only 75% of the day-14 CFU-c formed aggregates of three or more cells at day 7. This was a maximum estimate, since it presumed that all excess clusters went on to form colonies on day 14. This means that even if total aggregates (colonies and clusters) were counted on day 7, the total compartment of CFU-c would be underestimated.
Metcalf reported that in cultures of mouse bone marrow there is a continuous formation of colonies from cells that initiate proliferation late in the culture period. The present data suggest that a similar situation exists in human cultures and, furthermore, that the clusters that give rise to the late colonies are derived from a separate population of CFU-c. The reason why these progenitor cells proliferate more slowly during the first week of culture is not known, but it may be suggested that they initially lack the capacity of responding to CSA during these culture conditions.

The possibility that clusters scored on day 7 may derive from two different cell populations is of interest, since it raises the question of the nature of cells that form large numbers of clusters in cultures of some acute myeloblastic bone marrows.

Information about the properties of colony-forming cells was obtained by assessment of the proportion of cells in unseparated marrow that were sterilized after exposure to high specific activity \(^{3}\text{H-TdR}\). The results suggested that the three populations of CFU differed with respect to proportions of cells in S phase during normal conditions. On an average, day-14 CFU-c were less sensitive to \(^{3}\text{H-TdR}\) than day-7 CFU-c (\(p < 0.001\)), suggesting a slower turnover rate. In this context, Moore et al. found that 38% ± 14% of normal human CFU-c that formed colonies (>40 cells) on day 8 were in S phase. This value is not inconsistent with the present observation of 48% ± 4%.

Neutrophilic CFU-d seemed to be a population of cells with relatively low proliferative activity. This conclusion was based on the low average sensitivity to \(^{3}\text{H-TdR}\). It was also supported by the shape of the velocity sedimenting profile shown in Fig. 1, since S-phase cells, if present, would be expected to sediment at a rate between the peak sedimenting velocity (5.4 mm/hr) and 1.59 times this value (8.6 mm/hr). As can be seen, only a small proportion of the total CFU-d population was found in this area of the profile.

The fact that slowly sedimenting (G,) CFU were resistant to \(^{3}\text{H-TdR}\) (Fig. 3) supported the assumption that under the conditions of the experiments \(^{3}\text{H-TdR}\) was nontoxic to non-S-phase cells.

CFU-c are believed to be committed granulocyte-macrophage progenitor cells. Kinetic studies suggest that CFU-d are precursors of CFU-c. However, there is as yet no evidence that CFU-d represent multipotent stem cells, equivalent to CFU-s in mice. Alternatively, they may be intermediate cells, committed to granulopoiesis, but more immature than the cells detected in the CFU-c assay.

ACKNOWLEDGMENT

We would like to thank Sabariah Schrader for excellent technical assistance.
REFERENCES

26. Broxmeyer HE, Moore MAS, Ralph P: Cell-free granulocyte colony inhibiting activity


34. Iscove NN: Cell culture studies of hemopoietic progenitor cells from mouse and man. Thesis, Institute of Medical Science, University of Toronto, 1972, p 57


Diversity of human granulopoietic precursor cells: separation of cells that form colonies in diffusion chambers (CFU-d) from populations of colony-forming cells in vitro (CFU-c) by velocity sedimentation

N Jacobsen, HE Broxmeyer, E Grossbard and MA Moore