Polyamine Synthesis in Bone Marrow Granulocytes: Effect of Cell Maturity and Early Changes Following an Inflammatory Stimulus

By Warren H. Evans, Charles K. Grieshaber, William C. Miller, Shirley M. Wilson, and Harold A. Hoffman

Enriched fractions of mature and immature neutrophil granulocytes, isolated from guinea pig bone marrow, were assayed for ornithine decarboxylase activity and polyamine content. The results show that immature granulocytes contain at least ten times more ornithine decarboxylase activity and two times more spermidine than mature granulocytes. The incorporation of \(^{14}C\)-ornithine into putrescine and spermidine of intact immature granulocytes was three to four times and ten times, respectively, that of mature granulocyte preparations. Six hours after an inflammatory stimulus, transient increases of 14-fold and 3-fold in the activities of ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase, respectively, were observed in immature bone marrow granulocytes. At this time the incorporation of \(^{14}C\)-ornithine into putrescine and spermidine in bone marrow granulocytes from stimulated animals was 14 times that of cells from controls. A maximum increase in DNA synthesis in these cells during the inflammatory response occurred 6 hr after the maximum increase in the polyamine synthetic activity. Together these data suggest that polyamine synthesis in the granulocyte compartment of the bone marrow is associated chiefly with immature proliferating cells and that increased polyamine synthesis precedes increased granulocyte proliferation in the bone marrow following an inflammatory stimulus.

Granulocytic leukocytes in the bone marrow of normal individuals undergo a process of continual cell renewal that appears to be regulated by a feedback mechanism linked to the rate of loss of granulocytes in peripheral tissues. During inflammatory reactions, there is an increased destruction of these cells in inflamed tissues, and the bone marrow responds to this loss by an increase in the rate of proliferation and maturation of granulocyte precursors. At the present time, however, the biochemical mechanisms underlying the regulation of granulocyte production in both the normal and inflammatory states are poorly understood.

In recent years there have been many studies of the content and metabolism of polyamines in a wide variety of mammalian cells. Interest in this area has been kindled by the finding that the content and rate of synthesis of polyamines are generally much greater in tissues with high growth rates than in nongrowing tissues. Previous workers showed that bone marrow contains high levels of polyamines compared with most other tissues, but to our knowledge the content and synthesis of polyamines in specific types of bone marrow cells, such as the granulocytic leukocytes, have not yet been studied. Therefore as a first
step in investigating the possible involvement of polyamines in the regulation of
granulocyte proliferation in the normal and inflammatory states the present
study was aimed at determining whether or not bone marrow granulocytes
contain and are capable of synthesizing polyamines. Since these cells pass
sequentially through a proliferating phase and a nonproliferating phase during
maturation in the marrow, it was also of interest to ascertain if any differences
in polyamine content and synthesis existed between immature proliferating and
mature nonproliferating granulocytes. The effects of an inflammatory stimulus
on the metabolism of polyamines in bone marrow granulocytes are also re-
ported here.

MATERIALS AND METHODS
Isotopes and chemicals. The following isotopes were obtained from New England Nuclear:
l-[U-14C]-arginine, 295 mCi/mmol, S-adenosyl-l-[carboxy-L-14C]-methionine, 7.5 mCi/mmol,
l-[U-14C]-ornithine, 234 mCi/mmol, l-[U-14C]-ornithine, 59 mCi/mmol, was purchased from
Amersham Searle; [methyl-3H]-thymidine, 6 Ci/mmol, was acquired from Schwarz/Mann.
Sources of chemicals were as follows: l-arginine, Nutritional Biochemicals; thiosemicarbazide,
2,3-butanedione monoxime, cetyltrimethylammonium bromide (CETAB), and acetaldehyde,
Eastman Organic Chemicals; l-ornithine, pyridoxal-5'-phosphate, EDTA, putrescine dihydro-
chloride, spermine tetrahydrochloride, hyamine hydroxide, shellfish glycogen, and diphenylamine,
Sigma Chemical; dithiothreitol, S-adenosyl-l-methionine, Calbiochem; Tnis, Schwarz-Mann;
urea, J. T. Baker Chemical; Ficoll (average mol wt 400,000 daltons), Pharmacia; Instagel,
Packard Instruments. AG 50W-X8 cation exchange resin, 200-400 mesh, was obtained from Bio-
Rad Laboratories.
Preparation of granulocytes. Bone marrow cells were obtained from male strain 13 guinea pigs
(500-700 g) as described previously,6 except that in the present study the cells were subjected
to brief (60 sec) hypotonic shock to remove erythroid cells according to the method of Fallon
et al.7 The washed cells were designated as the mature granulocyte fraction (see Table 1 for
differential count). Enriched fractions of immature granulocytes were isolated from these suspen-
sions under sterile conditions by a modification of our previously described Ficoll density
centrifugation method.8 Briefly, 5 x 10^11 cells suspended in 25 ml of 17°C Ficoll (in 0.15
M NaCl-0.005 M KCl-0.01 M phosphate buffer, pH 7.4) were centrifuged at 200 g for 5 min and
then at 700 g for 50 min. The immature granulocytes, concentrated mainly in the supernatant
fraction, were collected and washed as described in the original procedure. Cells in the mature
and immature granulocyte fractions were 90%-95% viable on the basis of their ability to exclude
trypan blue. Neutrophil granulocytes were classed as mature (polymorphonuclears and bands),
intermediate (myelocytes and metamyelocytes), or early (promyelocytes and blasts).

Table 1. Polyamine Content and Ornithine Decarboxylase Activity of Enriched
Fractions of Mature and Immature Neutrophil Granulocytes

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Neutrophil Granulocytes (%)</th>
<th>Polyamines (nmoles/10^7 Cells)</th>
<th>Ornithine Decarboxylase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immature</td>
<td>Mature</td>
<td>Spermidine</td>
</tr>
<tr>
<td>A (immature)</td>
<td>61 ± 2* 22 ± 1 5 ± 1*</td>
<td>2.8 ± 0.2* 4.8 ± 0.2</td>
<td>20.4 ± 0.61</td>
</tr>
<tr>
<td>B (mature)</td>
<td>9 ± 2 22 ± 1 55 ± 2</td>
<td>1.4 ± 0.2 3.9 ± 0.4</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values for differential counts and polyamines represent means ± SE of four experiments. Enzyme activity
is expressed as pmoles CO2 formed/hr/10^7 cells; values are means ± SE of three experiments. Differences
between fractions were tested for significance using Student's t test on paired samples. Cell counts are ex-
pressed as percentages of each cell type in a total of 200 cells counted. Only percentages of neutrophil
granulocytes in the fractions are shown, since the small percentages of the remaining cell types (eosinophils,
basophils, and lymphocytes) were not significantly different (p > 0.05) between fractions A and B.
*p < 0.01.
†p < 0.005.
Inflammatory reactions were produced in guinea pigs using the method described by Fruhman for rats, consisting of injecting male strain 13 guinea pigs (650-750 g) intraperitoneally with sterile 0.5% glycogen in 0.9% saline. At various time intervals thereafter the animals were killed and the peritoneal fluid was removed and its cellular composition determined. Morphologic examination showed that the cells were 90-95%, polymorphonuclear leukocytes. The inflammatory reaction produced by the sterile glycogen solution could be attributed to the effects of both glycogen and saline. For this reason uninjected rather than saline-injected animals were used as controls in our studies.

**Enzyme assays.** For ornithine decarboxylase assays, bone marrow cells were disrupted by freezing and thawing and then homogenized in 50 mM Tris-HCl pH 7.5 containing 5.0 mM diethytoethanol and 0.1 mM pyridoxal-5'-phosphate. The homogenates were centrifuged at 25,000 g for 20 min, and the supernatants were removed and analyzed for ornithine decarboxylase activity by the radioassay procedure of Cavia and Webb. Enzyme units were calculated as pmoles CO₂ released/hr. A stoichiometric relationship between the release of CO₂ and the formation of putrescine from ornithine by ornithine decarboxylase was established using L-[U-¹⁴C]-ornithine as the substrate in the above assay. In three assays, the mean yield of CO₂ was 22 ± 1 pmoles/10⁷ cells, while that of putrescine was 18 ± 3 pmoles/10⁷ cells.

Adenosylmethionine decarboxylase activity was determined using a procedure similar to that described for ornithine decarboxylase; the only difference was in the composition of the incubation medium, to which 0.5 ml of the enzyme containing supernatant was added. The adenosylmethionine decarboxylase incubation medium contained 50 mM Tris-HCl pH 7.5, 0.2 mM EDTA, 5 mM putrescine dihydrochloride, 128 nM S-adenosyl-L-methionine, and 13 nM S-adenosyl-L-[carboxy-¹⁴C]-methionine (0.1 μCi).

Arginase activity was assayed as the rate of urea formation from L-arginine according to the method of Geyer and Dabich. Prior to assay, intact cells were lysed with 0.5% CETAB in 0.01 M phosphate buffer pH 7.0. CETAB at the concentration present in the lysates had no effect on the arginase activity of homogenates of marrow cells prepared in 0.34 M sucrose. Arginase enzyme units were expressed as pmoles urea formed/min/10⁹ cells.

All assays were carried out under conditions where product formation was linear with respect to incubation time and amount of enzyme.

**Determination of polyamines.** The concentration of polyamines in bone marrow cells was determined using a modification of the ion-exchange separation method reported by Inoue and Mizutani. Cells (5 x 10⁷) were extracted with 2% HClO₄, and the extract was applied to a column of AG 50W-X8. The HClO₄ was washed out with 5 ml 0.5 N HCl; putrescine and ornithine were eluted with 10 ml 2.0 N HCl, spermidine with 10 ml 3.0 N HCl, and spermine with 10 ml 6.0 N HCl. Each fraction was evaporated to dryness and resuspended in 0.1 ml 0.01 N HCl. Aliquots (0.05 ml) were subjected to high-voltage electrophoresis, and polyamine concentrations were determined as described. ¹⁴C-labeled polyamines were separated and counted in Instagel, using a Beckman LS-355 liquid scintillation spectrometer. All counts were corrected to disintegrations/min (dpm) by internal standardization.

**Isotope incorporation studies.** The medium employed for the radioisotope incorporation studies was serum-free Eagle’s Minimal Essential Medium (MEM) (NIH Media Unit) with no antibiotics added and supplemented with sodium pyruvate (1.0 mM), L-[U-¹⁴C]-arginine and L-[U-¹⁴C]-ornithine and [³H]-thymidine were added to the medium at a level of 1.0 μCi/ml. The final concentration of unlabeled L-arginine in MEM was 0.10 mM. Unlabeled L-ornithine was added in a final concentration of 0.10 mM only to the medium containing [¹⁴C]-ornithine. The cells (1 x 10⁷/ml) were incubated in Falcon tissue culture flasks at 37°C for 30-60 min under 95% air-5% CO₂. Following incubation, the cells were quantitatively removed from the flasks and washed twice with 2 ml 0.15 M NaCl-0.01 M phosphate buffer, pH 7.4. The final cell pellets were assayed for radioactive polyamines using the methods described above and for radioactive DNA as described previously.

**RESULTS**

**Ornithine decarboxylase activity and polyamine levels in enriched fractions of mature and immature granulocytes.** In guinea pig bone marrow, the immature proliferating granulocyte compartment consists of cells from the blast to the

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metamyelocyte stages of maturation, and the mature nonproliferating compartment consists of band and polymorphonuclear cells.\textsuperscript{12,13} The data in Table 1 show that ornithine decarboxylase activity of the fraction enriched in immature granulocytes is about 12 times that of the mature cell fraction; this difference presumably reflects the difference in the percentage of early cells (blasts plus promyelocytes) between the two cell preparations. This finding suggests that ornithine decarboxylase is most active during the proliferating phase of granulocyte maturation but decreases markedly in activity in the nonproliferating phase. The data also show that enriched fractions of immature granulocytes contain about twice as much spermidine as the mature cell fraction, with no significant difference in the spermine content between the two cell preparations. The concentration of putrescine was too low to be accurately determined spectrophotometrically in freshly isolated cell preparations.

**Synthesis of polyamines in enriched fractions of immature and mature granulocytes.** To determine whether or not the difference in ornithine decarboxylase activity between immature and mature granulocytes reflected a difference in the capacity for polyamine synthesis, we measured the rate of synthesis of putrescine, spermidine, and spermine in intact cells in vitro using L-[U-\textsuperscript{14}C]-ornithine as substrate. We also studied the rate of incorporation of L-[U-\textsuperscript{14}C]-arginine into polyamines, since others\textsuperscript{14} reported that granulocytes contained high levels of arginase, which converts arginine to ornithine. The results shown in Table 2 indicate that the rate of incorporation of radioactive ornithine into putrescine of the immature cell fraction was three to four times that of the mature cell fraction. The rate of incorporation of this substrate into spermidine of the immature cells was about ten times that in the mature cells. Both cell preparations incorporated only small amounts of label into spermine, and no significant difference between the two cell preparations could be detected.

Surprisingly, we found that no radioactive arginine incorporation into the polyamines of either immature or mature cells could be detected using the same incubation conditions in which radioactive ornithine was readily converted to polyamines. The possibility that arginase activity might be absent in immature cells was examined in an attempt to explain this finding. Enzyme assays indicated that the mean (±SE) arginase activity of the enriched fraction of immature granulocytes was 1.2 ± 0.1 units, which was only about one-fifth that of the mature cell fraction (5.5 ± 0.6 units); this difference presumably reflects the difference in the percentage of mature cells between the two cell preparations (see differential counts in Table 1). These findings suggest that most of the

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
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</thead>
<tbody>
<tr>
<td>A (immature)</td>
<td>360 ± 41\textdagger</td>
<td>174 ± 44*</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>B (mature)</td>
<td>99 ± 4</td>
<td>14 ± 8</td>
<td>4 ± 4</td>
</tr>
</tbody>
</table>

Values represent means ± SE of three experiments. The differential counts of the enriched immature and mature granulocyte fractions were essentially the same as those given in Table 1.

\*p = 0.05.
\daggerp < 0.001.
Table 3. Cellular Composition and Ornithine Decarboxylase and S-Adenosyl-L-Methionine Decarboxylase Activities of Guinea Pig Bone Marrow Following Intraperitoneal Inflammatory Stimulation

<table>
<thead>
<tr>
<th>Hours After Stimulation</th>
<th>Neutrophil Granulocytes</th>
<th>Lymphocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Ornithine Decarboxylase</th>
<th>S-Adenosyl-L-Methionine Decarboxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18 ± 2</td>
<td>17 ± 2</td>
<td>45 ± 2</td>
<td>4 ± 2</td>
<td>14 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>17 ± 3</td>
<td>19 ± 3</td>
<td>40 ± 4</td>
<td>5 ± 1</td>
<td>18 ± 3</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>6</td>
<td>11 ± 3</td>
<td>22 ± 2</td>
<td>40 ± 3</td>
<td>6 ± 1</td>
<td>18 ± 2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>12</td>
<td>11 ± 1</td>
<td>19 ± 2</td>
<td>39 ± 1</td>
<td>7 ± 2</td>
<td>21 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>20</td>
<td>13 ± 1</td>
<td>34 ± 1*</td>
<td>22 ± 2*</td>
<td>5 ± 1</td>
<td>22 ± 2</td>
<td>4 ± 1</td>
</tr>
</tbody>
</table>

Differential cell counts were performed as described in Table 1 and are means ± SE of five animals at zero time and three animals at other times. Enzyme activity is expressed as pmoles CO₂ formed/hr/10⁷ cells; the values represent means ± SE of duplicate determinations on three different bone marrow preparations for each time point. Data at zero time refers to uninjected controls. All data were obtained with unseparated cells.

*p < 0.05 significantly greater or less than zero time value.
†p < 0.001 significantly greater than zero time value.

arginase activity of bone marrow granulocytes is associated with cells in the mature nonproliferating compartment.

Changes in differential cell counts and polyamine-synthesizing enzymes in the bone marrow granulocyte compartment in response to inflammation. The cellular composition of guinea pig bone marrow following intraperitoneal injection of glycogen is shown in Table 3. There was no appreciable change in the percentage of either mature or immature neutrophil granulocytes 4–12 hr after injection. The greatest change in marrow granulocyte composition was observed between 12 and 20 hr. The mature cells decreased nearly 50%, with a corresponding twofold increase in the intermediate cell types. The other cell types present in the bone marrow, namely lymphocytes, eosinophils, and basophils, underwent little change throughout the 0–20 hr period.

The ornithine decarboxylase activity was relatively low in normal bone marrow granulocytes and was significantly increased 4 hr after stimulation (Table 3). The enzyme activity reached its maximum level 6 hr after injection of the stimulus and declined steadily over the next 14 hr. It is noteworthy that at the time of maximal ornithine decarboxylase elevation the cellular composition of the bone marrow granulocyte compartment remained essentially unchanged relative to controls. Unlike ornithine decarboxylase, the S-adenosyl methionine decarboxylase activity was not significantly elevated 4 hr after injection; however, the activity reached the maximum level 6 hr after stimulation.

In order to determine if the inflammation-induced elevation of ornithine decarboxylase activity took place in the proliferating granulocyte compartment, enriched fractions of immature granulocytes from control and 6-hr-stimulated guinea pigs were prepared by Ficoll density centrifugation. The differential counts of these two cell preparations were not significantly different and were similar to that of fraction A in Table 1. The mean ± SE (three experiments) ornithine decarboxylase activity of the stimulated immature cells (501 ± 73 pmoles CO₂/hr/10⁷ cells) was significantly elevated (p < 0.001) with respect to controls (34.3 ± 7.5 pmoles CO₂/hr/10⁷ cells). The magnitude of this stimulation (14-fold) was of the same order as that observed in the unseparated
granulocytes (see Table 3). Furthermore, after cell separation the enzyme activity increased in proportion to the enrichment of early cells in the stimulated marrow, that is, a fivefold enrichment in early cells was accompanied by a fivefold increase in ornithine decarboxylase activity. These data indicate that the increase in ornithine decarboxylase activity in guinea pig bone marrow granulocytes in response to an inflammatory stimulus most likely occurs in the immature granulocyte population.

**Polyamine synthesis in vitro in bone marrow granulocytes from normal and stimulated guinea pigs.** To determine whether or not polyamine synthesis was increased when the polyamine-synthesizing enzymes were elevated, bone marrow preparations from control and 6-hr-stimulated animals were incubated in the presence of L-[U-14C]-ornithine and the radioactivity (dpm/10^7 cells) of intracellular ornithine and of each polyamine was measured (Table 4). The

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity (dpm/10^7 Cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>2440 ± 217</td>
<td>74 ± 15</td>
<td>24 ± 1</td>
<td>14 ± 7</td>
</tr>
<tr>
<td>Stimulated</td>
<td>2912 ± 336</td>
<td>1021 ± 182*</td>
<td>328 ± 171</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

Values represent means ± SE of three determinations. Isolation of polyamines and radioactivity measured as described in Materials and Methods.

*p < 0.01.

**p < 0.001.
radioactivity of ornithine was not significantly changed in the bone marrow cells following stimulation. However, the radioactivities of putrescine and spermidine were significantly elevated (14- and 13-fold, respectively) after stimulation. The incorporation of radioactivity into spermine was unchanged over this time interval. Thus the increased ornithine decarboxylase and adenosylmethionine decarboxylase activities after inflammation were accompanied by increased incorporation of ornithine into the polyamines.

**Relationship between the stimulation of ornithine decarboxylase and adenosylmethionine decarboxylase activities to changes in DNA synthesis.** Changes in the level of DNA synthesis following the inflammatory stimulus are shown in Fig. 1. Since it is known that DNA synthesis occurs mainly in the immature cells, the levels of thymidine incorporation into DNA at various times after stimulation are expressed on the basis of immature cells. The ornithine decarboxylase and adenosylmethionine decarboxylase activities given in Table 3 are reexpressed in Fig. 1 on the basis of immature granulocytes for purposes of comparison with the level of DNA synthesis, since polyamine synthesis is also confined primarily to these cells. It is clearly evident that the maximal level of DNA synthesis occurred 12 hr after stimulation and was preceded by the peak of the polyamine-synthesizing enzymes, which reached maximal levels at 6 hr. A much smaller (22%) but consistent increase in DNA synthesis was also observed 4 hr after stimulation, but at present the significance of this increase is not understood.

**DISCUSSION**

The findings presented here strongly suggest that polyamine synthesis in bone marrow granulocytes is associated chiefly with cells in the immature proliferating compartment and that the cessation of cell division in mature granulocytes is accompanied by a marked decrease or loss of this synthetic function. The data also show that ornithine decarboxylase activity, adenosylmethionine decarboxylase activity, and polyamine synthesis increase rapidly and substantially in immature granulocytes in the first 6 hr following an inflammatory stimulus. A rapid response of ornithine decarboxylase to proliferation stimuli was observed in many other types of growing cells and is apparently related to the extremely short half-life of this enzyme. Our data also indicate that the increase in polyamine-synthesizing enzyme activities precedes the increase in DNA synthesis, which occurs in these cells during inflammation. It remains to be determined, however, if polyamines play an active role in the regulation of DNA synthesis and cellular proliferation in the bone marrow granulocyte compartment.

Previous workers showed that mature granulocytes isolated from blood contain the enzyme arginase. Our studies indicate that this enzyme activity is also found in mature granulocytes in the bone marrow but is either absent or greatly decreased in immature granulocytes. We also found that under conditions where ornithine was readily incorporated into the polyamines of immature granulocytes no significant arginine incorporation could be detected. It should be noted that the enriched fractions of immature and mature granulocytes used in this study readily incorporate 14C-labeled arginine into cellular proteins. Therefore it is not likely that the lack of incorporation of arginine into...
polyamines was due simply to the failure of the cells to take up this amino acid. These observations therefore suggest that in immature granulocytes little or no ornithine for polyamine synthesis is produced from arginine by the action of arginase. In this respect bone marrow granulocytes differ from other proliferating cells, such as those in mammary gland and prostate, in which arginase and ornithine decarboxylase act coordinately in the synthesis of polyamines from arginine. Mature granulocytes have a high arginase content but also fail to incorporate arginine into polyamines, an observation consistent with the finding that mature cells apparently lack ornithine decarboxylase activity. Taken together, the data suggest that arginase and ornithine decarboxylase activities function asynchronously rather than coordinately during granulocyte maturation.

Two mechanisms of regulation of proliferation in the bone marrow granulocyte compartment have been postulated, cell-mediated control and humoral control. Direct evidence for the operation of either of these mechanisms in vivo is lacking. The cell-mediated control hypothesis is based on the observation that depletion of mature granulocytes from the marrow occurs during the response to inflammation or infection, and this could stimulate proliferation of immature cells by negative feedback control. Our current biochemical data do not support this hypothesis, since the changes in the polyamine synthetic pathway and DNA synthesis during the inflammatory reaction occur well in advance of any significant decrease in the number of mature marrow granulocytes. It is more likely that the early biochemical changes in bone marrow granulocytes during inflammation are mediated by humoral regulators. In this connection it is noteworthy that the temporal pattern of changes in ornithine decarboxylase activity in granulocytes in response to inflammation is similar to that produced by hormonal stimulation in other tissues.

Recently, we developed a short-term tissue culture method for studying the rate of granulocyte production. With this method it should be possible to study the effects of various polyamines and inhibitors of polyamine synthesis on granulocyte formation. Such studies should be useful in obtaining greater insight into the function of polyamines in the processes of proliferation and maturation of bone marrow granulocytes.

REFERENCES

17. Evans WH, Grishaber CK: Unpublished observations
Polyamine synthesis in bone marrow granulocytes: effect of cell maturity and early changes following an inflammatory stimulus

WH Evans, CK Grieshaber, WC Miller, SM Wilson and HA Hoffman