DNA polymerase activities were assayed
in bone marrow cells and peripheral leukocytes from normal people and patients
with acute myelogenous, chronic lymphocytic, and chronic myelogenous leukemia.
Extracts of subcellular components were
fractionated by velocity sedimentation
through sucrose density gradients and
assayed using activated DNA as template.
Two major DNA-dependent DNA poly-
merases were found in human cells with
molecular weights of approximately
50,000 and 200,000 daltons, respectively.
The DNA polymerase of high molecular
weight is located in the soluble cyto-
plasmic fraction and is inhibited by
N-ethylmaleimide. The low molecular
weight polymerase is detected in extracts
of nuclei and in the soluble fraction. It is
resistant to inhibition by N-ethylmalei-
mide. In all cell types tested, total DNA
polymerase activities were much higher in
cytoplasmic than in nuclear extracts.
Lymphocytes purified from normal periph-
eral blood had three to four times as
much of both the high and low molecular
weight polymerase activities per cell as
purified granulocytes. Leukemic myelo-
blasts had 10 to 20 times as much cyto-
plasmic DNA polymerase activity as more
mature leukocytes from normal peripheral
blood. In general, immature granulo-
poietic cells contained higher total DNA poly-
merase activities than more mature
granulocytes, and the major increases in
polymerase activities were in the high
and low molecular weight cytoplasmic
enzymes rather than in the nuclear
enzyme.

THE ANALYSIS OF DNA polymerase (DNA nucleotidyl transferase,
EC 2.7.7.7) activities in eukaryotic cells is rather complex because of the
presence of several separate species of DNA-dependent DNA polymerase. The
purpose of this investigation was to make a quantitative assessment of DNA
polymerase activities in cells from normal and pathologic human blood and
bone marrow.

Earlier work from this laboratory using velocity sedimentation demonstrated
the presence of two major DNA-dependent DNA polymerase activities
in extracts from a variety of animal tissues. A high molecular weight (6-8S)
enzyme with a pH optimum of 7.2 was found only in the supernatant, and a
minor species with a low molecular weight (3.4S) and alkaline pH optimum
was found in both the supernatant and the nucleus. Using other analytic schemes,
multiple DNA polymerases have also been found in normal human lympho-

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cytes, HeLa cells and KB cells. Increases in the 6–8S supernatant polymerase activity in concert with an increase in DNA synthesis have been described in regenerating rat liver and during regrowth of mouse L cells.

Two distinct DNA-dependent DNA polymerases can be demonstrated in human hematopoietic cells. As in other animal cells, the major polymerase activity having a molecular weight of approximately 200,000 (6–8S) is a soluble enzyme found only in the supernatant. A minor activity, having a molecular weight of approximately 50,000, is found in both nuclear and supernatant fractions. For convenience in discussion we refer to these enzymes as maxipolymerase and minipolymerase, respectively. The results of this investigation demonstrate a higher level of supernatant DNA polymerase activity in immature granulocytes and leukemic myeloblasts than in mature granulocytes. Activity of the nuclear polymerase is low in all cell types and may be relatively invariant.

**MATERIALS AND METHODS**

Samples of peripheral blood and bone marrow were obtained both from hematologically normal individuals and from patients with several types of acute and chronic leukemia. Unless otherwise indicated, patients with leukemia did not have a history of antineoplastic chemotherapy. Routine diagnostic bone marrow aspirations and peripheral blood samples were anticoagulated with 5 mM EDTA, and nucleated cells were separated from erythrocytes by repeated gravity sedimentation in 0.5% dextran. Purified nucleated cells were washed in normal saline, counted, pelleted by centrifugation, and stored at −70°C. Differential cell counts on smears stained with Wright-Giemsa were done on both the original samples and on samples from which erythrocytes had been selectively removed. All cell counts were obtained by hemocytometer hand counting. Only those samples in which there was no appreciable aggregation of cells were used.

Lymphocytes and granulocytes were purified from peripheral blood of healthy donors. Dextran sedimentation was used to remove the majority of erythrocytes, and then the leukocyte-rich fraction was layered on an equal volume of Hypaque-Ficoll for centrifugal separation of lymphocytes from granulocytes. Lymphocyte fractions contained at least 90% lymphocytes with 10% or less monocytes, while the pellet of cells at the bottom of the centrifuge tube contained 95% neutrophils, 5% or less lymphocytes, plus varying, but small, numbers of mature erythrocytes. These cells were then washed once with RPMI-1640 tissue culture medium (Gibco, Grand Island, N.Y.), pelleted by centrifugation, and stored at −70°C. A differential and total cell count was made on each fraction. At least 10⁸ nucleated cells were required for reliable assays of polymerase activities. DNA polymerase activity was stable in cells stored at −70°C for at least several months.

All extraction procedures were carried out at 0–4°C. Nucleated cells from bone marrow aspirates or peripheral blood samples were homogenized in 1 volume of TKM-sucrose buffer, containing 50 mM Tris, pH 7.6, 25 mM KCl, 5 mM MgCl₂, and 0.25 M sucrose, with 35 strokes of a motor-driven Teflon pestle and glass homogenizer. Cell breakage was always greater than 98%. Cells were separated into soluble supernatant and nuclear extracts as previously described. A microsomal fraction was extracted with 50 mM Tris, pH 7.6, 0.5 M KCl, 1% Triton X-100, and 1 mM mercaptoethanol.

An aliquot (0.25 ml) of each dialyzed fraction was layered on a 5-ml 5%–20% sucrose gradient in 50 mM Tris, pH 7.6, 100 mM KCl, and 1 mM mercaptoethanol containing 100 μg/ml bovine serum albumin and centrifuged for 16 hr at 100,000 g in a Spinco SW 50.1 rotor. Fractions (0.25 ml) were collected from the gradients as described.

**Enzyme Assays**

Radioactive deoxynucleoside triphosphates were purchased from Schwarz/Mann, Orangeburg, N.Y. Unlabeled deoxynucleoside triphosphates were from P-L Biochemicals, Milwaukee, Wis.
Polydeoxynucleotides are standard preparations.* The template used for DNA-dependent DNA polymerase assays was activated calf thymus DNA. One unit of enzyme activity is defined as 1 nmole of radioactive deoxynucleotide incorporated per hour. Specific activity is expressed as units of activity per $10^8$ nucleated cells.

DNA-dependent DNA polymerase activities were measured by incorporation of $^3$H-dTTP into acid insoluble material using activated DNA as substrate. In all enzyme assays, $^3$H-dTTP was used at a specific activity of 500 cpm/pmole. Acid insoluble material from the reactions was spotted on Whatman GF/C glass fiber disks and processed as has been previously described. The use of bovine serum albumin and buffer A in the sucrose gradients gives some improvement in recovery at the low enzyme levels studied here.

Direct enzyme assays and sucrose gradient assays were carried out on every sample. It is important to note that while the two DNA-dependent DNA polymerases studied exhibit different pH optima (7.2 and 8.6), the reaction conditions are not mutually exclusive. The nuclear fraction contains only the minipolymerase, but the cytoplasmic fraction contains both the minipolymerase and the maxipolymerase. When these enzymes are distributed on a sucrose gradient, it is usually possible to obtain accurate estimates of the levels of each molecular species, provided the final activities of the enzymes are adequate for assay. In most experiments presented, at least 70% of the total activity found by direct assay was recovered from the sucrose gradients.

Calculation of approximate molecular weight was carried out using the method of Martin and Ames. Endogenous hemoglobin (MW 64,000) and lactate dehydrogenase (MW 140,000) activity were used as molecular weight markers on sucrose gradients.

Deoxyribonuclease assays were also carried out on a number of samples. The assay mixture contained, in 60 μl, 0.02 mM $^3$H-poly (dA·dT) (19.6 cpm/pmol), 0.1 M Tris, pH 9.0, 8 mM MgCl₂, and 1 mM 2-mercaptoethanol along with 10 μl of each sucrose gradient fraction. Incubations were at 35°C for 30 min, 1 hr, or 2 hr. Aliquots of the reaction mixture were spotted onto GF/C disks which were washed and counted.

In some experiments, extracts were assayed in the presence of N-ethylmaleimide. Extracts were incubated in ice for 10 min with 0.5 mM N-ethylmaleimide before addition of substrates.

RESULTS

Normal and AML Bone Marrow

Typical sucrose gradient patterns of DNA polymerase activities in the soluble fraction of bone marrow cells from a normal person and from a patient with acute myelogenous leukemia are shown in Fig. 1. The activity in a comparable AML nuclear extract is shown in Fig. 2. Normal bone marrow supernatant contains the maxipolymerase species, as might be expected in extracts of proliferating cells. A low level of minipolymerase activity is also normally seen in supernatant fractions. The molecular weights of the mini- and maxipolymerases were estimated to be 50,000 and 200,000 daltons, respectively. The supernatant extract from AML cells (75% leukemic myeloblasts) shows a five- to tenfold greater maxipolymerase activity and some increase in minipolymerase activity. The activity of the nuclear DNA polymerase is very low in samples of normal bone marrow, ranging from 0.03 to 0.17 nmoles dTMP incorporated per $10^8$ cells per hour by direct assay of nuclear extracts. This level of activity frequently cannot be detected after the dilution engendered by gradient fractionation, but when polymerase activity is seen, its molecular weight is approx-

*Abbreviations used for nucleotides and polynucleotides are those of the IUPAC-IUB commission. Other abbreviations: AML, acute myelogenous leukemia; AcDNA, DNase-activated DNA; MW, molecular weight; KP₁, 0.2 M potassium phosphate buffer, pH 7.6, containing 1 mM 2-mercaptoethanol. The terms max- and minipolymerase refer to DNA-dependent DNA polymerase activities with high and low sedimentation coefficients, respectively.
Fig. 1. DNA polymerase activities in the soluble supernatant extracts from human bone marrows. The marrow from the patient with acute myelogenous leukemia contained 75% myeloblasts with replacement of normal erythroid and myeloid elements. The normal marrow had a normal ratio of myeloid to erythroid cells with normal progression of maturation in all cell lines. For preparations of the supernatant extracts and analysis on sucrose gradients, see Materials and Methods.

Fig. 2. DNA polymerase activities in a nuclear extract from AML human bone marrow. Nuclei were prepared from marrow specimens described in Fig. 1. Because of the very low polymerase activities present in nuclear extracts from normal marrows, no activity was detectable on these gradients.
approximately 50,000. The maxipolymerase cannot be detected in nuclear extracts. In nuclei from leukemic marrow, the minipolymerase is also the only species present (Fig. 2). Microsomal extracts were assayed for both mini- and maxipolymerases, and, at the level of sensitivity of our assay, no appreciable activity was found.

Normal and AML Peripheral Blood

DNA polymerase activities in supernatant fractions from leukocytes in the peripheral blood of a normal person and a patient with acute myelogenous leukemia are shown in Fig. 3. Nuclear activity from the AML patient is shown in Fig. 4. Eighty-eight per cent of the peripheral nucleated cells in the leukemic patient were myeloblasts, presumably all leukemic cells. The specific activities per cell and intracellular distribution of the mini- and maxipolymerases in cells from leukemic blood were similar to those of leukemic bone marrow, as might be expected from the similarity of cell types present in the two samples. Polymerase activities in cells from normal peripheral blood are much lower than in cells from normal marrow. In Fig. 3 the normal peripheral maxi activity was higher than we usually observe and consistently heterogeneous. Table 2 gives over-all maxipolymerase activities which are lower in normal peripheral blood than in normal bone marrow. Since the nucleated cells from normal peripheral blood...
blood are a mixture of mature granulocytes and lymphocytes, whereas marrow contains many immature dividing cells, this result is expected. Cells from normal peripheral blood have extraordinarily low levels of the maxipolymerase in their supernatant, and the nuclear polymerase, when detectable, sediments with a molecular weight of 50,000. The polymerases from normal peripheral cells show more heterogeneity than usual on sucrose gradients with less clear separation into activities of high and low molecular weight. This may be an artifact associated with the low levels of activity present.

Normal Erythrocytes, Lymphocytes, and Granulocytes

The fact that blood and marrow samples are always a mixture of cell types requires that separate studies of the individual cell types be made. In this direction we note first of all that purified preparations of erythrocytes do not contain detectable DNA polymerase activities (Table 3). Our data are based on whole blood preparations with no separation of reticulocytes. It is entirely possible that the reticulocyte does contain DNA polymerase. Lymphocytes and granulocytes were purified from normal peripheral blood using Hypaque-Ficoll gradients. Nuclear and soluble supernatant fractions were prepared from each cell type, and DNA polymerase activities were assayed on extracts fractionated on sucrose density gradients (Figs. 5 and 6). The maxipolymerase is readily detected in supernatant fractions from lymphocytes and is much higher in activity in lymphocytes than in granulocytes. Maxipolymerase activity is not found in the nuclear extracts from either type of cell. Minipolymerase activity is present in the nucleus and cytoplasm of both lymphocytes and granulocytes and is the only detectable DNA-dependent DNA polymerase in the nucleus. The absence of maxipolymerase in nuclear fractions is not the result of inactivation of polymerase by Triton used in washing nuclear pellets (unpublished re-
Fig. 5. DNA polymerase activities in the cytoplasmic extracts of lymphocytes and granulocytes purified from normal human peripheral blood. Lymphocyte preparations contained over 90% lymphocytes with 10% or less monocytes. Granulocyte preparations contained over 95% granulocytes with 5% or less lymphocytes. Preparation of extracts and assay conditions are described in Materials and Methods. Minipolymerase was assayed in the presence of 0.5 mM N-ethylmaleimide.

Fig. 6. DNA polymerase activities in the nuclear extracts of lymphocytes and granulocytes purified from normal human peripheral blood. Preparation of extracts and assay conditions are described in Materials and Methods.
suits). These results on purified cells confirm those obtained using unfractionated cells from peripheral blood and add the finding that lymphocytes contain more of each polymerase activity per cell than mature granulocytes in all subcellular fractions.

**Quantitative Studies on Normal and Leukemic Cells**

Table 1 summarizes the numerical data from assays on our present series of leukemic and normal cells from bone marrow. The leukemic marrows were all from patients with acute myelogenous leukemia. Activity of the cytoplasmic maxipolymerase in 11 samples of normal bone marrow ranged from 1.2 to 3.4 nmoles dTMP incorporated \( 10^8 \) nucleated cells per hour, with a mean of 1.7. These data are from gradient summations, which we feel to be the most dependable method of quantitation. Recovery of activity from the gradients ranged from 60% to 100%. Total cell extracts or supernatant fractions from leukemic marrows consistently exhibit higher levels of soluble DNA polymerase activities than do extracts of normal marrows. Quantitative statements about the absolute level of the nuclear polymerase are not possible because of the low total nuclear activity present in the number of cells obtained from our usual marrow aspirates. In some cases the amount of activity that could be applied to gradients was so low that activity could not be detected after gradient fractionation. In general, however, minipolymerase activity was slightly higher in nuclei from leukemic than normal bone marrows.

### Table 1. Nucleotide Polymerizing Activities in Bone Marrow

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number Samples</th>
<th>Subcellular Fraction</th>
<th>Maxipolymerase</th>
<th>Minipolymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Direct</td>
<td>Gradient</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>Supernatant</td>
<td>2.6 (1.4–3.9)</td>
<td>1.7 (1.2–3.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0.45 (0.08–0.099)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microsome</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>4</td>
<td>Supernatant</td>
<td>7.8 (5.6–9.8)</td>
<td>5.4 (5–5.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microsome</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>4</td>
<td>0.2 M KPi</td>
<td>17.5 (11.3–22.6)</td>
<td>13.3 (7.3–20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>whole homogenate</td>
<td>0</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Specific activities are expressed as nmoles dTMP incorporated per \( 10^8 \) cells per hour. Mean activities are reported with ranges of activities for all samples given in parentheses. Nd indicates activity not detected on the gradient, although activity was detected in direct assay. Zero activity indicates that the specific activity was less than 0.02 nmoles dTMP incorporated per \( 10^8 \) cells per hour. Eight samples of marrow were obtained from seven patients with acute myelogenous leukemia, each containing over 70% myeloblasts. Most leukemic patients had not received chemotherapy; those that had were in relapse, and no differences between the two classes of patients were apparent. Whole cell extracts (in 0.2 M KPi) consistently exhibit higher enzyme activities than subcellular fraction extracts. We feel several factors, such as manipulative loss and enzyme inactivation during subcellular fractionation, contribute to the difference in activity.
Table 2. Nucleotide Polymerizing Activities in Peripheral Blood

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number Samples</th>
<th>Subcellular Fraction</th>
<th>Maxipolymerase</th>
<th>Minipolymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Direct</td>
<td>Gradient</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>Supernatant</td>
<td>0.46</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.1-0.99)</td>
<td>(0.18-1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.004-0.05)</td>
<td>(0-0.03)</td>
</tr>
<tr>
<td>Acute myelogenous</td>
<td>5</td>
<td>Supernatant</td>
<td>8.3</td>
<td>8.4</td>
</tr>
<tr>
<td>leukemia</td>
<td></td>
<td></td>
<td>(4.5-11.6)</td>
<td>(6.6-12.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.07-0.18)</td>
<td>(0-0.1)</td>
</tr>
<tr>
<td>Chronic lymphocytic</td>
<td>3</td>
<td>0.2 M KPi extract of whole homogenate</td>
<td>0.31</td>
<td>0.53</td>
</tr>
<tr>
<td>leukemia</td>
<td></td>
<td></td>
<td>(0.79-1.2)</td>
<td>(0.47-0.79)</td>
</tr>
<tr>
<td>Chronic myelogenous</td>
<td>2</td>
<td>0.2 M KPi extract of whole homogenate</td>
<td>0.67</td>
<td>0.43</td>
</tr>
<tr>
<td>leukemia</td>
<td></td>
<td></td>
<td>(0.57-0.77)</td>
<td>(0.34-0.52)</td>
</tr>
</tbody>
</table>

See the footnote to Table 1. Differential cell counts on samples: normal, 60% granulocytes, 40% lymphocytes; acute myelogenous leukemia, over 70% myeloblasts; chronic lymphocytic leukemia, over 80% lymphocytes; chronic myelogenous leukemia, over 98% granulocytic series with 36% mature neutrophils, 15% bands, 2% myeloblasts, and appropriate numbers of intermediate cell types.

Table 2 summarizes the results of experiments comparing polymerase activities in peripheral cells of leukemic blood with cells from normal peripheral blood. The activity of the high molecular weight DNA polymerase is elevated in samples containing leukemic myeloblasts (acute myelogenous leukemia), with a mean activity of 8.4 nmoles dTMP incorporated per $10^8$ cells per hour compared to a normal activity of 0.45. The activities in cells of peripheral blood from patients with chronic lymphocytic leukemia (over 80% lymphocytes) are only slightly higher than in those from patients with chronic myelogenous leukemia (70% metamyelocytes or more mature forms), but each is lower than the activities found in cells of patients with acute myelogenous leukemia (over 70% myeloblasts). The polymerase activities in lymphocytes of chronic lymphocytic leukemia are slightly lower than those present in normal purified lymphocytes (Table 3). The cell types present in chronic myelogenous leukemia are more heterogenous than in chronic lymphocytic leukemia and range from myeloblasts to mature neutrophils, with a predominance of the more mature cells. The activities of the maxi and mini DNA polymerases in cells from chronic myelogenous leukemia are twice as high as those present in the purified mature granulocytes (Table 3), probably reflecting a general correlation between increasing cell maturity (measured in part by inability to undergo cell division) and decreasing polymerase activities in myeloid cells. Within the granulocytic series, cells more mature than myelocytes cannot divide.14

Mature lymphocytes and granulocytes represent the predominant nucleated cell types present in normal human peripheral blood. Polymerase activities present in these two cell types are shown in Table 3. The specific activity (units per $10^8$ cells) of the supernatant maxipolymerase in lymphocytes is three-
### Table 3. Nucleotide Polymerizing Activities in Lymphocytes and Granulocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number Samples</th>
<th>Subcellular Fraction</th>
<th>Maxipolymerase</th>
<th>Minipolymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Direct</td>
<td>Gradient</td>
</tr>
<tr>
<td>Purified</td>
<td>2</td>
<td>Supernatant</td>
<td>1.3 (1.2-1.45)</td>
<td>1.0 (0.88-1.1)</td>
</tr>
<tr>
<td>lymphocytes</td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified</td>
<td>2</td>
<td>Supernatant</td>
<td>0.21 (0.13-0.29)</td>
<td>0.29 (0.19-0.39)</td>
</tr>
<tr>
<td>granulocytes</td>
<td></td>
<td>Nucleus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>2</td>
<td>Supernatant</td>
<td>0.0047 (0.0047-0.0052)</td>
<td>Nd</td>
</tr>
</tbody>
</table>

See footnote to Table 1. Purified lymphocytes and granulocytes were obtained by fractionation of normal peripheral blood on Hypaque-Ficoll gradients.

Fourfold higher than the same enzyme in granulocytes. The specific activity of the minipolymerase in the supernatant of lymphocytes is sixfold higher than in granulocytes. The nuclear activity is of low molecular weight and present at low levels in both cell types. In the two experiments shown, nuclear activity is slightly lower in granulocytes than in lymphocytes. Circulating red blood cells from whole blood do not contain detectable levels of either DNA polymerase.

**Effect of N-ethylmaleimide**

DNA polymerase activities from normal peripheral granulocytes and lymphocytes were assayed in the presence of N-ethylmaleimide to determine whether differential inhibition of activities occurred as has been previously reported. In both granulocytes and lymphocytes, the supernatant polymerase of high molecular weight is inhibited by 0.5 mM N-ethylmaleimide, whereas the nuclear polymerase activity does not change significantly.

**Nuclease Activities**

The possibility that the differences in polymerase activities observed among granulocytes, lymphocytes, and leukemic cells are due to differential exo-nuclease activities was examined by direct assay using $^3$H-poly (dA·dT). Low levels of exo-nuclease activity were detected after 30 min incubation at 35°C in most of the samples tested, however, at least 90% of the $^3$H-poly (dA·dT) remained acid insoluble in all samples. In fractions from leukemic blasts, about 15% of the radioactivity in the template was made acid soluble after a 2-hr incubation. Most of the detectable nuclease activity appears in fractions 5–10 on sucrose gradients and could selectively interfere with gradient assays of minipolymerase. All gradient assays are therefore incubated for 30 min to minimize the effects of the exo-nuclease. An excess of template is also present.

In addition to exo-nuclease assays, mixing experiments were performed with many samples to eliminate the possibility of inhibitory substances in normal cell populations or activators in leukemic blast cells. The results of mixing experiments are shown in Table 4. In all cases the calculated and experimental activities are nearly identical.
Table 4. Mixing Experiments to Detect Activators and Inhibitors of DNA Polymerase in Extracts of Cells

<table>
<thead>
<tr>
<th>Type and Amount of Extract Added (μl)</th>
<th>Polymerase Activity</th>
<th>Measured (units)</th>
<th>Calculated (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemic supernatant</td>
<td>Normal supernatant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>267</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>16.1</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>29.4</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>38.1</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>271</td>
<td>283</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>292</td>
<td>296</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>318</td>
<td>305</td>
</tr>
<tr>
<td>Lymphocyte supernatant</td>
<td>Granulocyte supernatant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>2.9</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>8.7</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>11.1</td>
<td>11.6</td>
</tr>
<tr>
<td>Lymphocyte nuclear</td>
<td>Granulocyte nuclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>0.22</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0.30</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>0.55</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Conditions for the assay of nuclear and supernatant enzymes were as described in Materials and Methods. Total reaction volume was 125 μl including tissue extracts as indicated in the table. Incubation was at 35°C, and 50-μl aliquots of the reaction mixture were removed at 10 and 30 min for determination of incorporation of nucleotide into DNA. Calculated enzyme units in the reaction are the sum of activities in the two extracts assayed separately and represent the activity that would be expected in the mixture if no inhibitors or activators were present.

DISCUSSION

The current study shows that the activities of both the nuclear and supernatant DNA polymerases are higher in normal bone marrow than in nucleated cells from normal peripheral blood. Bone marrow is composed of a large variety of myeloid and erythroid cells, many of which are capable of division. The majority of nucleated cells in normal peripheral blood are lymphocytes and granulocytes. The granulocyte is a terminally differentiated cell and is incapable of division. In contrast, while the lymphocyte does not normally undergo mitosis in the peripheral blood and shows little or no evidence of DNA synthesis as measured by incubation with 3H-thymidine or 32P, it can be stimulated to divide by a variety of agents including phytohemagglutinin. When cells of peripheral blood are separated, the granulocyte contains very low activities of both nuclear and cytoplasmic DNA polymerases when compared to lymphocytes. The levels of DNA polymerase in both the cytoplasm and nuclei of lymphocytes resemble those found in normal bone marrow and are approximately four times higher per cell than granulocytes. Our data on DNA polymerase levels in cells from normal peripheral blood and bone marrow suggest that cells which can undergo mitosis, or are doing so, have higher levels of DNA polymerase in both the cytoplasm and nuclei than do terminally differ-
entiated cells. The fact that lymphocytes contain detectable levels of DNA polymerase in the absence of DNA synthesis suggests that total DNA polymerase activities are not completely correlated with the rate of DNA synthesis in a cell. It is also possible that a slightly different form of maxipolymerase is present in those cells that are in these nonproliferative states.

In addition, we cannot rule out the possibility from this study that a subpopulation of peripheral lymphocytes is proliferative. The possibility that maxi and mini DNA polymerase levels vary at different stages of the mitotic cycle in normal cells of the hematopoietic system has not been examined in this study. Low DNA polymerase activity in granulocytes was observed previously by Rabinowitz. In his study a supernatant fraction from extracts of purified human granulocytes was assayed for total DNA polymerase activity. The exact nature of the template for the polymerase was not specified, but the mean incorporation of deoxynucleotide was 0.006 nmoles nucleotide per $10^8$ cells per hour. The activities that we observe using activated DNA as template are approximately 40-fold higher than this value.

Cell populations from the bone marrow and peripheral blood of patients with acute myelogenous leukemia have much higher levels of soluble supernatant maxipolymerase than do cells from normal marrow and peripheral blood, respectively. Minipolymerase levels in the soluble supernatant of leukemic cells also seem to be higher than in normal cells. Whether the level of nuclear minipolymerase is increased in leukemic cells is not clear, since low activities are present in all nuclear fractions, and gradient assays of nuclear polymerase are less reliable than those of soluble supernatant polymerase. Leukemic myeloblasts are poorly differentiated malignant cells that are generally capable of dividing, although they have lower mitotic and labeling indices than normal myeloblasts.

The maxi and mini DNA polymerase activities in extracts of cells (over 80% lymphocytes, less than 20% granulocytes) from the peripheral blood of patients with chronic lymphocytic leukemia were lower than activities observed in purified normal lymphocytes. This apparent decrease in DNA polymerase activities is probably due to the fact that the contaminating granulocytes have lower DNA polymerase activities per cell than lymphocytes. Lymphocytes from patients with chronic lymphocytic leukemia, like normal lymphocytes, show little or no evidence of DNA synthesis and metabolically seem similar to normal peripheral lymphocytes. Rabinowitz also observed similar DNA polymerase activities in supernatant fractions from normal and chronic leukemic lymphocytes, although in his experiments the rates of incorporation of nucleotide per cell were approximately one-eighth of the activities we observe.

Both the maxi and mini DNA polymerase activities in extracts of peripheral granulocytes from patients with chronic myelogenous leukemia are higher than in purified granulocytes from normal peripheral blood. Although preparations of cells from both normal and leukemic patients were over 95% granulocytes, the state of differentiation of the cells was markedly different. Granulocyte fractions from normal peripheral blood contain approximately 90% mature neutrophils and 10% bands. Neither of these cell types synthesizes DNA nor undergoes mitosis. In contrast, granulocyte fractions from patients with
chronic myelogenous leukemia contain approximately 36% mature neutrophils, with the remainder of the cells being less differentiated granulocytes, including 2% myeloblasts. Myelocytes and less mature granulocytes are capable of DNA synthesis and cell division. Our data suggest that maxi and mini DNA polymerase activities decrease in cells of the granulocytic series as the cells differentiate to form the mature polymorphonuclear leukocytes, and that high polymerase levels in leukemic cells are not chiefly related to their being malignant, but to their immaturity. Slight variations in sedimentation of minipolymerase are often observed. This may be due to aggregation of enzyme, slight degradation of the enzyme, or variations in the enzyme itself from one cell type to another. Ove, Kremer, and Laszlo reported that granulocytes from patients with chronic myelogenous leukemia had more DNA polymerase activity using native and denatured DNA as substrates than did normal granulocytes. The DNA polymerase activities observed in their studies were lower than those we observe, although their data do not permit calculations of activities per cell. The higher activities in our experiments would appear to be due to use of activated DNA as template and avoidance of precipitation procedures that may selectively inactivate DNA polymerase.

Sucrose velocity gradient fractionation is particularly useful in studies of DNA polymerase activities in human cells. It facilitates detection of different DNA polymerase species, both in whole cell extracts and in subcellular fractions, by adding molecular weight constraints to the usual methods of improving assay specificities such as adjustments of pH, template system, and divalent ion. Use of activated DNA as substrate for DNA polymerase provides a template superior to either denatured or native DNA, since multiple 3'-hydroxyl sites are available for enzymatic insertion of deoxynucleoside triphosphates. Since the replication and repair of DNA in eukaryotes appear to be extraordinarily complex processes involving several enzymes, it is of great importance that the various species be distinguished from one another and quantitated in different types of cells during different phases of replication. To accomplish this will require improvements in the specificity and sensitivity of polymerase assays so that quantitation of the different DNA polymerase activities can be done using small numbers of cells. These improved procedures could then be used to assay different cell types separated on albumin or ficoll gradients. A more careful analysis of the activity of maxi and mini in all cell types will also be required in order to confirm the generalities noted in this work.

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