Nonspecific Esterases of the Formed Elements: Zymograms Produced by pH 9.5 Polyacrylamide Gel Electrophoresis

By Joseph Yourno and Walter Mastropaolo

The formed elements of human blood each contain multiple isoenzymes of nonspecific esterase that hydrolyze short chain alpha naphthyl esters. Zymograms that are characteristic of each type of formed element are obtained by subjecting purified preparations of each to polyacrylamide slab gel electrophoresis at pH 9.5 and subsequent staining of the gels for esterase activity. The most prominent isoenzyme detected is a species of low mobility that is reactive with either acetyl or butyryl esters and is highly sensitive to inhibition by 40 mM sodium fluoride. Also detected are several major acetyl esterases and a single butyryl esterase, all of which are relatively fluoride resistant. The intercellular distribution of isoenzymes varies from element-specific to pancellular. The prominent fluoride-sensitive acetyl, butyryl esterase, is the major isoenzyme of monocyte zymograms, which is consistent with the well known cytochemistry of monocytes. Lesser but significant amounts (2%–3% of monocyte levels) of this isoenzyme were also detected in granulocyte zymograms. This system may prove useful in the study of differentiation of blood cells and in the classification of acute leukemias.

MATERIALS AND METHODS

Isolation of Formed Elements

Plateletpheresis red cell residues in acid citrate dextrose (ACD, Fenwal Laboratories, Deerfield, Ill.) prepared by the American Red Cross, Albany, N.Y., from normal donors were the source of mononuclear cells and platelets. The mononuclear cells and platelets were harvested by Ficoll-Hypaque density gradient centrifugation, ρ = 1.080, according to the method of Perper et al.19 Ficoll and Hypaque were supplied by Sigma Chemicals, St. Louis, Mo. Platelets and mononuclear cells were separated from each other by 4–6 cycles of differential low speed centrifugation (1000 × g × 10 min) in suspensions of Hank’s balanced salt solution, calcium and magnesium free (CMHBSS). Monocytes were isolated from suspensions of mononuclear cells in RPMI-1640 medium containing 20% fetal calf serum by 2 or 3 passages through nylon wool (Fenwal) columns by the method of Greaves et al.21

Red cells and granulocytes were isolated from buffy preparations prepared by the American Red Cross from normal donor fresh whole blood in citrate phosphate dextrose adenine (CPDA, Fenwal). Red cells and leukocytes were separated by differential sedimentation at room temperature of the buffy fraction mixed with an equal

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volume of CMHBSS, 3% in dextran (Sigma).20 The leucocyte fraction was washed with CMHBSS, resuspended in this same solution, and subjected to a Ficoll-Hyphae density gradient centrifugation as outlined above. The cell pellet was retained as the purified granulocyte preparation. The lower two-thirds of the red cell layer was collected and subjected to two further cycles of sedimentation in CMHBSS containing 1.5% dextran to obtain the final purified red cell preparation. B lymphocytes were obtained in one instance from a therapeutic leukapheresis specimen in acid citrate dextrose (ACD, Fenwal) from a patient with chronic lymphocytic leukemia. The leukapheresis specimen was subjected to a Ficoll-Hyphae density gradient centrifugation to obtain a purified preparation of lymphocytes. In a second instance, B lymphocytes from the same patient were similarly obtained from 4 ml of blood anticoagulated with ethylenediaminetetraacetic acid. Residual red cells in lymphocyte and granulocyte preparations were lysed by exposure to 0.83% ammonium chloride for 20 min at room temperature.20 Plasma was obtained as the supernatant of an aliquot of anticoagulated starting material, which was subjected to high speed centrifugation (15,000 g x 15 min). Purified cells and platelets were washed 2-4 times in 2-10 volumes of CMHBSS and centrifuged at 2200-5000 g for 15 min to obtain a final compact pellet for enzyme studies. The pellet was suspended in 5-9 volumes of 20 mM n-morpholino ethane sulfonic acid buffer (MES, Sigma), pH 6.4, containing 20% glycerol and stored at -20°C for enzyme studies. The isolation procedures were monitored by differential cell counts of air-dried, fixed smears stained by Wright’s stain or by nonspecific esterase cytochemistry. Cell and platelet counts were performed by hemocytometer. For greater precision in differential cells types present at 1% or less of the total, 12,000 cells were counted by the Miller Disc method. To minimize distributional error, counts were performed completely across the trailing edge, midpoint, and/or leading edge of the leucocyte smear. Confidence intervals for counts were calculated from the binomial distribution.

Cytochemistry

Esterase cytochemistries were performed with smears fixed in 38 mM Na citrate, pH 5.8-acetone-methanol, 4:6:1.1, for 30 sec at room temperature and incubated for 15 min at 37°C in the reaction mixture (Sigma Technical Bulletin no. 90, 1979). Solutions of alpha naphthyl acetate substrate (αNA, Sigma) or alpha naphthyl butyrate substrate (αNB, Sigma) and fast garnet GBC coupler (Sigma) were prepared as described by Yam et al.α The reaction mixture was buffered with 20 mM MES, pH 6.4, and in some cases contained 40 mM Na fluoride (Sigma). Cells were counterstained with methyl green solutionβ for 10 min at room temperature.

Surface Markers

Lymphocyte preparations were scored for sheep red blood cell receptors by the rosetting techniqueγ and for surface immunoglobulin with fluorescein-conjugated goat anti-human immunoglobulin F(ab)2 fragment (Cappel Laboratories, Cochranville, Pa.) by the method of Papamichail et al.δ

Enzyme Preparations

Purified cell or platelet suspensions, stored frozen in MES buffer with glycerol, were thawed on ice and subjected to two 15-sec bursts of sonic disruption at 23 W (Branson Sonifier, Model 200, Branson Sonic Power, Danbury, Conn.). Triton X-100 (Bio-Rad) was added to the treated preparation to a final concentration of 1%. For enzyme extraction, this non-ionic detergent proved superior to Na dodecyl sulfate (Bio-Rad), Na deoxycholate (Sigma), and cetyltrimethyl ammonium bromide (Sigma), all at 0.1%. After standing 5 min on ice, the suspension was centrifuged at 15,000 g for 15 min and the supernatant was collected as the enzyme preparation. The esterase activity in mononuclear cell extracts was stable for at least 3 mo of frozen storage and was unaffected by a single freeze–thaw cycle (Mastropaolo and Yourno, Analytical Biochemistry, in press). Spectrophotometric assay of nonspecific esterase at 37°C was performed at 235 nm with a reaction mixture containing 0.2 mM αNA or αNB in 10 mM MES buffer, pH 6.4 (W. Mastropaolo and J. Yourno, Analytical Biochemistry, in press). The reaction was monitored with a Gilford spectrophotometer, Model 252 (Gilford Instruments, Oberlin, Ohio).

Polyacrylamide Gel Electrophoresis

Vertical slab gel electrophoresis was routinely performed at pH 9.5α with running gels containing 7.5% polyacrylamide (Bio-Rad, Model 220, Bio-Rad Laboratories, Richmond, Calif.). Unless otherwise specified, 1–3 mU of αNB activity were applied to each well in a volume of 60 µl, 10% in glycerol (v/v), and 0.02% in bromphenol blue (w/v). Gels were subjected to 12 mA of current per slab at 4°C and the electrophoresis was terminated when the dye marker reached the anodal extremity of the gel. The gels were equilibrated at 4°C with 20 mM MES buffer, pH 6.4, for 30 min with buffer changes at 10-min intervals. For visualization of αNA esterases or αNB esterases, the gels were then incubated at room temperature with reaction mixture that was prepared as described above for cytochemistries, except that the concentration of substrate and coupler was increased to 50 mg/100 ml each. In some cases the equilibration buffer and reaction mixture contained 40 mM Na fluoride. The reaction was terminated at 60 min by immersion of the gels in 7.5% acetic acid (v/v). The gels were washed in 4% glycerol (w/v) and a permanent dried mount of each prepared with a vacuum slab gel dryer (Bio-Rad Model 224).

To study differential migration of isoenzyme species as a function of gel concentration, all preparations were also examined on gels containing 6.0% and 9.0% acrylamide. Experiments were performed otherwise as outlined above.

RESULTS

Homogeneity of Preparations of Formed Elements

Two or more preparations of each type of formed element were isolated and examined by Wright’s stain and cytochemistry (Table 1). Erythrocyte preparations (n – 6) and platelet preparations (n – 4) were essentially homogeneous in each case, with only rare contaminating leukocytes and erythrocytes seen on low power scan. Monocyte preparations were 90%–95% homogeneous, including a small fraction of degenerate to pyknotic cells testing cytochemically as monocytes. T-lymphocyte preparations exceeded 97% homogeneity, the great majority of which tested as T lymphocytes. While there may have been depletion of Fc-receptor-positive suppressor T cells by selective adherence to nylon wool, this was not examined. B-lymphocyte preparations consisted of more than 99% lymphocytes, with 95% small mature forms. Most of these cells demonstrated weak positivity for surface immunoglobulin, characteristic of many cases of chronic lymphocytic leukemia. Granulocyte preparations contained 98%–99% granulocytes, predomi-
NONSPECIFIC ESTERASES OF FORMED ELEMENTS

Table 1. Differential count of Purified Leukocyte Preparations*

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Monocytes (%)</th>
<th>Lymphocytes (%)</th>
<th>Granulocytes (%)</th>
<th>SRBC pos†</th>
<th>SIG pos‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutrophils</td>
<td>Basophils</td>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>90.0(10)§</td>
<td>7.0</td>
<td>—</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>95.1(10)</td>
<td>3.3</td>
<td>0.6</td>
<td>1.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>91.4(3)</td>
<td>6.0</td>
<td>1.0</td>
<td>1.6</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>0.4</td>
<td>99.6</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
<tr>
<td>1.0 ± 0.2</td>
<td>97.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.4</td>
<td>85</td>
</tr>
<tr>
<td>0.2 ± 0.1</td>
<td>98.1</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>92</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>0.3 ± 0.1</td>
<td>99.5</td>
<td>0.2</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>99.7</td>
<td>—</td>
<td>—</td>
<td>90**</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0.3</td>
<td>0.9</td>
<td>92.0</td>
<td>—</td>
<td>6.8</td>
</tr>
<tr>
<td>0.3 ± 0.1†</td>
<td>0.2</td>
<td>98.1</td>
<td>—</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1.8</td>
<td>96.6</td>
<td>—</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>0.3 ± 0.1</td>
<td>0.6</td>
<td>97.0</td>
<td>—</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>0.4 ± 0.1</td>
<td>0.3</td>
<td>97.7</td>
<td>0.4</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

*Wright stain and αNA stain, 500–1000 cell counts each unless otherwise indicated.
†Cells rosetting with sheep red blood cells.
‡Cells positive for surface immunoglobulin.
§Percent degenerate cells, more than 90% showing moderate to intense nonspecific esterase sensitive to fluoride inhibition.
[αNA or αNB stain with and without fluoride, 12,000 count, p < 0.05.]
††Wright stain, 12,000 count, p < 0.05.
**Weakly positive.


gel electrophoresis at pH 9.5. Table 2 summarizes the characteristics of major isoenzymes detected by this system. Zymogram patterns remained unchanged except for marked decrease in band intensity on incorporation of Triton X-100 (1%, v/v) into the gel matrix. Detergent was therefore routinely omitted. Zymograms were characteristic of each type of formed element examined and reproducible from preparation to preparation. Zymograms prepared by enzyme reaction at pH 5.8 (20 mM MES) were similar in all respects to standard zymograms prepared at pH 6.4, while zymograms prepared at pH 7.5 (20 mM Tris-maleate, Sigma) showed somewhat diminished intensity of all bands.

Zymograms of plasma controls showed multiple strong to moderate bands, more prominent with αNA substrate than αNB substrate. All are fluoride-sensitive except the acetyl esterase activity associated with albumin. None of these species appears to coincide with those in zymograms of the formed elements. With the exception of albumin, we have no information on the source of the esterolytic activities detected here in plasma.

The outstanding feature of the zymograms is the presence of two slow species, AB1 and AB2, which appear to be of a similar order of reactivity with αNA and αNB substrates and which are highly sensitive to inhibition by 40 mM fluoride. These isoenzymes are especially intense in monocyte zymograms. The AB2 isoenzyme is by a wide margin the most reactive among the isoenzymes detected by our system. Zymograms of granulocyte preparations showed the AB...
Fig. 1. Zymograms of nonspecific esterase from formed elements of blood, polyacrylamide gel electrophoresis, pH 9.5. Shown are zymograms in 7.5% gels obtained under four variations of substrate and inhibitor in the reaction mixture: a, pNA substrate; af, pNA and Na fluoride inhibitor; b, pNB substrate; bf, pNB and Na fluoride. Zymograms of each enzyme extract with and without fluoride were prepared in the same experiment with equal amounts of applied esterase. The following zymograms are shown: (1) plasma control, Alb, albumin; (2) platelets, 1.0 mU pNB activity each; (3) monocytes, 3.0 mU pNB activity each; (4) granulocytes, 3.0 mU pNB activity each; shown are zymograms of a preparation yielding moderately intense AB bands, 0.3% ± 0.1% monocyte contamination; (5) T lymphocytes, 0.2% ± 0.1% monocyte contamination, 3.0 mU pNB activity each; (6) CIL B lymphocytes, 0.3% ± 0.1% monocyte contamination, 3.0 mU pNB each; (7) red cells, 0.2 mU pNB activity each. Hb, hemoglobin. Esterase isoenzymes are labeled as follows: A1 through A10, isoenzymes predominantly with acetyl esterase activity; AB1 and AB2, isoenzymes with significant acetyl and butyryl esterase activity; B1, isoenzyme predominantly with butyryl esterase activity. The hemoglobin in red cell zymograms shows no esterase activity but is visualized because of its strong red color.

species at weak intensity (one preparation, 0.3% monocytes) to moderate intensity (four preparations, 0.3%–0.5% monocyte contamination). T-lymphocyte preparations (0.2% monocytes) and B-lymphocyte preparations (0.3% monocytes) showed only traces of the AB species; erythrocytes and platelets showed no detectable AB species.

To determine whether the AB isoenzymes visualized in granulocyte zymograms were genuinely from granulocytes or from contaminant monocytes, a titra-
tion was made of the AB isoenzymes of a granulocyte preparation and an autologous monocyte preparation from the same buffy coat. Parallel zymograms were made from serial dilutions of each extract, developed with αNB substrate. From total cell count and a careful differential count, the number of contaminant monocytes (0.4% ± 0.1% monocytes, p < 0.05) in the granulocyte preparation had been determined. Thus, monocyte equivalents in each dilution of the granulocyte extract were calculated. Similarly, the number of monocytes in the autologous monocyte presentation (58% ± 3% monocytes) was determined and monocyte equivalents in each dilution calculated. Dilutions of each extract were determined that yielded AB2 bands of equal intensity on the parallel zymograms. At these matching dilutions, the granulocyte extract contained only one-sixth to one-ninth the number of monocyte equivalents of the monocyte dilution. Stated otherwise, the granulocyte extract contained 6–9-fold greater AB activity than could be attributed to contaminant monocytes. From this experiment it is also possible to calculate the extractable AB activity per granulocyte relative to that per autologous monocyte. In this blood specimen the extractable AB activity per granulocyte was 2%–3% that per monocyte. In a similar comparative serial dilution experiment, the activity of the extractable AB isoenzymes in the T-lymphocyte preparation with 1.0% ± 0.2% contaminating monocytes could be attributed largely or exclusively to contaminating monocytes. Here the autologous monocyte preparation contained 90% ± 2% monocytes.

With αNB substrate, a relatively slow species, B1, which comigrates with AB1 and is relatively resistant to fluoride inhibition, is visualized. B1 is variably and weakly visualized with αNA substrate. With αNB as substrate, B1 is detectable at variable intensity in all preparations examined, being especially dense in erythrocyte zymograms, relatively weak in granulocyte zymograms, and of intermediate intensity in zymograms of other formed elements examined. Serial dilution experiments with three red cell preparations established that hemoglobin intensity by coincidence closely matched that of the B1 band on zymograms prepared with αNB substrate. Since the B1 species proved to be coextractable with hemoglobin, hemoglobin intensity served as a convenient indirect index of red cell contribution to B1 in zymograms of nonerythrocyte preparations. Even in granulocyte zymograms, which consistently yielded the weakest B1 band, the intensity of the B1 band and the lack of visualizable hemoglobin suggest that granulocytes contain at least fivefold the extractable B1 isoenzyme activity that can be attributed to any contaminating red cell material. The level of extractable B1 activity is correspondingly higher in other types of noneerythrocytic formed element, at least 20–80-fold that attributable to any contaminating red cells. The identity of this genuinely “nonspecific” pancellular species in all formed elements is suggested by its similar differential migration as a function of gel concentration (6.0%, 7.5%, and 9.0% polyacrylamide) in zymograms of different types of preparation.

With αNA substrate, 8–10 isoenzymes, all relatively fluoride-resistant, are visualized in addition to the AB species. The fastest species, A1 and A2, are unreactive or only weakly reactive with αNB. One or two species migrating even faster than A1 and A2 are variably detectable, being most intense in granulocyte preparations. Species A2 appears to be common to all formed elements examined; A1 to all except erythrocytes. Monocyte preparations contained a rather large fraction of contaminating lymphocytes, which show greater relative intensity for A1 and A2 isoenzymes.

### Table 2. Characteristics of Major Nonspecific Esterase Isoenzymes of the Formed Elements, pH 9.5, Polyacrylamide Gel Electrophoresis

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Mobility</th>
<th>Substrate Specificity</th>
<th>Fluoride Response</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>αNA</td>
<td>αNB</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>Fast</td>
<td>+</td>
<td>0 to ±</td>
<td>Res/pg</td>
</tr>
<tr>
<td>A2</td>
<td>Fast</td>
<td>+</td>
<td>0</td>
<td>Res/pg</td>
</tr>
<tr>
<td>A3</td>
<td>Intermediate</td>
<td>+</td>
<td>0</td>
<td>Res/pg</td>
</tr>
<tr>
<td>A4</td>
<td>Slow</td>
<td>+</td>
<td>0</td>
<td>Res/pg</td>
</tr>
<tr>
<td>A5–A9</td>
<td>Intermediate</td>
<td>+</td>
<td>0</td>
<td>Res/pg</td>
</tr>
<tr>
<td>A81</td>
<td>Slow</td>
<td>+</td>
<td>+</td>
<td>Sens/sg</td>
</tr>
<tr>
<td>AB2</td>
<td>Slow</td>
<td>+</td>
<td>+</td>
<td>Sens/sg</td>
</tr>
<tr>
<td>B1</td>
<td>Slow</td>
<td>+</td>
<td>±</td>
<td>Res/pg</td>
</tr>
</tbody>
</table>

*plt, platelets; mono, monocytes; gran, granulocytes; TL, T lymphocytes; BL, B lymphocytes; RBC, red cells.
†, reactive with substrate; ±, weakly reactive; 0, no detectable reactivity.
§, present in extract of formed element; 0, undetectable in extract or present at trace levels.

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Comparative serial dilution experiments with extracts of autologous monocytes (6% ± 1.5% contaminant lymphocytes) and T lymphocytes (97.6% ± 1% lymphocytes) established that the level of A1 and A2 isoenzymes in one monocyte preparation was at least 30-fold higher than that attributable to contaminating lymphocytes. In 4 of 5 granulocyte preparations examined, A1 was relatively more intense than A2, a pattern that has not been observed among other formed elements examined. Species A2 is clearly seen as a doublet in erythrocyte zymograms, and occasionally in leukocyte zymograms early in the enzyme reaction or when less enzyme is applied to the gel. The A1 and A2 isoenzymes are relatively weak in platelet zymograms. Again, the identity of these nonspecific pancellular species is suggested by their smaller differential migration as a function of gel concentration (6.0%, 7.5%, and 9.0% polyacrylamide).

With αNA substrate, a doublet of intermediate mobility, A3, which migrates just ahead of hemoglobin, and one of low mobility, A4, are visualized in erythrocyte zymograms. These acetyl esterases are relatively fluoride-resistant and both react poorly with αNB substrate. In addition, a series of four or five periodic slow to intermediate acetyl esterases, A5 through A9, all partially resistant to fluoride inhibition, are visualized in platelet zymograms. Other weak bands were noted. These include two faint bands consistently visualized either with αNA or αNB, which migrate just ahead of AB1 and AB2, respectively. These are seen in granulocyte preparations.

A faint and diffuse butyryl esterase activity of intermediate mobility is inconsistently visualized in T-lymphocyte and B-lymphocyte zymograms. One or two very weak acetyl esterases, which migrate somewhat more slowly than A2, were inconsistently detectable in platelet zymograms. Preliminary fractionation experiments (W. Mastropaolo and J. Yourno, unpublished data) suggest that the A2 and A1 species partially mask a rather faintly visualized species with both acetyl and butyryl esterase activity. This is best seen in zymograms developed with αNB. This species is present in B lymphocytes and appears to be present in zymograms of several other cell types as well.

DISCUSSION

Markert and Hunter originally enunciated the principle of differential gene activation in the nonspecific esterase system in their study of zymograms from various tissues of the mouse.26 These investigators showed by starch gel electrophoresis that individual tissues yield characteristic zymograms of αNB esterase and that isoenzymes vary in tissue distribution from more or less restricted to generalized. Li and coworkers originally reported on zymogram patterns of nonspecific esterase of human leukocytes.19 Isoenzymes of similar mobility were found among several types of formed element preparations, and a platelet-specific species was identified. The determination of isoenzyme distribution among the various formed elements was difficult, however, because of the relative heterogeneity of these preparations. Our results with nonspecific esterases of highly purified formed elements allow a less equivocal determination of isoenzyme distribution and reiterate the principle of differential gene activation in the differentiation of the various formed elements. The fast acetyl esterases, A1 and A2, appear to be common to all or most formed elements examined, likewise the butyryl esterase B1. The fluoride-sensitive AB species, in contrast, show a more limited cellular distribution with intense activity in monocytes. The characteristic nonspecific esterase cytochemistry of monocytes, therefore, would reflect the presence of the AB species in this cell that account for the bulk of zymogram nonspecific esterase activity. A “possible monocyte-specific” isoenzyme highly reactive against αNA substrate has been detected by isoelectric focusing18 and presumably corresponds to the AB species. While confirming the association of these powerful species with monocytes, our data suggest to the contrary that the AB isoenzymes are also present in granulocytes at relatively low levels, at least in neutrophils that were the major population of our granulocyte preparations. The extractable AB isoenzyme of granulocyte preparations ranged from scarcely above that of lymphocyte preparations to moderately high. The AB isoenzyme was undetectable in platelet and erythrocyte preparations, and the trace levels found in T lymphocyte and B lymphocyte preparations can largely, if not exclusively, be ascribed to contaminating monocytes. Hence, the AB isoenzyme gene system(s) appears to be essentially inoperative or operative at relatively low levels in formed elements other than monocytes, and to a more modest extent, granulocytes. The existence of a shared functional gene system for esterase isoenzymes in monocytes and granulocytes is eminently consistent with current concepts of myeloid differentiation. It is now apparent that neutrophils and monocytes are derived from a common committed stem cell, as evidenced by the appearance of mixed neutrophil-monocyte colonies in CFU-C culture27 and the close relationship of acute myeloblastic and monocytic leukemias.43 These findings can also explain, through partial depression of the AB gene system, the puzzling monocytoid pattern of nonspecific esterase cytochemistry occasionally seen in acute leukemias that are morphologically granulocyt-
A third group of isoenzymes, all acetyl esterases, appears to be element specific. This group includes species A3 and A4 in erythrocytes and species A5 through A9 in platelets.

Thus, slab gel electrophoresis at pH 9.5 yields esterase zymograms that are characteristic of each type of formed element, while disclosing certain genetic and developmental relationships among the isoenzymes of these elements. As regards the controversy over the presence of nonspecific esterase in B lymphocytes, our results are in agreement with reports of cytochemically detectable nonspecific esterase in these cells.9,11 including those of chronic lymphocytic leukemia,10 B-cell lymphomas, and myelomas.11 The data are also consistent with a previous report of the cytochemistry, and fine structure. Clin Hematol 1:49, 1972

The technique of isoelectric focusing is reported to make this distinction.18 Our system tends rather to emphasize the similarity of zymograms of B lymphocytes and T lymphocytes, presumably an expression of the close kinship of these cells. A possible explanation for these divergent data lies in the source of the cell used in each study. While Radzun et al. used tonsillar B cells,18 our study used malignant B cells of CLL. The resemblance of esterases in these cells to those of normal circulating B cells remains to be determined.

Several questions remain to be resolved. Among these are the anomalous cytological negativity of red cells despite cellular levels of nonspecific esterase, as determined spectrophotometrically and electrophoretically, comparable to that of lymphocytes and granulocytes (W. Mastropaolo and J. Yourno, unpublished data). Obviously we are seeing only those isoenzymes that our extraction procedure and zymogram system are capable of visualizing. We are now examining the isoenzymes of nonspecific esterase by additional methods of complement present data.

A fund of information exists on the isoenzymology of nonspecific esterase of human erythrocytes as detected by starch gel electrophoresis.31 Our results are not directly comparable since we used a different extraction procedure and gel system. The B1 species, however, shows many of the properties of the B isoenzyme that is present in the red cell of several different mammalian species.31 The three groups of acetyl esterases detected in erythrocytes by our system presumably correspond to the A1, A2, and A3 families of isoenzymes, and possibly carbonic anhydrase, described by Tashian et al.31 Since the pH 9.5 polyacrylamide slab gel system makes a clear distinction between myeloid cells and lymphocytes, we are investigating its utility in the classification of acute leukemias.

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Nonspecific esterases of the formed elements: zymograms produced by pH 9.5 polyacrylamide gel electrophoresis

J Yourn and W Mastropaolo