Comparison of Alkaline Phosphatase from Human Normal and Leukemic Leukocytes

R. H. Bottomley, C. A. Lovig, R. Holt, and M. J. Griffin

SUMMARY

The alkaline phosphatase of leukocytes from normal subjects, patients with chronic granulocytic leukemia, and patients with reactive granulocytosis have been characterized by means of agarose column chromatography, sucrose density ultracentrifugation, starch gel electrophoresis, Michaelis constant for \( p \)-nitrophenyl phosphate, heat inactivation, inhibition by \( L \)-phenylalanine, Ouchterlony double immunodiffusion precipitation, and immunoelectrophoresis using rabbit anti-leukocyte alkaline phosphatase serum. The same antiserum was used to quantitate the amount of alkaline phosphatase protein in purified preparations of the enzyme obtained from leukocytes of patients with chronic granulocytic leukemia, reactive granulocytosis, and normal individuals. Purified alkaline phosphatase from all three sources had similar biophysical and biochemical characteristics, but the enzyme specific activities were shown to be different using the antiserum and quantitative precipitation technics. This finding was corroborated by using disc gel electrophoresis which showed that similar amounts of enzyme protein had markedly different enzyme activity, i.e., the enzyme from chronic granulocytic leukemic leukocytes had low specific activity, the enzyme from normal leukocytes had intermediate specific activity, and the enzyme from patients with reactive granulocytosis had a very high specific activity.

INTRODUCTION

A decreased activity of leukocyte alkaline phosphatase (EC. 3.1.3.1) (34, 36) is found in patients with CGL.

Elevations of leukocyte alkaline phosphatase have been reported in several conditions associated with RG (34, 36). Variations in the activity of alkaline phosphatase from the normal may be attributed to either a quantitative difference in enzyme concentration or to qualitative changes in the enzyme which could either enhance or reduce enzymatic activity. In the present study, alkaline phosphatase from normal, CGL, and RG leukocytes have been characterized by biophysical, biochemical, and immunologic technics.

MATERIALS AND METHODS

Assay Procedures. Alkaline phosphatase was assayed at 37°C in a system consisting of 250 \( \mu \)moles Tris hydrochloride, pH 10.0, 1.0 \( \mu \)mole magnesium chloride, 8.0 \( \mu \)moles \( p \)-nitrophenyl phosphate, disodium salt (Sigma 104), and enzyme and water to a total volume of 1.0 ml. The change in optical density was followed continuously at 410 m\( \mu \) in a Beckman DU spectrophotometer (Beckman Instruments, Inc.) with Gilford attachments (Gilford Instrument Laboratories, Inc.) (15). A blank containing substrate but no enzyme was routinely included in the assay, and the O.D. of the blank was subtracted from the O.D. of the sample containing enzyme prior to calculation of the results. One unit of activity is defined as the amount of enzyme generating 1.0 \( \mu \)mole of \( p \)-nitrophenol per 60 minutes.

Cell Separation. Five hundred ml of blood were drawn from normal patients in heparinized plastic bags (Abbott Pliapak) and allowed to settle at room temperature in graduated cylinders. Smaller volumes of heparinized blood were obtained from patients with RG and CGL because of their elevated white blood counts. The supernatant plasma was removed and the leukocytes separated from the plasma by centrifugation at 2000 \( X \) g for 5 minutes. Contaminating red cells were then removed by lysing them with distilled water as previously described (2).

Enzyme Purification. A partial purification of all three types of leukocyte alkaline phosphatase was obtained by \( n \)-butanol extraction of whole leukocytes followed by dialysis. The cells were suspended in 9 volumes of 0.001 M Tris hydrochloride in 0.155 M NaCl, pH 7.5. One ml of \( n \)-butanol was added for each 3 ml of cell suspension. This mixture was then shaken vigorously at room temperature in a stopped glass centrifuge tube on a Vortex shaker for 2 minutes. All further operations were performed at 4°C. The tube was then centrifuged at 2000 rpm for 20 minutes in a refrigerated centrifuge, and the lower aqueous layer was removed by means of a syringe with a long needle. The cells in the butanol layer were then reextracted with...
9 volumes of Tris-saline solution and the centrifugation repeated. The two extracts were combined and dialyzed overnight against 0.05 M Tris, pH 7.5. After dialysis the partially purified enzyme was concentrated 20 fold by ultrafiltration using a collodion bag with a porosity of less than 15 µ (Kollodiumhulsen Gottingen, supplied by Schleicher & Schuell). The nonfiltrable solution, consisting of proteins of molecular weight 30,000 and greater, contained the enzyme; this step resulted in an overall increase in alkaline phosphatase specific activity (Table 1). Some of the experiments were carried out using this partially purified enzyme. The final step in purification was accomplished by placing 0.3 ml aliquots of concentrated, partially purified enzyme on a 1 x 2 cm column of Bio-Gel 1.5 M Agarose and eluting the enzyme with 0.05 M Tris hydrochloride, pH 7.5. Table 1 illustrates representative activities, recoveries, and purification of enzymes from normal, RG, and CGL leukocytes. Purification varied from 21.6 fold for the CGL enzyme to 52.0 fold for the normal enzyme.

Electrophoresis. Vertical starch-gel electrophoresis was carried out at 4°C in a discontinuous borate buffer system (26), pH 8.2, for five hours at 22.5 milliamperes constant current. The enzyme preparations were n-butanol extracts which had to be redialyzed and concentrated by collodion membrane filtration. Due to low activity, CGL leukocyte extracts were routinely concentrated five fold more than the enzyme from normal leukocytes. Longitudinal gel slices were stained directly for alkaline phosphate using α-naphthyl phosphate and fast blue BB (Borden Chemical Co.) as the coupling dye.

Heat Stability Studies. The stability of the various enzymes purified through n-butanol extraction, dialysis, and collodion membrane filtration was studied at fixed temperatures using a circulating water bath with automatic temperature control, and the temperature was monitored with a thermister-activated probe.

Kinetics. Quantitative inhibition studies were carried out on alkaline phosphatase purified through Step 2 by continuously monitoring the optical density at 410 µ in a Gilford spectrophotometer Model 2000. The substrate and inhibitors were dissolved in 0.165 M Tris hydrochloride, pH 10.0, containing 10⁻³ M MgCl₂. Since the inhibitors are buffers, after dissolving them in 0.165 M Tris, the pH was readjusted to 10.0. The pH was monitored throughout the reaction and did not vary more than ±0.05 pH units. Initial velocities were determined from slopes obtained during the first 10 minutes of hydrolysis, this accounted for less than 5% of the total hydrolysis. D-Phenylalanine (15 mM) was added to several other reaction mixtures as another control determination and produced velocities indistinguishable, within experimental error, from those obtained without added D-phenylalanine. Double reciprocal plots of enzyme activity versus substrate concentration were made with and without added L-phenylalanine using enzyme from all three cell types (21). The lines were determined by least squares analysis of the data, and the slopes, x intercepts, and y intercepts were determined by computer analysis using the statistical technic of Cleland (4). From these data apparent Kₘ's were determined with p-nitrophenyl phosphate as substrate.

Molecular Weight Determination. A measurement of the

### Table 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Normal</th>
<th>CGL</th>
<th>RG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>Yield (%)</td>
<td>Activity</td>
</tr>
<tr>
<td>Sonicate</td>
<td>0.35</td>
<td>100</td>
<td>0.083</td>
</tr>
<tr>
<td>Step 1, n-butyl alcohol extract</td>
<td>1.2</td>
<td>3.4</td>
<td>36</td>
</tr>
<tr>
<td>Step 2, membrane concentrated</td>
<td>9.9</td>
<td>29</td>
<td>46</td>
</tr>
<tr>
<td>Step 3, agarose column purified</td>
<td>18</td>
<td>52</td>
<td>29</td>
</tr>
</tbody>
</table>

Purification of leukocyte alkaline phosphatase. CGL, chronic granulocytic leukemia; FG, reactive granulocytosis.

Activity expressed as micromoles of p-nitrophenyl phosphate cleaved per 60 minutes per mg of protein.

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were compared to the RG or normal preparation. The alkaline
the cathodal band in the CGL leukocyte preparation as

cell type were applied to the starch-gel. Two bands of activity

Chart 4. Approximately equal amounts of enzyme from each

band was performed using the same technics utilized for

precipitates were suspended in 0.10 ml saline and assayed for alkaline phosphatase

precipitate washed three times with ice-cold saline. The

immunization with the enzyme. After incubation at 37° for

enzyme from normal, RG, and CGL leukocytes. Controls were

set up by using serum obtained from rabbits prior to

injections were given at 2-week intervals for three doses, and

the rabbits were bled 7 days later by cardiac puncture.

Immunological and Antibody-precipitation Studies. Immuno-
diffusion and immunoelectrophoresis were performed in

agar as described by Kabat and Mayer (18). Histochemical

staining of alkaline phosphatase activity in the cross-reacting

band was performed using the same technics utilized for

alkaline phosphatase staining of the starch-gel electrophoreto-
gram. Antibody-precipitation studies were performed by add-
ing 0.05 ml of antiserum to 0.05 ml of the Step 3-purified

enzyme from normal, RG, and CGL leukocytes. Controls were

set up by using serum obtained from rabbits prior to

immunization with the enzyme. After incubation at 37°C for

1 hour and at 4°C for 16 hours, the mixtures were then

centrifuged at 2500 X g for 30 minutes at 0°C and the precipitate washed three times with ice-cold saline. The precipitates were suspended in 0.10 ml saline and assayed for protein and alkaline phosphatase activity. Control tubes had no detectable precipitate or activity.

RESULTS

Kinetics. Charts 1–3 show the effect of varying substrate

centrations of the L-phenylalanine inhibition of alkaline

phosphatase from leukocytes from normal individuals (Chart

1), patients with CGL (Chart 2), and patients with RG (Chart

3). The majority of the slopes of the double reciprocal plots of

the inhibited reactions were parallel at substrate concentra-
tions from 1 X 10\(^{-2}\) to 1 X 10\(^{-4}\) M, within experimental

error, indicating noncompetitive inhibition as the major mode

of inhibition of all three enzymes with L-phenylalanine. Table

2 illustrates the \(K_m\) values of \(p\)-nitrophenyl phosphate for the

three enzymes, and they are the same within experimental

error.

Electrophoresis. A vertical starch-gel electrophoretogram

stained for alkaline phosphatase is illustrated in Fig. 1 and

Chart 4. Approximately equal amounts of enzyme from each
cell type were applied to the starch-gel. Two bands of activity

were found in each sample although there was relatively less of

the cathodal band in the CGL leukocyte preparation as

compared to the RG or normal preparation. The alkaline

phosphatase bands from all three cell types migrated approxi-

mately the same distance, suggesting no great difference in

these enzymes with respect to electric charge at pH 8.2. Other

starch-gel electrophoretograms using more concentrated en-

zyme preparations showed the slow moving isozyme with

much more clarity. Treatment of the enzyme preparations

with commercial neuraminidase from *Vibrio cholerae* by the

procedure of Moss et al. (25) afforded a slight retardation of

the anodal bands in all three cases (Fig. 1; Chart 4).
Leukocyte Alkaline Phosphatase

Heat Stability. n-Butanol extracts were studied at three temperatures: 48 ± 0.5, 51 ± 0.5, and 58 ± 0.5°C. At 48°C all three enzymes retained 76 ± 10% of their activity after 2 hours. At 58°C all three enzymes lost 100% of their activity after 30 minutes. At 51°C all three lost 35 ± 2% of their activity after 1 hour, 58 ± 3% of their activity after 2 hours, and 83 ± 6% of their activity after 6 hours.

Molecular Weight Determinations. Ultracentrifugation analysis of the three alkaline phosphatases purified through chromatography gave a S20,w value for CGL of 17.6 X 10⁻¹³ sec⁻¹, for the normal enzyme a value of 14.5 X 10⁻¹³ sec⁻¹, and for the RG enzyme a value of 15.8 X 10⁻¹³ sec⁻¹. The estimated molecular weights (22) using these figures are 410,000 ± 75,000 for the normal enzyme, 550,000 ± 75,000 for the CGL enzyme, and 470,000 ± 75,000 for the RG enzyme. Because of the uncertainty in the determination (± 1.5 X 10⁻¹³ sec⁻¹, for any sample) the differences between these various cell types are not considered significant. This relatively large molecular weight stands in contrast to that determined for other mammalian alkaline phosphatases, such as the calf intestinal enzyme (7) with an estimated molecular weight of 100,000 and the HeLa enzyme (M. J. Griffin, unpublished data) with an estimated molecular weight of 100,000.

Results of Immunodiffusion Studies. Fig. 2 illustrates the precipitation bands obtained when antinormal leukocyte alkaline phosphatase was placed in the center well of the immunodiffusion plate and purified normal, RG, and CGL alkaline phosphatase preparations were placed in the outer wells. A single precipitation line was formed with all three enzymes without evidence of spur formation suggesting immunologic identity of the alkaline phosphatase from all three sources.

Results of Immunoelectrophoresis. Fig. 3 illustrates the electrophoretogram on agar of normal, CGL, and RG alkaline phosphatase stained for alkaline phosphatase activity. In all of the experiments using agar electrophoresis, 15 microliters of CGL enzyme (0.8 unit/ml), 15 microliters of normal enzyme (4.6 units/ml), and 15 microliters of RG enzyme (35.8 units/ml) were placed in the sample wells. Equal amounts of CGL and normal leukocyte alkaline phosphatase protein were applied to the gel, but considerably smaller amounts of RG protein was present in the sample applied to the gel because sufficient amounts of the purified protein were not available to allow use of the enzyme at the same protein level as the other two enzymes. However, despite the lower protein concentration in the RG preparation, the greatest activity was seen in the RG enzyme and the lowest in the CGL as compared to the normal enzyme. Fig. 4 illustrates the cross-reacting bands that developed when these enzymes were allowed to diffuse against antinormal leukocyte alkaline phosphatase serum. After 5 days of diffusion, cross-reacting bands were seen between the normal and CGL enzymes and the antibody, but very little was seen with the RG enzyme at this relatively low protein concentration. The cross-reacting band seems heaviest between the CGL enzyme and the antibody despite the fact that the same amount of protein was placed in the CGL and normal wells, suggesting more cross-reacting protein in the CGL sample than in the normal one. Fig. 5 illustrates a replicate immunoelectrophoretogram stained for alkaline phosphatase activity. This figure illustrates much greater alkaline phosphatase activity in the cross-reacting band in the normal sample than in the case of the CGL enzyme despite the fact that there appeared to be a heavier protein precipitation band with CGL prior to enzyme staining. There appeared to be very little cross-reaction with the RG enzyme preparation at this protein concentration. Fig. 6 illustrates the same immunoelectrophoretogram stained for protein by the Amido-Schwarz stain after first being stained for alkaline-phosphatase. Here an additional cross-reacting band can be seen closer to the origin, which appears to have a line of identity with the faster-running alkaline-phosphatase band, despite the fact that they are migrating at different rates. These results indicate the homogeneity of these preparations in regard to alkaline-phosphatase activity.
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- Normal-0°C
- Normal-37°C
- Normal + Neuraminidase-37°C
- CGL-0°C
- CGL-37°C
- CGL + Neuraminidase-37°C
- Leukemoid-0°C
- Leukemoid-37°C
- Leukemoid + Neuraminidase-37°C
- Buffer

Figs. 1-7. CGL, chronic granulocytic leukemia; RG, reactive granulocytosis.

Fig. 1. Starch-gel electrophoretogram of alkaline phosphatase from normal, CGL, and leukemoid (RG) leukocytes, stained for alkaline phosphatase activity. The origin is to the left of the photograph and the anode to the right. Preparations subjected to electrophoresis included enzyme maintained at 0°C, enzyme incubated for 60 minutes at 37°C without added neuraminidase, and enzyme incubated for 60 minutes at 37°C with Vibrio cholera neuraminidase.

Fig. 2. Ouchterlony plate demonstrating the reaction of antinormal leukocyte alkaline phosphatase with normal, RG, and CGL leukocyte alkaline phosphatase. The center well contained 20 microliters of antinormal leukocyte serum, and outer wells contained 20 microliters each of purified (Step 3) normal (NOR), RG, and CGL alkaline phosphatase.

Chart 4. Diagram of a starch-gel electrophoretogram of alkaline phosphatase from normal, leukemoid (RG, reactive granulocytosis), and chronic granulocytic leukemia (CGL) leukocytes. The origin is at the left edge of the diagram with the anode to the right. The amount of activity in each band is depicted by the shading. Neuraminidase produced some slowing of the most anodal band of all three types of alkaline phosphatase.
as there are no additional cross-reacting bands seen except for the small cross-reacting band seen close to the origin, probably representing the slower isoenzyme seen on starch-gel electrophoresis.

Results of Immunoprecipitation Studies. Table 3 illustrates the specific activity of the antigen-antibody precipitate obtained when antinormal leukocyte alkaline-phosphatase serum was added to purified alkaline phosphatase from normal, RG, and CGL leukocytes. Approximately equal amounts of enzyme protein from each cell type were mixed with an equal volume of undiluted antiserum. Immunoprecipitation experiments indicated undiluted serum was near optimal in affording maximal complex precipitate formation with quantitative recovery of enzyme activity. The specific activities are quite different in each cell type and are similar to the relative activities observed in the whole cell sonicates and the purified enzyme. For example, the antigen-antibody complex using the enzyme from CGL leukocytes had the lowest specific activities (Table 3) as did the sonicate from CGL leukocytes (Table 1). The enzyme from normal leukocytes had intermediate specific activity, and the enzyme from the RG leukocytes had the highest specific activity both in sonicates and antigen-antibody precipitate (Tables 1, 3). Experiments 1 and 2 of Table 3 are repeats of the same type of experiment using purified enzymes.
from different individuals. Further substantiation of the immunoprecipitation results was obtained from acrylamide disc gel electrophoretic analysis of the Agarose column-purified enzymes. As Fig. 7 shows, no detectable contaminating proteins appear when the most highly purified enzyme preparations are stained for both enzyme activity and protein. This indicates these preparations and essentially homogenous and, therefore, represent adequate materials for comparing enzyme specific activities.

**Effects of Zn⁺⁺.** Attempts were made to activate the enzyme from CGL leukocytes by the addition of Zn⁺⁺ at 10⁻³, 10⁻⁴, and 10⁻⁵ M concentrations, a procedure which has been reported to activate E. coli apoalkaline phosphatase (31). It was found that Zn⁺⁺ inhibited the enzyme from CGL leukocytes at 10⁻³ and 10⁻⁴ M concentrations and had no effect at 10⁻⁵ M.

**Table 3**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Normal</th>
<th>RG</th>
<th>CGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>0.94⁺⁺</td>
<td>2.9</td>
<td>0.077</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>0.34</td>
<td>1.7</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Specific activities of the antigen-antibody complex obtained from purified normal, reactive granulocytosis (RG), and chronic granulocytic leukemia (CGL) alkaline phosphatase and rabbit antiserum made to normal leukocyte alkaline phosphatase.

DISCUSSION

Previous studies of human leukocyte alkaline phosphatase have been performed predominantly by histochemical technics as described by Gomori (14) and Kaplow (19). These studies have consistently shown that most CGL polymorphonuclear leukocytes had less histochemical alkaline phosphatase activity than normal cells (34). A biochemical assay was used by Valentine and Beck (36) with results similar to the histochemical observations. It has been suggested (1, 33) that the alkaline phosphatase activity in CGL leukocytes correlates with the observed loss of the long arms of chromosome 21 (Philadelphia chromosome). Another possible explanation for the decreased activity of this enzyme in this cell type is a decrease in the specific activity of the enzyme which might be due to a difference in its physical and biochemical characteristics. The present study was designed to investigate human leukocyte alkaline phosphatase from normal individuals, patients with CGL, and patients with granulocytosis, to determine whether the variation in activity could be correlated with differences in enzyme characteristics.

The Kₘ values using p-nitrophenyl phosphate as substrate were not significantly different for alkaline phosphatase from all three cell types and were in the same range reported for HeLa alkaline phosphatase (16). L-Phenylalanine inhibited the enzyme from all three types of leukocytes in an uncompetitive manner (coupling inhibition) (35). This type of inhibition, a function of both substrate and inhibitor concentration, has been reported for rat intestinal (8, 9, 12), human placental (9), and HeLa (5) alkaline phosphatase.

Disc gel electrophoresis and immunoelectrophoresis showed substantial homogeneity of the purified alkaline phosphatase from all three cell types. The disc gel results even without the immunoprecipitation data would suggest that the specific activities of the purified enzymes reflect the activity of the enzyme within the cell and represent a reason for the differing amounts of enzyme activity characteristic of the three cell types. All three enzymes are precipitated by antibody to normal leukocyte alkaline phosphatase, and the results of double diffusion analysis suggest this complex is specific for an alkaline phosphatase enzyme-protein complex. The antigen-antibody complex of these three enzymes have markedly different specific activities. If one assumes that the ratio of antigen to antibody in the three types of enzyme-antibody complex is approximately the same, then for all three enzymes the finding of markedly different specific activities in the alkaline phosphatase from these three cell types suggests three possibilities: (a) there are variable amounts of inactive alkaline phosphatase protein in these three types of leukocytes which retain immunologic identity and dilute the active alkaline phosphatase in the antigen-antibody complex; (b) in each cell type there is some alteration of all of the enzyme molecules which does not change the immunologic reactivity but does alter the specific activity of the molecule, or (c) leukocyte alkaline phosphatase is a heteropolymeric protein (aggregate) which contains varying amounts of active alkaline phosphatase protein.

Previous workers have suggested that the life span of the granulocyte might influence the level of alkaline phosphatase
(17). If the enzyme was synthesized in the myelocyte prior to
being released into the circulation, and if the enzyme in the
circulating granulocyte loses activity without loss of enzyme
protein as the cell ages, a large amount of inactive enzyme
protein would be found in a relatively aged cell such as a CGL
granulocyte. Mechanisms which might account for progressive
loss of enzyme activity could include loss of Zn\(^{++}\) from the
active site or conformational changes which affect enzyme
activity. Vallee (37) demonstrated that normal leukocytes
contained approximately 25 times as much zinc as red cells
and that a protein could be isolated from the leukocyte that
accounted for 81% of the intracellular zinc. Gibson et al. (13)
demonstrated low levels of zinc in chronic granulocytic
leukemocytes, and elevated levels in infection have been
reported (38). Trubowitz (32) demonstrated that purified
human leukocyte alkaline phosphatase contained substantial
amounts of zinc. These findings plus our own might suggest
that the activity of the enzyme is regulated by the Zn\(^{++}\)
apoenzyme ratio and that the changes in specific activity
observed in the present study are due to differences in zinc
content of the enzyme from the three types of leukocytes. In
the present study we were unable to activate the CGL enzyme
by the addition of Zn\(^{++}\). Trubowitz was able to partially
reactivate leukocyte alkaline phosphatase with Zn\(^{++}\) after
inactivating it with ethylenediaminetetraacetate (EDTA). Our
failure to activate the alkaline phosphatase from CGL leuko-
cytes suggests that the in vivo enzyme differs in some way
(such as configuration) from the EDTA-treated enzyme since
the EDTA-treated enzyme can be at least partially reactivated
while the CGL enzyme cannot. Proof of these various
alternatives will only be possible after sufficient amounts of
purified enzyme can be obtained to allow subunit characteriza-
tion and quantitation of the Zn\(^{++}\) content of the purified
enzyme from the three types of leukocytes.

Another aspect of mammalian alkaline phosphatase regula-
tion in vivo is suggested by the studies utilizing L-phenylalanine
as a stereospecific inhibitor. Leukocyte (23, 30), HeLa (6),
and mouse intestinal (24) alkaline phosphatases are induced by
adrenocortical hormones and they have been shown to be
inhibited by L-phenylalanine (5). On the other hand, other
mammalian alkaline phosphatase activities not induced by
glucocorticoids, such as kidney and fibroblast alkaline phos-
phatase, are relatively insensitive to inhibition by L-phenyl-
alanine (5). Therefore, this inhibition may be a distinguishing
characteristic of hormone-regulated alkaline phosphatases.

Starch-gel electrophoresis of CGL leukocyte alkaline phos-
phatase has been reported to give a pattern which differs from
the alkaline phosphatase of normal leukocytes (20, 28, 29).
The data reported in this paper show no significant difference
in the electrophoretic pattern of the high level RG cells, the
medium level normal cells, and the low level CGL leukocytes.
The observation that neuraminidase slows the migration of the
anodal bands of all three cell-type alkaline phosphatases
equally (Chart 4) would suggest that there is the same amount
of sialic acid on the isoenzyme from each of the three cell
types. Variations in migration of leukocyte alkaline phos-
phatases previously reported (27) may be related to differing
amounts of sialic acid residues (3). Heat stability curves carried
out on enzyme from all three cell types were almost identical,
indicating that the conformational resistance to irreversible
denaturation by heat of the three enzymes is approximately
the same.

The results described above would suggest that alterations in
the level of human leukocyte alkaline phosphatase are medi-
ated in some way through the activity of each enzyme
molecule and that these changes result in marked changes in
the specific activity of the enzyme molecules, but they do not
markedly alter electrophoretic migration, \(K_m\) for p-nitro-
phenyl phosphate, molecular weight, or heat stability. It is
possible that these changes could be related to increased or
decreased numbers of active sites on the molecule although the
possibility has not been directly tested. These results are similar
to the results obtained by Griffin and Cox (16) concerning the
mechanism of regulation of HeLa cell alkaline phosphatase by
hydrocortisone.

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Michaelis-Menten plots.

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