positive reactions only at toxic levels—greater than 4 mg/100 ml and at 150 mg/100 ml, respectively.

A series of 15 specimens tested by the screening procedure was rechecked by the ultraviolet method, and excellent correspondence as to the presence or absence of barbiturates was obtained. Only rarely did the clinician request that a quantitative test be subsequently performed.

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References

Absence of Measurable Leukocyte Alkaline Phosphatase Activity from Leukocytes of Patients with Chronic Granulocytic Leukemia

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Leukocyte phosphatase activity was determined over a broad pH range. Using this procedure, we found no alkaline phosphatase activity in leukocytes from three patients with chronic granulocytic leukemia. The low values usually reported for this disease are actually attributable to acid phosphatase activity, which is slight but measurable at alkaline pH.

Additional Keyphrases  sonication of cells  •  spectrophotometry  •  acid phosphatase activity of leukocytes

Decreased activities of leukocyte alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) have been reported in patients with chronic granulocytic leukemia (1, 2). Such activity measurements are useful in differentiating this disease from other leukemias (3). Such measurements are typically made at a single pH value, usually pH 10, whereas on measuring it over a broad range of pH we have demonstrated that leukocyte alkaline phosphatase activity is absent from the leukocytes of individuals with chronic granulocytic leukemia, rather than simply being decreased.

Materials and Methods

Leukocytes were isolated from both normal and leukemic blood and disrupted by sonication as previously described (4). Normal cells were obtained from apparently healthy volunteer subjects, leukemic cells from three patients with an unequivocal diagnosis of chronic granulocytic leukemia.

The phosphatase activity of the sonicates was determined as follows: 0.50 ml of substrate solution (0.015 mol of p-nitrophenylphosphate per liter) was mixed with 0.50 ml of the appropriate buffer:

(a) 2-Amino-2-methyl-1-propanol, 0.1 mol/liter, containing 0.001 mol of Mg\(^{2+}\) per liter, was used over the pH range 8.5 to 11.0.

(b) Tris(hydroxymethyl)alaminomethane, 0.1 mol/liter, containing 0.001 mol of Mg\(^{2+}\) per liter, was used over the pH range 6.5 to 9.0.

(c) Citrate, 0.1 mol/liter, was used over the pH range 3.5 to 6.5.

De-ionized water was added to give a final incubation volume (after the addition of sonicate) of 1.50 ml. All tubes were equilibrated at 37°C for 10 min. The reaction was then started by adding sonicate. Because of the relatively greater phosphatase activity in the acid range, we used less sonicate at the lower pH values. A typical curve was obtained by incubating 0.05 ml of normal sonicate (containing 0.075 mg of protein) in the pH range of 3.5 to 6.5, 0.10 ml of sonicate (0.15 mg of protein) in the pH range of 6.5 to 9.0, and 0.20 ml of sonicate (0.30 mg of protein) in pH range of 8.5 to 11.0. The incubation was ended after 30 min by adding 10.0 ml of 0.1M sodium hydroxide. The p-nitrophenol released
by the reaction was measured from its absorbance at 410 nm in a Beckman DB split-beam spectrophotometer; the instrument was zeroed on a control, consisting of a replicate to which the NaOH was added at the same time as the sonicate. The standard curve relating absorbance to concentration was linear to 1.0 absorbance unit.

We estimated the protein content of sonicates by the biuret method of Gornall et al. (5), with bovine serum albumin as a standard. All activities were expressed as μmoles of p-nitrophenol liberated per hour per mg of protein.

**Results**

Figure 1 illustrates the change with pH of phosphatase activity of cells derived from two normal persons and two of the patients with chronic granulocytic leukemia. The curves are representative of curves obtained for leukocytes from six normal subjects and our three patients. Alkaline phosphatase activity was totally absent from the leukemic cells. Although the values obtained at high pH are quite measurable, they all represent the overlapping tail of the acid phosphatase curve rather than any activity of alkaline phosphatase. There was no specific buffer effect, nor did sonicate from the patient contain inhibitor, as shown by the additive activity of a mixture of sonicate from normal and leukemic individuals.

**Discussion**

Generally, the alkaline phosphatase activity of the granulocytes in patients with chronic granulocytic leukemia is not increased after stimulation by pyrogen or adrenocortical hormones, whereas the alkaline phosphatase in leukocytes from normal subjects is definitely enhanced under these conditions (6–8). However, a number of patients with well-documented chronic granulocytic leukemia have been demonstrated to have an increased leukocyte alkaline phosphatase activity during acquired infection or after either steroid or pyrogen administration (9–11). Perillie (12) studied one such patient and demonstrated that increased leukocyte alkaline phosphatase activity appeared in circulating mature neutrophils 24 h later than in similar cells from the bone marrow, suggesting that these cells originate in the marrow. He further noted that the activity of leukocyte alkaline phosphatase increased with infection in only a relatively small number of cells from his patient, in contrast to the situation in normal cells, and so concluded that foci of normal myeloid tissue must be present if there is to be a response of leukocyte alkaline phosphatase in chronic granulocytic leukemia.

Our results support this hypothesis. The leukemic cells lack measurable alkaline phosphatase activity; it seems unlikely that such cells could somehow manufacture a normal enzyme as a consequence of infection. Rather, a population of normal cells with the normal complement of leukocyte alkaline phosphatase is probably released in response to the stimulus. Patients with long-standing leukemia might have no normal myeloid tissue, and hence could not increase their leukocyte alkaline phosphatase under stimulus.

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**References**