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Electrophoretic Heterogeneity of Leucocyte Alkaline Phosphatase in Normal Man and in Patients with Polycythemia Vera. (31437)

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Interest in the alkaline phosphatase activity of human polymorphonuclear leucocytes was stimulated by the now well-established finding that the activity of this enzyme falls to very low values in chronic myelogenous leukemia and rises to very high levels, well beyond the normal, in polycythemia vera(1). The speculation that the decrease in enzyme activity of the leukemic granulocyte is the result of partial deletion of the small acrocenter of chromosome 21, the Philadelphia chromosome, has been advanced(2,3). Evidence has also been presented(4,5) purporting to relate maturity and differentiation of the polymorphonuclear leucocyte with alkaline phosphatase activity. The potential for phosphatase synthesis is realized during differentiation of the granulocyte from its precursors, the myeloblast and myelocyte. Enzymes are now known to exist in multiple molecular forms and different isozyme patterns have been observed in different tissues (6). Heterogeneity of non-specific human serum alkaline phosphatase and of human placenta has been demonstrated(7). The purpose of the present investigation has been to describe the pattern of heterogeneity of normal human leucocyte alkaline phosphatase and to determine the influence of polycythemia rubra vera on this pattern.

Materials and methods. Five hundred ml of blood in ACD solution were obtained from 5 presumably normal donors immediately after removal in a commercial blood bank. The alkaline phosphatase assay of their leucocytes ranged between 29.6 and 83.0 mg phos/10^{10} polymorphonuclear leucocytes. In addition, 2 healthy, hematologically normal volunteers were phlebotomized of 500 ml of blood. The leucocyte alkaline phosphatase assay of these samples was 26 and 35 mg phos/10^{10} polymorphonuclear leucocytes. Similarly, 500 ml of blood were removed from 3 patients with well documented polycythemia rubra vera whose leucocyte alkaline phosphatase values were: 61, 192, and 465 mg phos/10^{10} polymorphonuclear leucocytes.

The leucocytes were isolated by means of differential sedimentation in 5% polyvinyl pyrrolidine and 2% bovine fibrinogen in normal saline(5). The leucocyte preparation was freed of most of the contaminating erythrocytes by exposure to hypotonic saline followed by restoration of tonicity with 3.5% saline (8). Six volumes of cold distilled water were added rapidly to 2 volumes of a saline suspension of the cells. After vigorous mixing for 30 seconds, tonicity was restored by rapid addition of 2 volumes of 3.5% saline. After one final washing with normal saline the leucocyte cell pack was resuspended in 1-2 volumes of water and stored at —20°C.

Upon thawing and homogenization of the cell pack in a tissue grinder, the enzyme was extracted by the method of Morton(9), as modified by Trubowitz *et al*(10). The extract

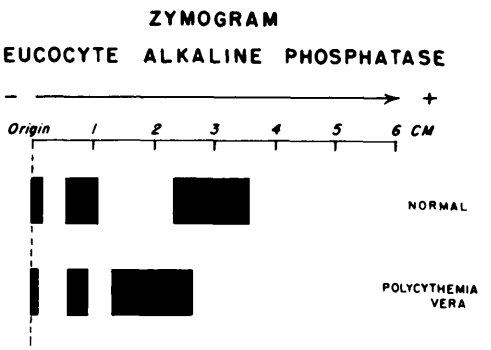

FIG. 1.

was taken through the first acetone precipitation and then redissolved in 0.1 M sodium hydrogen carbonate. The zymograms were developed by horizontal starch gel electrophoresis in a discontinuous system of buffers (11,12). The pH of the gel buffer was 8.65. The system was run at 200V for 16 hours at 4°C. Staining for enzyme activity was accomplished on the gel slices by the method of Boyer(7).

The results are summarized in Fig. 1. Three bands of enzyme activity were found in both normal and polycythemic subjects. In the normal, the bands were located at the origin, about the 1.0 cm line and about the 3.0 cm line. In the polycythemic, the initial two slow bands resembled the normal, but a cathodal shift in the third band was apparent, centering about the 2.0 cm line. Since the polycythemic cells were considerably richer in phosphatase activity (2-15 times) than the normal cells, it is unlikely that additional enzyme bands were missed.

The zymogram of normal leucocyte alkaline phosphatase shown above is quite similar to that reported by Robinson, Pierce and Goldstein(13). Three bands were identified with an identical migration pattern. The same investigators also reported the electrophoretic migration of leucocyte alkaline phosphatase obtained from chronic myelocytic leukemia; a decrease in intensity of the 2 slow bands and a cathodal shift of the major band to the 2.0 cm line. A faint fourth band at the 5.0 cm line was observed. Thus, except for the faint fourth band, the zymograms of the leucocyte alkaline phosphatase of chronic myelocytic leukemia and polycythemia rubra vera are quite similar.

The significance of these findings is not clear. If the zymograms described by Robinson *et al* and by us, do represent similar molecular species of leucocyte alkaline phosphatase, then the clinical suggestion of a relationship between chronic myelocytic leukemia and polycythemia rubra may be raised again.

Summary. The leucocyte alkaline phosphatase of 7 normal men and 3 patients with polycythemia rubra vera was examined by starch gel electrophoresis. Three bands of activity were seen in each group. A significant shift toward the cathode of the fastest band was noted in the polycythemic phosphatase.

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