TWO LEUKOCYTE ENZYMES WITH CATALASE ACTIVITY IN ACUTE LEUKEMIA[1]

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Variations in the levels of catalase activity in human leukocytes have been observed in certain pathological states (Kidson, 1962). Especially noticable are the very high levels present in myeloid leukemia, particularly the acute form, when compared to normal granulocytes. Leukemic levels are even higher when compared to young cells of the myeloid series from patients with infective leukocytosis.

During investigations into the mechanisms responsible for the high catalase activities of leukemic myeloid cells it was observed that lysates prepared from these cells showed two pH optima for catalase activity. The present report deals with the initial characterization in crude cell lysates of these two enzymes with catalatic properties and with the possible significance of the second enzyme in leukemia.

METHODS.

Leukocytes were prepared from normal and leukemic blood anticoagulated with EDTA 2.5% in 0.9% sodium chloride by a previously described (Marks et al., 1960) modification of the dextran flotation method of Skoog and Beck (1956). Contaminating erythrocytes were eradicated by differential lysis by

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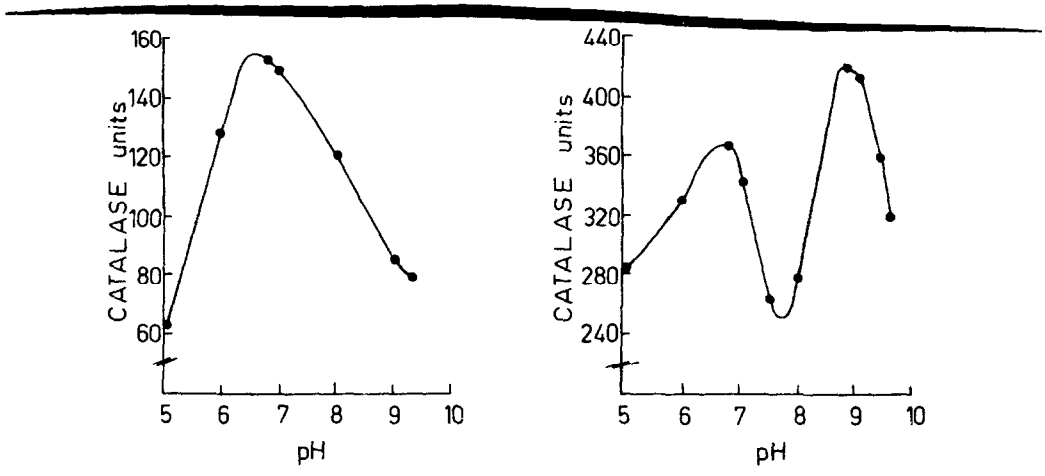
the method of Tomonaga et al. (1953). Catalase activity was assayed by Tomonaga's (1953) method, as previously modified (Kidson, 1962), but employing a 0.01 M glycylglycine-0.01 M phosphate buffer over the pH range 5.0 - 9.3. Michaelis constants (Km) were determined with this same buffer system, at pH 6.8 and 8.8 over a substrate concentration range of 0.01 M - 0.2 M hydrogen peroxide (H_2O_2); all reactions were carried out at 37°C. Thermal stability was examined over a range of 20°C - 60°C, using crude leukocyte lysates.

RESULTS

The normal pH curve for catalase activity in normal human leukocytes - mixed granulocytes and lymphocytes - was found to have an optimum peak at 6.8 (Figure 1a). A similar curve was obtained for erythrocyte catalase. In leukocytes from all five cases of acute myeloid leukemia examined, two pH optima were obtained, at 6.8 and 8.8 respectively (Figure 1b), indicating the presence of a second enzyme with catalase activity not apparent in the normal cells. The activity per cell of the second enzyme was, in all instances, significantly greater than that of the 6.8 enzyme. No second peak has been observed in chronic myeloid leukemia, or in any forms of lymphoid leukemia.



(a) (b)

Figure 1 pH curves for catalase activity.

(a) normal leukocytes

(b) leukocytes of acute myeloid leukemia.

Michaelis constants are listed in Table 1.

TABLE 1

Michaelis constants of normal and leukemic enzymes at respective pH optima.

Lysate	pH	Km (± S.D.) ($M \times 10^{-2}$ H_2O_2)
Normal	6.8	2.2 (± 0.2)
Leukemia (A)	6.8	1.9 (± 0.4)
Leukemia (B)	8.8	5.4 (± 0.5)

For the leukemic samples studied the Km values obtained at pH 6.8 (A) were not significantly different from the normal value of 2.4×10^{-2} (M H_2O_2). The Km of the leukemic pH 8.8 enzyme (B) was, however, significantly different from that at pH 6.8, the value of 5.4×10^{-2} confirming the distinctive nature of the second peak. Both leukemic catalatic enzymes appeared to be more stable to heat over the range tested, 20°C - 60°C, than the catalase from normal leukocytes; at 60°C at pH 6.8 the times for 50% inactivation were 20 minutes and 70 minutes for the normal and leukemic enzymes respectively.

DISCUSSION

The appearance of a second pH peak for catalase activity in lysates of leukocytes in acute myeloid leukemia, having a characteristic Km value different from that of the normal catalase indicates the presence in these cells of two distinct enzymes, conveniently labelled catalase A and B. The differences in thermal stability may also indicate a qualitative difference between the normal and leukemic A enzymes; however, in view of the significantly higher level of proteolytic activity in normal leukocytes as compared with those of acute myeloid leukemia (Frei et al., 1961) it is quite possible that

the greater instability of catalase in normal cell lysates is due to non-specific proteolysis and is not a distinctive property of the enzyme itself.

The biological significance of the second catalase in acute myeloid leukemia is at present uncertain. However, the possibilities of mutation at a normally "silent" gene locus, of mutation with loss of a normal inhibitor or of adaptive enzyme synthesis must be considered and are presently being examined experimentally.

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