Variations in Catalase Activity in Human Leukocytes

By CHEV KIDS0N

CATALASE is an enzyme found in most body tissues which has the capacity to release molecular oxygen from hydrogen peroxide (H₂O₂). It is concerned with peroxidation of substrates such as alcohol, phenol, formate and formaldehyde.¹

Catalase in blood has aroused considerable interest, the enzyme content being higher than that in other tissues except liver.² Most studies have concerned only the erythrocyte enzyme³⁴ because of earlier difficulties in separating leukocytes in sufficiently pure preparations for enzyme assay. The introduction of dextran methods⁵ of isolation of leukocytes from erythrocytes has enabled a more careful study to be made of the leukocyte enzyme.

Recently, Tomonaga et al.⁶ have investigated methods for studying leukocyte catalase activity, employing dextran separation followed by differential osmotic lysis of residual erythrocytes for preparing pure leukocyte samples. Applying these methods, Ichimaru⁷ has reported interesting alterations in the activity of this enzyme in leukemia, showing an increase in leukocyte catalase activity in chronic myeloid leukemia, very low levels in chronic lymphatic leukemia and a wide range of activities in acute leukemias.

The assessment of such findings raises the problem common to all studies of quantitative alterations of leukocyte enzymes in leukemia, namely the difficulty of interpreting comparisons made with mixed normal leukocyte populations. In the present study the preparation of both granulocyte-rich and lymphocyte-rich fractions of normal whole leukocyte populations has permitted a comparison of catalase activity of leukemic leukocytes with that of the normal cell prototypes. This has also permitted the question of cell age in relation to alterations of catalase activity in myeloid leukemia to be considered on the basis of studies on young leukocyte populations in infective states.

MATERIALS AND METHODS

Normal subjects: The initial study was of 10 normal subjects in good health who had values for hemoglobin, leukocyte counts and platelet counts within normal limits. In this group, leukocyte catalase activity was assayed on whole leukocyte populations.

A second group of 12 normal subjects was then examined, in which assays were performed on both granulocyte-rich and lymphocyte-rich fractions of leukocyte preparations. This group was used to assess the contributions made by normal granulocytes and lymphocytes to the activities of mixed cell populations, and to provide more suitable controls for comparison of catalase levels in leukemia and infection.

Leukemic subjects: A total of 18 patients with leukemia were investigated and included five with acute myeloid, three with chronic myeloid, five with acute lymphatic and five...
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with chronic lymphatic forms. In each leukemic group the predominating cell types were myeloid and lymphoid respectively, the picture in the acute leukemias in both groups being more varied with respect to cellular morphology and containing many more primitive cells than in the chronic leukemias. In the latter there were more than 80 per cent mature forms of the relevant type, as judged by morphology.

** Infective subjects:** Seven patients with infective states of varied etiology were studied. All subjects in this group had increased leukocyte counts, with more than 80 per cent neutrophils of which 20–60 per cent were band forms, indicating populations of young cells of the myeloid series.

** Leukemoid reaction:** One patient with a marked leukemoid reaction of 250,000 leukocytes per cu.mm.—dominantly mature neutrophils—secondary to carcinoma of the prostate was included in the study.

**Preparation of leukocytes:** Leukocytes were prepared by a previously described modification of the dextran method of Skoog and Beck. E.D.T.A. (ethylene-diaminetetra-acetic acid, disodium salt), 2.5 per cent in 0.9 per cent sodium chloride, was used as the anticoagulant in a concentration of 1 ml. per 10 ml. of blood. In those experiments involving separation of lymphocytes and granulocytes, a modification of the method of Ventzke et al. was employed. The dextran-separated leukocyte fraction was taken up in 0.5–1.0 ml. of autologous plasma, pipetted into a narrow siliconized tube, 80 mm. x 2.5 mm. diameter and centrifuged at 800 x g at 4 C. for 15 minutes. The cells separated into the following layers from above down: residual platelets, lymphocytes, granulocytes and residual erythrocytes. Slight contamination of the lymphocyte layer by platelets and of the granulocyte layer by erythrocytes was unavoidable. The plasma and platelets were pipetted off by very fine Pasteur pipette, then the lymphocyte and granulocyte layers removed separately with similar pipettes, the interface being discarded; the two leukocyte samples were suspended in 0.9 per cent saline. In all leukocyte samples, residual contaminating erythrocytes were eradicated by differential lysis by the method of Tomonaga et al.

**Assay of catalase activity:** Catalase activity was assayed with a slight variation of Saito's erythrocyte method as modified for the leukocyte enzyme by Tomonaga and co-workers. Leukocyte suspensions in 0.9 per cent saline were prepared to contain 10–15 x 10⁶ cells per ml. and 0.1 ml. of this suspension was added to 0.9 ml. of distilled water and rapidly mixed by pipette. Within three minutes this lysate was added, with mixing, to 5 ml. of N/10 H₂O₂ solution, buffered with M/150 phosphate at pH 6.8 (preheated in a water bath at 37 C.) Incubation was carried out in a water bath at 37 C. for three minutes and the reaction stopped by adding 4 ml. of 10 per cent (v/v) sulphuric acid. The residual H₂O₂ (a), was titrated against N/25 potassium permanganate solution; 5 ml. of N/10 H₂O₂ (b), and 0.1 ml. of lyzed leukocyte suspension (c), were similarly titrated.

The amount of H₂O₂ broken down during the three minute incubation, as measured by the difference, b— (c + a), and expressed as mEq. H₂O₂/10⁹ leukocytes (WBC), formed a measure of leukocyte catalase activity.

**Results**

**Normal whole leukocyte populations:** The results of the initial studies on whole leukocyte populations of 10 normal individuals are shown in table 1. Catalase activities ranged from 150–316 mEq. H₂O₂/10⁹ WBC, and the leukocyte populations varied in composition between 60–80 per cent granulocytes and 20–40 per cent lymphocytes.

**Separated normal granulocytes and lymphocytes:** Fractionation of leukocytes from 12 additional normal subjects yielded granulocyte-rich cell populations containing up to 85 per cent granulocytes, and lymphocyte-rich cell populations containing up to 95 per cent lymphocytes, the mean values being 80 per cent and 85 per cent respectively (table 2). Catalase estimations on these fractions gave mean values of 212 mEq H₂O₂/10⁹ WBC for granulocyte-rich
Table 1.—Catalase Activity in Normal Whole Leukocyte Population

<table>
<thead>
<tr>
<th>Leukocyte Morphology</th>
<th>Catalase Activity mEq. H₂O₂/10⁹ WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Granulocytes</td>
</tr>
<tr>
<td>Range (10 subjects)</td>
<td>60–80</td>
</tr>
<tr>
<td>Mean</td>
<td>69</td>
</tr>
</tbody>
</table>

Table 2.—Catalase Activity in Separated Normal Granulocytes and Lymphocytes

<table>
<thead>
<tr>
<th>Leukocyte Morphology</th>
<th>Uncorrected Catalase Activity mEq. H₂O₂/10⁹ WBC</th>
<th>Corrected Catalase Activity mEq. H₂O₂/10⁹ WBC</th>
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<tr>
<td></td>
<td>Granulocyte fraction % granulocytes</td>
<td>Lymphocyte fraction % lymphocytes</td>
</tr>
<tr>
<td>Mean</td>
<td>80</td>
<td>85</td>
</tr>
</tbody>
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populations and 103 mEq. H₂O₂/10⁹ WBC for lymphocyte-rich populations. These results were corrected for 100 per cent “pure” populations of the two cell types by solving simultaneous equations for the two lots of figures for each subject. This gave a mean normal granulocyte catalase value of 258 mEq. H₂O₂/10⁹ WBC and a mean normal lymphocyte value of 76 mEq. H₂O₂/10⁹ WBC, indicating a much higher level of catalase activity in normal granulocytes than in lymphocytes on a per cell basis.

Myeloid leukemia: The eight cases of myeloid leukemia all showed leukocyte catalase activities above the range for normal granulocytes as shown in figure 1, the mean values being 780 mEq. H₂O₂/10⁹ WBC for acute myeloid leukemia and 450 mEq. H₂O₂/10⁹ WBC for chronic myeloid leukemia. Some very high values were obtained in the acute group. These figures represent the activity of mixed cell populations, but in all cases there was a preponderance of cells of the myeloid series, predominantly mature neutrophils in the chronic group, with many more primitive forms—blast to band-neutrophil—in the acute group.

Lymphatic leukemia: The results in the 10 cases of lymphatic leukemia are shown in figure 1. The ranges of 52–153 mEq. H₂O₂/10⁹ WBC for acute lymphatic leukemia and 35–60 mEq. H₂O₂/10⁹ WBC for chronic lymphatic leukemia were lower than the range for normal whole leukocyte populations but as is evident from the graph they were not markedly different from that of normal lymphocytes. In fact they presented, together, a scatter approximating to the normal range. The cell populations of all cases of lymphatic leukemia contained more than 80 per cent of cells of the lymphatic series, the morphologic picture being much more varied in the acute group where the dominant cell type was the lymphoblast. It is evident from this small group of subjects that while catalase activity in lymphatic leukemic leukocytes was low, it was probably not altered from that of normal lymphocytes.

Infective leukocytosis: All seven cases of infective leukocytosis had increased total leukocyte counts, with more than 80 per cent neutrophils of which 20–50 per cent were band forms. All cases showed catalase activities below those of normal granulocytes as is evident from figure 1. The range of 82–141 mEq.
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Fig. 1.—Comparison of leukocyte catalase activity in normal granulocytes and lymphocytes, in leukemia and in infection. N.G. = normal granulocytes; N.L. = normal lymphocytes; A.M.L. = acute myeloid leukemia; C.M.L. = chronic myeloid leukemia; A.L.L. = acute lymphatic leukemia; C.L.L. = chronic lymphatic leukemia; I.L. = infective leukocytosis.

H₂O₂/10⁹ WBC, with a mean value of 117 mEq H₂O₂/10⁹ WBC was also below that of the normal mixed leukocyte populations studied.

Leukemoid reaction: The subject with the leukemoid reaction secondary to prostatic carcinoma had a leukocyte catalase activity of 236 mEq H₂O₂/10⁹
WBC, within the range of the normal granulocytes. The sample studied contained more than 90 per cent mature neutrophils.

**Discussion**

Comparison of catalase activity in normal whole leukocyte populations with that in separated granulocyte-rich and lymphocyte-rich fractions revealed two interesting points. First, the granulocyte-rich fractions had higher activities than the lymphocyte-rich fractions; this was always true for the two fractions from the one original mixed sample, although the ranges of values, as shown in table 2, revealed a slight overlap between individuals. On the basis of the values for pure granulocytes and lymphocytes, derived by solving simultaneous equations for the measured values, granulocytes had distinctly higher enzyme levels than lymphocytes. Secondly, normal mixed leukocyte samples, with more than 60 per cent granulocytes, reflected largely the activity of granulocytes, and formed a poor basis for comparison of lymphocyte activity in disease states.

Comparisons of leukocyte catalase activity in leukemia are therefore valid only in relation to their normal cell prototypes, not in relation to mixed normal leukocyte populations. Such comparisons do not, of course, overcome the difficulties of assessing accurately the significance of results in acute leukemias, where many immature cells are involved.

The series of cases of myeloid leukemia studied here revealed enzyme levels above those of normal granulocytes with some very high values in the acute group. It is of interest that the leukocytes of the one leukemoid state studied —where there was a very high neutrophil leukocytosis—had a normal catalase activity, showing a distinctly different behavior to the cells of chronic myeloid leukemia, despite the morphologic similarity of cell pattern. These findings confirm those of Ichimaru,7 and suggest that increased catalase activity is a feature of the abnormal leukocytes of myeloid leukemia.

In lymphatic leukemia, on the other hand, although the values observed for catalase activity were low compared to the normal mixed cell populations, comparison with normal lymphocytes suggested that there was probably no deviation from the normal pattern. Such a conclusion can at least be inferred for chronic lymphatic leukemia, where morphologic similarities of cell type were evident. The validity of the comparison with normal lymphocytes in the case of acute lymphatic leukemia is less sure, but no doubt, in the absence of a ready source of supply of normal lymphoblasts, such a comparison of immature, leukemic lymphoid cells with mature normal lymphocytes represents a more accurate appraisal than comparison with mixed normal leukocyte populations. The same probably applies to assessment of acute myeloid leukemia.

The results in this study suggest, therefore, that while increased catalase activity is a feature of acute and chronic myeloid leukemia, there is no significant alteration in the activity of the enzyme in lymphatic leukemia. The low levels reported by Ichimaru7 in lymphatic leukemia compared to mixed normal leukocyte populations probably represented activities within the range for normal lymphocytes.

Catalase estimations in infective leukocytosis revealed rather surprising
values, all being lower than the normal granulocyte range. As the presence of large numbers of band neutrophils in the samples studied was indicative of young granulocyte populations, this suggests that these young cells of the myeloid series had lower catalase activities than the mature cells rather than higher values as might be expected in young, metabolically active cells. Several workers,11-12 have suggested that the cells of myeloid leukemia have a shortened life-span and represent comparatively young cells, but the question of the age of leukemic leukocytes is by no means settled.13-15 If the cells of myeloid leukemia are young cells, then the finding of low catalase levels in young normal granulocytes serves to emphasize even more strongly the extent of the variations from the normal in myeloid leukemia.

**SUMMARY**

The enzyme, catalase, has been studied in normal leukocytes, and in leukocytes from patients with leukemia and infective states. Examination of both granulocyte-rich and lymphocyte-rich fractions prepared from normal leukocyte populations showed the catalase activity of normal granulocytes to be higher than that of normal lymphocytes.

In acute and chronic myeloid leukemia, catalase levels were found to be higher than the range for normal granulocytes, whereas in acute and chronic lymphatic leukemia, the levels were of the same order as those for normal lymphocytes.

Low catalase values were found in infective leukocytosis, suggesting that young granulocytes may have lower activities than the mature cells. The significance of the changes in leukocyte catalase activity in myeloid leukemia are discussed in relation to cell age.

**SUMMARIO IN INTERLINGUA**

Le enzyma catalase esseva studiate in leucocytos normal e in leucocytes ab patientes con leucemia e statos infective. Le examine de fractiones ric in granulocytos e de fractiones ric in lymphocytos preparate ab populationes de leucocytos normal monstrava que le activitate catalatic de granulocytos normal es plus forte que illo de lymphocytos normal.

In acute e chronic leucemia myeloide, le nivellos de catalase esseva plus alte que le spectro de valores pro granulocytos normal, durante que in acute e chronic leucemia lymphatic le nivellos esseva intra le spectro de valores pro lymphocytos normal.

Basse valores de catalase esseva trovate in leucocytosis infective, lo que pare indicar que juvene granulocytos ha plus basse activitates que le cellulas matur. Le signification del alterationes in le activitate catalatic del leucocytos in leucemia myeloide es discutite con referentia al etate del cellulas.

**ACKNOWLEDGMENTS**

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