Quantitative alterations in the purine catabolic enzyme, xanthine oxidase, have been observed in neoplasia and nonmalignant hyperplasia. A decrease in the xanthine oxidase level per cell during carcinogenesis in both liver and breast tumors has been reported (1, 3). Likewise, Bennett and associates1 have shown that several experimental tumors are low in xanthine oxidase activity. In contrast, the rapid cellular proliferation during liver regeneration is associated with a doubling of the cellular xanthine oxidase levels (4). An elevated xanthine oxidase level is therefore characteristic of normal hyperplastic liver, whereas diminished xanthine oxidase levels seem to be characteristic of rapidly growing hepatoma and of other neoplasms.

To determine whether the decrease in purine catabolic enzymes accompanying neoplasia is a general phenomenon, it is important to compare other rapidly growing normal tissues with their malignant counterparts. The experimental mouse ascitic leukemia L1210 provides just such an experimental opportunity. The xanthine oxidase and uricase activities of these leukemic cells were therefore compared with those of rapidly developing lymphocytes derived from normal mouse spleen and with normal polymorphonuclear leukocytes.

Since previous studies have demonstrated inhibition of xanthine oxidase by pharmacological levels of 8-azaguanine in vitro and in vivo (5, 9), the enzymatic basis underlying 8-azaguanine sensitivity and dependence of sublines of L1210 cells was also investigated. The hypothesis that differential 8-azaguanine sensitivity in the sensitive and dependent sublines is due to variations in the cellular levels of xanthine oxidase was tested.

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MATERIALS AND METHODS

Since enzyme estimates of tissue homogenates or extracts may not be identical with the actual functional level of the enzyme in the intact cell, a technic capable of measuring the functional activities of the purine catabolic enzymes, xanthine oxidase and uricase, in intact unhomogenized cells has been developed.2 Buffered leukocyte suspensions are incubated with xanthine-6-C14; the xanthine-6-C14 is converted by xanthine oxidase to uric acid, which is then oxidatively decarboxylated to allantoin by uricase, the number 6 carbon being released as C14O2. The rate of C14O2 appearance is, therefore, an expression of the sum of the intracellular xanthine oxidase and uricase pathways. By the addition of excess exogenous purified xanthine oxidase or uricase to the reaction mixture, it is possible to render the remaining enzyme rate-limiting in the over-all process. In this manner, measurements are obtained of the functional levels of combined xanthine oxidase and uricase and of the maximal activities of each of these individual enzymes within the intact cells.

8-Azaguanine-sensitive (S) and -dependent (D) sublines of the L1210 ascitic leukemia strain were obtained through the courtesy of Dr. Lloyd Law and serially transplanted in our laboratory in DBA/2 male mice (6). The peritoneal fluid from mice bearing these leukemia sublines was diluted with saline and provided leukemic lymphocytes. To obtain a preparation of nonleukemic lymphocytes, spleens were removed from normal nonleukemic DBA/2 mice, suspended in ice cold saline, gently squashed between two watch glasses, and passed through a gauze filter. To obtain a preparation of neutrophilic leukocytes, a saline suspension of heat-killed E. coli was injected into the peritoneal cavity of normal DBA mice; 24-48 hours later a leukocyte-rich ascitic fluid containing 60-90 per cent neutrophilic granulocytes was harvested and diluted with saline.

2 J. E. Ultmann and P. Feigelson, in preparation.
The L1210 ascitic fluid, the spleen suspension, and ascitic fluid of E. coli-treated mice were subjected to a brief hypotonic shock to lyse contaminating erythrocytes. Cold distilled water (1.6 volumes) was added to the isotonic (0.31 osmolar) cell suspensions, reducing the tonicity of the suspending medium to 0.12 osmolar. This was rapidly mixed and allowed to stand at 0°C for 30 seconds. An equal volume of 0.50 osmolar sodium chloride was added to each hypotonic suspension, thus restoring isotonicity. Ninety-nine per cent of the erythrocytes are thus differentially lysed, leaving the leukocytes undamaged, as evidenced by their normal morphology under the phase microscope and the unimpaired rate of incorporation of C14 precursors into the proteins and nucleic acids of these cells. The cell suspensions were then centrifuged at 500 g for 5 minutes in a refrigerated centrifuge. The supernatant was discarded, and the leukocyte pellet was suspended in a 0.2 M sodium phosphate buffer containing salts, pH 7.2 (8). Aliquots of this cell suspension were taken for estimations of leukocyte and red blood cell counts.

Two-ml. aliquots of each leukocyte suspension were placed in chilled Warburg vessels containing xanthine-6-C14 (10,000 counts/min) in 0.2 ml. (Isotopes Specialties Co.; specific activity, 0.4 mc/mmole). 8-Azaguanine and nonradioactive xanthine were added as indicated in the text. When xanthine oxidase alone was measured, ~.5 units of uricase (Worthington Biochemical Co.) was introduced into the flasks; when uricase alone was measured, ~90 units of purified bovine cream xanthine oxidase was added. The center well of the Warburg flask contained 0.~ ml. Hyamine hydroxide and 0.2 ml. toluene (7). The Warburg vessels were incubated at 38°C for 1 hour, after which time the metabolically formed C14O2 was released by tipping 0.8 ml. of 5.0 N H2SO4 from the side arm into the main chamber. Quantitative diffusion of the released C14O2 into the Hyamine hydroxide and 0.2 ml. toluene (7). The Warburg vessels were incubated at 38°C for 1 hour, after which time the metabolically formed C14O2 was released by tipping 0.8 ml. of 5.0 N H2SO4 from the side arm into the main chamber. Quantitative diffusion of the released C14O2 into the Hyamine hydroxide mixture in the center well was assured by shaking the flasks at 38°C for an additional hour following addition of the sulfuric acid. The Hyamine-toluene mixture containing trapped C14O2 was quantitatively transferred from the center well to a 5-dram liquid scintillation counting bottle, with 1.0 ml. absolute ethanol and 5.0 ml. 0.3 per cent 2,5-diphenyloxazole and 0.03 per cent 1,4-di[2-(5-phenyloxazolyl)]benzene in toluene. The radioactivity of the samples was measured in an automatic Tri-Carb Liquid Scintillation Counter, with an efficiency of 47 per cent and a probable counting error of less than 5 per cent.

RESULTS AND DISCUSSION

The blank radioactivity in the Hyamine hydroxide derived from xanthine-6-C14, incubated without cells, was never more than 40 counts/min. The addition of exogenous uricase had no effect on this blank. The addition of exogenous cream xanthine oxidase elevated the blank to 200 counts/min, suggesting that the xanthine oxidase was contaminated with trace amounts of uricase. The appropriate blank radioactivities were subtracted from the flasks containing cells.

Preliminary experiments were undertaken comparing cell preparations which had been osmotically shocked to remove contaminating red blood cells with unshocked suspensions containing red blood cells. These studies demonstrated that the shocking procedure had no demonstrable effect on the investigated enzyme activities of the leukocytes.

The validity of the xanthine oxidase assay procedure was further confirmed in the presence of exogenous uricase, C14O2 was evolved at a constant rate as a function of both time and L1210 D cell concentration (Charts 1 and 2).
The sensitivity of this assay may be evaluated. If it is assumed that the washed leukocytes were free of endogenous purines and that the sole source of xanthine was supplied by the exogenous labeled substrate, the shape of the line in Chart 2 indicates that C14O2 of 180 counts/min was produced by 10 × 10⁶ cells in 1 hour, corresponding to the oxidation of 41 × 10⁻¹² moles of xanthine.

Notwithstanding the high degree of sensitivity inherent in these assay systems, the physiological sum of the endogenous xanthine oxidase and uricase pathways of the splenic and leukemic lymphocytes were essentially at the threshold of detectability by this technic (Table 1). In the presence of excess uricase, however, when xanthine oxidase alone was the rate-limiting enzyme, the splenic lymphocytes showed significant activity, manifesting a xanthine oxidase activity twice that of the leukemic lymphocytes. Still greater differences were evidenced in the cellular levels of uricase (xanthine oxidase added in excess) between non-malignant splenic lymphocytes and leukemic lymphocytes; the uricase activity of normal lymphocytes was 5–10 times greater than that of either leukemic strain. Leukemic lymphocytes, therefore, contained markedly diminished levels of both the xanthine oxidase and uricase enzyme levels, as compared with nonmalignant splenic lymphocytes.

Since the over-all rate of xanthine catabolism in both the normal and the leukemic lymphocytes was slower than either xanthine oxidase or uricase measured alone, it is evident that each of these enzymes was present in effectively rate-limiting concentrations within these cells. The ratios of the cellular levels of uricase to xanthine oxidase were characteristic of each of the cell types studied; among the leukocytes studied, splenic lymphocytes alone possessed a greater uricase activity than xanthine oxidase activity.

Variations in the size of the endogenous xanthine pool would influence the measured C14O2 production from xanthine-6-C14. The possibility that differences in the size of this endogenous xanthine pool may be responsible for the observed differences in rates of C14O2 evolution was therefore excluded by the addition of an excess quantity (0.05 μmoles) of nonlabeled xanthine to the reaction mixture. As shown in Table 1, the relative levels of xanthine oxidase activity in the absence or presence of excess exogenous nonlabeled xanthine were comparable; thus, variations in endogenous xanthine pool do not underlie the observed differences in xanthine oxidase activity between

![Chart 2](chart.png)

**Chart 2.**—The catabolism of xanthine-6-C14 as a function of concentration of L1210 D leukemic cells.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>XANTHINE OXIDASE AND URICASE LEVELS IN NORMAL AND LEUKEMIC LEUKOCYTES</td>
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<tr>
<td><strong>LEEUCYTES</strong></td>
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<tr>
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</tr>
<tr>
<td>Splenic lymphocytes</td>
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<tr>
<td>Leukemia L1210 S</td>
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<tr>
<td>Leukemia L1210 D</td>
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<tr>
<td>Granulocytes</td>
</tr>
</tbody>
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* No supplemental enzymes.
† Xanthine oxidase added in excess.
‡ Uricase added in excess.
the normal and malignant tissues. It may be noted that $^{14}C_2$ production remained high in spite of the decreased specific activity of xanthine-6-$^{14}C$ due to the addition of cold xanthine. This may be interpreted as an increased degree of saturation of the enzyme system by the elevated substrate levels. Thus, an increased rate of metabolism of a larger amount of xanthine, although of lower specific activity, resulted in an output of similar levels of $^{14}C_2$.

The combined endogenous activity of xanthine oxidase and uricase within normal granulocytes indicate that these cells possess a capacity for xanthine oxidation which is tenfold that of either normal or leukemic lymphocytes. This greater metabolic activity of granulocytes is the resultant of xanthine oxidase activities which were 6 times, and uricase activities which were over twice, that of normal splenic lymphocytes. The ratio of uricase to xanthine oxidase activity within the granulocytic cells was close to one. Other differences between enzyme patterns of granulocytes and lymphocytes have been reported (10).

The diminished capacity for xanthine oxidation manifested by the leukemic lymphocytes, as compared with nonmalignant, although rapidly growing, splenic lymphocytes, is in agreement with reports that other malignant tissues have diminished xanthine oxidase activities (1, 3). Since decreased levels of uricase are also characteristic of the leukemic cells, it may be concluded that at least two purine-catabolizing enzyme systems are diminished in the leukemic lymphocytes as compared with their normal counterpart. These findings support the hypothesis that the enhanced anabolic activity characteristic of tumor cells may be in part the result of decreased competitive catabolic destruction of intermediates.

No statistically reliable differences are evident between the L1210-sensitive and -dependent strains with respect to their cellular xanthine oxidase and uricase levels. This, therefore, excludes the possibility that the 8-azaguanine dependence characteristic of the L1210 D strain is due to alteration in these enzyme levels. The demonstration of a pharmacologic effect of 8-azaguanine in vivo or in vitro on xanthine oxidase (9) does not constitute sufficient evidence for the establishment of this effect as the biochemical basis for the mechanism of action of this drug. It is probable that carcinostatic drugs exert many biochemical influences, some of which are of importance to the organism, but others of which exert little deleterious effect on cellular survival or multiplication. The enzymatic basis for resistance and tolerance is probably that recently described by Brockman et al. (2).

**SUMMARY**

Studies on intact cells indicate that ascitic leukemic lymphoid cells of mice contain diminished xanthine oxidase and uricase activities, as compared with normal lymphoid cells. This supports the hypothesis that diminished purine catabolic activity is characteristic of certain malignant tissues.

There were no significant differences between these enzyme levels in leukemic strains which are sensitive and dependent to 8-azaguanine.

Xanthine oxidase and uricase levels in normal granulocytes were several-fold those of normal lymphocytes.

**REFERENCES**

Cellular Xanthine Oxidase and Uricase Levels in Leukemic and Normal Mouse Leukocytes

Philip Feigelson, John E. Ultmann, Susanne Harris, et al.


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