Intracellular Distribution of Uricase and Catalase in Livers of Tumor-bearing Mice and Mice Given Injections of Aminotriazole

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That the catalase activity of the liver of the tumor-bearing animal is significantly decreased has been established beyond question (6). The activities of d-amino acid oxidase, arginase, esterase, and lipase are also diminished, although to a much lesser extent than catalase (6), while most other enzymes assayed have shown negligible changes in concentration. Uricase has not been studied so extensively as catalase, but there is evidence that the concentration of this enzyme may be lowered in the livers of animals with tumors. Lan observed a 50 per cent decrease in liver uricase in rats with transplanted Hepatoma 31, but only a slight decline after transplantation of Walker carcinosarcoma 256 (11). Mori and Shimojo found that the uricase activity of rat liver after induction of hepatomas by p-dimethylaminoazobenzene was only 23 per cent of normal (14); after the feeding of 2-acetylaminofluorene, Mori, Ichii, and Nagase reported a diminution of uricase to 14 per cent and of catalase to 2 per cent of control values (13). In the latter experiment, the loss of catalase was greater than that of uricase at various stages prior to the appearance of hepatomas.

Since uricase and catalase seem to be very closely associated in mouse liver (17), we were interested in studying the concentration and intracellular distribution of these two enzymes in livers of mice with tumors, to compare the rates at which the activities decreased and to see whether the disappearance of activity was also accompanied by shifts in the patterns of intracellular distribution. In addition, we were interested in finding out whether the potent inhibitor of catalase in vivo, 3-amino-1,2,4-triazole (8), had any effect on the concentration and distribution of uricase.

MATERIALS AND METHODS

The mice used in these experiments were CFl and CFW females, usually 8-10 months old. They were fed Rockland mouse diet and water ad libitum. The tumor-bearing mice included a series inoculated with the Hall carcinoma (7), some mice inoculated 18-21 days previously with Krebs-2 ascites tumors (which had begun to solidify), and a few animals with spontaneous mammary tumors. In the experiments with 3-amino-1,2,4-triazole, mice were given injections intraperitoneally of 2 gm/kg.

The technics of gradient centrifugation and construction of the distribution curves have been previously described (17, 18). In all experiments the mice were fasted 18-24 hours prior to sacrifice.

The assay for catalase was based on the perborate method of Feinstein (2). Five-tenths ml. of appropriately diluted tissue suspension was added to 2 ml. of a solution containing, per 100 ml., approximately 1.54 gm. of sodium perborate and 0.4 ml. of 85 per cent orthophosphoric acid (0.2 N NaBO3 • 4 H2O, 0.06 N phosphate, pH 7.0). After incubation at 37° C. for exactly 5 minutes, 2.5 ml. of 2 N sulfuric acid was added to stop the reaction. Then 0.5 ml. of the acidified solution was added to 10 ml. of accurately standardized 0.004 N KMnO4 in 1 N H2SO4, the residual permanganate color was measured in a Klett-Summerson colorimeter with a No. 54 filter. The colorimetric measurement of permanganate, first used in catalase assays by Goldblith and Proctor (4), is perhaps not so precise as titrimetric analysis, but for a large number of samples (fourteen assays in duplicate for each mouse) it is very convenient. One precaution which must be taken is to be sure that the perborate is slightly less than 0.2 N or the permanganate slightly more than 0.004 N, so that there will be a slight excess (about 0.001 milliequivalents) of permanganate in the blank tube. The method gives satisfactory results under conditions in which 10-60 per cent of the perborate is consumed.

Uricase was measured by the method of Schneider and Hogeboom (15). Originally, a phosphate buffer at pH 7.4 was used; more recently, however, we have used borate buffer at pH 9.0 to achieve more nearly maximum activity. We noted a very slight but consistent effect on the distribution pattern subsequently obtained; at pH 9.0 the distribution curve of activity in respect to particle size was displaced about 0.01-0.02 μ to the right of the curve at pH 7.4.

RESULTS

Intracellular relationships between uricase and catalase.—Greenfield and Price (5) demonstrated that, when rat liver was dispersed in a solution of

1 We are indebted to Mrs. Mildred Summers for supplying us with the tumor-bearing mice.

2 Dr. Robert N. Feinstein provided us with a generous supply of recrystallized aminotriazole.
sucrose and polyvinylpyrrolidone (PVP), nearly all the catalase was recovered in the mitochondrial fraction; previous investigators who had used solutions of sucrose alone had reported that rat liver catalase was distributed between the soluble and either the mitochondrial or microsomal fractions (1, 11, 14). Although we had observed a very close molecular weight, 40,000). In rat liver, there was apparently some solubilization of catalase even in the presence of PVP; adsorption of some of this extracted portion by microsomes (such extraction and adsorption presumably occurred in our earlier studies [18]) would account for the tendency of the uricase/catalase ratios to decrease with increasing

### TABLE 1

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Force (g)</th>
<th>Mouse Liver</th>
<th>Rat Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Catalase (per cent of total)</td>
<td>Uricase (per cent of total)</td>
</tr>
<tr>
<td>10</td>
<td>2,000†</td>
<td>6.5</td>
<td>5.8</td>
</tr>
<tr>
<td>10</td>
<td>6,500</td>
<td>25.4</td>
<td>25.9</td>
</tr>
<tr>
<td>10</td>
<td>15,500</td>
<td>16.5</td>
<td>18.1</td>
</tr>
<tr>
<td>10</td>
<td>25,000</td>
<td>16.5</td>
<td>18.1</td>
</tr>
<tr>
<td>40</td>
<td>25,000</td>
<td>22.6</td>
<td>22.8</td>
</tr>
<tr>
<td>160</td>
<td>25,000</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Supernatant</td>
<td></td>
<td>7.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Based on total enzyme sedimented; i.e., percentages of catalase are divided by (1 — 0.19), percentages of uricase divided by (1 — 0.039).
† International PR-1 refrigerated centrifuge, sediment washed once. All other centrifugations carried out with Servall SS-1, precipitates not washed.

### TABLE 2

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>Tumor size (cm)</th>
<th>Per cent of control activity†</th>
<th>Catalase‡</th>
<th>Uricase‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.45, 0.53</td>
<td>83.88</td>
<td>101.104</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.62</td>
<td>90</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.2</td>
<td>74</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.3</td>
<td>54</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>5.5</td>
<td>45</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

* Twenty-four mice were inoculated. The tumor failed to take in two, two others died (20 and 22 days), and the data from another one were rejected because of analytical deficiencies.
† Individual values are shown for entries with one or two mice; for the others, averages and ranges are given.
‡ Average of nine controls: 21.7 m.eq. perborate destroyed/5 min/mg N, σ = 4.0.
§ Average of ten controls: 96.3 μmoles uric acid destroyed/min/mg N, σ = 11.7.

association between catalase and uricase in mouse liver homogenized in sucrose solution (17), we considered it necessary to verify this observation using sucrose-PVP as a dispersing medium. Table 1 shows the close relationship between the two enzymes in arbitrarily selected fractions of both mouse and rat liver, homogenized in 20 per cent sucrose containing 10 per cent PVP (average sedimentation times and forces. In mouse liver, the agreement between uricase and catalase concentration was very close; these data confirm our previous findings obtained with sucrose solutions without PVP (17).

**Uricase and catalase in tumor-bearing mice.**—The activities of the two enzymes in livers of mice inoculated with the Hall carcinoma are listed in Table 2. The correlation between the age of the tumor and the average depression of enzyme activity was apparently rather good. However, there was considerable variation among the individuals within the groups, and among the mice the livers of which were fractionated the activities were higher in the two animals sacrificed 28 days after inoculation than in those killed after 21 or 23 days. This discrepancy is shown in curves 3 and 4 of Charts 1 and 2.

It is apparent that the activities of both catalase and uricase were diminished and that the loss of the former began earlier and was more pronounced; e.g., 23 days after inoculation, uricase was decreased by 30 per cent, catalase by 60 per cent. Chart 1 shows the distribution curves for catalase in the livers of normal mice and of mice at 1, 3, and 4 weeks after inoculation of the Hall carcinoma. Although there were appreciable changes in concentration, there was little alteration in the
shape of the distribution curves; the maxima lie within 0.02 μ of the control curve. Similar data are presented for uricase in Chart 2.

The average distributions for uricase and catalase in livers of five mice 18–21 days after inoculation with ascites tumors are shown in Chart 3.

We did not observe any significant changes in either the concentration or the intracellular distribution of total nitrogen in the livers of the tumor-bearing mice.

Effect of aminotriazole on distribution of uricase and catalase.—Attention has been called to the similarity between the effects of 3-amino-1,2,4-triazole and the effects of a tumor on the catalase activities of animal tissues (3, 8). Chart 4 shows the distribution patterns of catalase and uricase in livers of mice sacrificed 2½ and 16 hours after injection of aminotriazole. Catalase activities were 3.1 and 23.4 per cent of normal, respectively; in the former case, most of the activity can be accounted for by the catalase of the blood entrapped in the liver. Uricase activities were slightly lower than normal but still within the normal range. As in the case of the tumor-bearing mice, there were negligible changes in the shape of the uricase distribution curves. At 2½ hours after injection, a greater proportion of uricase was found to be completely sedimented—15 per cent, in contrast to 8 per cent in the controls. It is conceivable that this change may be the result of an in vitro effect of amino-

CHART 1.—Distribution of catalase in livers of CFW mice with Hall carcinomas. Curve 1: average of six controls; vertical lines indicate standard deviations. Curve 2: average of two mice, 7 days after inoculation. Curve 3: average of three mice, one at 21 and two at 23 days after inoculation. Curve 4: average of two mice, 28 days after inoculation. The ordinate, C/Ad, represents the fraction of enzyme activity found in a particular zone obtained by gradient centrifugation, divided by the difference between the extremes of particle size predicted for the zone (18). For the treated mice, C/Ad is calculated in Charts 1–4 on the basis of enzyme activity as a fraction of the total control activity, so that the decrease in concentration is reflected by the decrease in areas under the curves.

CHART 2.—Distribution of uricase in livers of CFW mice with Hall carcinomas. Curve numbers same as in Chart 1.

The average decrease in catalase was 55 per cent, in uricase 15 per cent. Also in Chart 3 are the distribution curves on one mouse with a spontaneous mammary tumor; the catalase activity was only 33 per cent of the control level, while the uricase activity was essentially normal. The distribution curve for uricase was virtually identical with the control curves for both uricase and catalase reported previously for CF1 mice (17).
triazole, which is present in the 1:10 homogenate of liver in appreciable quantity (0.0024 M, if one assumes equal distribution of the injected dose throughout the body), rather than to any in vivo effect.

DISCUSSION

We have previously reported that, in rats treated with carbon tetrachloride (19) and in hypoxic guinea pigs (10), there was both a diminution of uricase activity and an alteration in the distribution of the enzyme in respect to particle size, which observation seemed suggestive of a change in the physical characteristics of the uricase-containing particles. In the studies reported here, however, we have noticed no significant shift in the particle size associated with maximum uricase activity, even in those animals in which the uricase concentration was appreciably reduced.

The diminution of catalase in the livers of tumor-bearing animals is primarily a loss of active enzyme and is not the result of loss of the specific particulates with which catalase is associated, since we have shown that it is possible for catalase to be significantly reduced while the distribution and concentration of uricase are unaltered, e.g., in the mouse with spontaneous tumor and in the 1st week after inoculation with the Hall carcinoma. In the latter case, the uricase content of the liver subsequently decreases, although the distribution pattern remains the same. Whether this decrease represents the disappearance of specific particles or simply the loss of uricase from these particles has not been established. On the basis of the fact that in hypoxia and in carbon tetrachloride poisoning the loss of uricase is accompanied by marked changes in distribution, we favor the latter view; however, until unequivocal cytologic identification of these particles can be established and quantitative enumeration made, this question cannot be definitely answered.
That a substance can be extracted from tumor tissue which can decrease the concentration of catalase in the liver when administered to an animal has been demonstrated and amply confirmed (6). To our knowledge the effect of “toxohormone” on uricase has not been examined. Aminotriazole, which has an effect on catalase superficially similar to that of “toxohormone,” is virtually without effect on uricase in doses which inactivate nearly all the catalase in liver. It is possible, of course, that repeated injection or continuous infusion of aminotriazole into an animal might ultimately depress the level of uricase. Sugimura (16) has pointed out that there are fundamental differences between toxohormone and aminotriazole; however, there are certain discrepancies between his work and that of Feinstein et al. (3) which need to be reconciled.

One may argue, of course, that the decrease in uricase concentration in the tumor-bearing mice was secondary to the decline of the animal. However, there was no correlation between the appearance of the mouse and its liver uricase activity; the moribund mice did not necessarily have lower activities than healthy-looking animals. It may be relevant to add that in rats fed heavy water, the catalase activity of the liver was reduced by as much as 35 per cent, while the uricase activity was actually greater than normal, despite the fact that the animals were manifestly moribund (9).

It is clear that, despite the association of uricase and catalase with the same type of intracellular particulate, the two enzymes respond in different fashion both to the growth of a tumor and to the administration of aminotriazole, the decrease in uricase activity beginning later (if at all) and proceeding less extensively. It is also clear that the loss of these enzymes does not affect the sedimentability of the particles with which they are associated.

SUMMARY

1. The uricase activity of livers of tumor-bearing mice diminished with increasing tumor growth more slowly and ultimately less extensively than did the catalase activity. There was apparently no effect on the intracellular distribution of either enzyme.

2. Injection of 3-amino-1,2,4-triazole had no effect on uricase under conditions in which the liver catalase was reduced essentially to zero.

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