Catabolism of Xanthine and Uracil in Tumor-bearing Rats*

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SUMMARY

The urinary excretion of allantoin and uric acid was greatly increased in rats bearing Walker carcinoma 256. The increase was greater with more advanced tumor growth. A similar increase was also observed in the allantoin concentration of the plasma and liver of these rats. An increase in the RNA and DNA content and a decrease in the RNA/DNA ratio were found in the muscle of the tumor-bearing animals. Liver xanthine oxidase activity was increased in rats bearing Walker 256 and Guerin carcinomas and Novikoff and Morris 3683 hepatomas but was unchanged in rats bearing Jensen sarcoma, Morris 5123, and Morris 3924-A hepatomas, when compared with their respective controls fed ad libitum. Liver uracil reductase activity showed a decrease in rats bearing Morris 5123 and Morris 3924-A hepatomas but no change in rats bearing Walker carcinoma 256 and Novikoff and Morris 3683 hepatomas. These findings indicate that the effect of different tumors on a given enzyme in the host system need not be qualitatively uniform. Finally, unlike the other hepatomas tested, Morris 5123 hepatoma was found to possess xanthine oxidase activity approaching that found in a normal liver.

That the growth of a malignant tumor causes many disturbances in the metabolism of the host is well known. It can alter the concentrations of the substrates of an enzyme in the tissue, change the amount or the activity of the enzyme, and/or affect the end-product excretion in the urine. All these effects can be demonstrated when the degradation of purines and pyrimidines is studied in tumor-bearing animals, and these observations are presented in this report. Liver xanthine oxidase activity in tumor-bearing animals has been reported to be unaffected by tumor growth (5, 18). However, because of the changes observed in the tissue nucleic acid concentration and in the urinary excretion of uric acid and allantoin in rats bearing Walker carcinoma 256, it was considered desirable to re-examine liver xanthine oxidase activity in rats bearing this and other tumors. Of late, the enzymatic steps involved in the degradation of thymine and uracil in the rat have been elucidated (6, 13, 14, 20, 31), and the initial reduction of uracil to dihydouracil has been shown to be the rate-limiting step in the over-all degradation of uracil (6, 14). However, liver uracil reductase activity has not been studied in detail in tumor-bearing rats. A survey of this enzyme activity in the liver of these rats has hence been made and is also reported here. These results will provide a comparative study of xanthine oxidase and uracil reductase activities in the liver of rats bearing tumors of different origins in order to determine whether the effect of these tumors on the two enzymes is qualitatively uniform. In addition, it is of interest to note the difference in xanthine oxidase activity among the hepatomas used in this study.

MATERIALS AND METHODS

Animals.—Male Sprague-Dawley rats weighing 200–300 gm. were used to receive Walker carcinoma 256, Jensen sarcoma, Guerin epithelioma, and Novikoff hepatoma. Female Buffalo rats weighing 150–250 gm. were used to carry Morris hepatoma 5123 (Sublines B and D, 25th generation). Male A × C rats weighing 200–250 gm. were used to inoculate Morris 3683 and Morris 3924-A hepatomas. The Walker 256, Jensen, and Guerin tumors were obtained originally from the Upjohn Company, Kalamazoo, Michigan, and were maintained in this laboratory. The rats bearing the four hepatomas and the normal Buffalo and A × C strain rats were obtained through the courtesy of
Dr. Harold P. Morris of the National Cancer Institute. When these animals were received, they were put on a semisynthetic diet for a period of from 1 to 4 weeks, depending on the growth rates of the tumors. When possible, the tumors were allowed to grow to a size corresponding to 20–40 per cent of total body weight before the animals were sacrificed. The animals bearing the other tumors were also kept on the same diet. The composition of the diet was essentially the same as the one described previously (33) with the exception of replacing cod liver oil with vitamins A and D and of including vitamin B12.

Only in those experiments in which tissue and urinary constituents were determined were the control animals pair-fed to the animals bearing Walker carcinoma 256. In all other instances, the animals were fed the diet ad libitum. The procedure for urine collection and for estimation of tumor weight in vivo was the same as that previously described (33).

Analytical methods. -- Urinary allantoin was determined by the method of Young and Conway (34). Allantoin in the plasma was determined by the same method after the plasma was deproteinized with 2.5 times its volume of 5 per cent ZnSO4·7H2O and of 0.3 N Ba(OH)2. When allantoin was determined in the liver, a ZnSO4·Ba(OH)2 protein-free filtrate of the liver homogenate was prepared. After hydrolysis of allantoin in the filtrate with 0.5 N NaOH, it was found necessary to treat the alkaline hydrolysate with Norit to remove the pigment formed, which would otherwise interfere with the subsequent color development. Urinary uric acid was determined by the method of Dubbs et al. (11). Total nucleic acid as well as RNA and DNA was extracted and determined according to the method of Schneider (27). Lyophilized liver and muscle, prepared as before (38), were used for the determination of allantoin and nucleic acids.

Xanthine oxidase was assayed according to the method of Litwack et al. (23). Samples taken at 60- and 90-min. incubation times were analyzed, and the results were averaged. When it was not possible to run the assay on the same day, the liver was frozen immediately at −30° C. No appreciable loss of the enzyme activity was observed in 2 weeks at this temperature. Uracil reductase was assayed by a combination of the methods of Fritzson (15) and of Canellakis (6). The first method permits the use of liver homogenate rather than the soluble fraction prepared therefrom. This is a great advantage. The second method measures the C14O2 formation from uracil 2-C14 by a difference in C14 countings before and after the enzymic reaction. This procedure is more convenient and possibly more accurate than the one with uracil 6-C14 and isolating labeled β-alanine by chromatography for counting. The method actually employed in assaying uracil reductase activity in liver was as follows: Because of the instability of this enzyme (19), its activity was always assayed within a few hours after the animals were sacrificed. The liver homogenate was prepared in a 0.08 M phosphate buffer of pH 7.4 to contain 200 mg liver/ml homogenate. To the side-arm of a 25-ml. Warburg flask were introduced 2 μmole of triphosphopyridine nucleotide, 20 μmole of glucose-6-phosphate and 0.2 unit (21) of glucose-6-phosphate dehydrogenase dissolved in the phosphate buffer, each being contained in 0.2 ml. To the main chamber of the flask were added 100 μmole of nicotinamide, 5 μmole of adenosine triphosphate, 50 μmole of NaF, and 0.5 μmole of uracil-2-C14 containing 5 μc. of radioactivity, each being dissolved in 0.2 ml also. One ml. of the homogenate was finally added to the main chamber. The flask was incubated at 37° C. and flushed with N2 for 10 min. At the end of this period the system was closed, and the reduced-triphosphopyridine-nucleotide-generating system in the side-arm was tipped in. The reaction was allowed to proceed for 10 min. and was terminated with the addition of 0.2 ml. of 65 per cent TCA. A control flask was prepared in the same manner with the exception that TCA was added before the homogenate. The radioactivity in 0.1 ml. of the TCA filtrate was determined in an aluminum planchet with a Nuclear-Chicago gas-flow counter. The difference between the control and the reaction samples was due to the loss of C14O2, which was converted to μmole of uracil reduced in the final expression.

Materials.—All the chemicals used in this study were commercial preparations.

RESULTS

The daily urinary excretion of both allantoin and uric acid was determined in the rats bearing Walker carcinoma 256 and in the pair-fed controls during the entire period of tumor growth. The results are presented in Charts 1 and 2. The division of tumor-bearing rats into groups with increasing sizes of tumor is arbitrary and merely serves to correlate tumor growth with biochemical alterations. It is evident from the data that there was an increase in the excretion of both allantoin and uric acid in the tumor-bearing rats and that the increase was progressive with tumor growth. Furthermore, the control animals, which had various
degrees of restriction on food intake, excreted very constant amounts of the two compounds throughout the entire experimental period, indicating that, under the conditions of pair-feeding, dietary restriction had not been severe enough to cause a derangement in the over-all degradation of the purines.

The increased urinary excretion of allantoin by the tumor-bearing rats appeared to be a result of the increase in plasma allantoin concentration (Chart 3) which was paralleled by a similar increase in the liver (Table 1). In the plasma the increase also depended on the stages of tumor growth as it did in the urine.

The increase in liver nucleic acid of tumor-bearing animals observed in this study is consistent with the results of Cerecedo et al. (8), who showed an increase in both liver RNA and DNA of rats bearing Walker carcinoma 256. In addition, it is shown in Table 1 also that there was an increase in both muscle RNA and DNA of these tumor-bearing animals. The summation of the values for RNA and DNA agrees well with the value for total nucleic acid determined separately. Further-
more, despite the increase in the RNA and DNA content, the ratio of RNA/DNA in the muscle of tumor-bearing animals was decreased. It may be pointed out that the values are expressed on a dry weight basis. Although animals bearing different sizes of tumor (tumor weight from less than 10 per cent to more than 40 per cent of total body weight) were used, no clear-cut difference in either liver or muscle nucleic acid was observed among them, and the results were averaged as a single group.

The results on urinary and tissue allantoin suggest that there might be an increase in xanthine oxidase activity in the animals bearing Walker carcinoma 256, since uricase is not rate-limiting in the catabolism of xanthine to allantoin (3) and no change in uricase activity was observed in rats bearing this tumor (22). Table 2 shows the effect of Walker 256, Jensen, and Guerin tumors on the xanthine oxidase and uracil reductase activities of the host liver. There was an increase in xanthine oxidase activity in rats bearing Walker carcinoma 256. A similar increase was also observed in rats bearing Guerin epithelioma. On the other hand, there was no change in the activity of this enzyme in the liver of rats bearing Jensen sarcoma. The difference in the effect of the three tumors cannot be explained except to point out that Jensen is the fastest growing tumor of the three. It is of interest that uracil reductase was not altered significantly in the rats bearing Walker 256, indicating a lack of parallelism in the effect of the tumor on the enzymes catabolizing xanthine and uracil.

Recently, Morris 5123 hepatoma has created considerable interest because of its resemblance to...
normal liver in several enzyme patterns (18, 25, 26, 32). A comparison of this and other hepatomas concerning their effect on the catabolizing enzymes studied is made in Table 3. The values for the Sprague-Dawley controls are the same as shown in Table 2 and are transposed here for convenience of comparison. It is of interest that the control values for different strains of rats were relatively different. The A × C strain had higher xanthine oxidase activity and uracil reductase activity than that of either the Buffalo or the Sprague-Dawley strain, which also happened to have the lowest uracil reductase activity. Since the activity in both sexes of each strain was not determined it is not known whether the activity was dependent on the sex too. Fritzson (15) reported an average value of 0.75 μmole uracil reduced per gm. liver per 10 min. from six female rats of an unspecified strain. Although it is not possible to make a direct comparison of his value with any control value obtained in this study, they appear to be in general agreement. It can be seen that xanthine oxidase activity of the host liver was increased in rats bearing Novikoff hepatoma and Morris 3683 hepatoma when compared with that of their respective controls. No increase was observed in the liver of rats bearing Morris 5123 and Morris 3924-A hepatomas. On the contrary, the uracil reductase activity showed a decrease in the liver of rats bearing Morris 5123 and Morris 3924-A hepatomas and no change in the others. From these results it is obvious that tumor growth need not bring about a change in either xanthine oxidase activity or uracil reductase activity in the host liver. When a change was brought about, an increase in xanthine oxidase or a decrease in uracil reductase activity could be demonstrated. Concurrent changes in these two enzyme activities did not occur in the liver of tumor-bearing animals. In general, it may be stated that the effect of different tumors on the host system need not be uniform qualitatively. Such uniformity has been conceived, however, in

### TABLE 2

**XANTHINE OXIDASE AND URACIL REDUCTASE ACTIVITY IN THE LIVER OF NORMAL AND TUMOR-BEARING RATS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Xanthine oxidase*</th>
<th>Uracil reductase†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.9 ± 1.3 (7)</td>
<td>0.81 ± 0.17 (8)</td>
</tr>
<tr>
<td>Walker-bearing</td>
<td>12.7 ± 1.5 (9)</td>
<td>0.74 ± 0.16 (5)</td>
</tr>
<tr>
<td>Jensen-bearing</td>
<td>9.6 ± 2.5 (10)</td>
<td></td>
</tr>
<tr>
<td>Guerin-bearing</td>
<td>15.5 ± 2.1 (9)</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as μmoles xanthine oxidized/gm liver/hour.
† Expressed as μmoles uracil reduced/gm liver/10 minutes.
‡ The value after the ± sign shows the standard deviation from the mean.
§ The number in the parentheses indicates the number of animals used.
¶ The tumors ranged in size from 5 to 22 per cent of total body weight.
‖ The tumors ranged in size from 3 to 11 per cent of total body weight.
** The tumors ranged in size from 5 to 18 per cent of total body weight.
†† The tumors ranged in size from 18 to 30 per cent of total body weight.

### TABLE 3

**XANTHINE OXIDASE AND URACIL REDUCTASE ACTIVITY IN THE LIVER OF NORMAL AND HEPATOMA-BEARING RATS AND IN THE HEPATOMAS**

<table>
<thead>
<tr>
<th>Strain of rats</th>
<th>Group</th>
<th>Xanthine oxidase activity*</th>
<th>Uracil reductase activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Tumor</td>
</tr>
<tr>
<td>Sprague-Dawley, ♀</td>
<td>Control</td>
<td>8.9 ± 1.3 (7)</td>
<td>0.81 ± 0.17 (8)</td>
</tr>
<tr>
<td></td>
<td>Novikoff-bearing</td>
<td>12.9 ± 1.6 (6)</td>
<td>0.74 ± 0.16 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7 ± 0.6 (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buffalo, ♀</td>
<td>8.7 ± 1.1 (4)</td>
<td>1.16 ± 0.08 (4)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.8 ± 0.8 (5)</td>
<td>1.31 ± 0.10 (6)</td>
</tr>
<tr>
<td></td>
<td>Morris 5123-bearing</td>
<td>13.9 ± 1.0 (6)</td>
<td>1.17 ± 0.18 (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 ± 0.3 (5)</td>
<td>1.00 ± 0.14 (6)</td>
</tr>
<tr>
<td></td>
<td>Morris 3683-bearing**</td>
<td>16.0 ± 1.5 (8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.8 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morris 3924-A-bearing††</td>
<td>13.9 ± 1.9 (6)</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as μmoles xanthine oxidized/gm tissue/hour.
† Expressed as μmoles uracil reduced/gm liver/10 minutes.
‡ The value after the ± sign shows the standard deviation from the mean.
§ The number in the parentheses indicates the number of animals used.
¶ The tumors ranged in size from 5 to 22 per cent of total body weight.
‖ The tumors ranged in size from 3 to 11 per cent of total body weight.
** The tumors ranged in size from 5 to 18 per cent of total body weight.
†† The tumors ranged in size from 18 to 30 per cent of total body weight.
tumor-bearing rats with respect to liver catalase activity (17).

When the xanthine oxidase activity in the four hepatomas was examined, only Morris 5123 hepatoma showed an activity approaching that of a normal liver (Table 3). In the other three hepatomas, the activity was barely measurable. Actually in some of these determinations the activity was nearly zero. Uracil reductase activity has been reported to be present in Morris 5123 hepatoma and undetectable in Novikoff hepatoma (25).

**DISCUSSION**

The increase in the urinary excretion of allantoin by rats bearing Walker carcinoma 256 observed here has been observed also by Burton and Begg (5), although they did not correlate the increase to tumor size. The increase they reported was more than twice the highest recorded in this study on a comparable 25 per cent protein diet. Young and Dinning (35) showed that vitamin E-deficient rabbits had a much greater urinary excretion of allantoin than did their controls. It is conceivable that there are similarities in these two clinical conditions—malignancy and vitamin E deficiency—insofar as the loss of muscle mass and the degradation of purines are concerned. For, in addition to the similarity in increased allantoin excretion, there was an increase in both RNA and DNA content and a decrease in the ratio of RNA/DNA in the muscle tissue in vitamin E deficiency (35) as in tumor growth observed here. Perhaps both the increase in the content and the decrease in the ratio should be considered as results of an extensive and continuous muscle loss. It is reasonable to assume that, during the loss of muscle mass, the nucleic acids were degraded at a much slower rate than were other constituents of muscle, thereby resulting in an increase in the nucleic acid content. At the same time the muscle tissue should lose its cytoplasmic RNA faster than its nuclear DNA to account for a decrease in the RNA/DNA ratio. Zigman and Allison (36) have reported an increase in ribonuclease activity in tissues of tumor-bearing rats. The increase in the allantoin excretion by the rats with the most advanced tumor was about 300 moles/day. This amount can be contributed to the end product of purine catabolism. Second, an increased rate of turnover of nucleic acids in the tumor-bearing animals as reported by many workers (1, 10, 24) can also result in an increased formation of the end product.

The situation in the liver could be quite different from that in the muscle, for the liver mass was increased slightly during tumor growth. Burton and Begg (5) showed that the liver xanthine oxidase activity in tumor-bearing rats was the same as that in the control animals on a high protein diet. In the present study the animals were fed a 25 per cent casein diet ad libitum. Although the control value agrees well with that determined by these authors (5), the activity found in the host liver was not merely maintained at the normal level but was actually increased above the normal level. At least this is true in rats bearing Walker 256, Guerin, Novikoff, and Morris 3883 tumors. The increase in xanthine oxidase activity cannot readily explain the high urinary excretion of allantoin and uric acid, for an extremely small activity in vivo was sufficient to maintain normal urinary excretion (4). The mechanism of the increase in the enzyme activity in the host liver is made more obscure by the fact that in rats bearing certain types of tumor, such an increase in the liver was not observed. Of interest is the finding that only Morris 5123 hepatoma possessed xanthine oxidase activity comparable to that found in liver, whereas the other three hepatomas tested had no significant activity. This information is consistent with the evidence hitherto accumulated (25, 26, 32), which indicates that Morris 5123 hepatoma is more like normal liver tissue than is any other hepatoma.

The enzyme reducing uracil has been referred to as dihydrouracil dehydrogenase in the literature (15, 19). The name "uracil reductase" appears to be preferable, because it is short and, more importantly, it indicates correctly the direction of the enzymatic reaction studied. The reverse reaction to which the name "dihydrothymine dehydrogenase" can be aptly applied occurs to only an insignificant extent (15). A similar preference of "thymine reductase" to "dihydrothymine dehydrogenase" for the enzyme reducing thymine has been given (26).

That there is an inverse relationship between the capacity of a tissue to degrade uracil and its capacity to incorporate uracil into DNA has been proposed by Canellakis (7) and supported by others (16, 28, 29). The liver of a tumor-bearing rat appears to be under the influence of two opposite effects—that of the host system which is predominantly catabolic and that of the tumor itself which is primarily anabolic. It has been demonstrated
that there were an increased mitotic rate (2) and an increased incorporation of nucleic acid precursors into nucleic acids (1, 9, 10, 24, 30) in livers of tumor-bearing rats. These results indicate that the host liver was responding metabolically in an anabolic fashion despite the general catabolic effect noted in other tissues. In line with this thinking is the demonstration that the uracil reductase activity of the host liver was either lower than or the same as that found in the normal liver. A higher activity of the host liver has not been observed.

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