Tryptophan Metabolites in the Urine of Turkish Cows with Urinary Bladder Cancer*

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Industrial urinary bladder cancer in dye workers has been shown to be caused by exposure to certain aromatic amines (cf. 21 for review). Animal experiments have indicated that it is likely that the carcinogens are o-aminophenol metabolites (4, 5) and that these metabolites probably are carried to the urinary bladder by the urine (26, 41). Unidentified diazotizable aromatic amines in the urine of patients with spontaneous bladder cancer may also be responsible for this neoplastic disease, as suggested by Ekman and Strömbeck (19).

The known exogenous human bladder carcinogens (21) are chemically similar to several aromatic amines derived from tryptophan (14). The possible implication of tryptophan in bladder carcinogenesis was suggested by the findings of Dunning, Curtis, and Maun (18) and Dunning and Curtis (17), who showed that a high incidence of bladder tumors resulted when rats were fed 2-acetylaminofluorene combined with tryptophan or indole, while few bladder tumors were obtained with 2-acetylaminofluorene alone. Recently the mechanism of the carcinogenic activity of 2-acetylaminofluorene on the bladder was suggested to be the increased urinary excretion of 3-hydroxykynurenine and 3-hydroxyanthranilic acid (39). This suggestion was based on the observation that feeding 2-acetylaminofluorene to rats for 2–6 days decreased the extent to which the livers of these animals converted 3-hydroxyanthranilic acid to quinolinic acid. Presumably 3-hydroxyanthranilic acid and 3-hydroxykynurenine would accumulate under these conditions, but no evidence was presented to show that these compounds do accumulate in rats fed 2-acetylaminofluorene (39).

Recently the urinary excretion of tryptophan metabolites by patients with and without neoplasms of the urinary bladder has been investigated (7, 10, 11). In one study (10) abnormally large quantities of kynurenine, hydroxykynurenine, acetylkynurenine, and kynurenic acid were excreted in the urine of about one half of a group of 41 patients with bladder cancer, especially after the administration of supplements of L-tryptophan, while the remaining patients excreted normal quantities of these and other tryptophan metabolites before or after the ingestion of tryptophan supplements. In another study (7) it was reported that ten patients with bladder cancer excreted abnormally large quantities of anthranilic acid, hydroxyanthranilic acid, and hydroxykynurenine in the urine before and after the administration of 10 gm. of DL-tryptophan. Recently, a number of tryptophan metabolites have been tested for carcinogenicity by implantation in the bladder of mice (1, 6). 3-Hydroxykynurenine, 3-hydroxyanthranilic acid, the 8-methyl ether of xanthurenic acid, and 2-amino-3-hydroxyacetophenone were found to cause a significant number of tumors in mice (1).

In the light of these findings, it was of interest to study the urinary excretion of tryptophan metabolites by cows with bladder tumors as compared with normal cows. Neoplasms of the urinary bladder are very common in this species of animal and in water buffalo in certain districts of Turkey (30–32) and Formosa (20).

This paper presents the results of quantitative measurement of tryptophan metabolites in the urine of Turkish cows with bladder tumors as compared with normal cattle from the same area.

MATERIALS AND METHODS

It has been shown by one of the authors (30–32) that a large number of cattle and water buffalo in certain provinces of Turkey such as Samsun, Bolu, Ordu, Giresun, and Trabzon develop urinary bladder tumors of unknown etiology. The urine samples from these animals were collected under toluene. They were acidified to pH 3 with HCl to

* Supported in part by grants from the American Cancer Society; from the Elsa U. Pardee Foundation; from the Wisconsin Division of the American Cancer Society; and from the National Institute of Arthritis and Metabolic Diseases (Research Grant No. A-1469), Public Health Service.
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Received for publication November 25, 1958.
prevent the destruction of certain readily oxidizable tryptophan metabolites in alkaline bovine urine and to prevent the transmission to this country of any active viral agent with the urine. The urine samples were kept frozen before they were shipped to our laboratories by air mail express. Twenty-five urine samples from Turkish cows with urinary bladder tumors and fifteen samples from healthy Turkish cows were analyzed for a number of tryptophan metabolites and creatinine. The cattle were selected for these studies by veterinarians with considerable familiarity with clinical aspects of this disease as it occurs in Turkey. Thirteen of the 25 animals selected on a clinical basis for bladder cancer were autopsied following the urine collection, and a positive anatomical diagnosis was obtained in every case. Ten of the fifteen control animals were also autopsied following the urine collections and were found to be free of bladder tumors.

For purposes of comparison fresh urine samples were collected under toluene from a number of normal dairy cows of the University of Wisconsin Dairy Farm and analyzed promptly.

Kynurenine, N*-acetylkynurenine, o-aminohippuric acid, and anthranilic acid were measured by the method of Brown and Price (9). Hydroxykynurenine was estimated by the method of Brown (8). Kynurenic acid and xanthurenic acid were determined by the fluorometric method of Satoh and Price (40). Paper chromatograms were run on the aromatic amine fraction as qualitative checks on the quantitative procedure as described in a previous paper (33). Creatinine was determined by the method of Peters (35). Since it was not practical to collect 24-hour urine samples from cows, the metabolite content of the urine was expressed as milligrams per gram of creatinine. All data were subjected to statistical "t" test analysis (27), and probability (P) values were obtained.

RESULTS

Table 1 gives the average levels, range, and P values of the urinary excretion of various tryptophan metabolites and creatinine for the control and the diseased cows. It was found that the levels of metabolites varied greatly from one animal to another, especially in the diseased cows. The average values for the two groups of animals were very similar for most of the metabolites. Only acetylkynurenine was significantly elevated in the urine of tumor-bearing animals (P < 0.05). o-Aminohippuric acid excretion in the urine of the controls was significantly higher (P < 0.02) than in the urine from the cows with tumors. It was found that the duration of the disease, varying from 2 months to 6 years, had no effect upon the level of urinary excretion of these metabolites. The results presented in Table 1 indicate that normal American dairy cows excreted more of most of the metabolites than either group of Turkish cows.

TABLE 1

<table>
<thead>
<tr>
<th>Tryptophan metabolite</th>
<th>Control animals</th>
<th>Tumor-bearing animals</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-Aminohippuric acid</td>
<td>8.65 (3.20–25.99) t</td>
<td>5.40 (1.90–12.74) t</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>8.37 (0.53–22.00)</td>
<td>8.42 (0.54–43.84)</td>
<td>N.S. t</td>
</tr>
<tr>
<td>Acetylkynurenine</td>
<td>3.94 (0.47–8.40)</td>
<td>6.49 (1.39–23.00)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>3.16 (0.75–8.50)</td>
<td>3.70 (1.18–14.74)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Hydroxykynurenine</td>
<td>7.47 (1.50–15.16)</td>
<td>9.88 (3.09–24.95)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>3.75 (0.87–11.84)</td>
<td>4.71 (0.85–25.89)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>3.94 (1.35–10.55)</td>
<td>2.90 (0.90–7.90)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Creatinine (gm/l)</td>
<td>0.92 (0.23–1.57)</td>
<td>0.81 (0.23–2.45)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

No. animals: 15 25

* P = probability that this difference would occur by chance.
† Mean values, with ranges given in parentheses.
‡ N.S. = not significant.
§ Only twelve animals' urine analyzed.
# Only thirteen animals' urine analyzed.

DISCUSSION

The present study of the urinary excretion of tryptophan metabolites by normal Turkish cows and cows having urinary bladder tumors indicated that the differences between these groups were not statistically significant except for an elevation of acetylkynurenine and a lower level of o-aminohippuric acid in the urine of animals bearing bladder tumors. The absence of significant differences between the two groups may be due to the
fact that both groups of cows were from the same area and were eating similar forage. If the animals were eating the same foods, both groups might be expected to have similar amounts of metabolites in the urine. Thus, factors other than urinary tryptophan metabolites may determine the high incidence of bladder tumors in the cattle in these areas. Studies probably should be done to compare the tryptophan metabolism of normal cows from areas of low bladder tumor incidence with normal cows from areas having a high incidence.

It has been found that normal dairy cows of the University of Wisconsin excreted many of these metabolites in higher concentrations (Table 2) than did either the diseased or normal Turkish cows (Table 1); yet bovine bladder tumors in Wisconsin are apparently very rare. The differences in levels of excretion between Wisconsin (23), and in scleroderma (acrosclerosis) (38). Tuberculous patients who were given the pyridoxine antagonists, isoniazid or deoxypyridoxine, or human subjects with no known disease excreted more hydroxykynurenine than kynurenine in the urine (36, 37). Most patients with tuberculosis of the skin who were treated with isoniazid, normal subjects who received isoniazid, and pregnant women excreted a large quantity of xanthurenic acid in the urine after tryptophan supplementation (32). DL-Tryptophan supplementation increased xanthurenic acid excretion in the urine of pregnant and nonpregnant swine (16). It has also been shown that the administration of tryptophan to the dog and rat resulted in the excretion of considerable quantities of anthranilic acid and anthranilic acid glucuronide (9). Riboflavin deficiency in rats also resulted in a derangement of metabolism of tryptophan which brought about an excessive formation and excretion of anthranilic acid and its conjugated derivatives (12, 25). It was claimed by some workers (2, 34) that anthranilic acid was excreted by patients with congenital hypoplastic anemia. In the light of these findings and those of the present study, it would appear that abnormal tryptophan metabolism is not specific for any disease condition. However, the possibility still exists that some of these metabolites of tryptophan may be acting synergistically with unknown factors in the induction of mammalian bladder tumors. The apparent rarity of urinary bladder tumors in the cat (3) may be partly explained by the extremely low level of tryptophan metabolites excreted in the urine by this species (9). Studies from these laboratories emphasized that the dog, rat, cow, and man excreted relatively large amounts of certain tryptophan metabolites in the urine (9, 8, 33). These species, unlike the cat, develop spontaneous bladder cancer.

### Table 2

<table>
<thead>
<tr>
<th>Tryptophan Metabolite</th>
<th>Guernsey</th>
<th>Guernsey</th>
<th>Breton of Cow</th>
<th>Holstein</th>
<th>Holstein</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-Aminophthallic acid</td>
<td>23.19</td>
<td>14.90</td>
<td>17.50</td>
<td>13.40</td>
<td>17.32</td>
<td>17.17</td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>3.38</td>
<td>7.78</td>
<td>8.32</td>
<td>2.86</td>
<td>1.15</td>
<td>4.57</td>
</tr>
<tr>
<td>Acetyl-kynurenine</td>
<td>7.35</td>
<td>1.05</td>
<td>2.91</td>
<td>5.36</td>
<td>4.71</td>
<td>4.27</td>
</tr>
<tr>
<td>Kynurenine*</td>
<td>9.70</td>
<td>7.50</td>
<td>19.50</td>
<td>9.80</td>
<td>9.30</td>
<td>11.16</td>
</tr>
<tr>
<td>Hydroxyl-kynurenine*</td>
<td>6.90</td>
<td>9.20</td>
<td>21.50</td>
<td>29.40</td>
<td>6.82</td>
<td>14.76</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>4.41</td>
<td>2.40</td>
<td>6.70</td>
<td>5.47</td>
<td>7.62</td>
<td>5.92</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>9.07</td>
<td>8.20</td>
<td>21.21</td>
<td>22.90</td>
<td>25.91</td>
<td>17.17</td>
</tr>
<tr>
<td>Creatinine (g/mL)</td>
<td>0.902</td>
<td>0.907</td>
<td>0.172</td>
<td>0.387</td>
<td>0.952</td>
<td>0.672</td>
</tr>
</tbody>
</table>

* These values for the excretion of kynurenine and 3-hydroxykynurenine by American dairy cows have been reported elsewhere (23).

and Turkish cows may be due in part to differences in the quality and quantity of food available to the animals.

Previous studies (7, 11) suggested that most bladder tumor patients excreted abnormal amounts of a number of tryptophan metabolites. Recently it was disclosed that only half of the bladder tumor patients had abnormal tryptophan metabolism as expressed by elevated urinary levels of kynurenine, hydroxykynurenine, acetyl-kynurenine, and kynurenic acid (10). Abnormal tryptophan metabolism is not specific for subjects with tumors of the urinary bladder. Similar metabolic abnormalities were found in a number of patients with other human disease conditions, including certain other types of cancer (10). It has been shown that rats on a low pyridoxine diet excreted 3-hydroxykynurenine as an important metabolite after the administration of tryptophan (13). 3-Hydroxykynurenine was excreted in large amounts by patients with severe tuberculosis (24), in a large proportion of patients with fevers of varying etiology (15), in leukemia (28, 29), in diabetes metabolism of tryptophan which brought about an excessive formation and excretion of anthranilic acid and its conjugated derivatives (12, 25). It was claimed by some workers (2, 34) that anthranilic acid was excreted by patients with congenital hypoplastic anemia. In the light of these findings and those of the present study, it would appear that abnormal tryptophan metabolism is not specific for any disease condition. However, the possibility still exists that some of these metabolites of tryptophan may be acting synergistically with unknown factors in the induction of mammalian bladder tumors. The apparent rarity of urinary bladder tumors in the cat (3) may be partly explained by the extremely low level of tryptophan metabolites excreted in the urine by this species (9). Studies from these laboratories emphasized that the dog, rat, cow, and man excreted relatively large amounts of certain tryptophan metabolites in the urine (9, 8, 33). These species, unlike the cat, develop spontaneous bladder cancer.
SUMMARY

1. The urinary excretion of tryptophan metabolites was measured in urine samples from 25 Turkish cows with urinary bladder tumors and in urine samples of fifteen healthy Turkish cows from the same localities.

2. The levels of most tryptophan metabolites in the urine of the tumor-bearing animals were not higher than in the urine of the healthy animals. The elevation of N-acetyl-kynurenine in the diseased bovine urine was statistically significant. The cows without tumors excreted significantly more o-aminoohippuric acid than did the animals with tumors.

3. The possible role of urinary tryptophan metabolites in bladder carcinogenesis was discussed.

ACKNOWLEDGMENTS

The assistance of Professor David A. Grant and Mr. Wesley J. Hanecke, Department of Psychology, University of Wisconsin, Madison, Wisconsin, in making arrangements to obtain fully acknowledged.

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REFERENCES


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