The Induction of Neoplasms of the Urinary Bladder of the Cow and the Small Intestine of the Rat by Feeding Bracken Fern (*Pteris aquilina*)

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SUMMARY

Cattle were fed a diet containing bracken fern in the continuation of a study of the urinary bladder neoplasms produced under these conditions. One of the cattle died of a traumatic pericarditis and had no bladder lesion at 485 days. The other six animals fed bracken fern for from 510 to 1920 days all had gross bladder neoplasms. Four of the six animals with gross bladder neoplasms had transitional-cell carcinomas as well as hemangiomas. The remaining two animals had gross papillomas and hemangiomias, up to 3 cm in diameter. The cattle with bladder tumors all had gross hematuria for months or years before death. Although no control animals were autopsied during the past 2 years, the controls at that time were without tumors. During the 7 years of the study no control animal had had even microhematuria. Thirteen of ninety rats fed bracken fern mixed with a grain diet have died after 8 months and each animal had multiple adenocarcinomas of the small intestine. Six of these thirteen animals also had gross tumors in the urinary bladder. Since the study is in progress, no further information about these bladder lesions is available at this time.

INTRODUCTION

In certain parts of the world urinary bladder tumors are common in the cattle, and these neoplasms are associated with a syndrome known as chronic enzootic hematuria (7–10). Until recently the etiology of these naturally occurring bovine urinary bladder tumors was obscure (11). Chronic hematuria has been induced in cattle by feeding bracken fern (*Pteris aquilina*) (10, 11, 13), and a fatal poisoning has resulted from feeding the fresh or dried plant (2, 10, 11, 13). Acute bracken poisoning in cattle has been studied in detail in recent years (2–6). The toxic substance has been extracted from the bracken plant with boiling ethanol (6). Continuous feeding of a low level of bracken in the ration for months or years will result in the production of polypoid bladder lesions (13) and in some instances tumors (10, 11). In an earlier report by the present investigators, ten of eighteen animals fed bracken fern developed neoplasms in the urinary bladder after from 276 to 1192 days (11). This latter study was designed to simulate the field conditions which exist in the disease area of Turkey. Three of these lesions were malignant and the study has been continued. It now appears that most cows fed bracken fern for 3 years or more have developed carcinomas. One animal, however, was negative after 1920 days except for papillomas. Carcinomas have occurred in male and female animals. The possibility of a carcinogen in bracken was strongly supported by the findings of Evans and Mason (1) that rats fed bracken will develop intestinal adenocarcinomas. Evans and Mason (1) also reported that in a survey by Parker and McCrea (12) for bracken poisoning in sheep on the North Yorkshire Moors it was revealed that a number of older animals from areas infested with bracken died of the same type of tumor as that produced in rats fed a bracken diet (cited by Evans and Mason (1)). Preliminary results to be reported here confirm the findings of Evans and Mason (1) that rats develop multiple intestinal adenocarcinomas when they are fed bracken. Some of the rats fed bracken in this study also have developed large lesions in the urinary bladder, and these lesions are under further study.

MATERIALS AND METHODS

The cattle were of native stock and were obtained from areas near Ankara where the disease has not been observed. They were housed near Bolu, Turkey, to provide ready access to either fresh or dried bracken fern (*Pteris aquilina*) from the farms where the incidence of urinary bladder tumors was high. The animals were fed a dose of 200 to 300 gm of dried bracken daily, because previous experience (11) indicated that this dose was most suitable for producing neoplasms in animals weighing from 100 to 150 kg. The level of toxicity was monitored by inspection of the urine for microscopic hematuria, by platelet counts, and by total and differential leukocyte counts. Animals which became moribund were kept under constant observation in order that complete autopsies could be carried out before there was autolysis of the tissues. The urinary bladders were distended postmortem with 10%
formalin solution injected into the urethra. After fixation specimens of the bladder including multiple specimens of the tumors were systematically selected for histologic study. These tumors will be described in detail elsewhere.

The animals received a basic diet of timothy and alfalfa hay and a standard dairy feed supplement (1% salt, 27% corn, 27% bran, 27% oats, and 18% linseed oil meal). The chopped bracken was mixed with the diet and given to each test animal once a day. Otherwise, the experiment is a continuation of the study (11) which was started June 1, 1961.

The rats were albinos of both sexes and bred from local stock. They usually were started on experiment at the age of 49 days. They were fed pellets formulated from a grain mixture or the grain mixture containing 1/3 dried bracken fern by weight. The pellets containing the bracken were dark green in color and had the appearance of laboratory chows in common use in the U.S. There were 4 groups of animals as follows: Group 1, 90 rats fed bracken and given thiamine supplements; Group 2, 19 rats fed bracken; Group 3, 9 rats fed bracken (started at 30 days of age); and Group 4, 22 rats fed the control diet. The thiamine supplements were administered as required.

RESULTS

The animals which have died since the previous report (11) were all test animals and none of the controls has developed hematuria or any other clinical sign of neoplasia. The survival time of these seven cattle, the amount of bracken consumed per animal, and the first appearance of gross hematuria after the start of feeding are given in Table 1.

Bracken poisoning was associated with hematologic changes in these seven animals. The main feature was a progressive diminution in the number of white cells and platelets in the peripheral blood (Table 2). The change reached maximum proportions a few days before the death of the animal. As in the previous study there was a decrease primarily in the number of polymorphonuclear leukocytes.

Six of the seven test animals which have died in the past 2 years have had gross hematuria and the one which did not (No. 34) died from traumatic pericarditis. A piece of wire penetrated the wall of the reticulum and produced chronic fibrinous pericarditis with abscess formation. The animal had microhematuria starting 14 months after initiation of bracken feeding, but the bladder was essentially normal at the time of death.

The primary feature of bracken poisoning was multiple hemorrhages throughout the carcass, but the degree and location varied. Hemorrhages were observed in the subcutaneous tissue of the forearms (Cow 19), in the alimentary mucosa (Nos. 10, 19, 20, 22, 32, 951), and in the heart (Nos. 19, 20, 951). Free blood was found in the large intestine of some animals (Nos. 10, 19, 22, 951). In every bovine fed bracken, there were multiple submucosal hemorrhages extending throughout the urinary tract from the calyces of the kidneys to the urethra. The degree of hemorrhage varied from pinpoint lesions to confluent hemorrhages showing almost complete infiltration of the submucosa.

Tumors were observed macroscopically in six animals (Nos. 10, 19, 20, 22, 32, 951). The macroscopic appearance of these cases is described below.

Case 10. Hemangiomas, sessile nodules, and punctate hemorrhagic foci were distributed throughout the bladder. The wall of the bladder was moderately thickened. Histologic examination revealed that hemangiomas were the capillary type. The sessile nodules were histologically diagnosed as transitional-cell carcinoma. The tumors were contiguous and infiltrated the submucosa. No metastasis was detected.

Case 19. Five papillomas, hemangiomas, and hemorrhagic areas were situated at various sites in the mucosa of the urinary bladder. The vascular tumors were presented as a dark red, sharply defined patch as large as 1 by 3 cm in size. Blood could be expressed from the cut surface of hemangiomas. The papillomas were attached to the mucosa of the bladder by a short stalk and were cauliflower-like in shape. The bladder mucosa was thickened in some areas. The tumors were histologically diagnosed as papilloma, capillary hemangioma, and transitional-cell carcinoma infiltrating into the submucosa.

Case 20. Lesions in the bladder consisted of vascular growths, multiple sessile nodules, and papillomas distributed throughout the organ. The bladder was greatly thickened. The vascular growths varied in size and were firm in consistency (Fig. 1). Histologic examination revealed that these tumors were capillary hemangiomas. Papillomas were of the transitional-cell type. The sessile nodules consisted of transitional-cell carcinoma infiltrating into the submucosa.

Case 22. The bladder contained bloody urine. The wall of the organ was greatly thickened. A large papilloma was found in the fundus region (Fig. 2) of the bladder. The tumor was 2.5 cm in diameter and was attached to the mucosa by a short, broad neck. There were also hemangiomas varying in size and in random distribution. Some of these tumors infiltrated into the muscular coat and reached the subserosa.

Histologic examination of the tumors revealed that the tumor 3 cm in diameter was a papilloma and the hemangiomas were capillary types.

Case 32. The inside of the bladder was filled with hundreds of flattened, string-like cords of neoplastic tissue (Fig. 3) many of them about 2-3 cm long. At the beginning of the urethra there was a tumor about 1.5 cm in size, which caused retention of urine in the bladder.

The tumors were histologically diagnosed as papilloma, mucous adenocarcinoma, hemangioendothelioma, and transitional-cell carcinoma.

Case 951. Two papillomas were firmly attached to the bladder mucosa. They were cauliflower in shape. Extensive hemorrhage was present in the bladder wall. These lesions were transitional-cell papillomas.

The survival of the rats fed bracken fern was good. To date, thirteen rats from Group 1 have died 8 months after the start of the study and all of these had multiple adenocarcinomas of the small intestine (Fig. 4). Three animals died from Group 2, and one died from Group 4. None of these had gross tumors. Of the thirteen rats which died with intestinal adenocarcinomas, six had gross tumors in the urinary bladder. These lesions are under further study.
Table 1

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Survival time (days)</th>
<th>Bracken fern consumed (kg/animal)</th>
<th>First gross hematuria (days)</th>
<th>Histologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Fresh</td>
<td>Dried</td>
</tr>
<tr>
<td>10</td>
<td>1155</td>
<td>81</td>
<td>270</td>
<td>371</td>
</tr>
<tr>
<td>19</td>
<td>1572</td>
<td>104</td>
<td>324</td>
<td>402</td>
</tr>
<tr>
<td>20</td>
<td>1225</td>
<td>81</td>
<td>285</td>
<td>545</td>
</tr>
<tr>
<td>22</td>
<td>1110</td>
<td>54</td>
<td>225</td>
<td>335</td>
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<td>32</td>
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<td>50</td>
<td>143</td>
<td>420</td>
</tr>
<tr>
<td>34a</td>
<td>485</td>
<td>23</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>951</td>
<td>1920</td>
<td>144</td>
<td>542</td>
<td>60</td>
</tr>
</tbody>
</table>

Survival time, amount of bracken fern fed, date of gross hematuria, and the histologic diagnoses of bladder lesions in the cattle.

* Died of traumatic pericarditis.

Table 2

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Cell counts/cu mm</th>
<th>Differential leukocyte counts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC x 10^-3</td>
<td>Platelets x 10^5</td>
</tr>
<tr>
<td>10</td>
<td>4.5</td>
<td>3.24</td>
</tr>
<tr>
<td>19</td>
<td>3.2</td>
<td>3.8</td>
</tr>
<tr>
<td>20</td>
<td>6.5</td>
<td>3.2</td>
</tr>
<tr>
<td>22</td>
<td>8.3</td>
<td>2.4</td>
</tr>
<tr>
<td>32</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>34</td>
<td>8.3</td>
<td>2.3</td>
</tr>
<tr>
<td>951</td>
<td>9.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Leukocyte and platelet counts and differential leukocyte counts of the animals at a late stage of the toxic reaction to bracken feeding.

DISCUSSION

These results are an extension of the studies reported previously (11). Since the previous report, the technic of feeding the animals has improved; this accounts for the lower incidence of early mortality and the less severe bone marrow depression. Furthermore, only one animal developed an early gross hematuria and that was an animal which had been on experiment over 5 years (Animal 951) and died with the diagnosis of papilloma. In the earlier studies, gross hematuria was often the result of feeding too high a dose of the plant.

Six of the seven cattle to die since the previous publication had gross urinary bladder lesions and four of these were carcinomas. The one animal that died without any neoplastic change in the urinary bladder died of a traumatic pericarditis. This animal was ill for several weeks before death and accordingly, did not eat well. This illness was initially thought to be related to toxicity of the plant and the animal was not fed the bracken regularly.

The histologic appearance of the lesions developed by these animals was that expected in animals which develop carcinoma of the bladder under field conditions.

Although no controls were killed at this time, the four controls which have survived from the previous report are still without hematuria or other clinical signs of disease. In the 7 years this study has been in progress there has been no hematuria in the animals which were not fed the bracken fern.

The occurrence of adenocarcinoma in the small intestine of the rat confirms the finding of Evans and Mason (1). It was not expected, however, to find gross tumors in the urinary bladder. This finding is such a recent development that the histology of these lesions in the urinary bladder, of six of thirteen animals dying of adenocarcinoma of the small intestine, suggests that a high incidence of the bladder lesion will be obtained.

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REFERENCES

Neoplasms Induced by Bracken Fern

Fig. 1. Urinary bladder from Animal 20 showing the multiple sessile nodules, hemangiomas, and a few papillomas. Sections of these tumors included papilloma, capillary hemangioma, and transitional-cell carcinoma.

Fig. 2. Urinary bladder from Animal 22 showing a large papilloma and several capillary hemangiomas.

Fig. 3. Urinary bladder from Animal 32 showing the large number of cords of neoplastic tissue. Sections of this tumor included mucous adenocarcinoma and transitional-cell carcinoma.

Fig. 4. Ileum of a rat which had been fed bracken in the diet for 7.5 months. These lesions were adenocarcinomas.

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