Induction of Intestinal and Urinary Bladder Cancer in Rats by Feeding Bracken Fern (*Pteris aquilina*)[1]

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SUMMARY—Simultaneous intestinal and urinary bladder tumors were induced in rats by feeding them bracken fern (*Pteris aquilina*). Adenomatous polyps and adenocarcinomas developed predominantly in the ileum. These neoplasms were homogeneous and were sharply demarcated from adjacent normal mucosa. Urinary bladder tumors consisted of papillomas and carcinomas, and a high degree of malignancy followed the bracken feeding.—
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PAMUKCU (*1*) has reviewed epidemiologic studies relating chronic bovine hematuria to carcinoma of the urinary bladder. This work, in addition to that of Rosenberger and Heeschen (*2*) and Georgiev and Antonov (*3*), suggested that bracken fern might be a causal factor in the etiology of bovine bladder cancer in several regions of the world. More recently, a high incidence of chronic hematuria and urinary bladder carcinomas was induced in Turkish cattle fed bracken fern (*Pteris aquilina*) obtained from the disease areas (*4*, *5*). Although other factors cannot be excluded in the etiology of bovine bladder cancer, cattle fed bracken fern developed a condition indistinguishable from the syndrome so prevalent on farms in the mountains along the southern shore of the Black Sea.

Acute bracken fern poisoning in cattle has been known for many years. It is thought to be due to the cumulative effect of a toxic substance from the fern, since the animal must ingest the plant frequently for at least a few weeks. Typical symptoms include hemorrhages from the mucous membrane of the nose, conjunctiva, and vulva and clots of blood in the feces. An increased mucous discharge and pyrexia (107–109°F) are invariably observed during the acute stage (*6*).

Many workers have reproduced acute bovine bracken poisoning either by feeding the fresh fronds, sun-dried bracken, or powdered rhizomes mixed with an otherwise adequate diet (*1*, *7*), or by administering a fraction extracted from the bracken plant with boiling ethanol (*6*). A high level of bracken in the diet causes this hemorrhagic syndrome in cattle but does not cause neoplastic changes in the urinary bladder.

Recently, Evans and Mason (*8*) and Price and Pamukcu (*5*) induced intestinal adenocarcinoma in rats by feeding them bracken. Biologic tests (*3*, *9–11*), however, demonstrated that urine from cattle in regions where hematuria was

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prevalent, from cows fed hay from comparable districts of Bulgaria, or from cattle fed bracken fern contained some carcinogenic agent which induced tumors in the bladders of the calf, dog, rat, or mouse and tumors in the skin of mice.

Bracken fern has been accepted as a palatable human food in many countries (*12*); it is consumed as a salad green and as an asparagus-like vegetable when picked in the fiddlehead or crosier stage. Fernald and Kinsey (*13*) point out that pasture brake (*P. aquilina*) is used as human food, both cooked and uncooked, in some regions of New Zealand and Japan. However, only the young, green shoots are eaten by man, whereas the mature plant develops toxic principles. The toxic material includes a heat-stable and a heat-labile thiaminase which increases in concentration as the bracken crosiers begin to unfold, and then decreases through subsequent maturation stages of the bracken, including drying and weathering. The heat-stable factor, the identity of which is unknown, shows relatively little variation in the maturity or condition of the bracken (*14*). The thiaminase of bracken is significant only in monogastric animals and plays no role in the acute bracken poisoning of cattle (*7*). However, cattle eat large amounts of the young bracken shoots in the spring, so the carcinogenic material may be present in the plant at this stage of growth. Furthermore, the high incidence of human stomach cancer in Japan could be partially the result of bracken, since significant quantities of this plant are eaten in Japan (*15*).

The present investigation was undertaken to study the carcinogenic activity of bracken fern on the urinary bladder and on the alimentary tract of monogastric experimental animals (albino rats). This may be important in assessing the possible role of bracken in the etiology of cancer of the gastrointestinal tract of humans who consume bracken fern. The present report gives the preliminary results of these experiments. The study is continuing, and only the animals that have died to date are included in this article.

MATERIALS AND METHODS

Bracken fronds were collected in June 1967 from farms where the incidence of bovine urinary bladder tumors was high. The bracken was dried in the shade to preserve its natural dark green. It was then milled and mixed with a grain mixture (1:3 by weight). By use of steam and compression, the mixture was made into pellets that were immediately dried to avoid mold growth. Pellets containing bracken were dark green.

Albino rats of both sexes, bred from local stock, were used. A total of 112 rats, 49 days old, were divided into 2 groups: Group I consisted of 90 animals (38 males and 52 females) receiving the bracken-containing pellets, and Group II consisted of 22 animals that received the control diet consisting of the grain mixture only. Group I also received weekly subcutaneous injections of vitamin B_1 (2 mg in 0.2 ml physiological saline) to offset the thiaminase activity of the bracken in their diet.

The diet was fed *ad libitum* until the rat died or was killed, when a necropsy was performed. The urinary bladders were distended at postmortem examination with 10% formalin solution injected into the urethra. The intestinal specimens were also fixed with 10% buffered formalin solution. The sections were stained with hematoxylin and eosin and by special methods as required. The gross and microscopic appearance of the urinary bladder tumors was evaluated by the criteria of Bonser and Jull (*16*). Carcinomas not invading the muscular coat of the bladder were classified as Grade I, and those invading muscle were classified as Grade II. The incidence of carcinomas was used to assess carcinogenicity.

RESULTS

All rats from the 2 groups gained weight up to 3 months after initiation of feeding; there were no differences in body weight gain between the control group and the group fed bracken fern supplemented with thiamine.

Seventeen male and fourteen female rats from Group I died or were killed at different times during the study. Table 1 gives the time of death and corresponding tumor incidence. The results clearly show that intestinal and urinary bladder tumors were induced by bracken feeding after the first 29 weeks. The 3 animals that died earlier than 22 weeks had no tumors (table 1).

TABLE 1.—Time at which the 31 rats in Group I died or were killed and the corresponding incidence of macroscopic tumors of the urinary bladder or small intestine

Time elapsed after initiation of feeding (months)	Number of deaths		Number of rats having:			
			Intestinal tumors		Bladder tumors	
	Male	Female	Male (17)	Female (14)	Male (17)	Female (14)
7–8	3	5	3	5	1	1
8–9	1	2	1	2	1	1
9–10	11	4	11	4	4	2
10–11	2	3	2	3	—	—
Total	17/38	14/52	17/17	14/14	6/17	4/14

Intestinal Tumors

All 31 rats that died during the first 11 months had intestinal tumors, but there was no noticeable sex difference in the response rate. Postmortem examination revealed multiple tumors protruding into the lumen of the small intestine (fig. 1). The tumors, predominantly in the ileum, were 2–8 mm in diameter. Grossly, the small intestinal polyps and carcinomas were nearly all broad based; a few were sessile. None were ulcerated. Some tumors were dark red. In 2 cases the tumors appeared to have penetrated the bowel wall and protruded through the serosa. Adenomatous polyps could not be distinguished from adenocarcinomas by gross examination. In a few cases the tumors in the terminal ileum produced ileocecal intussusception. The feces in the colon were soft, but no diarrhea was noticed. Table 2 gives the types and incidence of tumors arising in the small intestine.

Histologically, the tumors of the small intestine were either adenomatous polyps (29%) or adenocarcinomas (71%), or a combination of both. The polyps presented a variety of histologic alterations. Most exhibited a moderate-to-marked glandular and cellular atypia. All had definite and at least moderate cellular atypism. Most polyps were glandular, but a few had papillary components. At the edge of the polyps and carcinomas, a transition from normal to abnormal epithelium was sharply distinguishable. Often the smaller polyps contained deep-lying glands—the most atypical glands present (fig. 2). Thus the genesis of the altered glands may be from below and not necessarily a surface phenomenon. However, in some cases, the polyps began with surface epithelial alteration. The diagnosis of carcinoma was not made unless invasiveness, varying from invasion into or through the muscularis mucosa, into the muscularis propria, and occasionally into the serosa, was demonstrated (figs. 3 and 4). The carcinomas were adenocarcinomas, some of which tended to produce excessive mucin. In 3 cases, lymph node metastases were present. In 7 cases, residual adenomatous polyps were in

TABLE 2.—Types and incidence of microscopic tumors arising in the small intestine and urinary bladder of 31 rats that died when fed bracken

Type of tumor	Male (17)	Female (14)	Total No. of tumors (%)
Small intestine			
Adenomatous polyp	4	5	29
Adenocarcinoma*	13	9	71
Urinary bladder			
Papilloma	4	4	26
Papillary			
a) Squamous cell carcinoma	3	1	13
b) Transitional cell carcinoma	4	5	29
Sessile			
a) Squamous cell carcinoma	1	0	3
b) Transitional cell carcinoma	2	1	10

*In 7 cases adenomatous polyps were also present.

the adenocarcinoma. However, evidence was not conclusive that there was a transition from polyp to adenocarcinoma in these rats.

Urinary Bladder Tumors

Urinary bladder tumors, the most frequent extraileic tumor, occurred in 81% of the rats autopsied (table 2). Fifteen bladder lesions were microscopic. Ten of thirty-one animals (32%) developed urinary bladder tumors which could be observed grossly after the distention of the urinary bladder (fig. 5). Some of these tumors were papillary and some were sessile.

The histologic changes in the rat bladders showed great variations, including hyperplastic, metaplastic, and neoplastic changes. The histologic features of the tumors are given in table 2. Tumors of the urinary bladder were either papillomas or sessile or papillary carcinomas, the latter type being observed in 13 cases (figs. 6–8). Fifty-five percent of the rats developed carcinomas. Of 17 carcinomas (Grade II), 6 penetrated the muscle wall (fig. 7), whereas the other 11 carcinomas (Grade I) were confined to the submucosa (fig. 6). No indication of metastasis was found during the 45 weeks of the experiment.

Two control animals died during the study, but they did not have a lesion in either the small intestine or the urinary bladder.

DISCUSSION

Spontaneous intestinal tumors in rats are rare, although Weisburger (*17*) reported inducing such tumors in rats by 2-fluoramine derivatives, 4-diphenylamine derivatives, 2- and 3-aminodibenzothiophene, 2- and 3-aminophenanthrene, 2-amino-9,10-dihydrophenanthrene, and 3-methyl-2-naphthylamine. Spjut and Spratt (*18*) induced colonic neoplasms in rats with 3,2′-dimethyl-4-aminobiphenyl. Tumors of the large and the small intestine have been induced by oral radioactive yttrium (*19*) which emits β-rays, and rats protected against lethal doses of X radiation by parabiosis have developed adenocarcinoma of the duodenum, jejunum, ileum, and colon. Recently, Evans and Mason (*8*) and Price and Pamukcu (*5*) induced intestinal adenocarcinoma in rats by feeding them bracken fern. The intestinal neoplasms induced with bracken were similar to those described by others (*17*, *18*) for the above carcinogens. Although urinary bladder tumors were not expected in rats, they were found at a relatively high incidence, and a high degree of malignancy was found after rats were fed bracken. The results indicate that the bracken fern contains substances carcinogenic for the urinary bladder of rats. The nature of the carcinogenic metabolites has not been elucidated.

Work is now in progress to determine the causative agent by assay of extracts prepared from the bracken fern.

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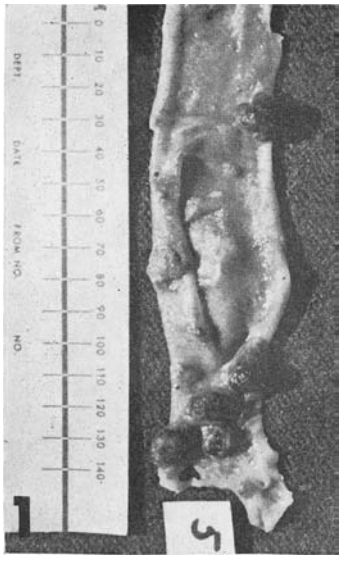
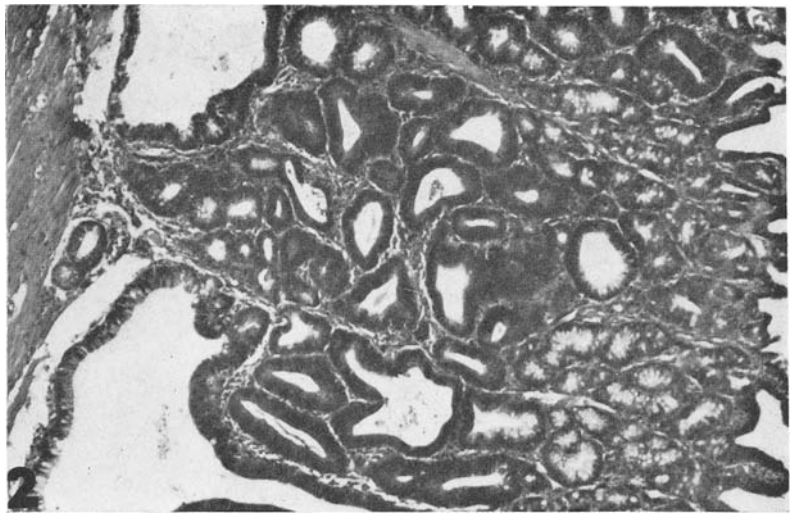
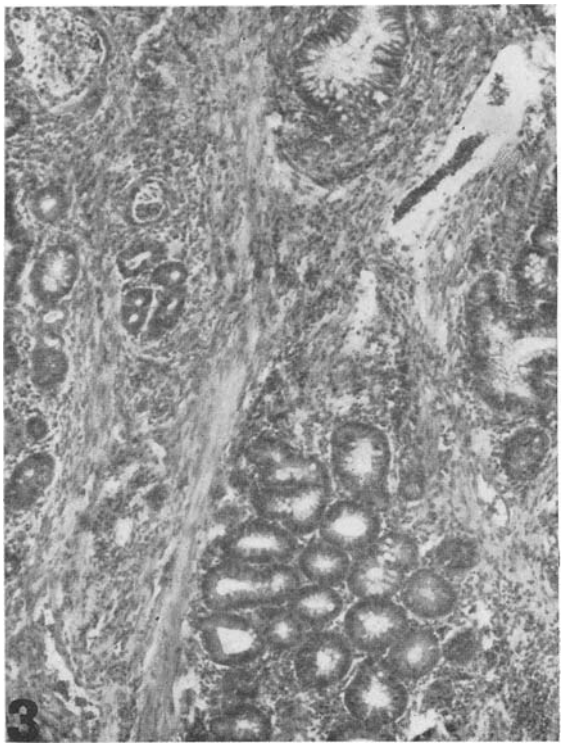






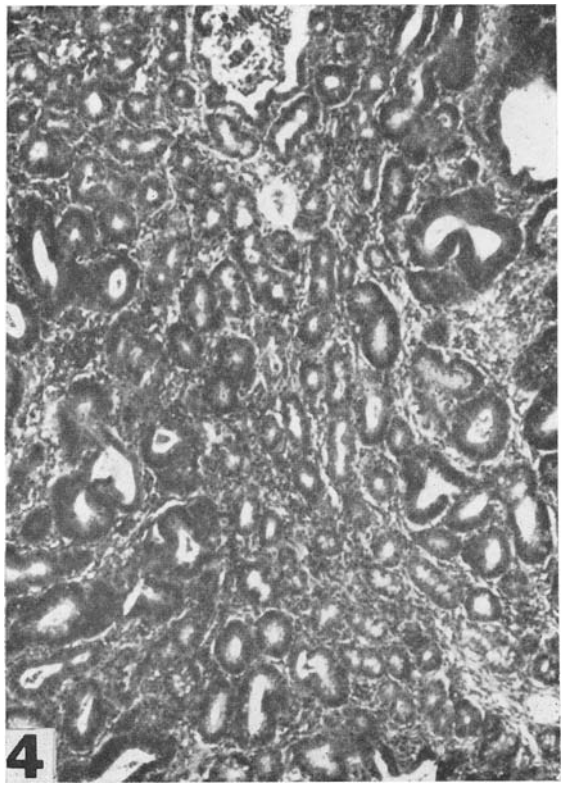
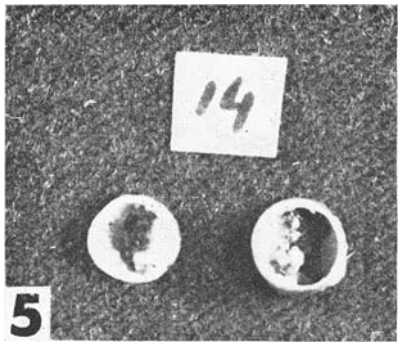


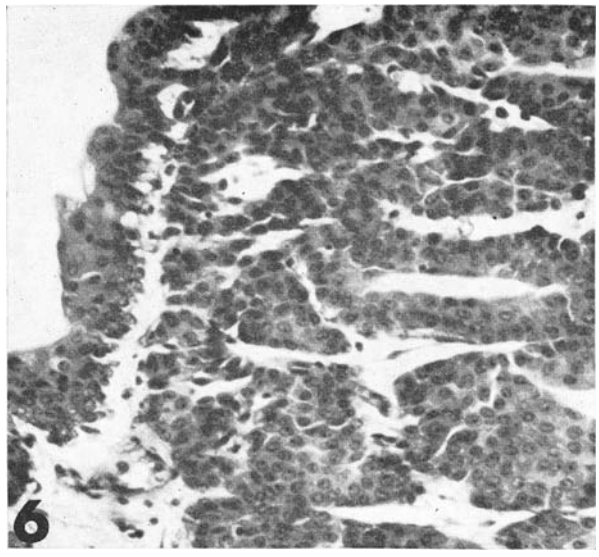
FIGURE 1.—Tumors of the ileum.

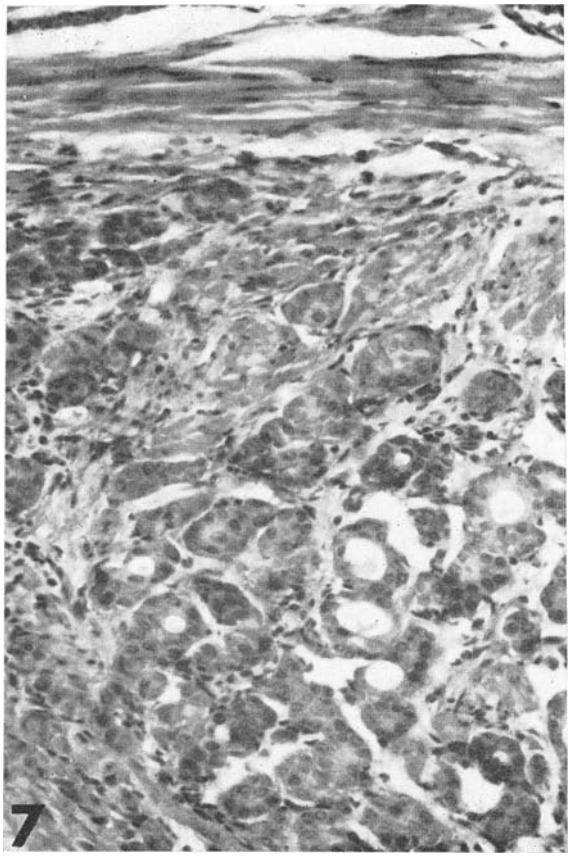
FIGURE 2.—Adenomatous polyp of the ileum. × 110

FIGURE 3.—Adenocarcinoma of the ileum. × 110

FIGURE 4.—Adenocarcinoma infiltrating the wall of the ileum. × 110







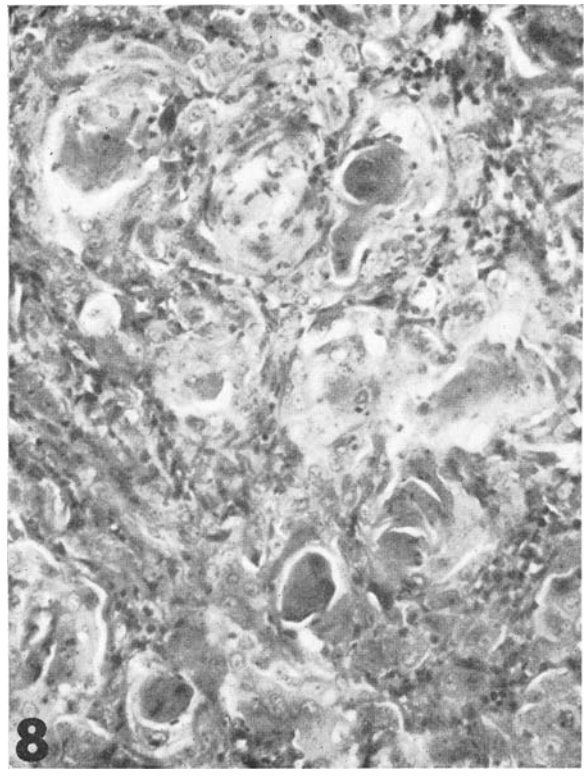


FIGURE 5.—Tumors of the urinary bladder.

FIGURE 6.—Transitional cell carcinoma of the urinary bladder. × 110

FIGURE 7.—Invasion of squamous cell carcinoma into the muscular wall of the urinary bladder. × 110

FIGURE 8.—Squamous cell carcinoma with pearls in the urinary bladder. ×110