A Urinary Bladder Tumor Induced by a Bovine
Cutaneous Papilloma Agent*

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Tumors of the urinary bladder in cattle are common in certain parts of the world. The disease is sometimes referred to as chronic enzootic hematuria (16, 17) and is descriptive of the usual manifestation of the natural disease. In endemic areas, which are usually restricted to mountainous regions, the incidence in cattle older than 2 years of age varies from farm to farm and has been reported to be as high as 90 per cent (9, 16, 17). The disease has been recognized in the Pacific Northwest coastal areas of the United States and Canada, Formosa, India, Turkey, Yugoslavia, Italy, France, and Germany. The etiology of the naturally occurring urinary bladder tumor in cattle is not known. Numerous factors have been suggested to be of causal significance in the disease. These include insufficient food, ingestion of plants containing oxalic acid, poisonous plants, deficiency of lime or excess of molybdenum in the soil, and schistosomal, bacterial, mycotic, or protozoal infections (cf. 16 for review). None of these factors has been established by convincing evidence.

Kalkus (11) produced the disease in a cow by injecting into her urinary bladder a suspension of hemangiomatous lesions from a bovine urinary bladder. An attempt at serial passage of the disease to another cow failed. The natural disease encountered by Kalkus had the character of a multiple hemangiomata with a well developed connective tissue stroma. Some of the lesions were pedunculated tumors. Kalkus mentioned observing papillomas in the bovine urinary bladder but considered them to be a separate entity.

The structure of many spontaneous bladder tumors in cattle does not differ materially in pathology from the industrial bladder cancer in dye workers. Industrial bladder cancer has been shown to be caused by exposure to certain aromatic amines (cf. 10 for a review). Animal experiments have indicated that it is probable that the bladder carcinogens are o-aminophenol metabolites of aromatic amines (3, 4) and that these metabolites probably are carried to the urinary bladder by the urine (12, 20). The known urinary bladder carcinogens (10) are chemically similar to several aromatic amines derived from tryptophan (8). An elevated level of certain tryptophan metabolites, such as 3-hydroxykynurenine and 3-hydroxyanthranilic acid, has been found in the urine of patients with spontaneous carcinoma of the urinary bladder (5, 6). A study has been made of the urinary excretion of tryptophan metabolites by Turkish cows with and without bladder tumors. The differences between these groups were not statistically significant except for an elevation of acetylkynurenine in the urine of animals bearing bladder tumors (18).

As far as the authors are aware, no viral agent has been recovered from naturally occurring bovine bladder tumors. However, in cutaneous bovine papillomatosis a viral agent has been described (7). This agent, which can produce warts with both epithelial and connective tissue proliferation in the skin and fibropapillomas of the genital mucosa in cattle (13), can also produce a sarcoma-like lesion in the skin of the horse (15). The effect of the bovine wart agent in the urinary bladder was not known.

Preliminary studies reported here have concerned a possible interplay between an agent in
On cystoscopic examination some calves were found to have a reaction at the injection sites in the urinary bladder at first examination 14 days after exposure. In one case the bladder was normal when examined 14 days and 28 days after exposure, but a tumor was found at 69 days. The reactions seemed to begin at one or both sites of injection, and later in some instances the two lesions became confluent. The early lesions were a flat type of bullous edema and were limited to the area immediately around the site of injection. The rest of the bladder wall appeared normal. Later there was a local formation of polyps which projected into the lumen of the bladder. A fairly sharp border was maintained between the site of the lesion and the normal bladder mucosa. In most instances the growth reached an estimated maximum diameter of about 30–50 mm. In one calf the tumor was estimated at 40 mm. in diameter at 51 days and maintained this size for at least 300 days.

The inocula were placed in the skin as well as in the wall of the urinary bladder to provide information about relative susceptibility of the calves as well as the tissue exposed. Warts developed in the skin of twelve calves in 33–88 days. Warts developed in eleven of the thirteen calves which had reactions in their urinary bladders. One of the two calves with no reaction from papilloma agent in the urinary bladder developed warts. Three calves which were exposed by laparotomy and one exposed by transurethral approach to 3-hydroxykynurenine and 3-hydroxyanthranilic acid have shown no reaction in the urinary bladder at 170 days post-injection and are still under observation.

Pathology.—Six calves with tumors of the urinary bladder have been examined by necropsy from 40 to 81 days after inoculation. The growths at the sites of inoculation were from 20 to 30 mm. in diameter and consisted of marked thickening of the wall with considerable projection into the lumen of the bladder. The mucosal aspect was covered with translucent yellowish polyps up to 5 mm. in diameter and 10 mm. in height. Near the center of most of the tumors was a crater-like central area 3–8 mm. in diameter, devoid of polyps and red in color from congestion and hemorrhage (Fig. 1). The hemorrhagic central areas coincided with the sites of injections as marked by the carbon particles in the underlying fibroma-like tissue. In some cases, the growths from the two inoculation sites coalesced. Cross-sections through the growths revealed a mass of firm tissue which extended under the base of the tumor. Histologically, the polyps were long, edem-
formation or break in the continuity of the mucosa. From the epithelium (Figs. 4 and 5).

In the older tumors the fibroma-like tissue extended into many of the polyps, replacing the edematous tissue (Fig. 3). At the interface of the fibroma-like tissue and the transitional epithelium there was a marked disturbance. The basement membrane of the epithelium was no longer evident, and there was intermingling of the fibroma-like tissue with projection of growth from the epithelium (Figs. 4 and 5).

In one case there was a fibroblastic reaction only in the wall of the bladder, with no polyp formation or break in the continuity of the mucosa.

No histologic evidence of metastases was found in the lymph nodes draining lymph from the bladder. Structures resembling inclusion bodies were seen in the nuclei of a few fibroblasts in the fibroma-like areas of some cases. In one case acidophilic cytoplasmic bodies were evident in some of the proliferating epithelial cells. A careful study of these structures has not been made, and their significance is not known.

The warts produced in the skin had hyperplasia of both the epithelium and the connective tissue of the corium. The fibroblastic proliferation was quite similar to the fibroblastic proliferation observed in the urinary bladder. However, the basement membrane of the epithelium was clearly evident and distinctly separated the epithelial and fibroblastic elements. The wart might more properly be considered a fibropapilloma (Fig. 6).

**DISCUSSION**

It is evident that a growth can be induced in the urinary bladder of susceptible calves with an agent in the common bovine cutaneous papilloma. The tumor could be induced with the wart agent alone. Thus far there is no evidence that addition of 3-hydroxykynurenine or 3-hydroxyanthranilic acid to the bovine papilloma extract at the time of inoculation had any influence on the development of the resulting tumor. Five of the thirteen bladder tumors were induced with papilloma agent alone, six were induced with papilloma agent plus 3-hydroxykynurenine, one with papilloma agent plus 3-hydroxyanthranilic acid, and one with papilloma agent plus both 3-hydroxykynurenine and 3-hydroxyanthranilic acid. One calf which received 3-hydroxykynurenine and 3-hydroxyanthranilic acid in its urinary bladder and three calves which received 3-hydroxykynurenine in their urinary bladders have thus far shown no evidence of tumor development.

The growths of the tumors appeared to be progressive, though at a rather slow rate. The initial development of bullous edema and the formation of polyps which were evident in some cases as early as 14 days were striking. The cystoscopic appearance of these growths was that of a papillary tumor of a benign character. In those tumors which had grown for 80 days there was histologic disturbance at the basement membrane which might be evidence of early malignant change in the epithelium. Whether or not chemicals in the urine might affect the experimentally induced tumor in these calves is unknown. Because 3-hydroxykynurenine and 3-hydroxyanthranilic acid have been considered carcinogenic by some workers (1) and they occur in urine of normal cattle (18, 19), such action by these chemicals is possible.

Polypoid growths similar to those observed in the induced bladder tumors have not been commonly described among the natural diseases of cattle (16, 17). The natural tumors of cattle and water buffalo are composed of either epithelial or mesoblastic elements or both. In one series of 50 cases (16) one half were either epithelial or mesenchymal in structure and the other half had neoplasia of both elements. In another series of 61 cases (17) about three-fourths had neoplasia of both elements. The natural tumors were invasive, destructive, and occasionally metastatic. The naturally occurring tumors were found in animals 4 years of age and older (16, 17). Perhaps the polypoid character of the relatively young experimental tumors will change with the passage of time.

The tumors induced in the bladder by the bovine papilloma extract tended to exhibit a definite invasiveness of the fibroma-like element, and only continued observation will establish their true character. The cutaneous wart produced by the bovine papilloma agent is known to be a benign and self-limiting lesion, even though both epithelial and connective tissues are affected (2). The connective tissue response in the horse from the same agent is somewhat more anaplastic (15), and the natural disease of equine sarcoid is often progressive (14).

The existence of bovine cutaneous papilloma agent in natural tumors of the bovine urinary bladder is not known, and the role of the agent in such tumors must be speculative at this time.
SUMMARY

The submucosal injection of suspensions of a bovine cutaneous papilloma induced a growth of the urinary bladder in thirteen of fifteen calves. The growths were composed of polyps of the mucosa and a fibroma-like tissue in the submucosa. Lesions examined at about 80 days showed irregularity at the juncture of the fibroma-like tissue and epithelium which suggested an early stage of malignancy. No metastases have been observed. These findings are preliminary, and the study is being continued.

REFERENCES


Fig. 1.—Mucosal aspect of the urinary bladder of a calf with papillary growths at the two sites of injection with bovine cutaneous papilloma material 79 days previously. The central dark craters were the points of injection. The injections were made through a suprapubic eystostomy with incision of the bladder wall between the points of injection.

Fig. 2.—Fibroma-like growth in wall of urinary bladder 72 days after injection of bovine cutaneous papilloma material. Carbon particles which were mixed with inoculum are evident. The large vesicular nuclei of the young fibroblasts are apparent. Relatively few mitotic figures could be found in the fibroma-like proliferation which was well supplied with capillaries. Mag. X880.

Fig. 3.—Section through the base of the polyps of a bladder tumor of a calf 48 days after its induction with bovine wart material. There is edema in the ends of the polyps. The invasion by the fibromatous growth into the polyps is marked on the right side of the photomicrograph and is in an early stage in one polyp on the left. The transitional epithelial covering of the edematous portion of the polyps is essentially normal, whereas the epithelium in juxtaposition to the fibromatous proliferation is increased in thickness. Mag. X16.

Fig. 4.—Cross-section of a polyp from the tumor illustrated in Figure 1. The fibroma-like growth fills the central portion. The epithelium is increased in thickness, and the base of the epithelium is no longer discrete. The area marked is enlarged in Figure 5. Mag. X16.

Fig. 5.—Enlargement of the area marked in Figure 4 to illustrate intermingling of fibroma-like tissue and epithelium with no evidence of a basement membrane. Mag. X380.

Fig. 6.—Epithelial margin of a papilloma of the skin induced 48 days previously by intradermal injection of bovine cutaneous papilloma material. This calf also developed tumors at the inoculation sites in the urinary bladder. The sharp border between the growth of epithelium and the fibroma-like growth in the corium is in contrast with the relations illustrated in Figures 4 and 5 of the urinary bladder tumor. Mag. X110.
fibroblasts had an anaplastic appearance with edematous tissue (Fig. 3). At the interface of the corium. The fibroblastic proliferation was quite similar to the fibroblastic proliferation observed in the induced bladder tumors. The nuclei of the fibroblasts in the fibroma-like tissue were large, though mitotic figures were rare. Structures resembling inclusion bodies were seen in the nuclei of a few fibroblasts in the fibroma-like tissue. Cytoplasmic bodies were evident in some of the fibroblasts.

The growths of the tumors appeared to be invasive, destructive, and occasionally metastatic. The warts produced in the skin had hyperplasia or mesenchymal in structure and the other half were neoplasia of both the epithelium and the connective tissue. The wart might more properly be considered a fibropapilloma (Fig. 6). The naturally occurring tumors were found in cattle (16, 17). The natural tumors of cattle and water buffalo are composed of either epithelial or mesoblastic elements or both. In one series of 61 cases (17) about three-fourths had neoplasia of both elements. In another series of 50 cases (16) one half were either epithelial or mesoblastic in structure and the other half were neoplasia of both elements. In one series of 50 cases (16) one half were epithelial or mesoblastic in structure and the other half were neoplasia of both elements. The natural tumors were induced with the bovine papilloma agent plus S-hydroxykynurenine. One calf which received S-hydroxykynurenine in its urinary bladder has shown no evidence of tumor development. No histologic evidence of metastases was found in the lymph nodes draining lymph from the bladder and three calves which received 3-hydroxykynurenine and 3-hydroxyanthranilic acid in their urinary bladders have shown no evidence of tumor development.

The naturally occurring tumors were found in cattle and water buffalo. The tumors induced in the bladder by the bovine papilloma agent plus $-hydroxykynurenine, one with papilloma agent plus S-hydroxykynurenine and one with papilloma agent plus both $-hydroxykynurenine and S-hydroxyanthranilic acid, were induced with the wart extract. The induced tumor in these calves is unknown. Because the tumors induced with the wart extract tended to exhibit a definite change in the epithelium. Whether or not chemicals such as $-hydroxykynurenine have been considered carcinogenic by some workers (1) and they occur in urine of normal cattle (16) which was analyzed for chemicals but did not have tumors.

DISCUSSION

The incidence of bovine cutaneous papilloma is quite high in cattle 4 years of age and older (16, 17). Such action by these chemicals is possible. Perhaps the existence of bovine cutaneous papilloma in normal cattle might affect the experimentally induced tumors. The tumors induced with the wart extract tended to exhibit a definite change in the epithelium. Whether or not chemicals such as $-hydroxykynurenine and S-hydroxyanthranilic acid have been considered carcinogenic by some workers (1) and they occur in urine of normal cattle (16) which was analyzed for chemicals but did not have tumors.

The tumors induced in the bladder by the bovine papilloma agent plus $-hydroxykynurenine in normal cattle have shown no evidence of tumor development. The tumors induced with the wart extract have shown no evidence of tumor development.

The tumors induced in the bladder by the bovine papilloma agent plus S-hydroxykynurenine in normal cattle have shown no evidence of tumor development. The tumors induced with the wart extract have shown no evidence of tumor development.

The tumors induced in the bladder by the bovine papilloma agent plus 3-hydroxykynurenine and 3-hydroxyanthranilic acid in normal cattle have shown no evidence of tumor development. The tumors induced with the wart extract have shown no evidence of tumor development.
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