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
cutaneous bovine papilloma and 3-hydroxykynurenine and 3-hydroxyanthranilic acid in the causation of bladder tumors. These metabolites occur in normal bovine urine (18, 19) and cause a significant number of bladder tumors in mice (1). This report of preliminary results indicates that bovine wart material can produce a tumor in the urinary bladders of calves.

**MATERIALS AND METHODS**

Sixteen female and three male calves of dairy stock ranging from 2 to 3 months of age were used. The inoculum of wart agent was prepared as a 10 per cent isotonic saline suspension of bovine wart tissue which had been stored in 50 per cent glycerin at about 4°C. A small amount of powdered carbon was added to each inoculum to mark the site of injection. The standard dose per injection site was approximately .05 ml. of 10 per cent bovine papilloma suspension, approximately 5 mg. of 3-hydroxyanthranilic acid or approximately 2 mg. of 3-hydroxy-L-kynurenine. The chemicals were ground to a fine powder with an agate mortar and pestle, following which this powder was suspended in either isotonic saline or a suspension of bovine wart tissue. The materials were sometimes used singly and sometimes in combination. Duplicate injections were made of the inocula in the submucosa of the urinary bladder either by a suprapubic cystostomy or, in females, transurethrally with a #28 Stern-McCarthy working element with attachable needle. Injections were in the anterio-ventral area of the fundus when done by cystostomy and in the lateral walls of the fundus just anterior to the trigone by the transurethral route. The same preparation as used in the wall of the urinary bladder was also placed both intradermally and on scarified skin of the same animals. Most of the calves were examined cystoscopically at intervals, starting 14 days after injection.

**RESULTS**

Thirteen of fifteen calves which received bovine papilloma agent either alone or in combination with a chemical developed tumors of their urinary bladders. The method of introducing papilloma agent seemed to be of little importance, since eight tumors occurred in ten calves exposed by laparotomy and in each of five calves given injections by the transurethral route. Five of six calves developed tumors following exposure to bovine papilloma material alone. Eight of nine calves developed tumors when the papilloma material was mixed with either or both 3-hydroxykynurenine or 3-hydroxyanthranilic acid.

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-
atous, finger-like processes covered with essentially normal transitional epithelium. In some areas the number of layers of epithelial cells was increased. The fibroblastic reaction in the submucosa was particularly conspicuous in the older tumors. The fibroblasts had an anaplastic appearance with large nuclei, although mitotic figures were rare and considerable collagen was present (Fig. 2).

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-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

fibroblasts had an anaplastic appearance with tendons into many of the polyps, replacing the particularly conspicuous in the older tumors. The edematous tissue (Fig. 3). At the interface of the fibroma-like tissue with projection of growth formation or break in the continuity of the basement membrane of the epithelium was no longer evident, and there was intermingling of the fibroma-like tissue and the transitional epithelium there was a marked disturbance. The asbestos. The fibroblastic proliferation was only in the wall of the bladder, with no polyp number of layers of epithelial cells was increased.

The fibroblastic reaction in the submucosa was at the time of inoculation had any influence on metastases was found in the lymph nodes draining lymph from the bladder and three calves which received 3-hydroxyanthranilic acid. One calf which received S-hydroxykynurenine in its urinary bladder is not known, and the role of the agent must be speculative at this time.

The naturally occurring tumors were found in susceptible calves with addition of 3-hydroxykynurenine and 3-hydroxyanthranilic acid to the bovine papilloma extract tended to exhibit a definite invasive, destructive, and occasionally metastatic. No histologic evidence of metastases was found in such tumors must be speculative at this time.

It is evident that a growth can be induced or mesenchymal in structure and the other half of the corium. The fibroblastic proliferation was 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 these structures has not been made, and their significance is not known.

In one case there was a fibroblastic reaction quite similar to the fibroblastic proliferation observed in the naturally occurring tumors. The wart might more properly be considered a fibropapilloma (Fig. 6). The warts produced in the skin had hyperplasia or mesoblastic elements or both. In one series of 50 cases (16) one half were either epithelial and connective tissues are affected (~). In another series the natural tumors were inducing a growth. The connective tissue response in the horse from the papilloma agent plus 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.

The cutaneous wart produced by the bovine papilloma agent is known to be a benign, self-limiting lesion, even though both epithelial and connective tissues are affected (~). It is possible that a growth can be induced in the urinary bladder of susceptible calves with the wart agent alone. Thus far there is no evidence that the papilloma agent plus $-hydroxykynurenine, one with papilloma agent alone, six were induced with the wart agent alone. Five induction of the resulting tumor. Five of the thirteen bladder tumors were induced with the wart agent plus 3-hydroxykynurenine or 3-hydroxyanthranilic acid. One calf which received S-hydroxykynurenine in the urinary bladder is not known, and the role of the agent in such tumors must be speculative at this time.
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