Papilloma-like Virus from Bovine Urinary Bladder Tumors

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SUMMARY

Suspensions of 6 spontaneous bovine urinary bladder tumors produced fibropapillomas of the skin and vagina as well as polypoid growths with fibroma in the urinary bladders of test calves. The induction period ranged from 25 to 111 days. Three serial animal passages of one isolate which was filterable did not increase the activity of the virus. The infective agent resembles the bovine wart virus in its behavior in test calves and may be a causative factor in the etiology of spontaneous bladder tumors or it might simply be a passenger virus. Material from 10 other spontaneous bovine urinary bladder tumors were inactive.

Neoplasia of the urinary bladder is commonly associated with chronic enzootic hematuria (also called haematuria vesicalis cancerogenes bovis) of cattle which is recognized as a disease entity in various parts of the world. The neoplasms consist of either or both mesenchymal and epithelial elements and can be designated according to their histologic appearances as hemangioma, hemangiendothelioma, sarcoma, papilloma, papillary carcinoma, adenocarcinoma, squamous cell carcinoma, and other similar terms to suit the cytologic structure. The character of the tumors appears to vary somewhat with different geographical regions. A greater variety and more malignant tumors have been reported from Yugoslavia (11) and Turkey (18, 19, 20). The bladder tumors in the Pacific northwest coast of the United States and Canada are usually hemangiomas (10, 22). In Japan the tumors were hemangioma, fibroma, papilloma, and adenoma (6, 13). The geographically localized occurrence of chronic bovine enzootic hematuria was recognized early (3, 10) and confirmed by others (4, 8, 21). The endemic areas are usually wooded regions in mountainous country. The disease occurs in cattle older than 2 years and the incidence on some farms has been reported to be as high as 90% by Georgijev (7). The etiology of chronic enzootic hematuria and the associated bladder tumors is not known.

Bracken fern (Pteris aquilinum) has been associated with so-called “hematuria farms” in various countries. Hematuria but no tumors developed in 4 cows fed bracken fern at low levels for about 15 months (23). Oral administration of a mixture of carcinogenic hydrocarbons to a calf for a year and a half resulted in papillomatosis of the bladder in which the oncogenic action of the hydrocarbons was attributed to orthohydroxylated metabolic products of the aminated hydrocarbons (12). Kalkus (10) observed hematuria and diffuse hemorrhagic areas in the submucosa of a urinary bladder of a cow into which he had previously injected a suspension of hemangiomatic bladder material from a naturally occurring case. An attempt at serial passage to another cow failed.

Fibromas and polyps have been produced in the urinary bladder of cattle by injection of a suspension of bovine cutaneous papilloma into the wall of the urinary bladder (15). The tumors and polyps thus produced in the urinary bladder regress, although at certain stages, they are similar to the naturally occurring tumors in bovine enzootic material (2).

In our experience, the first evidence of a viral agent in the hemangiomatic and metaplastic changes of the urinary bladder came from material collected in the state of Washington in 1959 and preserved in glycerin. The suspension of the bladder specimen produced polypoid tumors in the urinary bladder similar to those obtained with material from cutaneous bovine papillomatosis (2, 15) but did not at this stage cause papillomas on the skin of the same calves. Production of fibropapillomas of the skin and vaginal mucosa with this material (isolate 272) has been reported in connection with other work (16). Suspensions of naturally occurring bladder tumors from cattle in Turkey have also produced fibropapillomas in the skin and vaginal mucosa of test calves (20, 21). The purpose of this report is to present additional evidence of a transmissible agent in naturally occurring tumors of bovine urinary bladders.

MATERIALS AND METHODS

The experiments were conducted at the University of Wisconsin with material from the state of Washington...
TABLE 1
SALIENT FEATURES OF 16 NATURALLY OCCURRING BOVINE URINARY BLADDER TUMORS USED FOR TRANSMISSION TRIALS

<table>
<thead>
<tr>
<th>Case</th>
<th>Origin</th>
<th>Age (yr)</th>
<th>Duration (yr)</th>
<th>Metaplasia</th>
<th>Neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glandular</td>
<td>Squamous</td>
</tr>
<tr>
<td>1</td>
<td>Washington</td>
<td>8½</td>
<td>?*</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2*</td>
<td>Panama</td>
<td>5</td>
<td>4 mo +</td>
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<tr>
<td>3</td>
<td>Bolu</td>
<td>6</td>
<td>1½</td>
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<tr>
<td>4</td>
<td>Samsun</td>
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<tr>
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<td>Bolu</td>
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<td>British Columbia</td>
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<td>Bolu</td>
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<tr>
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</tr>
<tr>
<td>16</td>
<td>Samsun</td>
<td>8</td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Information not available.

We are indebted to Col. C. A. Gleiser of the U. S. Army Mission to Panama for this material.

(Cases 1, 8), British Columbia (Case 7) and Panama (Case 2) and at the University of Ankara with material originating in Bolu and Samsun, Turkey (Cases 3–6 and 9–16).

The bladder tumors used for the transmission experiments came from cattle raised in areas where chronic enzootic bovine hematuria was common. The clinical and pathologic features were varied in the 16 cases (Table 1, Figs. 1–10). The material was preserved in 50% glycerin with physiologic saline solution and kept at 4°C until used in the preparation of suspensions. In one instance the tissue had been frozen and shipped in Dry Ice. Inoculums were prepared as a 10% suspension of tumor material in physiologic saline.

The 61 calves used for test animals were 2–5 months old (except as indicated) and obtained from herds of cattle presumably free of cutaneous papillomatosis (warts). From 0.5 to 2.0 ml of inoculum was injected into the submucosa of the urinary bladder of the calves with the aid of a cystoscope or by a suprapubic cystostomy. The inoculum, in approximately 0.2 ml amounts, was also injected intradermally in the skin and vaginal wall and rubbed into scarified skin. The inoculation sites of the vagina and skin were observed at frequent intervals for development of lesions. The urinary bladders of certain calves were examined periodically with a cystoscope.

In some trials the susceptibility of the calves to bovine papilloma virus was tested by simultaneous inoculation of skin and vagina with suspensions of bovine warts which were capable of producing warts in susceptible calves. The inoculation sites were different from those used for urinary bladder tumor suspensions.

Attempts at serial passage were made with materials from 2 bladder tumors.

RESULTS

Of the 16 naturally occurring bladder tumors (Cases 1–6, Table 1) 6 were transmissible; they produced lesions in the skin, vagina and urinary bladder of test calves (Tables 2, 3). The results of the experiments with these 6 cases are given in detail.

Case 1.—The tumor (Figs. 1, 2) suspension (isolate 259) was injected into Calves 48 and 49. At cystoscopy, 3 months later, the inoculation sites in the urinary bladders of both calves were covered with numerous polyps up to 1.5 cm in length and breadth. Necrotic and hemorrhagic areas could be seen in the mass of polyps. The urine in the bladders contained blood clots and shreds of tissue. Hematuria continued in Calves 48 and 49. At necropsy, the lining of the bladder was 15 mm thick at the inoculation sites (Fig. 16). The affected epithelium was hyperplastic and elevated into short papillae. Hemorrhages were evident in the submucosa and between the transitional epithelial cells. Glandular metaplasia (Fig. 15) was evident and there were numerous thin-walled vascular channels in the edematous lamina propria and submucosa. The inoculation sites of the skin and vagina were negative.
For serial passage, inoculums were prepared from the affected parts of the bladder of Calf 49 (designated as isolate 272). Calves 45 and 46 received unfiltered isolate 272. Short polyps and hemorrhagic areas of the urinary bladder mucosa were observed 36 and 189 days after inoculation of Calf 46. At cystoscopy done 295 and 385 days later, the bladder mucosa was normal. The urinary bladder of Calf 45 remained normal as noted by repeated cystoscopy up to 189 days after inoculation. The inoculation of the skin and vagina of both animals produced no change.

Isolate 272 material filtered through a Millipore HA (45 mfilter) filter (Millipore Filter Corporation, Bedford, Mass.) was inoculated into Calves 69 and 70. Lesions similar to those in the bladder of Calf 49 were noted in both calves, 97 days after inoculation. The lesions persisted in Calf 70 to 363 days, but were definitely regressing at cystoscopy 478 days after inoculation. The inoculation sites of the skin and the vagina were negative in both calves. Calf 69 was killed 303 days after inoculation. The bladder mucosa at inoculation sites was elevated with underlying dense fibrous tissue. Numerous small elevations of the mucosa were noted in other areas. Histologically, there was an adenomatous type of glandular metaplasia with fibromatous supporting tissue (Fig. 17). The fibroma had a somewhat anaplastic appearance and infiltrated among bundles of the smooth muscle of the bladder wall (Fig. 18). Only a moderate angiomatic change was found which could be considered as an ectasia.

At a later date, isolate 272 was used in a cross-immunity experiment which has been previously reported (16). Three calves had received prior intradermal injections of formalinized 272 isolate, and 3 weeks later were challenged with active 272 suspension. All 3 calves developed fibropapillomas at the inoculation sites of the skin (Fig. 12) and vaginal mucosa, and only 1 of the 3 developed a fibroma at the inoculation site in the urinary bladder. Two other calves received only the active 272 material and both developed typical fibropapillomas at the inoculation sites of the skin and one, at the inoculation site of the vaginal mucosa while the urinary bladders of both remained negative.

The fibroma and metaplastic epithelium of urinary bladder (Figs. 17, 18) induced in Calf 69 (designated isolate 286), represented a third passage of the original field isolate 259. Calves 92 and 93 received nonfiltered inoculums from isolate 286 and developed short polyps of the bladder mucosa with hemorrhage and some necrosis, 58 and 106 days, respectively, after inoculation. The lesions were definitely regressing 300 days after inoculation. A small fibropapilloma developed in the skin of 1 calf but the inoculation sites in the vaginal mucosa were negative. The same inoculum which had been filtered through a Millipore HA filter was injected into 2 other calves and all sites (urinary bladder, skin, and vaginal mucosa) remained negative.

Thus of 15 susceptible calves receiving material derived from the original isolate 259, 6 developed polyoid, metaplastic, and fibromatous lesions in their urinary bladders, 2 had only polyps and 7 had no reaction. Six calves had fibropapillomas of the skin and 9 were negative. Fibropapillomas of the vaginal mucosa occurred in 4 calves.

Case 2.—A suspension of the urinary bladder hemangioma and papilloma was injected into 3 calves. At 49 days there was a small nodular lesion in the skin of 1 of the 3 calves which had regressed 101 days after inoculation. The urinary bladders were examined cystoscopically at 21, 49, 101, and 209 days after inoculation. A hemorrhagic elevation of bladder mucosa was noted 101 days after inoculations in only 1 calf.

Case 3.—The suspension of hemangioma (Fig. 5) was injected into 3 calves. A bovine wart suspension was also inoculated into different sites of the skin and vaginal wall of the 3 test calves. Twenty-five days later nodular fibropapillomas developed at the cutaneous sites of inoculation with bladder tumor suspension in the skin of 2 animals, one of which also had a fibropapilloma in the vagina at 39 days. Both of these calves developed similar lesions at all the sites of inoculation with bovine wart material. The growths reached maximum size at 95 days and then regressed. One calf had no reaction at any inoculation site. All 3 calves were killed 180 days after inoculation and none had lesions in their urinary bladders.

Case 4.—Three calves were inoculated with bladder papilloma and bovine wart suspension. Two calves developed fibropapillomas of the skin and vaginal wall from both inoculums. These lesions were apparent at 45 days, reached maximum size at 100 days and then regressed. One calf showed no reaction at any site of exposure. All calves were killed at 122 days and one (Calf 27) had a fibroma in the submucosa of the urinary bladder and polyps of the mucosa. In this area the epithelium was thickened and elevated in short papillae. There were a few areas of glandular metaplasia.

Several of the fibropapillomas produced on the skin of Calf 27 with bladder tumor suspension were removed at about 105 days for serial passage (Fig. 11). A suspension of the skin fibropapilloma was injected into 3 test calves and 72 days later 2 calves had developed fibropapillomas in the skin and vaginal wall. One calf showed no reaction. The 3 calves were killed for examination of their urinary bladder at 144 days. In 1 calf the mucosa of the bladder was red and covered with a polyoid growth. The underlying bladder wall was thickened with fibroma.

Case 5.—Three calves received the bladder papilloma material and bovine wart suspension. Fibropapillomas of the skin and fibromas in the vaginal wall were produced with the bladder tumor suspension and the bovine papilloma material in 2 of 3 calves. The incubation period of the skin and vaginal tumors was 45 days in 1 calf and 53 days in a second calf. The animals were killed at 154 days. One of these calves had polyoid and hemorrhagic lesions confined to the ventral part of the bladder. The polyps surrounded a necrotic crater under which was a fibroblastic proliferation in the submucosa. The epithelial lining was thickened and elevated in short papillae, nests, and glandular metaplasia.

One calf was negative to all inoculations.

Case 6.—Three calves were inoculated with the mixed
tumor of the bladder (Figs. 7–10) and bovine wart suspension. All 3 calves developed skin fibropapillomas from both inoculums. Vaginal fibropapillomas became apparent in only 2 calves. The induction period was 55, 62, and 73 days in the 3 calves. The 3 animals were killed 160 days after the inoculation. One calf had a polyoid growth in the urinary bladder with an underlying fibroblastic proliferation in the mucosa (Fig. 13). The epithelial lining was thickened with development of Brunn's nests. Some polyps were filled with blood (Fig. 14).

**DISCUSSION**

Urinary bladder tumor suspensions produced fibropapillomas of the skin (10/17 calves), fibropapillomas in the vaginal wall (7/17 calves), and polyoid growths with fibromas in the urinary bladder (5/17 calves). Bovine cutaneous papilloma (wart) materials have been found to produce essentially similar lesions in the skin, vagina, and urinary bladder of the bovine (2, 15, 16). This may suggest that the agents derived from urinary bladder tumors and bovine warts are similar. The association of an agent similar to the bovine cutaneous papilloma virus with naturally occurring bladder tumors in cattle has been suggested in previous reports (16, 20, 21). The bovine wart virus may be a passenger in the naturally occurring bladder tumors.

Not all bladder tumors used for the preparation of inoculums were active. Only 6 of the 16 spontaneous tumors produced lesions in the skin, vaginal wall, or urinary bladder of test calves. The 10 nontransmissible tumors may have contained a low concentration of infective virus which was insufficient to cause lesions in the 9 calves susceptible to wart virus (Table 2). Variations of infectivity have been demonstrated in cutaneous papillomas of cattle (14).

The infectivity of the tumor materials was not dependent on the histologic features of the tumors tested. The 6 active tumors included papillomas, hemangiomas, and a carcinoma (Table 1). Similar types of neoplasias were represented among the 10 tumors in which transmission trials were negative. The active tumor materials came from 3 widely scattered areas of the world, namely the state of Washington, Turkey, and Panama, where chronic enzootic hematuria is endemic.

The 2 attempts at serial passage did not increase infectivity of the agent but rather served to demonstrate variations in results that were obtained with material from a single source (Tables 2, 3).

The somewhat erratic response of calves to wart virus make them undesirable as test animals in critical experiments. It is difficult to obtain known susceptible calves. Whether this is due to immunity from previous exposure, or to natural variation in susceptibility is not known (17). An attempt was made to determine the susceptibility of some of the test calves in these experiments by inoculations with active bovine wart material at the same time as inoculations with the bladder tumor material. A transfer of wart virus to the site of inoculation with bladder tumor material, thus causing a lesion which could be erroneously attributed to the bladder tumor material should be considered. This seems invalid for 2 reasons. Test calves which received bladder tumor material from Cases 1 and 2 (Tables 2, 3) did not receive bovine wart material and still the bladder tumor material produced lesions. Secondly, if there had been transfer of papilloma virus to the site of bladder tumor material inoculation, then some of the 9 calves developing warts from wart material should have been expected to develop fibropapillomas at the sites where they received bladder tumor materials and in fact these were negative (Cases 9–15, Table 2).

Further characterization of the papilloma-like virus
found in bovine bladder tumors is needed. There is a lack of a suitable assay procedure. Transformation of cells in tissue cultures by the bovine wart virus has been demonstrated (1, 25, 26). This method of assay might be applicable. Cultures of skin from bovine embryos transformed by bovine papilloma virus failed to fluoresce (L. K. Smithies and C. Olson, unpublished data) when stained with a conjugated antiserum which will demonstrate infective virus in bovine papillomas (24). The hamster will develop a fibroma after subcutaneous injection of bovine papilloma virus (5, 9) as will certain strains of mice (5). The hamster is being used as a test animal for the bovine wart virus although the incubation period of 4—14 months for development of fibrosarcoma is relatively long (W. L. Wooding and C. Olson, Fibrosarcomas induced in hamsters by bovine cutaneous papilloma material, manuscript in preparation).

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Fig. 7.—Case 6. Branched crypts lined with columnar epithelium lie under the transitional epithelial layer. There is also chronic inflammatory infiltration and adenoma. H & E, X 120.

Fig. 8.—Case 6. Islands of squamous-cell carcinoma in the submucosa of the urinary bladder. H & E, X 120.

Fig. 9.—Case 6. Squamous cell carcinoma in the region of the tunica muscularis of the urinary bladder. Mitotic figures and an intranuclear inclusion body (see arrow) are present. H & E, X 120.

Fig. 10.—Case 6. Metastatic squamous-cell carcinoma in the iliac lymph node. H & E, X 175.

Fig. 11.—Cutaneous fibropapillomas of Calf 27 induced with intradermal inoculations of a suspension of bovine spontaneous urinary bladder papilloma (Case 4). Inoculated 94 days previously.

Fig. 12.—Cutaneous fibropapilloma induced with intradermal injection of isolate 272 (Case 1). Inoculated 150 days previously. Note the fibroma under the hyperplastic epithelium. H & E, X 40.
FIG. 13.—Polypoid lesion of the urinary bladder induced by a submucosal injection of a suspension of a mixed tumor (Case 6). Inoculated 160 days previously.

FIG. 14.—Polyps and solid ingrowths of transitional epithelium into subepithelial tissues (Brunn's nest) with fibrosis of the urinary bladder shown in Fig. 13. H & E, × 43.

FIG. 15.—Transitional-cell hyperplasia and glandular metaplasia of the urinary bladder (Calf 49). Inoculated 189 days previously with a suspension of spontaneous urinary bladder hemangioma (Case 1). H & E, × 97.

FIG. 16.—The mucosal aspect of the urinary bladder of Calf 49. The mucosa of ventral portion of the organ was thickened with scattered petechial hemorrhages. Inoculated 189 days previously. Histology of the lesion is shown in Fig. 15.

FIG. 17.—Transitional-cell hyperplasia, branched crypts lined with columnar epithelium in urinary bladder of Calf 09 induced with filtrate of experimentally produced urinary bladder lesion. Inoculated 303 days previously. H & E, × 40.

FIG. 18.—Fibroma infiltrating around muscle bundles in the wall of same urinary bladder as shown in Fig. 17. H & E, × 100.
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