The ability of the bovine cutaneous papilloma agent to induce tumors in the bovine urinary bladder as well as in the skin and vagina was previously reported (7, 8). The induced tumors were benign in histologic appearance and no metastases were observed.

This paper reports the histology of bladder tumors examined at intervals varying from 15-500 days after induction with the papilloma agent.

MATERIALS AND METHODS

Five different cutaneous papilloma isolates (215, 219, 233-A, 247, and 260) were used for inoculation of the skin and urinary bladder in 59 nonvaccinated and twenty vaccinated calves 2—6 mo. of age. Some of the nonvaccinated and all of the vaccinated calves were part of a previously reported experiment on immunity (7). Inocula were prepared as a 10% isotonic saline suspension of bovine wart tissue which had been stored in 50 per cent glycerine at 4°C. Duplicate injections of approximately 0.5 ml of inoculum were made into the submucosa of the urinary bladder either by suprapubic cystostomy or, in female calves, transurethrally with a cystoscope and a No. 28 Stern-McCarthy working element. The inoculum used in the urinary bladder was also inoculated intradermally and on scarified skin of the same animal. Female calves were examined cystoscopically at monthly intervals to study tumor growth and regression. Bladder lesions in 35 nonvaccinated calves were examined histologically 15–500 days after inoculation.

Twenty calves were vaccinated with formalinized cutaneous papilloma suspensions to test the ability to immunize of wart isolates 215 and 260. Ten calves received vaccine prepared from material 215 and ten received vaccine prepared from material 260. The vaccines were prepared by making a 5% suspension of ground wart material and adding formalin to make a final concentration of 0.5%. The vaccination procedure consisted of 0.5 ml vaccine intradermally which was repeated ten days later. Twenty days after completion of vaccination the urinary bladder, skin, and other tissues were challenged with inocula prepared from materials 215 and 260. The methods of inoculation were as in the unvaccinated calves. Five of each group of ten calves vaccinated with materials 215 and 260 were challenged with material 215 and five with material 260. All animals were examined cystoscopically at monthly intervals and all vaccinated calves were killed 117–150 days from the time of challenge.

RESULTS

Nonvaccinated calves.—The five cutaneous papilloma isolates used in inoculation were active and produced growths in either skin or urinary bladder. One isolate, 247, did not produce bladder lesions but did cause cutaneous papillomas. Forty-eight of the 59 calves developed bladder tumors which were detected cystoscopically, and 38 grew warts at the skin injection sites (Table 1).
TABLE 1

Comparison of Regression of 48 Bladder Tumors and 38 Skin Tumors Produced by Inoculation of Cutaneous Papilloma Materials in 59 Nonvaccinated Calves

Since some tumors regressed and other tumor-bearing calves were killed, the number with tumor at the beginning of each observation period is smaller than in the preceding period.

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Observation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-60</td>
</tr>
<tr>
<td>Bladdera</td>
<td>1/49b</td>
</tr>
<tr>
<td>Skin</td>
<td>3/23</td>
</tr>
</tbody>
</table>

* Results are based on cystoscopic observations.
* Numerator = Number of animals in which the tumor regressed. Denominator = Number of animals with tumor in each observation period.
* These calves had small lesions which were observed cystoscopically 1038 days after inoculation.
* No skin growth lasted longer than 362 days.

Thirty-seven calves in which tumors were observed cystoscopically were killed. The bladder tumors in 35 of these calves were examined at necropsy 15-500 days after inoculation.

The majority of the bladder lesions were composed of polypoid projections of mucosa arranged about the rim of a crater void of mucosal projections (Fig. 1). In some lesions the polypoid projections were located about the rim of a crater void of mucosal projections (Fig. 2). The polyps of each tumor were of various sizes but in the majority of the lesions the polyps did not exceed 1.5 cm in length and 5 mm in diameter. The maximum size of mucosal projections was found in a tumor 374 days old which had projections measuring 1-4 cm in length and 3-10 mm in diameter. The majority of lesions less than 180 days old had polyps which were pliable and slightly translucent, whereas in tumors older than 180 days the polyps were more rigid and opaque. The base of the lesions in cross section consisted of dense tissue and often was sharply delineated from normal bladder wall (Fig. 2).

Table 2

<table>
<thead>
<tr>
<th>No. of calves</th>
<th>Duration (days)</th>
<th>Epithelial changes (Per cent involvement)</th>
<th>Subepithelial changes (Per cent involvement)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Polyplastic transformation</td>
<td>Thickened epithelium</td>
</tr>
<tr>
<td>Nonvaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15-60</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>14</td>
<td>61-120</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>13</td>
<td>121-180</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>181-500</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>Vaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>117-150</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

The dense tissue in the base of all lesions consisted of a compact mass of fibroblasts. From 60 to 180 days after inoculation this fibromatos tissue began to infiltrate the edematous tissue of the polyps (Fig. 3). Although the fibromatos reaction was located primarily within the lamina propria and submucosa, fibroblasts were observed penetrating between muscle bundles deep in the bladder wall. In a lesion of 500 days duration, fibroblastic tissue was present within the polyps and extended to the serosal surface of the bladder (Fig. 4).

The histology of the polypoid projections of mucosa varied with their age. In general the epithelium was thicker than normal and often the basal layer of cells was indistinct where transitional epithelium and fibromatos tissue were in juxtaposition (Fig. 5). Within areas of thickened epithelium small intraepithelial cysts were often present and were most frequently observed in tumors older than 60 days (Table 2). Subepithelial cysts were present within the polyps of many tumors and were most common in growths more than 180 days old. These cysts were observed within nests of transitional epithelium (Brunn’s nests) apparently isolated from the surface (Fig. 6), or in the process of becoming isolated. These cysts frequently contained material which stained red with mucicarmine stain. Glandular and squamous metaplasia were observed in the epithelium of the polyps of many tumors. Glandular metaplasia was observed only in polyps of tumors more than 60 days old and was seen in 66% of the tumors more than 180 days old (Table 2). The glandular epithelium was composed of columnar cells, many of which were goblet-shaped and filled with material that stained like mucin. Columnar cells occasionally were present within buds of transitional epithelium (Fig. 7), but more commonly were present lining crypts of mucosa. In cross section these crypts had the appearance of glands (Figs. 8, 9). Squamous metaplasia was observed less commonly in the epithelium of the polyps than was glandular metaplasia. It was observed most often in epithelium which was in direct contact with fibromatos tissue that had infiltrated the polyps and in epithelium lining a central crater present at the base of some lesions. The stroma of the polypoid projections of tumors less than 180 days old was largely lamina propria;
evidence of chronic inflammation, as represented by edema and infiltration with round cells, was present. In tumors older than 180 days the stroma of the polypoid projections was largely of fibromatous tissue with mature collagen often present. In some projections which had been infiltrated with fibromatous tissue there was an increase in the number and size of blood vessels. This increase in vascularity was located most often near the junction of fibromatous reaction and transitional epithelium (Fig. 10).

The polypoid projections of the tumors in five calves regressed to the point where they were no longer apparent by cystoscopy (Table 1). Regression was a gradual process in which the projections first lost their pink edematous appearance and became firm and white. At subsequent examinations the polyp became shorter, more blunt, and finally assumed an appearance resembling normal bladder mucosa. Cystoscopic examination of a bladder tumor at 500 days revealed the polyp to be much shorter than previously noted and, at necropsy, a thickened fibrous mass was still present in the bladder wall (Fig. 4). In three animals, short firm polypoid projections of mucosa were observed cystoscopically 1038 days after inoculation.

The cutaneous papillomas of twenty calves regressed spontaneously 35–362 days after inoculation (Table 1). But regression of cutaneous papillomas did not coincide with regression of the polyps of the bladder lesions.

**Vaccinated calves.**—Three of the ten calves given injections of vaccine prepared from material 260, and seven of the ten calves receiving vaccine prepared from material 215, developed bladder tumors when challenged. The growths developing in the urinary bladders of these calves varied in size and appearance and all but one were smaller than growths of similar duration in the nonvaccinated control calves. Two of the tumors consisted only of fibromatous masses in the submucosa which were not apparent on gross examination. The majority of the remaining growths in the vaccinated group were crater-like lesions with a firm fibrous base surrounded by short bulbous polyps. Microscopically the epithelial and subepithelial alterations were similar to those observed in tumors seen in the nonvaccinated control animals (Table 2). Squamous metaplasia of the epithelium was a more common finding in bladder lesions of vaccinated than in nonvaccinated animals. This metaplastic alteration was observed primarily in the central part of the lesion which was not covered with polyps. Areas of ulceration within the base of the crater were rarely observed.

**DISCUSSION**

The most constant feature of the tumor induced in the urinary bladder of cattle was a fibromatous mass which developed at the site of submucosal injection. This tissue resembled the fibromatous tissue seen in the base of the bovine cutaneous papilloma, and vaginal tumors of cattle induced with the bovine cutaneous papilloma agent (1, 5). Mitotic figures were rarely seen in the fibromatous element, although it did exhibit a definite invasiveness. In tumors with a duration of less than 60 days the fibroblastic tissue was generally confined to the area of the injection site. In older tumors the fibroblast extended into the edematous polyp, and in a tumor of 500 days duration the entire anterior third of the bladder was composed of dense fibromatous tissue. There was no evidence to indicate that the fibromatous tissue would become malignant, since the tissue appeared to become less cellular and more mature with age.

The projections of mucosa formed by elevations of the edematous lamina propria in some respects resembled the papillae seen in naturally occurring papillary hyperplasia.
FIG. 7.—Section through a polyp of the tumor also illustrated by the photomicrograph in Fig. 5. The proliferation of the epithelium was toward the lamina propria and appeared to be in process of becoming isolated from the surface epithelium. Arrow points to gland-like arrangement of columnar cells within the proliferating bud of cells. X 400.

FIG. 8.—Section through a portion of tumor described in Fig. 5. A large portion of the epithelium contained metaplastic columnar cells. The metaplastic epithelium has proliferated toward the lamina propria. At the bottom of photomicrograph there is dense fibromatous tissue. X 30.

FIG. 9.—A higher magnification of a different area of the tumor illustrated in Figs. 5, 7 and 8, depicting the changes of cystitis glandularis. The nests of metaplastic epithelium were composed of columnar and goblet-shaped cells. X 100.

FIG. 10.—Hemangiomatous areas in urinary bladder of non-vaccinated calf which was inoculated with papilloma material 81 days prior to necropsy. Numerous thin-walled vascular channels were present where thickened epithelium was in junction with the fibromatous tissue of tumor base. X 110.

FIG. 11.—Submucous cavernous hemanginoma in urinary bladder of cow, from the state of Washington, with enzootic hematuria. The thin-walled vascular channels are similar to those in the experimentally induced lesion illustrated in Fig. 10. X 300.

FIG. 12.—Papillary adenoma in urinary bladder of cow from Japan with enzootic hematuria. The cyst-like spaces around periphery of the lesion were lined with columnar epithelium. Fibrous connective tissue was present in submucosa. X 6. (Tissue section through courtesy of Dr. Yutaka Fujimoto, Hokaido Univ., Japan.)

FIG. 13.—Transitional cell adenocarcinoma in urinary bladder of cow from Turkey with enzootic hematuria. Benign glandular metaplasia and malignant changes were present in the epithelium. Dense fibromatous tissue was present in the submucosa. X 50. (Tissue section through courtesy of Dr. M. Pamukcu, Univ. of Ankara, Turkey.)
of the bovine bladder (2). The mucosal projections produced by the papilloma agent were, however, more massive and the reaction could appropriately be considered polypoid hyperplasia. Papillary hyperplasia is believed to be a response to an inflammatory reaction or chronic irritation which produces edema in the bladder submucosa and causes a proliferation of the bladder mucosa (6). In the experimentally induced tumors the fibromatous mass in the bladder wall may have supplied the stimulus necessary for this process to occur.

The number and length of the mucosal projections were related directly to the size of the fibromatous mass in the tumor base and its depth of penetration into the bladder wall. This observation was most evident in tumors of vaccinated calves. The mucosal projections of these tumors were few and small; similarly, the fibromatous reaction in the wall of the bladder was smaller than in nonvaccinated calves. Immunization would seem to limit the extent of the fibroblastic reaction and thereby might limit accompanying inflammatory response. Age of the tumor was also a factor determining the size of the mucosal polyps. The longest polyps were in tumors older than 120 days. However, in those tumors in which fibrosis of the polyps was extensive, cystoscopic examinations prior to necropsy indicated that the polyps had ceased growing in length and in number.

Although the primary lesion in the experimentally induced tumor was due to a proliferation of fibroblasts in the submucosa of the bladder, the transitional epithelium displayed marked changes. The sequence of epithelial alterations in areas of polypoid hyperplasia was a thickening followed by glandular metaplasia, and often the development of Brunn’s nests. Glandular metaplasia was not observed in tumors less than 60 days of age but appeared to increase progressively in older tumors. Glandular metaplasia in tumors more than 180 days old was similar to naturally occurring glandular metaplasia of the bovine bladder, although growths resembling mucinous adenomas of the bladder were not present (2). Squamous metaplasia was observed in tumors of various ages. Similar epithelial alterations have been observed in the urinary bladder of man (3, 6, 10). Cattle with enzootic hematuria have glandular and squamous metaplasia of the bladder epithelium as well as hemangiomatous areas and fibromatous changes in the submucosa (Figs. 11–13) similar to the bladder lesions produced experimentally with cutaneous papilloma material. Progressive neoplasia of epithelial or mesenchymal elements as seen in enzootic hematuria (4, 9) was not observed in our experimentally induced lesions which did regress. Glandular metaplasia in the bladder of man is regarded by some as a precancerous change which can progress to adenocarcinoma, although others consider it simply cellular metaplasia secondary to chronic irritation (3, 10). The similar lesion observed in the present experiments may be different or it may require some additional stimulus for malignancy. Martinic (4) observed in Yugoslavia that half the cattle which had to be slaughtered because of hematuria and 80% of those that died from the disease had pronounced malignant alterations of the urinary bladder epithelium. This strongly suggests an additional factor which requires time for its action.

REFERENCES
Histopathology of Urinary Bladder Tumors Induced by Bovine Cutaneous Papilloma Agent

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