Bladder Calculi and Urothelial Hyperplasia with Papillomatosis in the Rat following Insertion of Chalk Powder in the Bladder Cavity with Subsequent Trauma of the Bladder Wall

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SUMMARY

A suspension of chalk powder was injected into the cavity of the urinary bladder of Fischer 344 rats. Three weeks later rats were divided into 4 major groups and were given a submucosal injection. One group received a suspension of viable Chapman 4909 tumor cells, the 2nd group received a suspension of frozen-killed 4909 tumor cells, the 3rd group received a suspension of normal rat spleen cells, and the 4th group received cell-free fresh tissue culture medium. After 3 additional weeks urolithiasis was recognized in each experimental group. The incidence of calculi in the groups as listed above was 14 of 17, 6 of 11, 6 of 11, and 2 of 15, respectively.

In control studies inocula consisted of tumor alone, i.e., without chalk powder. Inoculation of the 4909 rat bladder cancer cell line into the lumen of urinary bladders of rats did not result in any calculi after 3 weeks but did produce intramural tumor nodules and hyperplastic changes in adjacent host urothelium in 2 of 10 rats. The tumor inoculated in the submucosa of the bladder produced calculi and papillomas in 2 of 7 rats, and it produced intramural tumor nodules with adjacent hyperplasia of urothelium in all 7 rats.

Over 90% of the rats with large urinary stones, i.e., 35 mg or more, had urothelial changes consisting of both hyperplasia and papillomatosis. For the rats with calculi smaller than 35 mg/rat, hyperplasia was seen in only 25% and no papillomas were observed.

These observations indicate that in the Fischer 344 male rat the formation of calculi in the urinary bladder in association with particles of chalk powder is enhanced by manipulation of the submucosa of the bladder wall by a number of materials. Large calculi themselves, in the absence of neoplastic cells, stimulate the formation of papillomas.

INTRODUCTION

A number of investigators have reported that in the laboratory rat hyperplasia, papillomas, or a combination of the 2 regularly precede the appearance of carcinoma (4, 5, 10). The interrelationships of calculi, hyperplasia, papillomas, and eventual carcinoma are poorly defined. In an earlier publication we described a model system in which a heterotopic bladder in rats was produced outside of the urinary stream (8). We are now studying responses to conditions favoring the appearance of hyperplasia, papillomas, and carcinomas in the heterotopic bladder and in the in situ bladder. The work reported here is 1 step in a broad investigation on urothelial neoplasia (6, 11).

MATERIALS AND METHODS

Animals. Fischer 344 male rats, weighing approximately 150 g each, were obtained from NIH. Rats were placed in individual hanging stainless steel cages and fed Purina laboratory chow and tap water ad libitum.

Intravesicular Inoculation of Chalk Powder. Blackboard chalk dust was gathered, suspended in tap water, and sterilized by boiling for 30 min. The final suspension used for inoculation was approximately 25% by volume in water.

Invasvesicular Inoculation of Chalk Powder. Blackboard chalk dust was gathered, suspended in tap water, and sterilized by boiling for 30 min. The final suspension used for inoculation was approximately 25% by volume in water.

Injection was performed on rats anesthetized with i.p. pentobarbital sodium in a dose of 5 mg/100 g of rat body weight. On each anesthetized rat the abdominal hair was removed, and under aseptic conditions the abdomen was opened by a suprapubic incision so that the urinary bladder was exposed. As the fundus of the bladder was held gently with small forceps, the urine in the bladder was removed with a 1-ml syringe fitted with a 27-gauge x 0.5-inch needle. Then a 0.2-ml suspension of the chalk powder was inoculated into the collapsed vesicle.

Submucosal Injection of Cells or Cell-free Medium. Three weeks after the intravesicular insertion of chalk powder, the bladder was exposed again using the procedure described for the 1st series of inoculations. The submucosa of the collapsed bladder was injected with 0.2 ml of either living tumor cells, frozen-killed tumor cells, living spleen cells, or fresh tissue-culture medium. The suspensions of cells were approximately 1 x 10⁶ cells/rat.

Live Cancer Cells. Dr. W. H. Chapman graciously supported by NIH Research Grants CA 14137 and CA 17772.

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provided us with carcinoma cell line 4909 (9). The cell line grows in Fischer 344 rats as a transitional cell carcinoma. In monolayer tissue culture it grows as a pleomorphic population of epithelial cells. We found that s.c. transplantability of the line, either from rat to rat or from tissue culture to rat, is 100% in Fischer 344 rats. The tumor grows invasively, eventually causing death of the host. The inoculum used in these experiments was a suspension of cells prepared with EDTA-trypsin (Grand Island Biological Co., Grand Island, N. Y.) from monolayer cultures.

Dead Cancer Cells. A suspension of 4909 cells in MEM-BSS* was alternately frozen at -8° and thawed 3 times.

Spleen Cells. The spleen of an adult Fischer 344 rat was minced as finely as possible in MEM-BSS. After centrifugation, a cell suspension of approximately 5 x 10⁶ cells/ml medium was prepared for inoculation in MEM-BSS.

Techniques of Observation. At the termination of the experiments the animals were killed with ether, and the urinary bladders and ureters were fixed in 10% formaldehyde. The 1st step in this procedure was to ligate the urethra and inject fixative into the bladder until it was in a normal distended form. The urethra was then cut below the ligature, and the bladder was put into fixative for several days, at which time the bladder was carefully cut in a sagittal plane and the inner surface was studied with a magnifying glass. The calculi were removed and weighed. For light microscopic studies the hemisected bladders were immersed in decalcifying solution (Harleco, Philadelphia, Pa.) for 16 hr; then they were sliced further and embedded in paraffin with cut edges down. Sections were stained with hematoxylin and eosin. Many sections were prepared from both halves of the bladder as skipped sections, with some sequences of serial sections.

RESULTS*

 Five experimental groups, labeled A, B, C, D, and E, were studied as summarized in Table 1.

 Animals of Group A had the chalk suspension inoculated in the bladder lumen and live cancer cells injected into the submucosa. There were 2 schedules. In Group A-1, 10 rats had tumor cells injected into the wall of the bladder at the same time that chalk was placed in the lumen. In these rats, killed 3 weeks later, 2 had small calculi and no tumors. The remaining 8 without calculi were found to have tumor growth in the wall. Seven of this group had hyperplasia of the normal urothelium in the vicinity of the tumors. In Group A-2, 17 rats had chalk placed in the bladder cavity, and cancer cells were inoculated in the submucosa 3 weeks later. The animals were killed after an additional 3 weeks. Tumor was found in the wall of all 17 animals, with hyperplasia of host urothelium in every bladder. Calculi were seen in 14 rats, and multiple bladder papillomas were seen in 11 rats (Figs. 1 to 6).

 In Group B all rats were given chalk powder injections in the bladder cavity and then they received a submucosal inoculum 3 weeks later. Group B-1, 11 rats, received dead cancer cells as the 2nd injection. Of these, 6 rats had bladder calculi, and 5 of the 6 had both urothelial hyperplasia and multiple papillomas. Group B-2, 11 rats, received a suspension of spleen cells as the 2nd inoculation. Six of these animals were found to have bladder calculi, urothelial hyperplasia, and multiple papillomas (Figs. 7 to 11). Group B-3 received tissue culture medium as the 2nd injection. In this group, consisting of 15 rats, 2 rats were found to have calculi and hyperplasia, and 1 of the 2 also had papillomas. None of the animals in Group B showed any evidence of carcinoma.

 A control group of rats, Group C, was inoculated only with a suspension of live cancer cells. Two routes of inoculation were used. The 10 rats of Group C-1 received the inoculation into the bladder cavity and the 7 rats of Group C-2 received the inoculation in the submucosa of the bladder wall. All these animals were killed and examined 3 weeks after inoculation. In 2 rats of Group C-1 and 7 of Group C-2 intramural tumors were found. In each case the tumor on the mucosal surface appeared to represent the periphery of the subjacent tumor mass growing in the substance of the wall. In Group C-1, where tumor was injected directly into the urinary bladder cavity, no scattered foci of tumor transplants were found lining the cavity. The 2 tumors that were found intramurally in this group were solitary, probably needle track implantations.

 In all rats where live tumor cell injection was followed by the appearance of tumor growth, including the C-1 group where tumor cells were inoculated into the bladder cavity, the tumors were intramural. There was no unequivocal evidence that tumor cells implanted successfully from the lumen of the bladder onto the mucosal surface. On the other hand, there was obvious evidence of invasion of hyperplastic host urothelium by tumor cells from adjacent submucosal tumor masses.

 Group D consisted of 10 rats inoculated in the lumen of the bladder with chalk. Only 1 animal had a calculus 3 weeks later, and this was relatively small. There were no urothelial abnormalities in that rat or in the other 9.

 Group E consisted of 10 rats inoculated in the wall with tissue culture medium. Neither calculi nor urothelial abnormalities were observed 3 weeks after inoculation.

 The incidence and size of the calculi is seen in Chart 1, and the relationship of size of calculi to accompanying epithelial hyperplasia or papillomas is seen in Table 2. Papillomas were common in the presence of large calculi with or without the accompaniment of growing tumor (25 of 26). Papillomas were absent where there were intramural tumors without calculi (0 of 18).

 We observed a high incidence of hyperplasia of host urothelium 3 weeks after the intramural injection of Chapman tumor 4909. When these injection-treated animals were modified by a prior injection of chalk powder in the bladder

*The abbreviations used are: MEM, minimal essential medium (Eagle's); BSS, balanced salt solution (Hanks').

Subsequent to the submission of this manuscript we observed diffuse bladder papillomatosis and multiple bladder calculi 3 weeks after the fundus of the bladder was traumatized by being clamped several times with a hemostat.

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Rat Bladder Papillomas following Trauma and Calculi
Table 1

Inoculation into the urinary bladder of chalk powder, cancer cells (dead and alive), and spleen cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculum</th>
<th>Total length of experiment (wk)</th>
<th>Rats with stone/no. of experimental rats</th>
<th>Epithelial changes in urinary bladder</th>
<th>Growth of inoculated cancer</th>
</tr>
</thead>
</table>
| A     | Chalk powder with living cancer cells  
1. Added at the same time  
2. Added 3 wk later | 3/10                            | 7/10*                                    | 0/10                                  | 8/10*                       |
| B     | Chalk powder with addition 3 wk later of  
1. Dead cancer cells  
2. Rat spleen cells  
| C     | Cancer cells only  
1. In the bladder cavity  
2. In the bladder wall | 6/15                            | 2/15                                    | 2/15                                  | 1/15                       |
| D     | Chalk powder                        | 3/10                            | 2/10*                                   | 0/10                                  | 2/10*                       |
| E     | Culture medium in the bladder wall  | 3/10                            | 0/10                                    | 0/10                                  | 0/10                       |

* Seven with hyperplasia were of the group of 8 with growing transplanted cancer.

Hyperplasia in same 2 animals that had growth of transplanted cancer.

A-C = Stones found in all experimental groups.  
D = Stones found in all experimental groups except D.

![Chart 1. Urinary stone weights in experimental rats of all groups in which stones were found.](image)

DISCUSSION

Hyperplasia and papillomatosis are significant histopathological findings in the human urinary bladder since they are common precursors of carcinoma (3). Chemical carcinogens in rats have produced these same proliferative changes preceding the development of urothelial cancer (4, 5, 10). Proliferative responses of the bladder have also been observed in rats following various types of mechanical irritation such as foreign bodies inserted into the bladder, and large calculi that formed spontaneously within the vesicle (7). Possible involvement of genetic factors is emphasized by the observation that the brown Norway rat has a high incidence of calculi, followed by transitional cell and squamous cell carcinoma of the bladder and of the ureter (1).

Although there is no reported correlation between bladder calculi and bladder carcinoma in man, a coincidence has been reported of calculi and carcinoma higher in the urothelial tract, i.e., in the renal pelvis. In a review of over 100 cases, Gahagan and Reed (2) described the occurrence of squamous cell carcinoma of the renal pelvis in regular association with chronic inflammation. They reported the finding of large calculi in the renal pelvis in about one-half of the cases with cancer. For the human renal pelvis and the rat bladder, the apparent coincidence of calculi and cancer may be attributable to the ratio of the size of the calculi to the size of their urothelial container. Relatively large calculi in a relatively small sac may have greater influence by way of trauma or otherwise on the surrounding urothelial cells.

We have reported here a high coincidence of large bladder calculi, hyperplasia, and papillomatosis appearing within 6 weeks in rats receiving intraluminal chalk dust and intramural trauma. A number of questions for future investigation are raised. We would like to study the histopathological and ultrastructural evolution of these proliferative reactions and the biological nature of the altered urothelium. Does its histological resemblance to early stages in carcinogenesis of rat bladders exposed to human carcinogens reflect a similar process in response to chalk dust and injury? We do not
Rat Bladder Papillomas following Trauma and Calculi

Table 2
Relationship of alterations in host urothelium to size of bladder calculi

<table>
<thead>
<tr>
<th>Status of urothelium</th>
<th>Calculi (17 rats)</th>
<th>Calculi intramural tumor (16 rats)</th>
<th>No calculi but intramural tumor (18 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 35 mg</td>
<td>&gt; 35 mg</td>
<td>&lt; 35 mg</td>
</tr>
<tr>
<td>Normal</td>
<td>3/4</td>
<td>1/13</td>
<td>2/3</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>1/4</td>
<td>12/13</td>
<td>1/3</td>
</tr>
<tr>
<td>Papillomas</td>
<td>0/4</td>
<td>12/13</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* See Table 1: Groups A-1, B-1, B-2, B-3, and D.
* See Table 1: Groups A-2 and C-2.
* See Table 1: Groups A-1, A-2, C-1, and C-2.
* Weight of calculi/rat bladder.

know whether the changes we have seen have been reversible or are a stage in carcinogenesis.

ACKNOWLEDGMENTS

We wish to acknowledge the participation of Ruth Bender and Carol Brown for histology, of Bruce Grant for medical illustration, and of Alla Bachtalowsky for assistance in preparing the manuscript.

REFERENCES


Figs. 1 to 5. Photomicrographs of a rat urinary bladder at 3 magnifications following the injection of chalk powder into the cavity and the subsequent intramural inoculation of transplantable rat bladder cancer 4909.

Fig. 1. Low magnification of about one-third of the circumference of the bladder. *Left*, site of submucosal tumor inoculation, which has given rise to a focus of infiltrating carcinoma seen as a raised nodule. *Center (left to right)*, broad zone of thickening of the bladder wall composed in large part of a thickened hyperplastic urothelium that appears as many elongated papillary processes extending into the lumen. *Right*, many elongated slender papillary processes have fused with one another producing an elaborate branching pattern. The muscular and fibrous components of the bladder wall are prominent. H & E, × 52.

Fig. 2. The nodule of tumor growth is seen at higher magnification. The overlying urothelium of the host is hyperplastic. In the center of the field the continuity of the host urothelium is interrupted and replaced by carcinoma cells at the margin of the tumor. H & E, × 52.

Fig. 3. A segment of the hyperplastic and papillary host bladder urothelium from Fig. 1 seen with higher magnification. The papillary processes have distinct stromal cores. The muscular and fibrous components of the bladder wall are prominent. H & E, × 52.

Fig. 4. Higher magnifications illustrating details of the altered urothelium. The urothelium is 8 to 10 cells thick. The luminal surface of the thickened urothelium is covered by a horizontal monolayer of flattened urothelial cells, the so-called "umbrella cells." The vascular stromal cores of the papillary fronds are thin, but distinct. H & E, × 326.

Fig. 5. Elongated slender papillary processes have fused with one another producing an elaborate branching pattern. H & E, × 52.

Fig. 6. Bladder stone. Note the irregular, spongy, pitted surface of calculus observed in this study. × 23.

Figs. 7 to 11. Photomicrographs of a rat urinary bladder at 3 magnifications following the injection of chalk powder into the cavity and the subsequent intramural inoculation of live spleen cells.

Fig. 7. Low magnification of a bladder with extensive thickening of the wall. Striking diffuse papillary pattern involving most of the circumference of the bladder lumen. Many papillary processes touch one another in a lacy pattern. H & E, × 13.5.

Fig. 8. A nodule of bladder wall protruding into the lumen. The lining epithelium is thickened and arranged into many papillary processes. Where the bases of papillary processes meet, the epithelium suggests an almost glandular arrangement. The stroma is edematous and infiltrated with leukocytes. H & E, × 52.

Fig. 9. Elongated slender papillary processes have fused with one another producing an elaborate branching pattern. H & E, × 52.

Fig. 10. Organoid association of papillary processes is seen in greater detail, and the connecting delicate vascular cores are also evident. The epithelium is from 6 to 12 cell diameters thick. H & E, × 326.

Fig. 11. A discrete papillary process is seen to the *left* of the field, and a smaller process to the *right*. Part of the superficial surface of the urothelium here and in Fig. 10 is covered with a horizontal monolayer of cells. H & E, × 326.
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