Urological Research 4, 55-61 (1976)

Urological Research

Denudation of the Urinary Bladder Mucosa in the Cat by Formaldehyde

R. Pust, M. Butz, A. Rost, S. Ogbuihi, and B. Riedel

Department of Urology, Klinikum Steglitz, Free University of Berlin

Received: December 16, 1975

Summary. Chemical Stripping of the urinary bladder mucosa was studied in 38 cats using 5 to 25% formaldehyde solutions. The contact time varied from 1 to 20 min. With a 20% solution and contact time of 1 min, total denudation was possible without necrosis of subepithelial layers. In such cases, complete reepithelialisation and normal bladder dynamics were seen within 3-4 weeks after formaldehyde instillation. Signs of formaldehyde intoxication due to vesical resorption were not observed.

Key words: Urinary bladder, Mucosal denudation, Formaldehyde instillation.

Thirty percent of patients with transitional cell papilloma of the bladder have multiple tumours. (19, 45) In spite of therapy, such as open surgery, transurethral resection or coagulation, there is a high rate of recurrence (2, 21, 45) and malignant change (2, 27, 33, 44) is possible many years after onset of the therapy. Conservative management of recurrent papilloma after transurethral procedures has been tried with a variety of chemical agents that destroy the mucosa to different degrees. Carbolic acid has not been proved effective (17). Cytostatic substances such as podophyllin (10, 38, 42) and Thio-TEPA (18, 20, 43) revealed better results. With the latter, recurrency of the tumor occurred only in about 30%. Even lower recurrency rates have been reported with Mitomycin ^{R} C (31) and Epodyl (39) therapy.

According to recent observations (7, 22, 23, 35, 37) papillomata occur as the result of a change in the whole bladder mucosa. Thus, a therapy that completely destroys the mucosa could have a clinical application for multiple low grade bladder tumours.

Superficial necrosis of the coagulation type can be achieved with formaldehyde solution (6, 12, 34). It has already been successfully instilled to stop bleeding from inoperable carcinoma of the bladder or secondary to radiation or cyclophosphamide therapy (5, 11, 41, 44). Clinical signs of intoxication have not been described. In such emergency cases, the coagulation of blood vessels of submucosal or even the muscle layers is wanted.

We have studied the time dependent effect of different formaldehyde concentrations on the transitional epithelium of the bladder of the cat, as its structure is similar to that of human urinary bladder (4).

MATERIAL AND METHODS

Laparatomy was performed in 38 adult female domestic cats (1.8-3.9 kg) under Nembutal^{R} anaesthesia (30 mg/kg body weight i. m.). The bladder was emptied by puncture with an 0.5 mm Braunule^{R} and then 12-15 cc of formaldehyde solution were instilled. Commercial aqueous formaldehyde solution (38%) was itself diluted to 5, 7.5, 10, 15, 20 and 25%, and buffered with $NaHCO_3$ solution (pH 7).

Contact times of between 1 and 20 min were used and then the solution was completely aspirated and the bladder washed three times with physiological saline. Final emptying was by compression of the bladder in order to wash the bladder neck and urethra.

The puncture hole was closed by a catgut

Fig. 1. (5%, 20 min), 9 days postop. No transitional epithelium, haemorrhagic necrosis of the whole bladder wall, some intact muscle fibres. v. Gieson stain, (x 100)

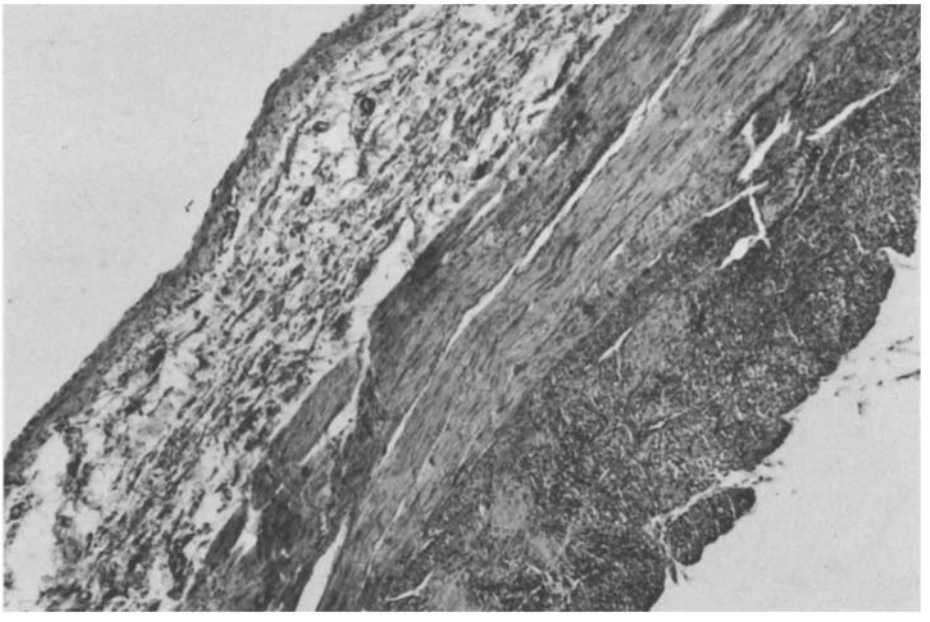

Fig. 2. (20%, 1 min), 8 days postop. Superficial protein precipitation of the epithelium, oedema of the submucosa. v. Gieson stain, (x 100)

stitch. Gentamycin was administered for 3-5 days postoperatively (3 mg/kg body weight i. m.). Intravenous urograms and histological examinations were performed 1-2 weeks, 4-6 weeks, 3 months, 6 months and 12 months or longer after operation. In another 3 cats formalin was instilled through the upper ureters via bilateral ureterotomy. In these cases x-ray and histological examinations were carried out 7 days, 4 weeks and 12 weeks after operation.

RESULTS

No clinical signs of general intoxication, such as lacrimation, bronchospasm, vomiting, tachapnoea or tachycardia, were observed.

The histological examinations between 7 and 14 days after formaldehyde instillation revealed the following results:

5% solution, 5 min contact time:

The transitional cell epithelium looks almost normal and is not demarcated from its base.

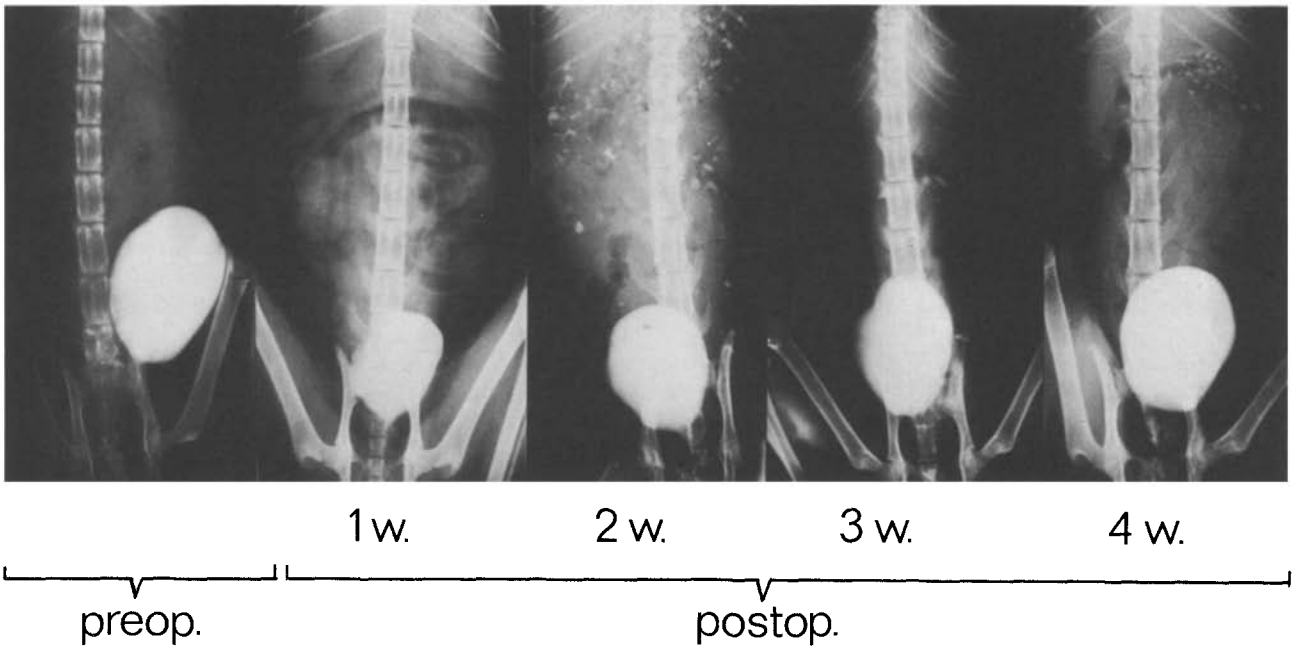

Fig. 3. (20%, 1 min), i. v. cystogram

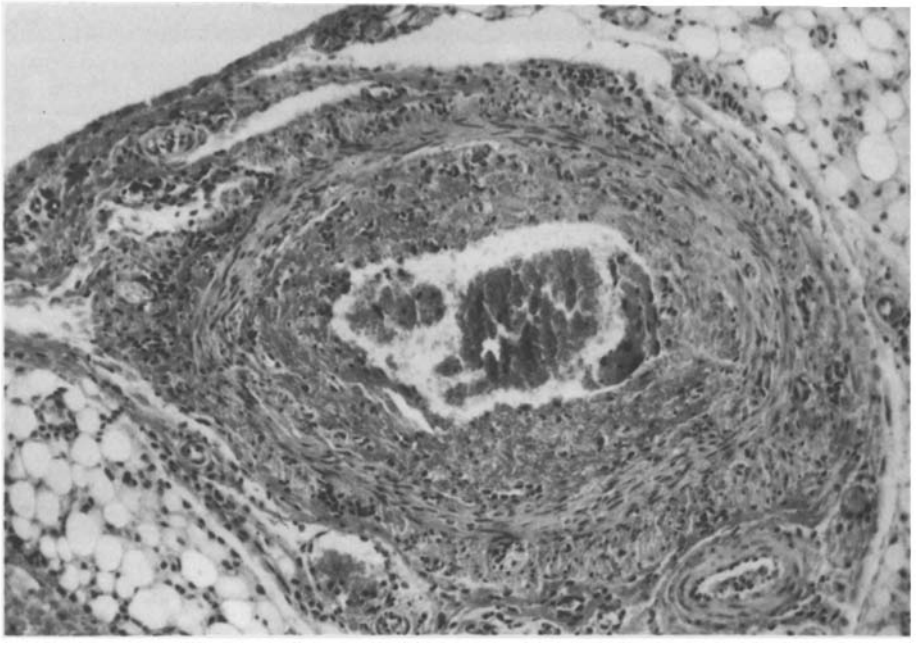

Fig. 4. (20%, 1 min), 7 days postop., ureteral instillation. Cross section of the distal ureter. Mucosal denudation, subepithelial oedema. v. Gieson stain, (x 115)

Some chromatin-dense nuclei can be seen. Slight subepithelial oedema and leucocytic infiltration occurred. Similar alterations could be seen in the interstitial layers of the tunica muscularis.

5% solution, 20 min contact time:
With this contact time complete necrosis of the epithelium was achieved. Necrosis includes the submucosal and muscular layers. A number of muscular fibres survived (Fig. 1).

7.5% solution, 5 min contact time:
The transitional epithelium is damaged to various degree with loosening and detachment of cells. The majority of cells show caryorhexis or have pyknotic nuclei. The submucosa is oedematous, with cellular infiltration and scattered haemorrhage. The deeper muscular layers show loosening of the connective tissue and leucocytic infiltration.

10% solution, 5 min contact time:
Complete necrosis of the epithelium including parts of the submucosa was achieved. The

Fig. 5. (10%, 3 min), 42 days postop. Thin epithelial coating, multiple connective tissue fibres in the submucosa. H.E. stain, (x 100)

oedema extended in to the muscular layers. The whole bladder wall shows slight cellular infiltration.

15% solution, 5 min contact time:
The destruction of the epithelium is similar to the 10% solution but within the submucosa a small homogenous layer of dense necrotic tissue can be seen. The adjacent inner submucosa shows loosened collagenous connective tissue. The muscular interstitium is infiltrated by oedema, the muscle itself is not affected.

20% solution, 1 min contact time:
The whole transitional epithelium is destroyed and replaced by a thin homogenous, condensed layer which is equivalent to the coagulated cellular proteins. The submucosal layer is oedematous but there is no cellular infiltration. The muscular layer remained unaltered (Fig. 2).

25% solution, 1 min contact time:
The protein coagulation zone was broader and extended to the submucosal layers which are haemorrhagic. Little cellular infiltration of all layers can be seen. The muscular fibres look normal, the interstitium is extensively dilated by edema.

Depending upon the degree of subepithelial destruction and the time-dependent demarcation of the necrotic material, the formation of new transitional epithelium is generally completed within 3 to 14 weeks after formaldehyde instillation.

In the group treated with 5% concentration for a 20 min period, 75% mortality due to severe haemorrhagic cystitis was observed. The surviving animals had about 60% reduction

Fig. 6. (10%, 5 min), 26 days postop. Cross section of the trigone and parts of the posterior bladder wall. New transitional epithelium originating from the trigone. H.E. stain, (x 185)

Fig. 7a. (20%, 1 min), 21 days postop. Complete coating of transitional epithelium. Deeper layers not affected v. Gieson stain, (x 100)

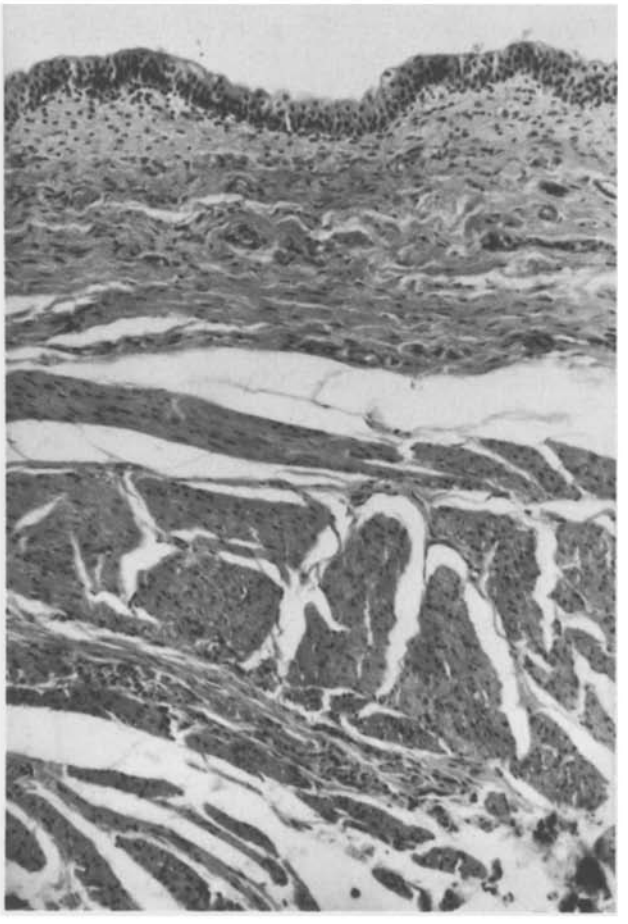

Fig. 7b. (20%, 1 min), 12 months postop. Normal aspect of all bladder layers. v. Gieson stain, (x 100)

of the bladder capacity due to scarring and shrinkage after 8 weeks. X-ray examination showed residual urine without exception. Histologically, incomplete, extremely thin, transitional epithelial layers could be seen after 12 to 16 weeks. Even in these cases there was no obstruction of the ureteral urinary output (6 months follow-up).

In all surviving cats the mucosal parts of the trigone and urethra were unaltered, probably due to reflex contraction of the trigone when exposed to formaldehyde.

The best functional final results were observed in the group treated with 20% concentration for 1 min when elasticity and bladder capacity is restored within 3-4 weeks (Fig. 3). This is in good agreement with the histological findings (Fig. 7a). In the group where formaldehyde (20%, 1 min contact time) was instilled via bilateral ureterotomy, alterations of the

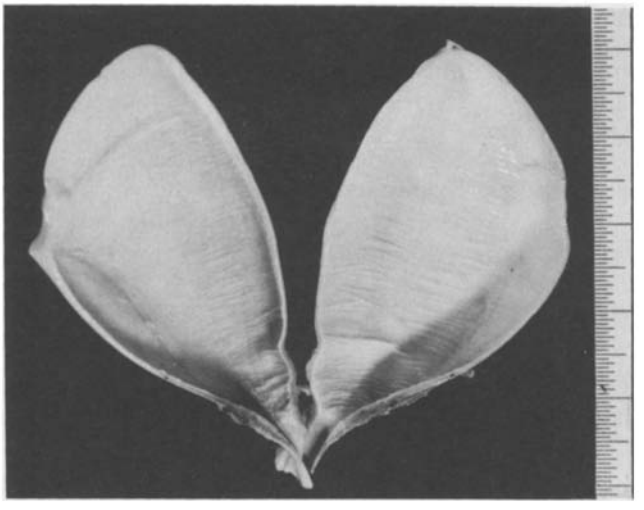

Fig. 8. (20%, 1 min), 8 days postop. Typical so-called greyish-white aspect of the bladder mucosa

ureteral wall, such as selective mucosal denudation and loosened structure of deeper layers can be seen (Fig. 4). Excretory urography, 1 week, 2 weeks and 3 months postoperatively, showed no ureteral obstruction.

DISCUSSION

The complete mucosal denudation of the urinary bladder can be chemically achieved by formaldehyde instillation. Using a 20% solution for 1 min, a superficial quantitative protein precipitation results; the so-called formaldehyde type of fixation (6, 12, 34). Further penetration of formaldehyde into the submocosal and muscular layers is prevented by this coagulation zone and the reactive edema.

The process of new epithelial coating is generally complete within a period of 3 to 14 weeks. The recovery time mainly depends on the depth of the necrosis. A randomly superimposed bacterial urinary infection enhanced the healing process (13, 14-16, 21, 28). The new mucosa often is not as thick as the original (Fig. 5).

The mechanism of reepithelisation is not yet fully elucidated (9, 13, 29, 30). Multifocal mesenchymal metaplasia as described by various authors (3) seems to be unlikely if a complete denudation is achieved. Our histological examinations show rests of unaltered original epithelium only in the trigonal and urethral regions (Fig. 6). It can be speculated that these areas are the origin of reepithelisation (8, 9, 40).

Mechanical denudation has been tried in animals and man by several authors (1, 3, 13-16, 21, 40). There was a high rate of failure due to scarring and shrinkage of the bladder. In some cases, upper urinary obstruction and even uraemia resulted (15).

We never observed ureteral lesions when filling the bladder directly with formaldehyde solution. Reflex contraction of the trigone probably protects the ureteral orifices. In man, vesico-uretric reflux should be excluded by prior cystography (32).

When using chemical agents, possible vesical reabsorption must be considered. Intravenous injection of 0.2 M formaldehyde solution in dogs and cats causes acute acidosis accompanied by vomiting, tachypnoea and tachycardia (24-26). It is quickly oxidised to formic acid which is an irreversible process. Formaldehyde given orally could not be detected quantitatively in plasma; small amounts were found in erythrocytes (25). In all cases the formic acid is quickly eliminated as CO_2.

If further experiments safely exclude vesical resorption of formaldehyde and possible toxic side effects, clinical application for the treatment of superficial bladder tumours would be possible.

REFERENCES

1. Akbari, A., Ward, J.N., Draper, J.W.: A technique for removing the mucosa from the urinary bladder. Investigative Urology 1, 429 (1964)
2. Albertini, v., A.: Histologische Geschwulstdiagnostik. Stuttgart: G. Thieme Verlag 1955
3. Baker, R., Manted, W.C., Dipasquale, N.: Regeneration of transitional epithelium of the human bladder after total surgical excision for recurrent, multiple bladder cancer: apparent tumor inhibition. Journal of Urology 93, 593 (1965)
4. Battifora, H., Eisenstein, R., MacDonald, J.D.: The human urinary bladder mucosa. An electron microscopic study. Investigative Urology 1, 354 (1964)
5. Brown, R.B.: A method of management of inoperable carcinoma of the bladder. Medical Journal of Australia 1, 23 (1969)
6. Burck, H.G.: Histologische Technik. Stuttgart: G. Thieme Verlag 1973
7. Cifuentes Delatte, L.: Remarques sur les papillomes de la vessie Journal d'urologie et de néphrologic 64, 280 (1958)
8. Conolly, J.G., Jewett, M.A.S., Murray, H.: The bladder volume in dogs following removal of vesical mucosa. Investigative Urology 7, 487 (1970)
9. Conolly, J.G., Morales, A., Minnaker, L., Raeburn, H.: The origin of regenerating bladder mucosa after partial mucosal stripping. Investigative Urology 8, 481 (1971)
10. Druckworth, D.A.: Treatment of papillomatosis with podophyllin. Journal of Urology 64, 740 (1950)
11. Firlit, C.F.: Intractable hemorrhagic cystitis secondary to extensive carcinomatosis; Management with formalin solution. Journal of Urology 110, 57 (1973)
12. French, D., Edsall, J.T.: The reaction of formaldehyde with amino acids and proteins. Advances in Protein Chemistry 2, 277 (1945)
13. Hansen, R.I., Lund, F., Wanstrupp, J.: Regeneration of bladder epithelium after mucosal denudation (stripping) in guinea pigs. Scandinavian Journal of Urology and Nephrology 3, 208 (1969)
14. Harada, N., Yano, H., Ohkawa, T., Misse, T., Kurita, T., Nagahara, A.: New surgical treatment of bladder tumours: mucosal denudation of the bladder. British Journal of Urology 37, 545 (1965)
15. Harada, N., Kusunoki, T.: Results of the mucosal denudation for bladder tumors: an interim report. Journal of Urology 99, 725 (1968)
16. Harada, N., Kusunoki, T.: Results of the mucosal denudation for bladder tumors: an interim report. Journal of Urology 99, 725 (1968)
17. Jewett, H.J.: Section: Tumors of the blad-

der, Vol. 2, in Urology, ed. by Campell, M. F. and Harrison, J. H., Philadelphia: W. B. Saunders 1970
18. Jones, H. S., Swinney, J.: Thiotepa in the treatment of tumours of the bladder. Lancet II, 615 (1961)
19. Kaplan, J. H., McDonald, J. R., Thompson, G. J.: Multicentric origin of papillary tumors of the urinary tract. Journal of Urology 66, 792 (1951)
20. Kierfeld, G., Mellin, P.: Thiotepa zur Rezidivprophylaxe papillärer Blasentumoren. Zeitschrift für Urologie 66, 569 (1973)
21. Lund, F.: Mucosal denudation (stripping) for bladder papillomatosis. Scandinavian Journal of Urology and Nephrology 3, 208 (1969)
22. Lunglmayr, G., Breitenecker, G., Pecherstorfer, M.: Die Bedeutung proliferativer und metaplastischer Urothelveränderungen für die Karzinomentwicklung in der Harnblase. Urologia internationalis 27, 24 (1972)
23. Lunglmayr, G., Regele, H.: Autoradiographische Untersuchungen zur Proliferation von papillären Tumoren der Harnblase. Urologe 8, 67 (1969)
24. Malorny, G., Rietbrock, N., Schneider, M.: Schicksal des resorbierten Formaldehyds. Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie 247, 381 (1964)
25. Malorny, G., Rietbrock, N., Schneider, M.: Die Oxydation des Formaldehyds zu Ameisensäure im Blut, ein Beitrag zum Stoffwechsel des Formaldehyds. Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie 250, 419 (1965)
26. Malorny, G.: Kinetik der Formaldehyd- und Ameisensäureoxydation bei verschiedenen Tierspecies. Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie 255, 42 (1966)
27. Melicow, M. M.: Tumors of the urinary bladder: a clinico-pathological analysis of our 2.500 specimens and biopsies. Journal of Urology 74, 498 (1955)
28. Mostofi, F. K.: Potentialities of bladder epithelium. Journal of Urology 71, 705 (1954)
29. Oehlert, W., Büchner, Th.: Mechanischer und zeitlicher Ablauf der physiolog. Regeneration im mehrschichtigen Plattenepithel. Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie 125, 374 (1961)
30. Oehlert, W., Doermer, Lesch: Die Steuerung der Regeneration im mehrschichtigen Plattenepithel. Verhandlungen der Deutschen Gesellschaft für Pathologie 50, 90 (1966)
31. Mishina, T., Oda, K., Murata, S., Ooe, H., Mori, Y., Takahashi, T.: Mitomycin-C bladder instillation therapy for bladder tumors. Journal of Urology 2, 217 (1975)
32. Orlin, J. J.: The role of cystography in Thio-TEPA-toxicity, Journal of Urology 108, 257 (1972)
33. Pyrah, L. N., Raper, F. P., Thomas, J. M.: Report of a follow-up of papillary tumours of the bladder. British Journal of Urology 36, 14 (1964)
34. Raida, H.: Formaldehydwirkung auf überlebende Organe. Zeitschrift für die gesamte experimentelle Medizin 549 (1924)
35. Riedel, B.: Experimentelle Untersuchungen an Harnblasentumoren. Habilitationsschrift Berlin (FUB) 1971
36. Riedel, B., Krafft, P., Pust, R., Heinau, H., Richter-Reichhelm, M., Rall, E., Rost, A., Knuth, O., Vincke, E., Kelâmi, A.: Rezidivprophylaxe des Blasenpapilloms mit Triäthylenthiophosphoramid (Thiotepa) Urologe 14, 87 (1975)
37. Riedel, B., Piper, R.: Frühveränderungen der Harnblasenschleimhaut der Ratte bei der Kanzerogenese mit N-Butyl-N-butanal (4)-nitrosamin (Ein Beitrag zur Frage der multizentrischen Entstehung von Blasentumoren) Urologia internationalis 28, 322 (1973)
38. Richardson, E. J.: The use of podophyllin in the treatment of papillomatosis of the bladder. Journal of Urology 82, 234 (1959)
39. Riddle, P. R.: The management of superficial bladder tumours with intravesical Epodyl. British Journal of Urology 45, 84 (1973)
40. Sanders, A. R., Schein, C. J., Orkin, L.: Total mucosa denudation of the canine bladder: Experimental observations and clinical implications, final report. Journal of Urology 79, 63 (1958)
41. Shah, B. C., Albert, D. J.: Intravesical instillation of formalin for the management of intractable hematuria. Journal of Urology 110, 519 (1973)
42. Semple, J. E.: Papillomata of bladder treated with podophyllin; preliminary report. British Medical Journal 1, 1235 (1948)
43. Veenema, R. J., Dean, Jr., A. L., Uson, A. C., Roberts, M., Longo, F.: Thiotepa bladder instillations: therapy and prophylaxis for superficial bladder tumors. Journal of Urology 101, 711 (1969)
44. Wallace, D. M.: Tumours of the bladder. Edinburgh, London: Livingstone 1959
45. Zingg, E. J., Büsser, E.: Klinik des Blasenpapilloms. Urologe 7, 82 (1968)

Dr. R. Pust
Klinikum Steglitz der
Freien Universität Berlin
Urologische Klinik und Poliklinik
Hindenburgdamm 30
D-1000 Berlin 45