Experimental Pyelonephritis: Characteristics of Infection in Rats Following Reduction of Bladder Capacity.* (30463)

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Certain abnormalities in structure or function of the urinary bladder seem to increase susceptibility of rats to pyelonephritis. For example, the incidence of pyelonephritis following instillation of bacteria into the bladder can be substantially increased by procedures such as massaging(1) or placing a foreign body in the bladder(2,3,4). The capacity of the urinary bladder is an important variable that has not been studied in relation to susceptibility to pyelonephritis in rats.

The experimental studies reported here were designed to evaluate the incidence and characteristics of spontaneous kidney infection in rats subjected to surgical reduction of bladder capacity.

Methods. Sprague-Dawley rats, weighing 200-250 g were anesthetized with pentobarbital and the bladder exposed through a suprapubic incision. One of the following procedures was performed: 1. surgical resection of the upper ⅓ of the bladder (cystectomy type A); 2. surgical resection of the upper ½ of the bladder (cystectomy type B); 3. surgical resection of the upper ⅔ of the bladder (cystectomy type C). Fig. 1 shows the different types of cystectomy. In addition, a group of control rats was subjected to cystostomy followed by closure. In all animals the ureters were left intact and suture of the bladder was done with surgical silk 000. Groups of 10-12 rats were sacrificed weekly for 4 weeks following each of the different operative procedures. At time of sacrifice urine was aspirated from the bladder through a large midline abdominal incision and streaked on blood agar and desoxycholate agar plates. The kidneys were removed aseptically and transected using sterile precautions. Half of each kidney was homogenized separately in saline solution and the number of bacteria was determined by making serial dilutions and pour plates. The presence of more than 100,000 bacteria in a kidney was considered to indicate the presence of pyelonephritis. The other half of each kidney was fixed in 10% formalin and sectioned for histologic study. All bladder calculi were collected.

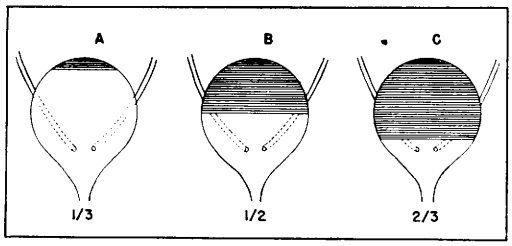

Fig. 1. Types of cystectomy performed.

Results. The incidence of spontaneous pyelonephritis is shown in Table I. Infection was infrequent (2 of 42 rats) in the first 2 weeks following cystectomy type A or B, but increased to 40% (16 of 40 rats) during the third and fourth weeks. This was similar to the incidence of infection developing after simple cystostomy: one of 20 rats sacrificed during the first 2 weeks, and 4 of 10 sacrificed in the third and fourth weeks had pyelonephritis. In contrast, infection was much more frequent following cystectomy type C. Pyelonephritis was observed in 60% (12 of 20) of the animals in the first 2 weeks following the surgery and increased to 80% (16 of 20) in the third and fourth weeks.

Forty-six animals developed pyelonephritis following the different types of cystectomy.

TABLE I. Pyelonephritis in Rats Subjected to Different Types of Cystectomy.

Cystectomy	1st wk	2nd wk	3rd wk	4th wk	Total (%)
Type					
A	1/10	0/10	4/10	3/10	8/40 (20)
B	1/10	0/12	3/10	6/10	10/42 (24)
C	5/10	7/10	8/10	8/10	28/40 (70)

No. of rats with pyelonephritis/No. of rats sacrificed.

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TABLE II. Frequency of Bladder Lithiasis in Rats Subjected to Different Types of Cystectomy.

Cystectomy	1st wk	2nd wk	3rd wk	4th wk	Total (%)
Type					
A	0/10	0/10	4/10	6/10	10/40 (25)
B	0/10	0/12	4/10	8/10	12/42 (29)
C	3/10	8/10	10/10	10/10	31/40 (78)

No. of rats with lithiasis/No. of rats sacrificed.

A significant titer of staphylococcus (greater than 100,000) was isolated from the kidneys of 30 rats, proteus from 7, a coliform organism from 7 and a streptococcus from 2. In each of these rats the bladder urine revealed the presence of the same organism isolated from the kidneys. The infection was bilateral in 38 rats and unilateral in 8. When infection was bilateral, the same bacteria were always recovered from both kidneys.

The frequency of urinary bladder calculi is shown in Table II and Fig. 2. The finding of calculi was closely correlated with the presence of infection. Thirty-six of 46 (78%) rats with pyelonephritis had bladder calculi, and pyelonephritis was present in 36 of the 53 (68%) rats with urolithiasis. Animals subjected to cystectomy type C demonstrated a higher incidence and earlier onset of infection and calculi than rats subjected to cystectomy type A or B. It is of interest that urinary lithiasis was found in all animals with infection due to proteus.

Kidneys from animals with infection showed the typical histological changes of acute pyelonephritis as described by Cotran *et al*(4).

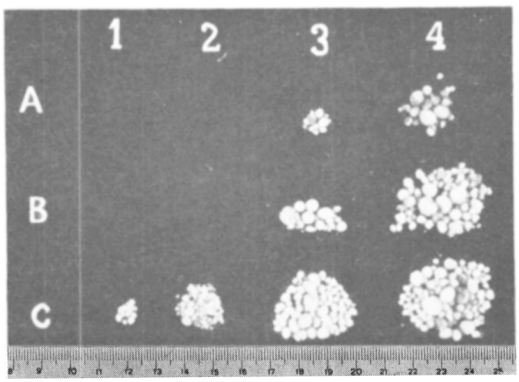

FIG. 2. Bladder calculi collected from rats sacrificed 1, 2, 3 and 4 weeks after cystectomy type A, B and C.

Discussion. It is likely that urinary tract infection in some of the animals was related to contamination of the bladder at time of surgery. However, it is noteworthy that infection was observed in only 2 of 42 rats during the first 2 weeks following cystectomy type A or B but increased to 16 of 40 three to four weeks after surgery. This delay in onset of infection suggests a source unrelated to surgery in many animals. It is likely that in these rats, bacteria invaded the urinary bladder from the urethra. Pyelonephritis could then be produced by passage of bacteria to the upper portions of the urinary tract through the vesico-ureteral reflux usually present in rats(1,3,5). The higher incidence of pyelonephritis following the more radical cystectomy (⅔ of the bladder) may in part be related to the greater contamination and trauma produced at time of surgery. However, it is also likely that more extensive amputation of muscles and nerves results in a more marked disturbance in bladder motility with urinary stasis and reflux to the kidneys.

There was a striking association of pyelonephritis and bladder calculi especially in the presence of infection caused by proteus. Infection with proteus organisms and the resultant alkalinization of the urine predisposes to formation of calculi(4). It is likely that the calculi served to maintain infection in the bladder increasing the possibility of ascending infection and pyelonephritis.

Summary. The incidence of spontaneous pyelonephritis and bladder calculi was studied in rats following cystectomy. 1. Rats were subjected to surgical resection of ⅓ or ½ of the bladder. Spontaneously developing pyelonephritis was infrequent (2 of 42 rats) in the first 2 weeks following surgery but increased to 40% (16 animals of 40) in the third and fourth weeks. When the cystectomy was more radical (⅔ of the bladder), the incidence of spontaneous infection increased to 60% (12 of 20 rats) during the first two weeks and to 80% (16 of 20) during the third and fourth weeks after surgery. 2. The infecting organisms isolated

were staphylococcus, proteus, a coliform organism or streptococcus. 3. The incidence of bladder calculi was higher in instances of more radical cystectomy (⅔ of the bladder), and followed closely the incidence of infection. Urinary calculi were always present when proteus was the infecting agent.

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Hamster Ascitic Fluids Containing Complement-Fixing Antibody Against Virus-Induced Tumor Antigens. (30464)

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Virus-specific, complement-fixing (CF) antigens have been demonstrated in tumors produced by adenoviruses(1), SV 40(2), Schmitt-Ruppin strain of Rous virus(3) and polyoma(4). Animals bearing these tumors develop the corresponding CF antibodies. Since the CF antigens appear to be highly specific and are present in both *in vitro* and *in vivo* transformed cells, they have provided an additional parameter in virus-tumor studies. Up to the present the only source of antibody to these antigens has been the serum of tumor-bearing animals. Because the animals involved are generally small rodents (hamsters and mice) there is a limited volume of usable antiserum readily available. Investigators studying other types of antibody produced in mice were confronted with similar problems of small volumes and were able to resolve them by techniques developed by Munoz(5) and Lieberman *et al*(6,7). These workers were able to obtain high titer antibody to various antigens in the ascitic fluids of mice which had been given intraperitoneal injections of adjuvant. By similar procedures, we have been able to produce relatively large quantities of ascitic fluids containing CF antibody to antigens in tumors induced by SV 40, adeno-18 and polyoma viruses as reported here.

Materials and methods. Tumors. A transplantable SV 40 virus-induced hamster tumor obtained from Dr. Bernice Eddy was used during initial studies, and a similar tumor produced by inoculation of suckling hamsters with a large-plaque strain of SV 40 virus derived in this laboratory was subsequently studied. This latter tumor has been designated as the SV 40-LP-T. A polyoma tumor was similarly produced after inoculation of suckling hamsters with polyoma virus. The adeno-18 hamster tumor was obtained from Dr. Wallace Rowe. All of these tumors were readily transplantable by subcutaneous (s.c.) inoculation of adult hamsters and were free of demonstrable infectious virus.

CF test. The technique of the test has previously been described(4). Four to eight units of antigen in the form of whole tumor emulsions or suspensions of washed cells grown in tissue culture were used for titration of antibody. End-points represent the highest dilution of serum or ascitic fluid giving 3 or 4 plus fixation. Positive control sera were included in every test. Titrations given in Table I were obtained in tests using SV 40 transformed human diploid culture cells (W1 18 Va 2)(8) as antigen.

Results and discussion. Preliminary experiments were conducted with the SV 40 tumor obtained from Dr. Eddy. Hamsters were inoculated s.c. with 10^6 trypsin-dispersed cells. After 2-3 weeks when the tumors had attained a size approximately 5 to 10 mm, they