Virchows Arch. path. Anat. 342, 103—108 (1967)

An Experimental Model of Ascending Pyelonephritis in the Rabbit

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Received October 10, 1966

In recent years considerable attention has been devoted to problems of experimental pyelonephritis. The inflammatory renal process was usually produced by a haematogenous injection of bacteria. According to most authors, however, greater importance is attached to the ascending route of infection from an infected bladder. A considerable number of experiments dealt with ascending pyelonephritis (VIVALDI et al., 1959; IMURA, 1961; KEITH et al., 1962; ANDERSEN and JACKSON, 1961; DAVIS and COIL, 1961; HEPTINSTALL, 1964). Most of them were performed in rats, where vesicoureteral reflux is a normal phenomenon (SUNSHINE, 1964). These conditions are different from those prevailing in man. In rabbits the vesicoureteral reflux is not a constant feature; it was found only if the bladder pressure increases to 35—40 cm H_2O (PRÁT and HATALA, 1966). We attempted, therefore, to produce ascending pyelonephritis in rabbits after injecting E. coli suspension into the bladder. Our previous work (HATALA and PRÁT, 1967) had shown that E. coli infection of the bladder does not affect intact rabbit kidneys; in experiments presented in this article one of the kidneys was damaged by temporary obstruction of the ureter.

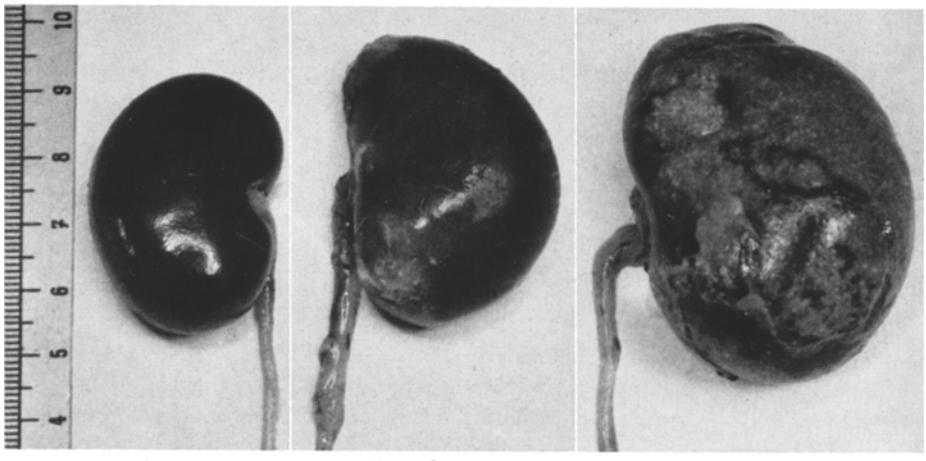
Methods

The experiments were carried out on full grown male rabbits, chinchilla strain, weighing 2,500 to 3,500 g. Before the experiment catheterized bladder urine from each animal was bacteriologically examined to rule out spontaneous bacteriuria. Rabbits with a positive bacteriological finding (more than 10^3 bacteria/ml of urine) were not used in our experiments.

Experimental procedure: under general pentothal anesthaesia a left-sided ureteral ligature was made. The passage through the ureter was renewed after removal of the ligature 40 hrs later. Details on this method were described previously (PRÁT et al., 1959). Four hours after removal of the ureteral ligature a catheter was introduced and the bladder was emptied. Subsequently an E. coli suspension in saline was injected through the catheter into the bladder. To one group of animals 10 ml suspension, to the second group 23 ml suspension were administered; the total number of bacteria was equal in both groups. After removal of the catheter the bladder was gently squeezed several times through the abdominal wall, and at the same time to prevent the escape of urine from the urethra, the external urethral meatus was compressed. After seven days the animals were killed by i. v. administration of a penthotal solution. In the control group the same experimental procedure was applied with one exception only: instead of an E. coli suspension 23 ml sterile saline were injected into the bladder.

On necropsy the abdominal cavity was opened under sterile conditions; first of all urine was withdrawn from the bladder by puncture for bacteriological examination. Next, both kidneys and the urinary bladder were removed. The kidneys were bisected: the upper and lower pole were subjected to bacteriological examination, the central part of the kidney to

histological examination. Urine and renal tissues were examined by a quantitative bacteriological dilution method ("streak plate") and the number of microorganisms per ml of urine or gram of homogenized renal tissue was assessed. In all positive E. coli cultures a serological



a b c

Fig. 1. a Normal right kidney; b Focal pyelonephritis in the left lower pole; c Suppurative left pyelonephritis

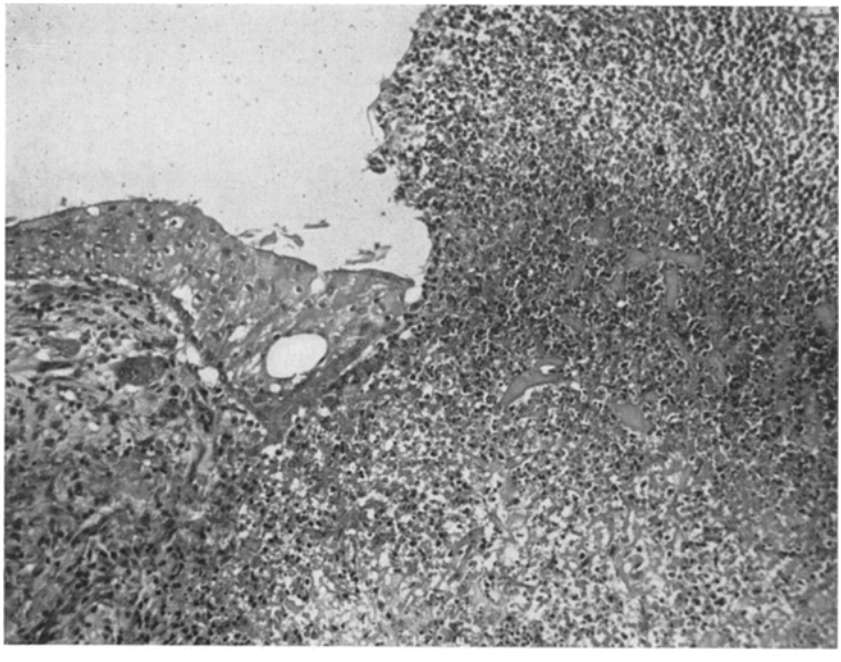


Fig. 2. Ulcerous pyelitis (H.E., × 120)

examination was made, using the method of cover slip agglutination with specific anti-OB serum to establish the identity of the detected E. coli strain with that used to produce bladder infection.

A suspension of bacteria in saline was prepared in a 18-hour agar medium with the haemolytic variant of the E. coli strain, serotype 026:B6; details were described in a previous paper (HATALA and PRÁT, 1963). The suspension was diluted to contain about $6 \cdot 10^9$ of living bacteria.

The exact number of bacteria in the suspension was checked by subsequent quantitative cultivation.

Evaluation of results. Histological findings in the kidneys were divided into three grades: 1. The most significant finding was a picture of acute purulent pyelonephritis and perinephritis (Fig. 1 c). 2. In some cases there were only focal inflammatory changes in the renal parenchyma (focal pyelonephritis) (Fig. 1 b); sometimes only the surface of the papilla or the pelvic wall were affected (Figs. 2 and 3). 3. Negative histological finding: small round-cell infiltrates in

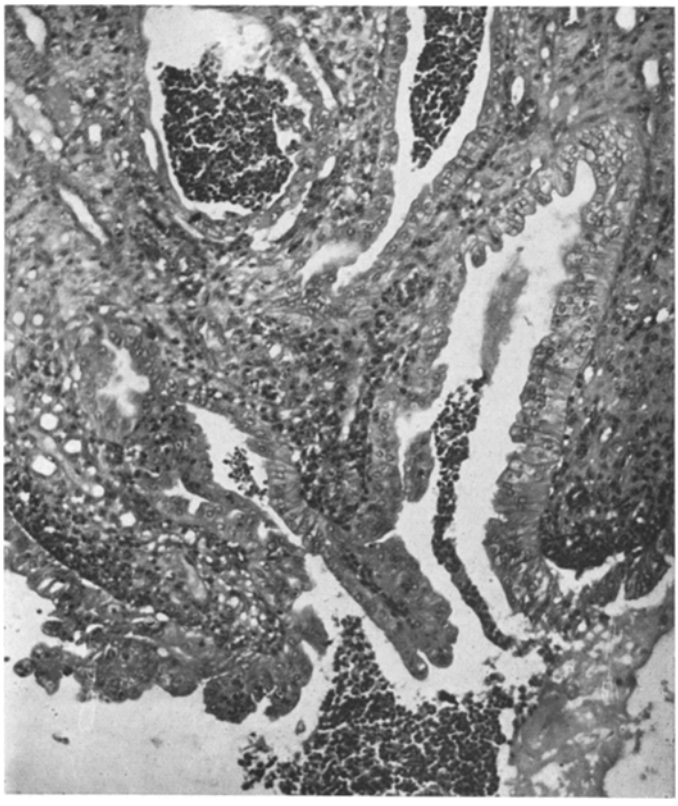


Fig. 3. Renal papilla with pus in efferent ducts (H.E., × 120)

the parenchyma or small focal fibrosis was considered a negative finding because they can be found also in intact rabbits (PRÁT et al., 1965). These findings were much more marked in rabbits of the control group where only sterile saline was injected into the bladder. Because necrosis of the papilla was also found frequently, it can be assumed that these morphological changes were caused by the penetration of the bladder content into the renal pelvis. A rather frequent finding was catarrhal-purulent cystitis.

A simple preliminary experiment was made to find out if the left ureter (after temporary ligature) was patent at the time of E. coli injection into the bladder. Through a bladder catheter an indigocarmin solution (23 ml) was injected into the bladder of three rabbits four hours after removal of the two-days ligature of the left ureter. Subsequently the animals were killed and after opening the abdomen it was investigated whether the blue coloured liquid penetrated through the ureters to the renal pelvis. In two animals both ureters were filled up to the kidneys; the site of previous ligature on the left ureter was readily visible. In one case the ureters were not coloured by the liquid but after compression of the bladder the blue liquid filled both ureters.

Results

Results of bacteriological and histological examinations are shown in tables 1 and 2. The bacteriological findings show the relationship between positive results in the renal tissue as well in urine and the size of bladder filling. The bacteriological

Table 1. *Results of bacteriological examination (injection of E. coli into the bladder 4 hrs*

Experimental group	Total No. of animals	Left kidney (No of E. coli/g)		
		negative	below 10^3/g	10^3/g and above
10 ml of E coli suspension	13	4	3	6
23 ml of E. coli suspension	18	3	0	15
Controls: 23 ml saline	8	8*	0	0

* In one case Str. faecalis was found in numbers of 10^6/g

Table 2. *Results of histological examination*

Experimental group	Total No. of rabbits	Left kidney			Right kidney			Bladder		
		negative	+	++	negative	+	++	negative	+	++
10 ml of E. coli suspension	13	8	1	4	13	0	0	8	4	1
23 ml of E. coli suspension	18	1	4	13	17	1	0	12	4	2
Controls: 23 ml saline	8	2	5	1	7	1	0	7	0	1

Kidneys: + = inflammation of pelvis or focal pyelonephritis; ++ = purulent pyelonephritis. Bladder: + = catharral-purulent inflammation; ++ = ulcerophlegmonous inflammation.

finding is more positive in the left kidney (after temporary ureteral ligature). All control animals are without E. coli infection. A similar relationship between the size of bladder filling and the incidence of purulent pyelonephritis is shown by the histological findings. A severe degree of renal inflammation was found only in the left kidney, which was damaged by urinary stasis after the ureteral obstruction. The character of inflammatory changes demonstrates the ascendent spread of inflammation into the renal parenchyma. The inflammatory process always involves the pelvic wall and the papilla; across the necrotic papillary tissue the inflammatory process spreads to the renal parenchyma and the renal cortex as well as the capsula.

Discussion

Bacteriological and histological findings showed that the presented method is suitable for creating a laboratory model of ascending acute purulent pyelonephritis in the rabbit. The typical inflammatory process developed only in the left kidney the ureter of which was temporarily ligatured. A further important factor which renders the penetration of bacteria from the bladder into the kidney possible is the volume of suspension, used to infect the bladder. It can be assumed that

during greater distension of the bladder wall the vesicoureteral reflux occurs more frequently than when a small volume of suspension is used. Histological findings in the control group, where 23 ml of saline are used, showed that the liquid penetrated into the pelvis and there the raised pressure damaged the surface of

after removing of the ligature of the left ureter. The animals were killed after 7 days)

Right kidney (No. of E. coli/g)			Urine (No. of E. coli/ml)			Haemoculture	
negative	below 10^3/g	10^3/g and above	negative	below 10^4/ml	10^4/ml and above	negative	positive
7	2	4	4	1	8	13	0
6	3	9	3	3	12	16	2
8*	0	0	8*	0	0	8	0

of tissue in the left kidney and 10^7/ml of urine.

the papilla; necrosis of the papillary mucosa promotes the penetration of infection into the renal tissue. Leucocytic infiltration in the pelvic wall and on the surface of the papilla in uninfected controls was not produced by bacteria because bacteriological findings were negative. Inflammatory infiltrates are most probably a reactive inflammation caused by mechanical injury of the epithelium when the liquid from the bladder reaches the renal pelvis.

Summary

A model of acute ascending purulent pyelonephritis was produced in rabbits by injection of an E. coli suspension into the urinary bladder with subsequent squeezing of the bladder wall. An important factor influencing the penetration of bacteria from the bladder into the renal pelvis was a sufficient volume of the bacterial suspension. Penetration of bacteria into the renal parenchyma and their multiplication in the renal tissue was rendered possible by temporary ligature of the ureter prior to the injection of bacterial suspension.

Experimentelles Modell einer ascendierenden Pyelonephritis beim Kaninchen

Zusammenfassung

Das Modell einer akuten ascendierenden eitrigen Pyelonephritis wurde bei Kaninchen mittels Injektion einer E. coli-Suspension in die Harnblase und nachfolgender Kompression der Blasenwand erzeugt. Ein genügend großes Volumen der Keimsuspension ist ein wichtiger Faktor, um das Eindringen der Keime aus der Blase in das Nierenbecken zu bewirken. Das Eindringen der Keime in das Nierenparenchym und ihre Vermehrung im Nierengewebe wird durch eine temporäre Harnleiterligatur vor der Keiminjektion in die Blase ermöglicht.

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