

EXPERIMENTAL PYELONEPHRITIS
ASCENDING INFECTION OF THE RAT KIDNEY BY ORGANISMS
RESIDING IN THE URETHRA*

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DURING an experiment performed in the female rat to study infection of the kidney by organisms introduced via the urethra it was noticed that when very small numbers of *Escherichia coli* were injected suspended in saline the organisms cultured from the kidney were frequently of a different species from that injected (Heptinstall 1964a). The most likely explanation was that the injection fluid during its passage along the urethra was picking up organisms normally resident in this structure and transmitting them to the kidney.

The present experiments were designed to test this and it was found that injection of sterile saline up the urethra gave rise to an infection of the kidney with the causative organism identical with that found in the urethra.

MATERIALS AND METHODS

Female Wistar rats of 200–240 g. were used and injections up the urethra made under light ether anaesthesia using a 2 ml. all glass syringe with the hub of a 21 gauge needle inserted at the external urethral meatus, the shaft having been broken off (Andersen and Jackson, 1961). The injections consisted of 1.5 ml. of sterile saline and after injection the external urethral meatus was lightly compressed between the rubber covered blades of artery forceps and the bladder gently compressed 20 times by grasping it between the fingers and thumb through the thickness of the abdominal wall. This procedure has been shown to aid reflux of fluid up the ureter from the bladder (Heptinstall 1964a). Culture of the urethra was performed by inserting a slightly flattened platinum loop up this structure after cleaning the external meatus with surgical spirit, and streaking the loop directly on to blood agar and desoxycholate agar plates. Kidneys were removed aseptically and after their surfaces had been washed with sterile saline were ground up in glass tubes by teflon pestles attached to a small electric motor. Appropriate dilutions were cultured in duplicate on blood agar and desoxycholate agar to give whole kidney organism counts. No attempt was made to determine the type of *Proteus* species when this was encountered and in the text will be referred to merely as *Proteus*. Operations for ligating the ureter were performed through the loin under ether anaesthesia, the occlusion being made near the pelvi-ureteric junction using fine silk.

RESULTS

(1) *Effect of injection of sterile saline up urethra*

In this part of the experiment the urethra was cultured and 1.5 ml. of saline injected up the urethra followed by squeezing the bladder 20 times. After 4 days the animals were killed and both kidneys removed and cultured. The

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TABLE I.—To show Results of Culture of Urethra before Injection, and Culture of Right and Left Kidneys 4 Days after Injection of 1.5 ml. Sterile Saline up Urethra Followed by 20 Squeezes of Bladder

Number of rat	Culture of urethra	Culture of kidneys (total organisms)	
		Right	Left
967	Pr. +, S.N. ±	7×10^4 Pr.	8×10^2 Pr.
968	S.N. +	0	0
969	Pr. ++, S.N. ±	1.2×10^3 Pr.	1.61×10^5 Pr.
970	E.C. +, S.P. ±	0	1.2×10^3 E.C.
971	E.C. +, S.N. ±	0	0
972	E.C. +++, S.N. ±	2.72×10^5 E.C.	10^5 E.C.
973	Pr. ++, S.N. ±, E.C. ±	4.8×10^3 Pr.	10^5 Pr.
974	Pr. +, S.N. ±, E.C. ±	1.4×10^3 Pr.	1.2×10^3 Pr.
979	S.N. ++	7.9×10^3 S.N.	0
980	S.P. ++, Dip. ±	8×10^4 S.P.	10^3 S.P.
981	E.C. ++, S.P. ±	3.6×10^3 S.P.	10^3 S.P.
982	Pr. ++, E.C. +++	7.6×10^3 Pr.	4.8×10^4 Pr.
983	Pr. ++++, S.N. ±	1.04×10^7 Pr.	4.68×10^6 Pr.
984	Pr. ++++	3.7×10^3 Pr.	6×10^5 Pr.
1007	E.C. ++++, S.P. +++	3.9×10^5 E.C. 7×10^4 S.P.	1.3×10^3 S.P.
1008	S.N. ++	0	2.4×10^4 S.N.
1009	S.N. ++, Ent. ++	0	6×10^2 S.N.
1010	E.C. ++++, S.N. +	6.2×10^4 E.C.	3×10^3 E.C.
1238	E.C. ++++, S.P. ++	2.3×10^3 S.P.	10^2 S.P.
1239	Ent. ++	7.8×10^4 Ent.	2.7×10^6 Ent.
1240	E.C. ++++	2.7×10^4 E.C.	4×10^2 E.C.
1241	Ent. ++++	6.9×10^5 Ent.	1.07×10^6 Ent.

S.N. = Coagulase negative staphylococci. S.P. = Coagulase positive staphylococci. E.C. = *Esch. coli*. Dip. = Diphtheroids. Ent. = Enterococci. Pr. = Proteus. Graded from ± to +++.

results are shown in Table I. From this it will be seen that several different organisms were normally found in the female rat urethra and that as many as 3 different species could be recovered from the same animal. A study of the organism counts of the kidneys reveals that there was a considerable variation in response, from animal number 983 with numerous abscesses visible to the naked eye to number 971 which showed no organisms in either kidney. This difference in response is probably a function of the degree of reflux which takes place up the ureters. It is noteworthy however that irrespective of the number of bacteria in the kidneys the species was identical with that recovered from the urethra. It is also of interest that the organisms cultured from any particular kidney were almost exclusively of a single species and that usually in any one animal the same species was found in both kidneys.

(2) Culture of kidneys from untouched rats

In order to eliminate the possibility that the kidneys of normal rats might harbour the different species of organisms encountered in the first part of the experiment, and also to determine the number of such organisms which might be present, both kidneys were removed from a group of 36 untreated rats and cultured. It was found that usually the kidneys were sterile but that coagulase positive and coagulase negative staphylococci were found in numbers of $<10^3$ in a certain proportion of kidneys. No other organism was encountered.

(3) *Effect of injection of sterile saline up urethra followed by permanent occlusion of left ureter*

It has been shown that very severe renal infection may be produced by organisms introduced into the pelvis of the kidney when the ureter is subsequently ligated (Heptinstall 1964b). It was felt that this would be a convincing way of demonstrating the presence of organisms in the kidney and seeing how they compared with those derived from the urethra. Precisely the same procedures were carried out as described above (1) except that immediately after the injection was made the left ureter was occluded through the loin. The right ureter was not touched and the animals were killed after 4 days and both kidneys cultured.

TABLE II.—*To show Results of Culture of Urethra before Injection and Culture of Right and Left Kidneys 4 Days after Injection up Urethra of 1.5 ml. Sterile Saline and 20 Squeezes of Bladder*

Left ureter was occluded immediately after injection of saline and remained occluded for 4 days.

Number of rat	Culture of urethra	Culture of kidneys (total organisms)	
		Right	Left
1035	S.N. +, Ent. ++, E.C. ±	1.4 × 10 ⁴ E.C. 2 × 10 ³ Ent.	3.68 × 10 ⁷ E.C.
1036	Pr. +, E.C. ++, S.N. +	10 ⁶ Pr.	4.8 × 10 ⁷ Pr. 6 × 10 ⁶ S.N.
1037	E.C. ++, S.P. +	1.3 × 10 ³ S.P.	3 × 10 ⁷ S.P.
1038	E.C. ++, Ent. +	7 × 10 ³ E.C.	1.4 × 10 ⁷ E.C.
1039	Ent. ++, S.P. ±	4.6 × 10 ⁵ Ent.	1.05 × 10 ⁷ Ent.
1053	E.C. ++++, S.N. ++++	9 × 10 ⁵ E.C.	5.6 × 10 ⁷ E.C.
1054	S.P. ++	1.1 × 10 ⁶ S.P.	4.2 × 10 ⁶ S.P.
1055	S.P. ++++, Pr. ±	5.2 × 10 ⁴ S.P.	6.12 × 10 ⁷ S.P.
1056	Pr. ++, E.C. +, S.N. ±	8.2 × 10 ⁶ Pr. 7 × 10 ⁵ S.N.	1.97 × 10 ⁷ Pr. 6.2 × 10 ⁶ S.N.
1057	Pr. +++	2 × 10 ⁶ Pr.	3.89 × 10 ⁷ Pr.
1242	S.N. +, Dip. +	1.1 × 10 ³ S.N.	7.46 × 10 ⁷ S.N.
1243	0	0	2 × 10 ³ S.N.
1244	Pr. ++++, S.N. ±	8 × 10 ⁶ Pr.	1.85 × 10 ⁷ Pr.
1245	S.N. ++	1.8 × 10 ³ S.N. 1.4 × 10 ³ S.P.	3.87 × 10 ⁵ S.N.

S.N. = Coagulase negative staphylococci. S.P. = Coagulase positive staphylococci. E.C. = *Esch. coli*. Dip. = Diphtheroids. Ent. = Enterococci. Pr. = *Proteus*. Graded from ± to +++.

The results are shown in Table II and it will be apparent that the right kidneys showed substantially the same picture as the right and left kidneys in Table I but that the left kidneys had counts of a considerably higher order as anticipated. However the same species was found in both right and left kidneys and this correlated well with the species isolated from the urethra. In animal number 1035 enterococci were found in addition to *Esch. coli* in the right kidney, whereas they were absent from the left; coagulase negative staphylococci were found as a second organism in the left kidney of rat number 1036, being absent from the right.

(4) *Effect of permanent occlusion of left ureter in normal rats*

In order to exclude the possibility that the results of the last experiment might be due to an undue proliferation of organisms normally resident in the

kidney, a group of 10 normal rats was taken and after culturing the urethra the left ureter was occluded immediately. The animals were killed after 4 days and the kidneys cultured.

It was found that only 2 occluded left kidneys had high counts (6×10^5 in one and 5.2×10^5 in the other), the organism being a coagulase negative staphylococcus in each case. The same organism was present in small numbers in the opposite non-occluded kidney in each of these two rats. In the other 8 rats, cultures were sterile or showed only few staphylococci on both sides.

DISCUSSION

It is quite clearly demonstrated from experiments 1 and 3 that injection of sterile saline up the urethra can cause infection of the kidney. The evidence that the organisms come from the urethra is good but before presenting this some notice of previous work would be helpful. During the past 2 yr. we have been investigating various aspects of ascending infection (Heptinstall, 1964*a* and *b*) and in these experiments it has been shown that reflux of solutions up the ureter occurs with great frequency when amounts of 0.5–1.5 ml. are injected up the urethra. Reflux occurs more frequently when the larger dose is injected, and this can be still further increased by gentle squeezing of the bladder through the abdominal wall. When testing the thesis that organisms found in the kidney following urethral injection of sterile saline come from the urethra, it is clearly important to use the method giving maximum reflux and for this reason the injected amount was 1.5 ml. coupled with 20 squeezes of the bladder.

The evidence that the organisms come from the urethra seems acceptable. First there is good correlation between the various species isolated from the urethra and the species found in the kidney. This is clearly shown in experiments 1 and 3 in which the organism responsible for infection in the kidney never differed from that found in the urethra. In experiment 3, use was made of the fact that organisms delivered to the renal pelvis will affect the kidney more severely when the ureter is subsequently ligated. This is a very striking effect and elsewhere it has been shown that organisms reaching the renal pelvis will cause widespread abscess formation within 1 or 2 days if the ureter is subsequently ligated after introducing *Esch. coli* from below (Heptinstall 1964*b*). When the right and left kidneys in experiment 3 are compared it will be apparent that the same species is present in both kidneys but that much higher counts (with almost constant abscess formation) are present in the left. This suggests that a similar species was delivered to both kidneys from below, the subsequent obstruction accounting for the greater counts in the left kidney.

Second, it might be argued that the organisms responsible for the renal infection are normally present in the kidney and that they are merely stimulated to proliferate by reflux of sterile saline, either because of local damage inflicted by the refluxing fluid (forcible reflux of fluid can cause haemorrhages in the fornices) or in the case of the left kidneys of experiment 3 by obstructing the ureter. Experiment 2 in which kidneys from normal rats were cultured shows that staphylococci are the only organisms found and that these are present only in small numbers. Except in the animals in experiments 1 and 3 where the staphylococcus is the organism responsible for infection, the concept that infections are caused by normal residents of the kidney is untenable. The likelihood that

normal inhabitants of the kidney can give rise to infection when the ureter is obstructed was tested in experiment 4. No excessively high organism counts were found in the obstructed left kidneys and in two with moderately raised counts staphylococci were cultured. It should be mentioned that in some rats encountered in previous experiments tying the ureter of an apparently normal rat kidney led to a pyelonephritis with staphylococci and this has also been recorded by Guze and Beeson (1956a). Thus although renal infection consequent on injection of sterile saline up the urethra followed by tying off the left ureter might be explained by proliferation of organisms normally resident in the kidney in the case of staphylococci, there is nothing to suggest that infection by other species could take place by this means.

Certain points of importance emerge from this study. The first is the great ease with which the urethral organisms become transported to the kidney, the main factor of course being the frequency and facility with which reflux up the ureter occurs in the rat. What is rather impressive is the way in which first, sufficient organisms reach the kidney pelvis to cause infection of the kidney, and second the dramatic way in which the organisms delivered to the kidney can cause an extremely florid pyelonephritis which virtually destroys the kidney when the ureter is subsequently ligated. It is tempting to draw parallels with the human in this situation. The presence of a bacterial flora in the urethra of presumably healthy people is now well established (Helmholz, 1950; Shackman and Messent, 1954) as is the fact that these organisms can be transmitted to the bladder and cause infection of the urine by catheterization (Guze and Beeson, 1956b). Reflux in the human is clearly not so common as in the rat but there is increasing evidence to show that it is considerably more frequent in association with infections than was at one time supposed (Bumpus, 1924; Forsythe and Whelan, 1958; Hodson and Edwards, 1960; Hutch, Miller and Hinman, 1963). The present experiments lend weight to the idea that reflux in the human could serve as a potent means by which organisms in the urine can reach the kidney. The manner in which a severe pyelonephritis follows obstruction of the rat ureter after organisms have been delivered to the renal pelvis is also paralleled by the greater incidence of pyelonephritis in patients with obstruction (Bell, 1942).

A second interesting fact which emerges from this study is the way in which infections induced in the kidney by injections of sterile saline are usually caused by a single species, and this occurs in rats which have as many as 3 different species in the urethra. This could possibly be explained by the urethral organisms being present in different numbers so that the predominant organism only was carried up to the kidney. This in general is true for when the relative numbers of urethral organisms (Tables I and II) are compared with the organism responsible for the kidney infection it is generally found that the urethral organism present in greatest numbers is that found in the kidney. This however is not invariable, for example in Table II rat 1035 showed the organism responsible for infection in the left kidney to be *Esch. coli* whereas only few of this species was found in the urethra. Similarly, rat 1036 showed renal infection by *Proteus* and coagulase negative staphylococci whereas *Esch. coli* was the predominant urethral species. Alternatively one particular species of organism could have a special propensity for growing in the kidney and the results are suggestive that *Proteus* infections will usually predominate if present, but the numbers are too small to state this with any certainty. The dosage effect however is clearly important for in several

hundred rats studied by the urethral injection of high concentrations of *Esch. coli*, additional kidney infection by *Proteus* has occurred very infrequently, and although urethral cultures were not made in these rats it is inconceivable that this species was not present in the urethra of a large number of animals. However, when very dilute cultures of *Esch. coli* are injected *Proteus* infection is commonly found. From this it might be inferred that high initial numbers of *Esch. coli* in the kidney can suppress *Proteus* infection.

SUMMARY

It has previously been observed that when a very dilute culture of *Esch. coli* is injected up the urethra, infection of the kidney is frequently caused by organisms other than *Esch. coli*. To test whether these organisms have their origin in the urethra and are carried up to the kidney during the injection, a sterile saline solution was injected up the urethra after culturing this structure. Kidneys were examined bacteriologically after 4 days.

It is found that renal infection may be produced by such means and that there is a good correlation between the organisms cultured from the urethra and those from the kidney.

The renal infection can be considerably accentuated if one ureter is ligated immediately following the urethral injection, the organism responsible being the same as that found in the opposite non-obstructed kidney.

The frequency with which vesico-ureteric reflux occurs in the rat is an important factor in the production of these ascending infections.

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