ASCENDING URINARY TRACT INFECTION IN RATS CAUSED BY *PROTEUS MIRABILIS*

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خمج المسالك البولية الصاعدة في الجرذان
المتسبب بالبروتيس ميرابيليس *PROTEUS MIRABILIS*

طور المولفون نموذجاً لاحداث خمج، المسالك البولية غير الانسدادي في إناث الجرذان، لدراسة امراضية هذه النوعية من الاخماج الكلوية من نوع *Proteus Mirabilis* حيث تم تلقيح مثانة الجرذان بمرق نقيع الدماغ والقلب والذي يحتوي على خلايا بكتيرية حية ثم جمعت عينات من الدم والبول كما تم استئصال الكلى وذلك في الايام ٢، ٤ وفي الاسابيع ١-٨ لدراسة الخمج الكلوي وتقدير الوظائف الكلوية. وقد اوضحت النتائج تفاوت واستدامة الخمج البكتيري طوال فترة الدراسة، كما ارتفع تركيز يوريا الدم بصورة معنوية بعد يومين من التلقيح واستمر في الزيادة حتى نهاية التجربة. كما إزداد مستوى كرياتينين المصل في الأسبوع الثالث، واستمرت مستوياته بالارتفاع حتى نهاية التجربة وعلى العكس منه انخفض مستوى الكرياتينين وبصورة معنوية في اليوم الرابع واستمر مستواه بالانحدار حتى انتهاء الدراسة.

Key-Words : Uninarytract, Rats, *Proteus mirabilis*.

ABSTRACT

A model for ascending unobstructed urinary tract infection was developed in female rats to study the pathogenesis of urinary tract infection by *Proteus mirabilis*. Urinary bladders of rats were inoculated by I. V. cannula with 0.5 ml of brainheart infusion broth containing approximataly 3 x 108 living bacteria. Kidney, blood and urine samples *collected* at days 2, 4 and weeks 1-8 intervals were studied for renal infection as well as renal function tests.

The results indicated the variation and persistence of *Proteus* infection in rats kidney during the experiment. Blood urea nitrogen was significantly increased after 2 days and continued to increase throughout the experiment. Serum creatinine increased signticantly on 3rd week and increased steadily to the end of experiment. Creatinine clearance fell markedly at 4th day and continued to fall thereafter.

INTRODUCTION

In studies of pathogensis of bacterial infections attention has been paid to kidney infection in man caused by *Escherichia coli, Proteus, Klebsiella,* Staphylococci, and (1-3). *Proteus mirabilis* is a frequent cause of these infections, especially in rehabilitation hospitals and persons with structural abnormalities of the urinary tract (4). In addition, *Proteus* often infects elderly patients (5), children and infants (6). *Proteus* is commonly localized in the kidney of patients with urinary tract infection [UTI] (7), and generally produces a more severe disease than *E. coli* and other bacteria owing to the production of urease (8, 9) and other virulence factors (10, 11).

In Iraq bacteriological investigations for renal infections demonstrated that *Proteus* was the second major genus causing UTI *P. mirabilis* was the most common species in these infections (12, 13, 14). To our knowledge there have been no studies on the effect of strains *of P. mirabilis* from Iraq on the kidney. The present study was undertaken therefore to investigate the role of *P. mirabilis* isolated from Iraqi patients with UTI in an attempt to understand how the ascending UTI with this bacteria can affect the urinary system.

MATERIAL AND METHODS

Animals: Ninety young adult female rats 150-180 gm/and bred in the department were fed Laboratory chow and water *ad lib.* The animals were housed in plastic cages and transferred to metabolic cages 24hr prior being sacrificed, for colelction of all urine passed.

Bacterial inoculum: The bacterial inoculum was from a strain of *P. mirabilis* freshly isolated from the urine of a patient with UTI. The bacteria were cultured in Brain-heart infusion broth for 18hs at 37C° prior to bladder inoculation.

Induction of pyelonephritis: Under ether anesthesia, the external urethra was swabbed with 70% alcohol and the inoculum was infused through urethra into the bladder using an I. V. cannula (22G x 25mm). To facilitate the entrance of the cannula, the ventral lip of the urethra was grasped with sterilized straight forceps and gently extended in a caudal direction. This tended to straighten the urethral passage and prevent the inoculum leackage upon injection. With practice, this process was accomplished with the minimum of trauma.

Each animal in the experimental group was inoculated with 0.5 ml of broth containing approximately 3×10^8 (log 8.5) viable bacteria. Control animals were inoculated with the same volume of sterile broth. Six experimental and three control animals were sacrificed at 2 and 4 days and at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after inoculation.

Collection of samples: At sacrifice and after light ether anesthezia, blood was obtained from the animal by the cardiac puncture. The abdominal region was swabbed with 70% alcohol and the kidneys were removed aseptically and divided in two halves. One half from each kidney was reserved for future pathologic study while the other halve was homogenised in 5 ml distilled water to prepare ten-fold serial dilutions. The dilutions were plated on MacConkey agar (Oxoid 115) and incubated for 24-48hs at 37°. The number of bacteria was expressed as the logarithm of colony forming units (log CFU) per gram fresh kidney weight.

The impairment of renal function was investigated by determination of blood urea nitrogen (BUN) using the diacetyl monoxime method and by estimation of serum and urine creatinine (SC & UC) using the Jaffe method (15). Creatinine clearance (CC) was measured as a glomerular filtration rate (GFR) from the equation:

$$CC \ (\ /ml \ / \ min \ ) = \frac{UC \ x \ urine \ vol/min.}{SC}$$

The geometric means of results were used for statistical analysis. The differences between experimental and control groups were evaluated for significance with Student's-t-test and the data were expressed as the mean ± SD of the mean.

RESULTS

Evolution of infection: The viable count obtained from animal kidneys infected with *P. mirabilis* are given in Fig. 1. Infection was established in 100% of the animals as determined by recovery of the bacteria from the kidneys 2 days to 8 weeks after infection. The frequency of bacterial infection varied throughout the experiment. There was significant reduction (p< 0.01) in the number of bacteria isolated from the kidney on the 2nd day; the count was log 5.4 ± 0.78 compared to bladder inoculation (log 8.5). On the 4th day, the number of bacteria increased sharply [log 10.3 ± 0.15] (P < 0.05) and remained around this level for

about 7 days (P >0.05). On the 2nd day it remained at this level for about 7 days (P > 0.05). On the 2nd week a significant decline in kidney bacterial content was observed [log 5.93 ± 2.21] (P < 0.05) and counts remained approximately at this level to the end of the experiment (P > 0.05).

The kidneys of control animals were clear of *P. mirabilis*, but few animals contained *E. coli* or *Bacillus* which might been due to contamination during handling of animals at the end of the experiment.
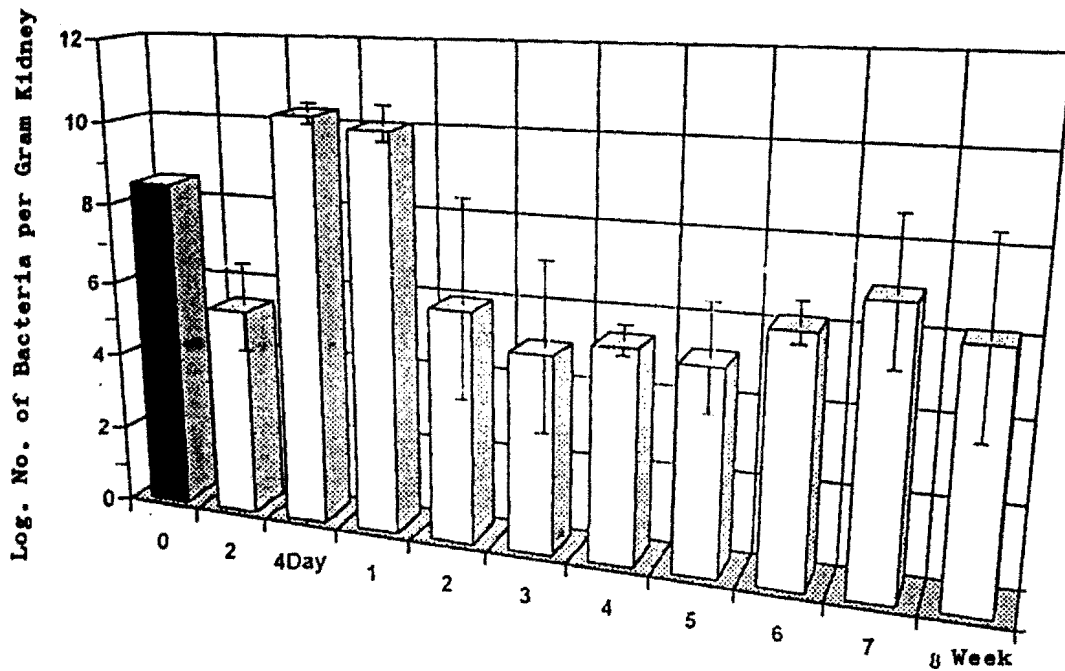
Fig (1) Bacterial counts in rats kidney from 1 day to 8 weeks (▢) after bladder inoculation (■) with 3 x 108 *Proteus mirabilis*. The results are shown as mean ± IS.D.

Kidney functions: Experimental animals showed progressive increase in BUN (Fig 2). On the 2nd day BUN increased sharply to 42.61 ± 2.13 mg/dl compared to the control values: 30.23 ± 3.51 mg/ dl (p < 0.05) and continued to rise over the 2nd week, reaching 72.64 ± 7.97mg/dl (P < 0.001). This level of BUN remained approximately constant over the following weeks without significant differences between the treated groups (P > 0.05).

Serum creatinine levels also rose on the 2nd day (Fig 3), with insignificant increases (P >0.005), reaching 0.74 ± 0.05 mg/dl compared to control values (0.7 ± 0.02 mg/dl, (p < 0.001). This increase continued throughout the experiment, reaching 1.06 ± 0.08 mg/dl (P < 0.001) on the 8th week. However, no significant differences (P > 0.05) were found between the treated groups.
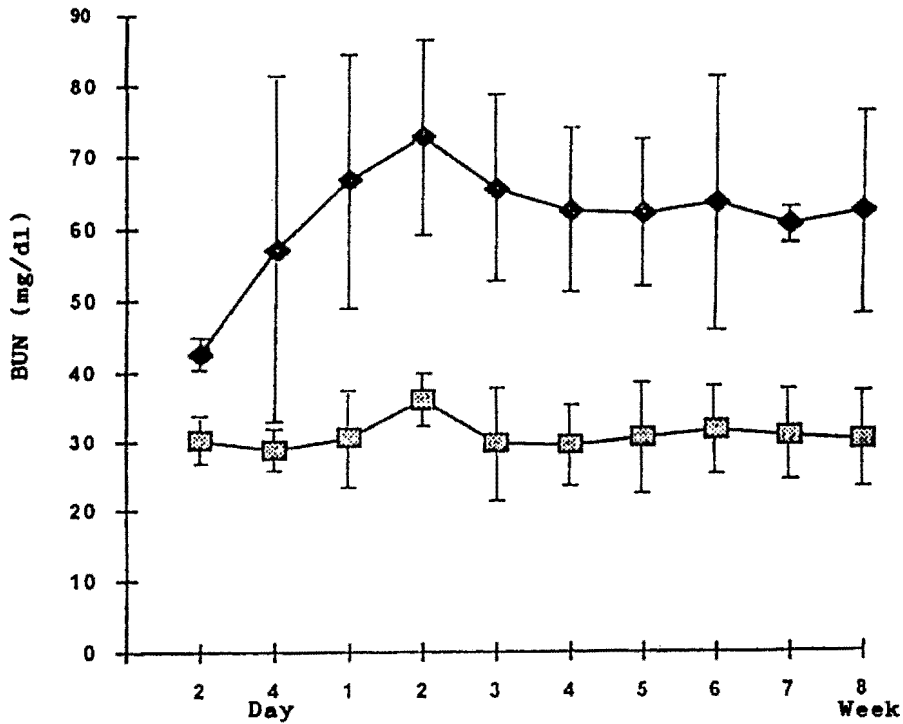
Fig (2) Blood urea nitrogen (BUN) levels in rats from 1 day to 8 week after transurethral infection with *Proteus mirabilis* (◆) compared to control levels (❑) . The results are shown as mean ± IS. D.

Creatinine clearance decreased markedly and significantly (P < 0.05) in treated animals on day 4, reaching 0.6 0.25ml/ min compared to the control (1.02 ± 0.13 m//min). Creatinine clearance continued to fall significantly (P < 0. 001), reaching 0. 44 ± 0.14ml/min on the 1st week and then remained more or less constant. No significant differences (P > 0.05) were observed between treated groups (Fig 4).
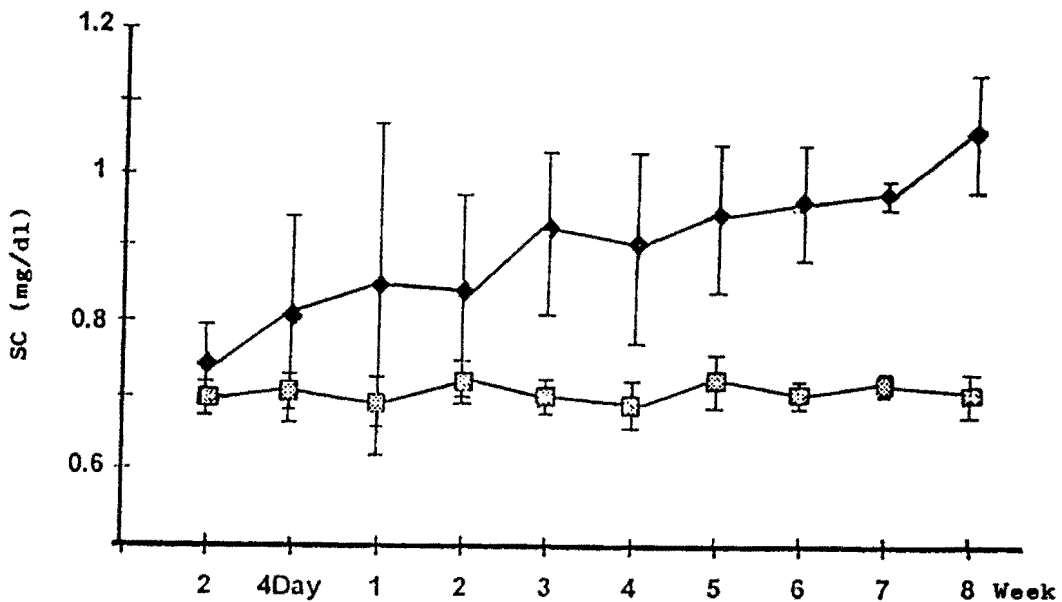

Fig (3) Serum creatinine (SC) levels in rats from 1 day to 8 weeks after transurethral infection with *Proteus mirabilis* (◆) compared to control levels (❑). The results are shown as mean ± IS. D.
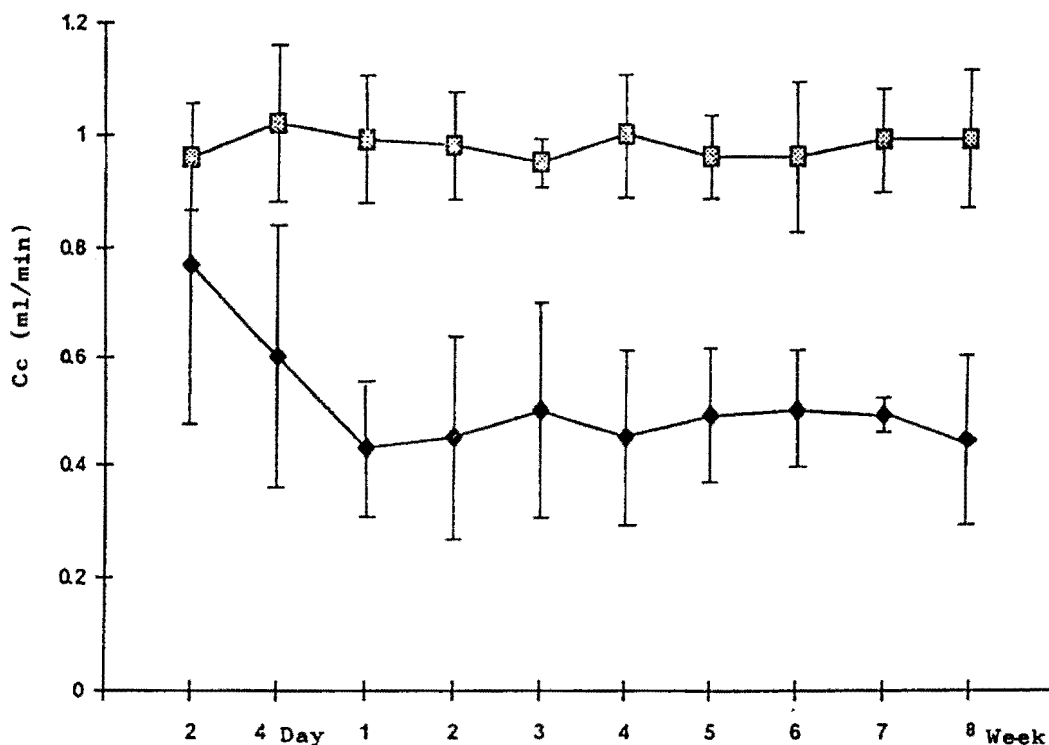
Fig (4) Creatinine clearance (Cc) levels in rats from 1 day to 8 weeks after transurethral infection with *Proteus mirabilis* (♦) compared to control levels (❑). The results are shown as mean ± IS. D.

DISCUSSION

Several procedures have been developed to study the pathophysiology of kidney damage and other aspects on the host-parasite relationship in UTI. These procedures use the hematogenous routes (16, 17, 18), ascending routes (19, 20) and direct routes (21) to induce experimental pyelonephrities. Many animal species used in these studies do not readily develop UTI, therefor most experimental models stipulate a mechanical manipulation in the urinary tract to ensure a high frequency of pyelonephritic infection. Examples of such manipulation are bladder or kidney massage (16, 21), abdominal operation, often with temporary ureteral ligation or puncture of the kidney or bladder wall (18, 20, 21) and introduction of foreign bodies into the bladder (23, 24).

In the present study, ascending UTI was employed without mechanical manipulation and with little trauma or injury to the urinary tract. The subsequent pyelonephritis persisted for 8 weeks confirming the ability of *Proteus* infection to exist for an extended period in renal tissue. Earlier studies have shown that *P. mirabilis* maybe recovered from animal kidneys infected by cardiac puncture for up to 15 weeks (22, 25). Thomas and Tang (26) showed that the kidney of rats infected with *P. mirabilis* by ascending routes

contained only 1% of the inoculated bacteria on the 6th day and were free of bacteria on the 4th week. The difference between this result and ours may be due to differences in the virulence of strains used. The persistence of *P. mirabilis* renal infection has been related by many studies in tissue culture to the intracellular invading ability of this bacteria (27, 28).

The present study has shown early reduction in the number of bacteria ascending to the kidney on the 2nd day after inoculation. This early reduction might be related to the bladder voiding fallowing the inoculation (29.30). Also, the inability of *P. mirabilis* to adhere to the bladder urothelium may assist the voiding of the bacteria from the bladder (1, 2, 31). The bacteria count reaches a maximum on the 4th day. This is consistent with observations on *E. coli* which was recovered in a higher number after 3 days of I. V. inoculation (32), and on *P. mirabilis* after 5 days of ascending inoculation (33). This elevation in bacterial numbers might be the result of spread and expansion of the bacterial invasion toward the renal medulla and cortex. Brooks (34) state that the earliest lesions in kidney were a bacterial colonies distributed in medulla, while cortical lesions were not observed until the 4th day (35). Many studies showed that the bacterial invasion of the pelvic

epithelium is the initial step in renal infection, for reasons which include poor blood circulation, higher concentration of urea, higher osmolalitty and lower PH, all of which tend to impair phagocytosis as well as inhibition of the complement effect (34, 36, 37).

The bacterial count fell significantly on the 2nd week. This fall was in agreement with the finding of Niekel et al. (38) and Brook et al. (20) in their studies on P. mirabilis and E. coli respectively. They showed that the reduction in bacterial count was associated with domination of inflamatory cells (lymphocytes and plasma cells) in kidney tissue and circulating antibody in the blood. This reduction may indicate that the infection was again restricted to the pelvic and medulla as suggested by Black (39), who studied bacterial distribution in kidney tissue by a flourescent technique, and found that when infection became chronic, the flourescent bacteria were always observed in pelvis while cortex and medulla contained only a flourescent amorphous in the marcophage assosiated with chronic inflamatory response.

The most impressive changes caused by ascending infection of P. mirabilis were related to reduced performance of kidney function. There were early increase in BUN, SC and reduction in CC.

The observations on BUN are in agreement with the reported azotemia developed in 1st week in animals inoculated with P. mirabilis (22, 40). Henry (41) postulated that crystal deposition and hyaline casts that occlude the renal tubules may increase the hydrostatic back pressure and this may reduce urine flow and allow increased urea reabsorption.

Serum creatinine concentration also increased progressively, but was less sensitive for renal functional changes and did not rise significantly until the 3rd week (P < 0.01), but then continued throughout the period of the experiment. These results are similar to those of Bergeron

et al. (42) in animals infected with E coli and suffering from severe destruction in many parts of the kidney. Arean (43) pointed out that renal insufficiency observed in Guinea pigs inoculated with Leptospira icterohaemorrhagiae precedes the interstital nephritis and is more intimately related to damage of tubular epitelium than to a primary inflammatory response of the interstitium. We observed that the CC fell markedly on the 4th day and reached the lowest level in the 1st week. This is in agreement with Gilbert (44) in a study on rats infected with E. coli. Bank and Aynedjian (45) also reported a significant decrease in GFR of animals infected with P. mirabilis and suffering from severe destruction in interistium.

It would appear from above observations that the mean of the renal function of treated animals reflect significant alteration from those of control. However, the wide range of standard deviation indicates that many of these animals possessed functional values near the range of the controls (Fig 2, 3, 4). It is possible that although bacterial infection induces many morphological changes (46), there are considerable proportion of intact nephrons possessing a compensatory hypertrophy to increase the GFR, as an adaptational response to compensate the altered degenerated nephrons (47).

Our observations on experimental P. mirabilis pyeloneephritis need further biochemical, ultrastructural and pathological studies. These are in progress. In general, the experimental method used was reliable, simple and required no special manipulations in the urinary tract. The development of the experimental disease resembled that of ascending UTI in man. Therefore, this method should be suitable for studies on the immune response, the protective effect of vaccination, the role of different virulence factors in establishing the infection and effect of antibiotics treatment.

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