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The Significance of the Bacterial Count in Pyelonephrititis due to Pseudomonas Aeruginosa in Animal Experiments

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Summary. In 22 rabbits it was possible to produce haematogenous pyelonephritis of the left kidney by means of a 48-hour ligature of the left ureter and a single intravenous injection of 1 ml of a pseudomonas aeruginosa suspension with 10^{11} living bacteria. In the follow-up study the antibiotic treatment with carbenicillin and gentamicin showed a high degree of effectiveness against the micro-organism, the level of the urinary bacterial count being taken as the parameter for the histopathological changes. Bacterial counts in renal tissue of up to 10^{4} bacteria/ml in histopathological and urinobacteriological residual findings after a 12-day period of treatment indicate the importance of a long-term antibiotic treatment of pyelonephritis.

Key words: Haematogenous pyelonephritis, Urinary bacterial count, Bacterial count of renal tissue, Histopathological findings.

Introduction

Pseudomonas aeruginosa pyelonephritis is characterized by its pathogenicity and its frequent resistance to therapy. Information as to the degree of clinical renal infection is obtained by urinary bacterial count. In most cases, however, this method does not reveal the true histopathological changes in the kidney.

Acute pyelonephritis was produced in the experimental animal by pseudomonas aeruginosa. In follow-up studies, bacterial counts were made from the renal pelvis and bladder urine of untreated and chemotherapeutically treated animals. The results of the bacteriological investigation of the urine were subsequently compared with the histopathological findings and the bacterial count of the renal tissue.

Material and Methods

1. The animals used for the experiments were 22 rabbits, on which we carried out the following operations and investigations

a) 1st Operation. Transabdominal preparation of the left kidney and the left ureter, culture of the bladder urine, ligature of the left ureter, biopsy of the left kidney. Closure of the wound. Intravenous injection of 1 millilitre (ml) of physiological saline solution with 10^{11} living pseudomonas aeruginosa bacteria per ml.

b) 2nd Operation (after 48 hrs). Re-exploration. Puncture of the renal pelvis for bacterial demonstration and count, culture of the bladder urine, removal of the ureteric ligature, and biopsies from both kidneys.

c) After manifestation of the pyelonephritis, the first group was treated with carbenicillin[1] (200 mg per kg body weight per day) and the second group with gentamicin (7 mg per kg body weight per day), in both cases for a period of twelve days. In each group one control animal received no antibiotic treatment.

d) As a bacteriological follow-up study we carried out suprapubic aspirations at intervals of two days up to the eigth day for the purpose of bacterial count and demonstration.

e) 3rd Operation. On the twelfth day after slipping of the ureteric ligature, a leftsided nephrectomy was performed for the purpose of histopathological examination and bacterial count in the renal

1 We used Microcillin of the Bayer AG

tissue. In addition a final bacteriological examination of the bladder urine was carried out.

2. Bacteriology

a) For the experiment a bacterium of the species pseudomonas aeruginosa from the urine of patient was used, which in the resistance test [1] had proved to be moderately sensitive (mE)[2] to carbenicillin and fully sensitive (E)[3] to gentamicin. During the period of the experiment the bacterium was transferred onto blood culture plates and 48 hrs before each intravenous injection was enriched in 5 ml of nutrient broth.

b) For the bacterial demonstration from the punctate, Endo plates were used. The bacterial count was performed by means of a dilution series using graduated pipettes. From the dilution series, 0.1 ml was smeared onto Endo-Agar and after 24 hours of aerobic cultivation at 37° C the individual colonies were counted and the total number of bacteria calculated.

c) For the tissue bacterial count, a segment of the medulla of the renal cortex weighing approximately 1.1 to 1.2 g was homogenized under sterile conditions in 5 ml of physiological saline solution. The bacterial demonstration and count were then carried out with the homogenized tissue in the manner already described.

Results

1. Clinical development and therapy

In 17 of the 22 animals we were able, by haematogenous means, to produce an acute pyelonephritis. After the 48-hour-ligature of the ureter, single or clustered micro-abscesses could be seen macroscopically in the congested left kidney.

In the first group, which had been treated intraperitoneally with carbenicillin at a daily dosage rate of 200 mg per kg body weight, two animals died from the effects of an intercurret general sepsis on the second and third day after commencement of therapy.

In the second group of seven animals, which had received gentamicin intraperitoneally at a daily dosage rate of 7 mg per kg body weight, three animals died: one from the effects of sepsis on the second day and the two others on the second and third day after the commencement of a therapy following the perforation of an abscess in connection with persistent ureteric obstruction.

In five animals we were unable to produce pyelonephritis due to reduced virulence of the bacterium or of the ureteric ligature.

Of the untreated control animals in the two groups one died on the first and the other on the second day after the second operation from the effects of a fulminant sepsis on the basis of a macroscopic phlegmonous-purulent pyelonephritis.

2. Bacteriological findings

In all rabbits the primary culture of the bladder urine showed no bacteria.

Before slipping the 48-hour ligature of the ureter we found - with the bladder urine partly sterile, partly slightly infected - a bacterial count of pseudomonas aeruginosa in the renal pelvis of 10^9-10^{11} bacteria per ml. Of the ten antibiotically treated animals the bacterial count in the bladder urine of the carbenicillin group showed a reduction of bacteria to 10^0-10^2 per ml after 8 days, and in that of the gentamicin group after 6 days. On performing the third operation after 12 days, we found that with continued treatment in one animal of the carbenicillin group and two animals of the gentamicin group the bladder urine was sterile. In five animals of the carbenicillin group and two of the gentamicin group, however, we were still able to observe sporadic growth of pseudomonas aeruginosa with a bacterial count of 10^1-10^2. In all treated animals the bacterial count of the renal tissue still showed 10^3-10^4 bacteria per gramme of tissue (Fig. 1).

3. Histopathological findings

After a 48-hour ligature of the ureter with simultaneous pseudomonas infection the histopathological finding at this stage was an acute focal pyelonephritis in the region of the boundary between the medulla and the renal cortex (Fig. 2). After a 12-day period of treatment with carbenicillin or gentamicin, only very sporadic histopathological findings of leucocytic infiltration of the boundary between medulla and the renal cortex were obtained (Fig. 3). In the two untreated control animals a post-mortem histopathological examination showed numerous confluent pyelonephritic foci ascending from the medulla into the cortex as in a phlegmonous pyelonephritis (Fig. 4).

Discussion

1. With a 48-hour ligature of the ureter the bacterial count required to produce pyelonephritis with certainty by intravenous injection of 1ml of a watery suspension with virulent pseudomonas aeruginosa bacteria is in the region of 10^{10}-10^{11} bacteria per ml. With less than 10^7 bacteria per ml the renal pelvis urine was found to be sterile, while in the kidney there was no histopathological evidence of pyelonephritis. With bacterial counts of over 10^{12} per ml or with a second injection on

1 PDM Test Plates, Messrs. A.B. Biodisk, Sweden; Germany: Messrs. Medac GmbH, 2 Hamburg 36.

2 mE = 256 - 33 ug/ml carbenicillin.

3 E = 1 ug/ml gentamicin.

the following day the animals die of sepsis within a few hours.

2. In the treatment of the pseudomonas aeruginosa pyelonephritis, a favourable influence on the inflammatory processes was achieved by the use of carbenicillin and gentamicin. Here it is to be noted, however, that in the resistance test in vitro the bacterium was fully sensitive to gentamicin but

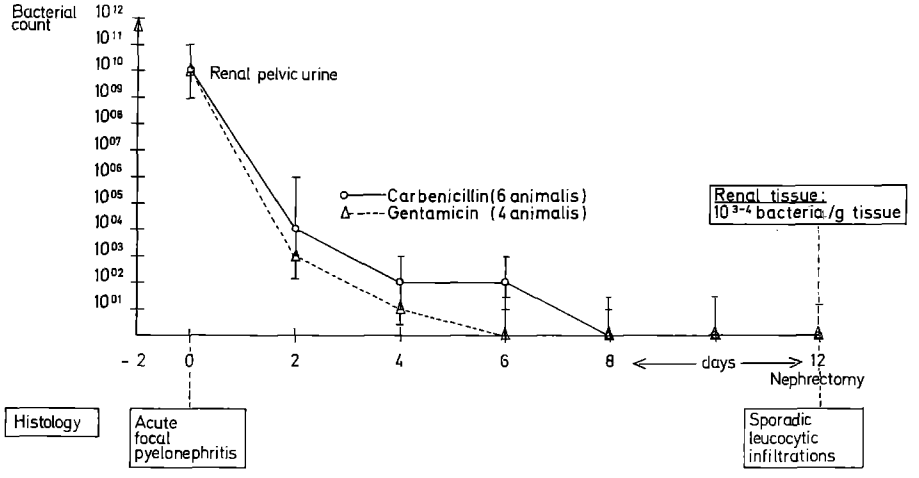

Fig. 1. Relations between the bacterial count of the renal pelvic urine, bladder urine, and renal tissue on the one side and the histopathological findings on the other during a 12-day period of observation under therapy

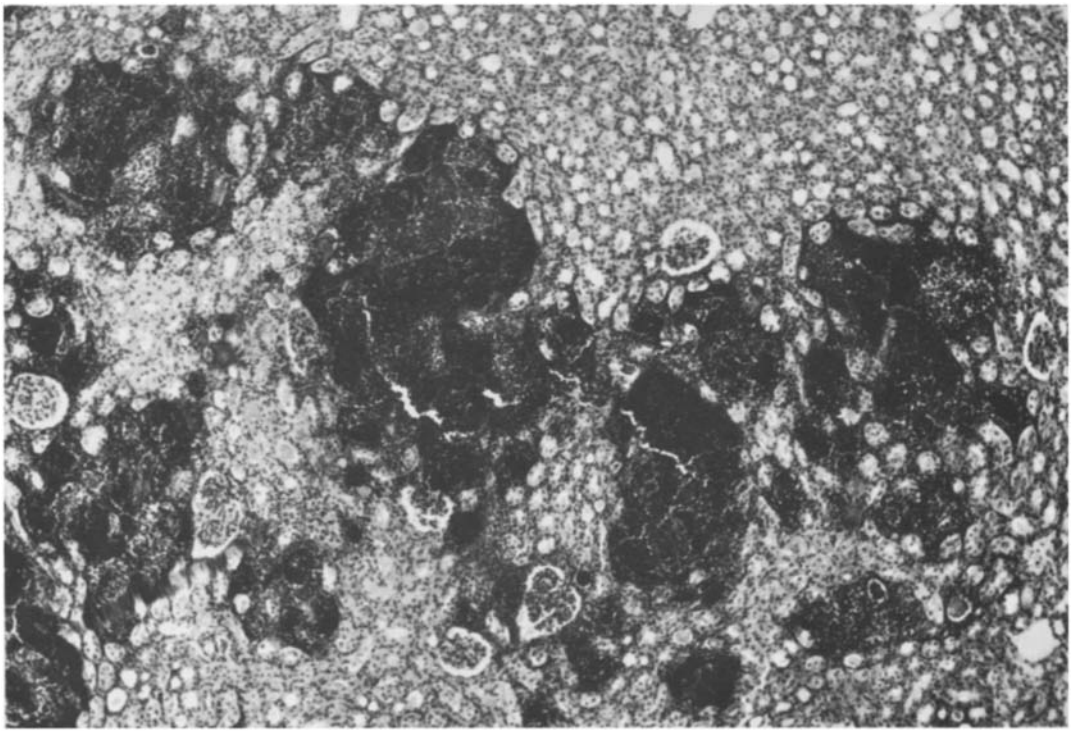

Fig. 2. Acute focal pyelonephritis after 48-hour ureteral ligature and intravenous application of a pseudomonas aeruginosa suspension of 1 ml 10^{11} living bacteria

only moderately sensitive to carbenicillin. Where ureteral obstruction is persistent or the general condition of the animal is poor, it is possible that, in spite of the antibiotic treatment, a urosepsis which is not capable of being influenced, may develop.

3. In experimental pseudomonas aeruginosa pyelonephritis there is a relation between the uri-

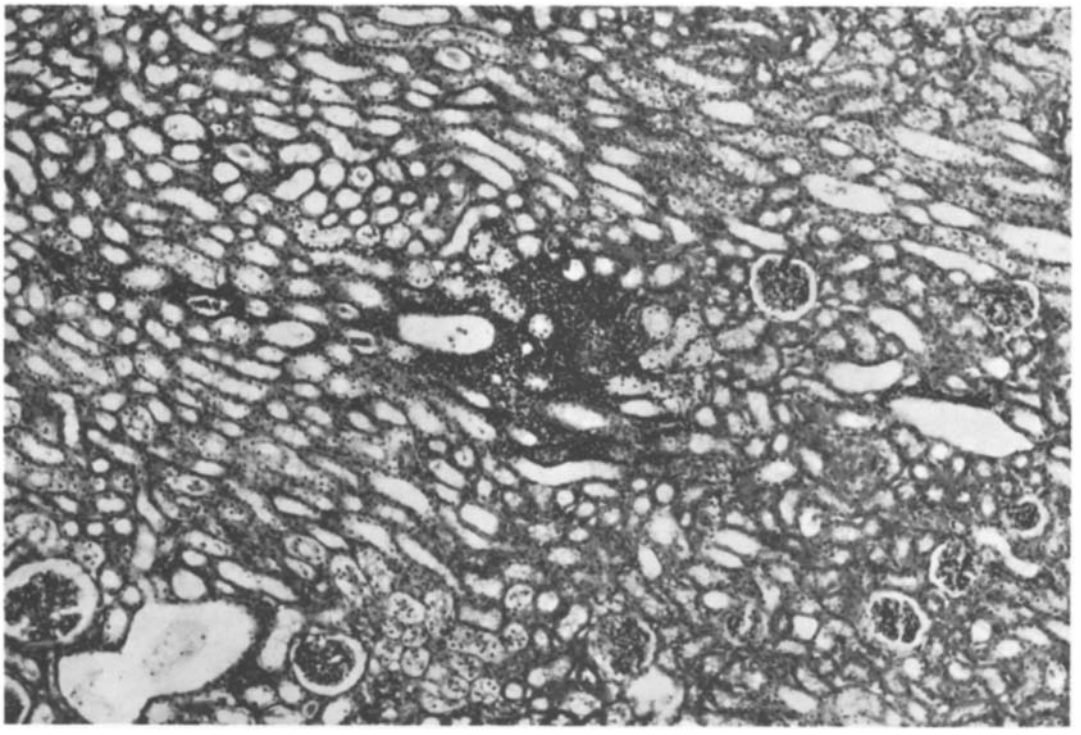

Fig. 3. Sporadic leucocytic infiltrations of the boundary of the medulla of the renal cortex after 12-day treatment with carbenicillin (200 mg per kg body weight per day) or gentamicin (7 mg per kg body weight per day)

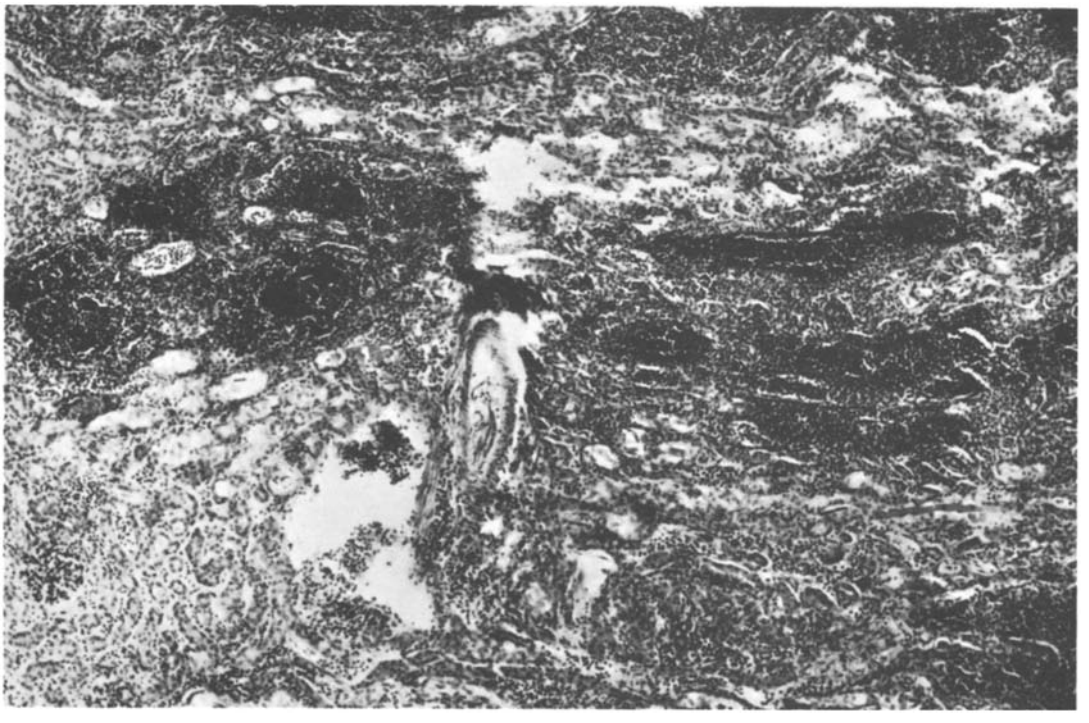

Fig. 4. Phlegmonous pyelonephritis in an untreated animal two days after slipping the ureteral ligature

nary bacterial count and the histopathological findings. The level of the urinary bacterial count serves as parameter for the degree of severity of the histopathological changes.

4. Bacterial counts in renal tissue of 10^3-10^4 bacteria per ml and histopathologically determinable leucocytic infiltrations of the boundary between the medulla and the renal cortex with sterile or only slightly infected bladder urine after a 12-day treatment period show the necessity of improved antibiotic treatment.

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References

1. Brainerd, H.J., Cecil, L.M.: Observations on the pathogenesis, course and treatment of non-obstructive pyelonephritis. Ann. intern. Med. 45, 232 (1956)
2. Caselitz, F.H.: Erzeugung von Infektionen durch Pseudomonadaceae und Achromobactergruppen. In: Handbuch der experimentellen Pharmakologie XVI/10, 1-38. Berlin-Heidelberg-New York: Springer 1966
3. Erlanson, P., Jonsson, G.: Bacterial aspects of chemotherapy of surgical urinary infections. Occurence of resistance to chemotherapeutic agents. Acta. chir. scand. 106, 399 (1953)
4. Prat, V.: Die Infektionswege. In: Losse/Kienitz, Die Pyelonephritis, 9-19. Stuttgart: G. Tieme 1966
5. Thelen, H., Rother, K., Sarre, H.: Experimentelle Untersuchung zur Pathogenese der pyelonephritischen Schrumpfniere. Urol. int. 3, 359 (1956)
6. Wiesmann, E.: Bakteriologie. In: Handbuch der Urologie, Bd. IX/1, 7-21. Berlin-Heidelberg-New York: Springer 1964

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