# LABORATORY AIDS IN THE DIAGNOSIS AND TREATMENT OF URINARY TRACT INFECTIONS

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PYELONEPHRITIS and related infections of the urinary tract are among the most frequently undiagnosed and most difficult to manage of all infectious diseases. A few selected data will indicate the magnitude of this problem: (1) pyelonephritis is the commonest renal lesion found at autopsies, and has been reported in up to 20 per cent of consecutive necropsies;[1,2] (2) the clinical diagnosis of urinary tract infection had been made in only 20 per cent of those patients who at necropsy were found to have active pyelonephritis, and the diagnosis is missed equally often whether massive or minimal lesions are present.[2] This disparity between clinical diagnosis and autopsy findings indicates that overt clinical symptoms of pyelonephritis are frequently absent.

The natural course of pyelonephritis has been described as a series of separate episodes of infection. Now, evidence suggests that pyelonephritis is a disease continuum, and in many cases the episodes of acute infection are linked by an asymptomatic but active infection that is constantly taking its toll on renal function.[3] The initial event in the relentless course of chronic pyelonephritis may occur during childhood. Macaulay and Sutton,[4] as well as others,[5,6] reported that chronic infection developed in from 30 to 60 per cent of children who had urinary tract infection in childhood. The recent studies by Kass[7] on the occurrence of true bacilluria (100,000 or more organisms per milliliter of urine) in asymptomatic patients have shown a high incidence of asymptomatic pyelonephritis in the general population.

It becomes readily apparent that one cannot depend on typical symptoms and signs alone to make the diagnosis of pyelonephritis. The purpose of this paper is to review briefly the pathogenesis and factors that influence the course of pyelonephritis and to present the laboratory methods that may be of some value in the diagnosis and treatment of pyelonephritis.

## Pathogenesis

Major gaps exist in our knowledge of the pathogenesis of pyelonephritis. We do know that bacteria may reach the kidneys by four possible routes; some experimental evidence as well as clinical data supports this knowledge.[8]

1. *Hematogenous route.* There is little question that coccal infections of the kidney result from hematogenous involvement from a primary focus such as a caruncle. It is unlikely, however, that organisms from the intestinal tract enter the blood stream and lodge in the kidneys, because coliform organisms are rarely found on blood cultures, and one must wonder why only the renal parenchyma

would be involved. Bacteremia is frequently associated with instrumentation on the lower urinary tract,[9] and may initiate renal infection, particularly when the kidney has been damaged either by pre-existing parenchymal disease or some type of obstructive uropathy.

Experimental pyelonephritis may be produced in the laboratory animal by massaging the kidney and then injecting organisms intravenously.[10] Similar infections have been produced in the partially obstructed kidney.[11] Recently, Guze, Goldner, Finegold, and Hewitt[12] reported the production of pyelonephritis in unobstructed kidneys in rats, using *Streptococcus faecalis.* This finding in the experimental animal would lend credence to the belief that in man the hematogenous spread of organisms can produce pyelonephritis in normal kidneys if a sufficient number of bacteria localize therein.

2. *Ascending route.* Contamination of the bladder by organisms entering through the urethrae undoubtedly occurs, particularly in females, who have short urethrae. Upper urinary tract infection may occur by way of ureterovesical reflux secondary to inflammatory changes at the ureterovesical junction, or because of obstruction at the outlet of the bladder, which thereby necessitates increased intravesical pressure in voiding. Recently, Vivalde, Cotran, Zangwill, and Kass[13] produced in rats the experimental prototype of this route of infection; they used a strain of *Proteus vulgaris.*

3. *Lymphogenous route.* There are no well-defined direct lymphatic connections between the colon and the kidneys.[14] Lymphatic drainage of the ureter is segmental, and whether bacteria may or may not ascend via the lymphatics from the bladder to the kidneys is not known. Recently, Murphy[15] placed carbon particles in the bladders of dogs and studied the migration of these particles at various levels of intravesical pressure. He found that if the intravesical pressure remained at 40 cm. of water, the carbon particles remained in the bladder; but, if it was increased to 70 cm., carbon particles were seen in the periureteral tissues and in the lymph nodes around the renal pelvis.

4. *Direct extension.* Direct extension of infection to the kidneys from surrounding inflammatory tissue undoubtedly occurs, but probably only rarely and does not play a significant role in the pathogenesis of pyelonephritis.

## Factors That Influence the Course of Pyelonephritis

*Specific nephrogenicity of bacteria.* Little is known about the specific nephrogenicity of certain bacterial strains but, knowing that in nearly all types of infection there are strains of high and low virulence, it is thought that this principle may apply to bacteria that enter the urinary tract.

*Genetic predisposition.* The frequent occurrence of urinary tract infection in children with congenital abnormalities of the urinary tract is well known. Recently, certain microscopic congenital abnormalities were reported,[16] such as congenital immaturity of a nephron and microscopic dysplasia. These abnormal foci may be areas that are particularly susceptible to the infecting organism.

*Specific host susceptibility.* Host resistance plays an extremely important part in urinary tract infections, as most infections are a delicate balance between states of host resistance and growth of organisms. The presence of potent nonspecific antibacterial substances has been demonstrated in the normal urine.[17] Rowley[18] recently demonstrated that complement fixation may be an important natural inhibitor of bacterial multiplication. The fourth component of the complement is inactivated by ammonia. Since the renal medulla produces ammonia from glutamine, this may explain why the medullary areas of the kidneys are especially susceptible to infection. There is no specific evidence of renal damage due to specific antigen-antibiotic reactions or an autoimmune phenomenon, but in sensitized animals, bacterial endotoxin will produce severe renal lesions.[19]

*Functional defects.* Certain functional demands placed on the kidneys would seem to increase their susceptibility to infection. The breakdown products of many drugs are excreted by the kidneys, and there is some evidence to suggest that the presence of these products may increase susceptibility to the infecting organism.[20]

*Acquired structural defects.* The structural changes associated with obstructive uropathy or renal parenchymal disease of an acquired nature predispose the urinary tract to infection.

## Laboratory Aids in Diagnosis

The patient with pyelonephritis whom the physician is usually called upon to treat is the one who has overt signs of urinary tract infection such as urgency, frequency, dysuria, pain in the costovertebral angle (with or without chills and fever). The diagnosis in such a case is not difficult to make because unmistakable urinary abnormalities usually are present. An asymptomatic patient with urinary abnormalities presents the greatest problem in diagnosis. In such a case it may be necessary to utilize several laboratory procedures to rule out or to confirm the diagnosis of pyelonephritis.

*Urinalysis.* The first morning-voided specimen is the best to examine, as it has been in the bladder overnight and any bacteria present will have multiplied.[2] Proteinuria is usually not so severe in pyelonephritis as it is in glomerulonephritis, because there is little initial glomerular damage. Pyuria is frequently present, although recognition of it is not necessary for a diagnosis of pyelonephritis. Pyuria, arbitrarily defined as the presence of more than from 3 to 5 leukocytes per high-power field in the urine, was present in 84 per cent of 253 randomly selected patients with more than 100,000 bacteria per milliliter of urine.[21] In patients with chronic pyelonephritis the incidence of pyuria is between 30 and 50 per cent.[7] Few casts are usually found in the urine, for although the tubules are dilated and filled with colloidal material they are also obstructed, and the casts cannot enter the urine. The gram stain or methylene-blue stain of the urinary sediment, for bacteria, is an extremely valuable diagnostic procedure. It

is usually positive for bacteria when 100,000 or more organisms are present per milliliter of urine;[2] but, unfortunately, an intrinsic error of from 20 to 30 per cent exists even in the most skillful hands.[21] The Addis count is a good tool for quantitating the sediment and may be helpful in some cases in differentiating glomerulonephritis, nephrosclerosis, and pyelonephritis.[22]

*Glitter-cell phenomenon.* Sternheimer and Malbin[23] developed a special stain (gentian violet-safranin) that facilitates recognition of glitter cells. These cells are polymorphonucleated leukocytes that stain a pale blue and contain granules in the cytoplasm which exhibit Brownian movement. Normal polymorphonucleated leukocytes immersed in a hypotonic solution will become glitter cells, and when returned to isoosmolarity they again become normal. It would seem that the osmolarity of the urine plays a role in the formation of the glitter cell. However, osmolarity is not the only factor in the production of glitter cells, as hypertonic urine may contain them, and it is undoubtedly a combination of hypotonicity and decreased resistance of the leukocyte to changes in osmolarity that produces the characteristic leukocyte.[24] Glitter cells are frequently present in the urine of patients with pyelonephritis, but they may also be seen elsewhere in the urinary tract in inflammatory disease such as prostatitis, so they are not pathognomonic for pyelonephritis. The presence of glitter cells should make one think of pyelonephritis, but their absence should not exclude consideration of this diagnosis.[25]

*Griess's test.* The majority of organisms producing urinary tract infection will reduce to nitrites the nitrates normally present in the urine. Griess's test will show the presence or absence of such organisms. The Griess agent contains sulfanilic acid, acetic acid, and alpha-naphthylamine. When the agent is added to the urine containing nitrite, it rapidly turns red because of the formation of azo-alpha-aminonaphthaline parabenzine-sulfonic acid. Uninfected urine contains no nitrite and does not change in color. To be reliable, a screening test must be sensitive and must elicit a low incidence of false-positive, and false-negative reactions. The chief drawback with Griess's test is its insensitivity:[26] a significant bacilluria can be present and a Griess's test may be negative.

*Urine cultures.* In the standard culture of the urine there is always the problem of determining whether or not the organism obtained in the culture is a contaminant or a true pathogen. The introduction of the quantitative urine culture, a technic that has long been in use by the United States Public Health Service for bacterial counts in contaminated water, presents a method by which true bacilluria can be differentiated from contamination.[27]

The technic is roughly as follows: a 1-ml. specimen of urine is appropriately diluted (depending on the number of organisms counted on the stained sediment) and is incorporated into a pour plate of 9 ml. (or more depending upon the dilution) of a general culture medium such as trypticase soy agar. After 24-hour incubation the colonies are counted and, assuming that each organism is viable and produces a colony, a quantitative estimation of the number of bacteria per

milliliter of urine is calculated. In careful studies it has been found that significant bacilluria is present when 100,000 or more bacteria are found in 1 milliliter of urine. When less than 1,000 organisms per milliliter of urine are present, they are contaminants; organisms present in the range from 1,000 to 10,000 per milliliter are of variable significance.[7] Certain limitations must be recognized, for even in the presence of severe pyelonephritis, bacterial counts less than 100,000 per milliliter of urine may be obtained if: (1) a bacteriostatic agent is in the urine; (2) the patient is in the midst of a water diuresis; (3) the discharge of bacteria into the urine from the kidneys is small in number and multiplication in the bladder has not had time to take place; and (4) there is obstruction of the ureters and no organisms enter the urine in the bladder.

*Antibody-hemagglutination response.* The diagnosis of specific infectious diseases (not in the urinary tract) has been accomplished by the identification of antibody titers against the etiologic agent. This method has not been successfully used in urinary tract infections either because the antibodies were not formed or the methods used were not sensitive enough to register the antibody response. Erythrocytes modified by bacterial antigens are more sensitive agents for the detection of bacterial antibodies than is the standard agglutination test. The antibody-hemagglutination method has been used in patients with urinary tract infections with some success. Erythrocytes from the patient are mixed with a dilution of a specially prepared bacterial antigen which is adsorbed on the erythrocyte. Then the hemagglutination test is performed, using the patient's serum at various dilutions from which the antibody titer is obtained. The technic may be useful in urinary tract infections to identify the specific antibodies isolated on cultures, thus confirming the pathogenicity; and also in subsequent antibody titers to evaluate the effectiveness of therapy.[28]

*Needle biopsy of the kidney.* Although pyelonephritis is frequently due to a focal lesion and sometimes does not involve the entire kidney, valuable information may be obtained by biopsy of the kidney with the Vim-Silverman needle. At the time the renal biopsy specimen is obtained, a culture is also made from the needle. This technic may be extremely useful in selected patients.[29]

## Laboratory Aids in Treatment

The key to successful therapy of pyelonephritis is the selection of the appropriate antimicrobial agent. It is well at this time to review the various methods of determining in vitro sensitivity, and the reliability of the tests in determining in vivo effectiveness.[30,31]

The agar diffusion method of determining sensitivity consists of placing an antibiotic-impregnated disk on the surface of an agar plate cultured with the specific microorganisms. The antibiotic diffuses over the surface of the plate, and the size of the clear zone surrounding the organic colony is used as a measure of the inhibitory power of the antibiotic being tested. The individual disks contain various concentrations of the antibiotic, registering various degrees of sensitivity.

The chief drawback of this method is the inaccuracy that is largely due to a failure to standardize the concentration of antibiotics in the disk. Moreover, the matrix in which the antibiotic is contained has variable binding and releasing properties, so that diffusion is not uniformly even. There is a great need to standardize these disks, and Branch, Starkey, and Power[32] are working toward this goal.

The agar dilution method involves the use of various concentrations of antibiotics that are incorporated into pour plates. Several organisms can then be tested on one plate. This method is accurate but, unfortunately, is cumbersome and therefore not in widespread use.[33]

The tube dilution method utilizes a series of tubes containing various dilutions of antibiotics in a broth medium. The tubes are inoculated and incubated for a 24-hour period. A lack of growth is indicated by absence of turbidity. This is by far the most accurate method at the present time to determine sensitivity of a patient to various antibiotics.

The rapid hemoglobin-reduction method[34] of determining sensitivity is a technic that consists of seeding the organisms in a tube of melted and cooled brain-heart infusion agar plus dextrose. This solution is poured into Petri dishes containing a thin layer of heart infusion plus 20 per cent citrated blood. Impregnated disks are placed on the surface and the preparation is incubated. Prior to the appearance of visible growth, most pathogenic strains of organisms will develop relatively intense reducing conditions within the medium, which are reflected in the reduction of the hemoglobin and therefrom a change in the color of the blood. Inhibition of growth causes no change in color of the blood, and zones of inhibition are therefore identifiable. Results are available in from two to four hours after inoculation.

There are many factors present in vivo that influence the effectiveness of antibiotics: (1) the rapidity with which the bacterial population multiplies; (2) the physical and chemical natures of the environment in which the organisms live, because blood, exudate, and other tissue fluids affect the drug; (3) the amount of the drug in the area in which the bacteria are located; and (4) the extent of the antimicrobial defenses of the host. In spite of these many factors, a correlation between in vitro sensitivity and in vivo results is surprisingly accurate, particularly in acute, uncomplicated infections, and it becomes progressively less accurate in chronic and complicated infections.[35]

## Conclusion

The discrepancy noted—between the incidence of severe pyelonephritis demonstrated at autopsy and that of the antemortem diagnosis of pyelonephritis—suggests the existence of a large number of patients with asymptomatic pyelonephritis. The incidence of this disease and the difficulty with which the diagnosis

is made in these asymptomatic patients clearly indicate that all diagnostic procedures available should be utilized. A brief review of the laboratory aids in the diagnosis and treatment of pyelonephritis is presented, and it is hoped that the utilization of these tests will facilitate the detection of asymptomatic pyelonephritis and assist in the therapy of this important renal disease.

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