Modern Bacteriological Concepts in the Treatment and Diagnosis of Urinary Tract Infection.
References are indicated in the text by bracketed numbers, e.g. "(14)", and are listed at the end in alphabetical order.
The urinary tract is subject to infection by many different micro-organisms and throughout its length. The most important infections at the present time are those caused by the Gram negative bacilli. Other important infections are renal tuberculosis, gonorrhea and perinephric abscesses, which are usually caused by Staphylococci. In each of these infections the bacteriological laboratory has a part to play in the diagnosis. Renal tuberculosis is now rare, but the possibility must always be borne in mind. The only satisfactory way of establishing the diagnosis in this case is culture of a series of overnight urine specimens and examination of the Ziehl-Neelsen stained smear for the presence of acid- and alcholofast bacilli. The diagnosis of gonorrhea is usually evident on clinical grounds, but it is important to establish it firmly by taking a suitable specimen and growing the organism to study its morphology.

The infections caused by the Gram negative bacilli are: cystitis and pyelonephritis. Pyelonephritis is very common; it has been reported by Kass (13, 14) and a number of others (6, 20, 25) as being present in 15 - 20 % of post mortem examinations. It is repeatedly emphasised by these writers that only 20 - 30 % of the patients found to have pyelonephritis at post mortem were diagnosed as having it during life. Pyelonephritis is the commonest underlying lesion in uraemia and also appears to have some association with the occurrence of hypertension in later life. Certain factors are known to be associated with the high incidence of pyelonephritis. These are: diabetes mellitus, pregnancy and instrumentation of the urinary tract. Anatomical abnormalities of the urinary tract, such as horseshoe or polycystic kidneys, and physiological abnormalities such as vesico-ureteric reflux
Two are also known to predispose to this condition.

In over 40% of pyelonephritis cases, the infecting organism is found to be *Escherichia coli*. When other organisms such as the various species of *Proteus* and *Pseudomonas pyocyanea* are present, they are usually associated with some abnormality of the urinary tract, with instrumentation of it or with a mixed infection.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>498</td>
<td>(71%)</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>93</td>
<td>(13%)</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><em>Enterococci</em></td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><em>Ps. pyocyanea</em></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><em>Coliforms</em></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><em>Paracolons</em></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>Myco. tuberculosis</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>696</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE I.** Total positive urine cultures.
From Murdoch et al (24)

Previous antibiotic therapy may also be responsible for the appearance of these organisms. It has been shown that most of the *Escherichia coli* infections of the urinary tract are caused by a few serotypes, namely 02, 04, 06 and 075. (23, 26) Turck and Petersdorf are of the opinion that as these serotypes are more common in non-enteric infections than the other serotypes of *Escherichia coli*, they are probably more pathogenic than the other varieties (33). Mond et al (23) also suggest
that some serotypes have a specific affinity for the urinary tract.

The usual criterion for the diagnosis of urinary tract infection is the finding of bacteriuria. This is indicative of the multiplication of bacteria in the urine. Bladder urine is a very good culture medium for most Gram negative organisms, but is sterile in the normal person. This sterility is thought to be maintained by the presence of a certain degree of anti-bacterial activity within the bladder walls and also by the fact that the periodic emptying of the bladder, if complete, will wash away any bacteria which do accumulate. Thus, incomplete emptying of the bladder predisposes to infection. Infection may be localised to the lower urinary tract, producing the condition of cystitis, or, as Hanley prefers to call it, "the urethral syndrome". Upper urinary tract infection, pyelonephritis, produces actual damage to the renal tissue and is more significant than lower urinary tract infection in this respect. Patients with pyelonephritis frequently have a history of previous cystitis and Hanley (8, 9) has shown that vesico-ureteric reflux does occur, although transiently, in patients with cystitis. This could provide a route of infection from the bladder to the kidneys. There has been some controversy about the route by which infection reaches the kidneys. It has been shown experimentally on rats that both the ascending and haematogenous routes of infection are possible. Kass (17) has shown that quantitative culture of urine is of value in differentiating true bacteriuria from contamination of the specimen during collection. Examination of post mortem specimens has shown that more than 100,000 \(10^5\) bacteria per ml. of urine is associated with the presence of active pyelonephritis as judged by the presence of
polymorphonuclear leucocytes in the interstitial tissue of the kidney.

When the common urinary pathogens are cultured in vitro using sterile urine as the culture medium, they multiply rapidly. Variations in pH and specific gravity within the physiological ranges have little effect on the rate of growth. It may be assumed that similar multiplication can occur in vivo. In another study, Kass (13) shows that women can be divided into two groups on the basis of quantitative culture of their urine: one with counts of less than $10^4$ organisms per ml. and the other with over $10^5$ organisms per ml. The two groups overlap slightly between these values. The organisms present in the lower count specimens are usually diphtheroids, Staph. albus and other skin commensals; these organisms are thought to be contaminants. Esch. coli and the other urinary pathogens are rarely found in these low count specimens. When they are found, it is not usually in pure or even predominant culture. The high count specimens, on the other hand, frequently contain Esch. coli in pure culture and only rarely do the commensal organisms occur. High and low counts are usually easily reproducible from subsequent specimens unless treatment has been instituted. Specimens in the range $10^4 - 10^5$, where there is some doubt as to the significance of the quantitative count, usually fall into one or the other group when a second specimen is examined. Kass therefore suggests that quantitative bacterial counts in urine of over $10^5$ organisms per ml. should be considered significant and indicative of urinary tract infection.

One of the difficulties in obtaining a satisfactory specimen for quantitative bacteriological culture is ensuring
that it is free from contamination by skin commensal organisms. This is particularly difficult in women, and since the majority of patients with pyelonephritis are women, it constitutes a problem. The cleansing of the vulva with chlorhexidine or some other antiseptic solution before the urine is passed is the usual method of trying to ensure a satisfactory specimen. Murdoch et al (24) have shown that there is no statistical difference between the cleansing method and the non-cleansing or clean catch method described by Turner, in terms of obtaining either true or false positive results. Falsely low counts may be obtained when there is some antibacterial activity in the urine due to treatment or the rate of urine production is high with the result that the bladder is emptied frequently and there is insufficient time for multiplication to occur. (12) It might be suggested that in such situations, where the patient is producing large quantities of urine, the level of significance of bacterial counts may need to be revised.

Since urinary tract infections, particularly pyelonephritis, are so frequently asymptomatic, it will be appreciated that quantitative bacterial culture is of value in the diagnosis of previously unsuspected infections. Urinary tract infections are a well known complication of pregnancy. Various authors (11, 16, 34) report finding asymptomatic infection, as judged by the presence of significant bacteriuria, in between 5 and 11% of pregnant women at term. Further, quantitative bacterial counts can be used epidemiologically; for example, most pregnant women who develop bacteriuria have done so by about the second month of pregnancy (15, 17), at which time the radiographic changes in the urinary tract characteristic of pregnancy have not developed. This would tend to invalidate the theory that hormones in pregnancy cause dilatation of the
ureters and hence a greater tendency to reflux and ascending infection. Similarly, it is unlikely that by this stage the uterus should have become so large as to cause any significant obstruction of the ureters.

Kaitz and Hodder (11) have studied 50 pregnant women with insignificant bacteriuria (less than $10^5$ organisms per ml.), obtaining urine specimens at 12 - 16, 20 - 24, and 32 - 36 weeks gestation. In one case only of the 50 did the patient develop significant bacteriuria during this study. So it seems that if significant bacteriuria is not established before the second month, it is unlikely to occur during the later months of pregnancy.

Pregnant patients with bacteriuria are not significantly different from non-bacteriurics in respect of either their residual urine volume or their glucose tolerance tests. Nor is there any change in the normal vaginal flora in these patients. (15). There is no significant difference in the haematological indices of the two groups, and the prematurity and miscarriage rates are the same. However, they do show a drop in the urine concentrating ability early in pregnancy, which may indicate, even at that early date, of renal medullary damage (17). Asymptomatic significant bacteriuria has been demonstrated in all the high risk groups, i.e. diabetics, women with genitourinary anomalies such as cystocele, and in about 95% of all patients with an indwelling catheter which has been in situ for over 24 hours (13). Brumfitt, Davis and Rosser (5) found a higher incidence of significant bacteriuria in a group of patients who had been catheterised (9.1%) than in an uncatheterised group (4.7%). The incidence was even higher in a group of patients catheterised for some obstetric reason (22.8%) and
highest of all (41\%) in a group catheterised for urinary retention, which is a condition associated with an increased incidence of infection. This high incidence was found even when the bladder urine was sterile at the time of catheterisation.

There are two common ways of performing quantitative bacterial counts on urine. In one, 1 ml. of urine is added to the culture medium when it is poured and the number of colonies grown after 24 hours' incubation at 37°C is noted. This is the "pour plate" method. In the other method, serial tenfold dilutions of urine are made and a number of 0.1 ml. drops are allowed to fall on to the culture medium. After 24 hours' incubation at 37°C, the number of separate bacterial colonies grown from each dilution of urine can be noted. Knowing the dilution, the bacterial count can therefore easily be calculated. As these methods are very time-consuming, other quantitative methods have been suggested: for example, a standard size of loop can be used to plate-out uncentrifuged urine. This method is not so accurate as those previously described.

Leigh and Williams (21) have suggested a simpler method of performing quantitative counts. They use strips of sterile filter paper, \(\frac{1}{4}\)" wide with a \(\frac{1}{8}\)" 'foot' bent over at one end. This foot is dipped in the urine, drained by touching the side of the container and touched to the culture medium. They find that the number of organisms remaining on the surface of the paper depends on the porosity of the paper and is a constant for any one type of paper. Therefore, if a standard variety is used, the technique itself is standardised. They have plotted calibration curves for a number of urinary pathogens using quantitative counts done in the normal manner as a standard. It was found that all bacilli gave similar curves with quantitative counts of over \(10^5\) giving more than 25
colonies per inoculum by the filter paper method. This method is easy and quick to use and is also economical, as ten urines can be cultured in duplicate on the same plate. The significant level of 25 colonies can be easily counted in $\frac{1}{4} \times \frac{1}{2}$ area inoculated. It is noted that counts of over $10^6$ give confluent growth. Comparing this method with standard quantitative counts, it gives 0.7% false positives and 6% false negatives. It may therefore be useful as a screening test for large numbers of urines although final diagnosis should continue to be based on the standard methods of quantitative counting.

Sanford et al (27) suggest that direct microscopy of a stained urine smear may be of value in assessing the presence of bacteriuria. They find that if 1 - 2 organisms are seen per oil-immersion field then the quantitative culture usually shows more than $10^3$ organisms per ml., and that more than $10^4$ organisms per ml. must be present before organisms are always seen on microscopic examination. This may be of some value in obtaining a rapid answer but can never be more than an impression as there is no finding consistently associated with the level of bacteriuria normally considered significant.

There is a need for some quick and easy test to demonstrate the presence of bacteriuria. Several such chemical methods, depending on the metabolism of the infecting organism's producing some change in a reagent have been suggested. Examples of these are the nitrite, the T.T.C. and the catalase tests.

The nitrite test is based on the conversion, by bacterial action, of nitrates to nitrites, the latter not being normally found in the urine. As originally described, this test consists
of adding the reagents alphanaphthylamine and sulphanilic acid to urine. If a pink colour is produced, there is nitrite present in the urine in a quantity greater than 1 mcg. (10). The accuracy of this test is dependent on the amount of nitrate originally present in the urine and available for metabolism. Smith and Schmidt (31) have obtained 92% accuracy using this test; they report this as being more accurate than originally claimed. They also note that the accuracy of the test is improved by the use of overnight urines; presumably there is more nitrate available in such specimens. Sleigh (30) has suggested a modification of this test, involving incubation of the urine with added potassium nitrate for 6 hours at 37°C. He reports that over 90% of urines negative to the standard nitrite test but with significant bacteriuria gave a positive modified test. All specimens which gave positive standard tests also gave positive modified tests. He claims that the modified test is about 97% accurate, giving about 1.6% false positives; Brumfitt and Percival (6), however, find that this test is only 61% accurate.

The T.T.C. test depends on the reduction by actively respiring bacteria of the colourless, soluble compound triphenyl tetrazolium chloride to the insoluble red compound triphenyl formazan. This reaction only occurs in alkaline solutions and therefore the reagent is made up in a saturated solution of disodium hydrogen phosphate. The reagent and urine are incubated together for 4 hours at 37°C and examined for the presence of a red precipitate. When the urine is highly coloured, it can be centrifuged after incubation and the supernatant fluid replaced by water. The test is not influenced by the presence of excess urobilinogen, nor by glucose or ketones. Simmons and Williams (29) report this test as being
94% accurate, with 2% false postives.

The catalase test consists of dipping a filter paper disc into urine and placing it in a test-tube containing hydrogen peroxide. Catalase produced by the bacteria on the filter paper disc reduces the hydrogen peroxide and the oxygen produced causes the disc to float to the surface. If flotation occurs in under 1 hour, the test is considered positive. Braude and Berkowitz (2) found that the test was positive in 80% of infected urines but also in 6 out of 12 non-infected urines. They point out that catalase is found in renal tissue as well as being produced by bacteria and therefore positive reactions may be found in other renal diseases.

These three screening methods give different degrees of accuracy in different hands. Kincaid-Smith et al (19) compared the three, using quantitative counts as a standard and found that the T.T.C. test is the most accurate.

<table>
<thead>
<tr>
<th>T.T.C.</th>
<th>Nitrite</th>
<th>Catalase</th>
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<tbody>
<tr>
<td>Accuracy</td>
<td>86%</td>
<td>35%</td>
</tr>
<tr>
<td>False Negatives</td>
<td>14%</td>
<td>65%</td>
</tr>
<tr>
<td>False Positives</td>
<td>8.3%</td>
<td>0.9%</td>
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TABLE II. Comparison of three screening tests for bacteriuria. From Kincaid-Smith et al (19)

None of these tests shows any consistency in results and therefore standard quantitative counting would still seem to be the diagnostic method of choice.
The urinary white cell excretion rate has been used as an index of infection of the urinary tract. More than 400,000 (4 x 10^5) cells per hour is considered abnormal. This excretion rate is found to be associated with more than 10 white cells per cu. mm. of urine. Less than 3 white cells per cu. mm. of urine indicates that the excretion rate is less than 4 x 10^5 white cells per hour. Between 3 and 10 white cells per cu. mm. of urine, the excretion rate may be above or below 4 x 10^5 white cells per hour; this uncertainty is produced by the varying volumes of urine which may be formed in one hour. (22). This test is subject to considerable observer error and gives a false positive rate of about 14% and a false negative rate of 48% (18). A modification of this test, provoking the excretion of white cells by the oral administration of 40 mg. of Prednisolone has been suggested. A positive result to this test is taken as being a 100% rise in excretion rate, ending above 4 x 10^5 white cells per hour. Although this test is difficult to interpret, it may be useful when other tests prove inconclusive.

Significant bacteriuria is only indicative of some infection within the urinary tract; it gives no indication of the site. The infection may be limited to the lower urinary tract or there may be renal involvement, i.e. pyelonephritis, even when only lower tract symptoms are present. Percival, Brumfitt and De Louvris (25) have studied serum antibody levels in urinary tract infections. They have shown that titres of over 1:640 are indicative of pyelonephritis. They studied 3 groups of patients: one group with no urinary tract symptoms and no bacteriuria, acting as a control group; a second group with symptoms of pyelonephritis and significant bacteriuria; and a third group with significant bacteriuria and lower tract or no
symptoms. In all cases, blood was examined for antibodies to the infecting organism (to any organism in the case of the first group) at the time of diagnosis and again 12 - 17 days and 6 weeks later. 95% of the control group were found to have titres of less than 1:320 and the remaining 5% had titres of 1:320. 93% of the pyelonephritic group had titres of over 1:320 and 83% were over 1:640. The authors therefore assume that the normal titre is less than or equal to 1:160. 18% of the asymptomatic group had titres of over 1:320, indicating renal tissue involvement. Operative specimens of renal tissue were obtained in 8 patients in the pyelonephritic group. In each of these cases, organisms were isolated from the renal tissue, the organisms found being those against which antibodies were detected. Histological examination of these specimens showed either acute or chronic pyelonephritis in 7 cases of the 8. The 8th case had a staghorn calculus and in this case, the titre was 1:160. Intravenous pyelography confirmed the presence of chronic pyelonephritis in 78% of the group clinically diagnosed as having pyelonephritis. It also showed the presence of chronic pyelonephritis in 4 out of 5 patients in the asymptomatic group who had titres of over 1:320. If the first blood specimen was obtained early enough in the course of the disease, it was possible to demonstrate a rising titre. Clinical and bacteriological cure of the disease was followed by a fall in antibody titre; where bacteriuria is persistent even after treatment, the titres remain high. On the basis of this study, titres of over 1:640 are considered definitely abnormal and indicative of renal tissue damage. Titres of under 1:160 make the diagnosis of pyelonephritis unlikely, although it was found in 2 such cases during this study. A titre of 1:320 is inconclusive but should arouse suspicion of renal tissue damage. It is not understood why antibodies are produced only
when renal tissue is involved in infection, but it is suggested that as bladder infection tends to be superficial and restricted to the trigone, there may be insufficient stimulus to antibody production.

Antibody studies have confirmed that medullary involvement is established early in pregnancy, as was suggested by Kass (17). Brumfitt and Percival (6) have shown that about $\frac{1}{3}$ of patients with asymptomatic significant bacteriuria of pregnancy have antibody titres of over 1:640 and Mond et al. (23) have shown significant antibody levels in 26% of patients with lower tract symptoms.

Sanford and Barnett (28), studying experimental pyelonephritis in rats, have shown that pre-immunisation to Esch. coli can produce a slight amelioration of both the incidence and severity of pyelonephritis produced by both the haematogenous and ascending routes.

The successful treatment of urinary tract infections by chemotherapeutic and antibiotic drugs is dependent on a number of factors. Firstly, the organism must be sensitive to the drug being used. This is determined in the laboratory by sub-culture of the organism isolated from the urine specimen. A simple means of determining whether or not an organism is sensitive to a drug is to place a filter paper disc impregnated with the drug on a culture plate inoculated from the primary culture. After overnight incubation at $37^\circ C$, the plate is examined for the presence of a zone of inhibited growth around the filter paper disc. If this is present, the organism is assumed to be sensitive to the drug. The size of the zone of inhibition is proportional to the sensitivity of the organism, and also to the solubility of the drug. A more accurate means of determining the sensitivity of an organism is to culture it in test-tubes to which doubling dilutions of antibiotic solution
have been added. The sensitivity of the organism is expressed in terms of the smallest amount of antibiotic which will inhibit its growth: the minimum inhibitory concentration (M.I.C.). The second requisite for satisfactory chemotherapeutic treatment is that the organism must be sensitive to the selected drug in concentrations which are easily obtainable in renal tissue and in urine, and this concentration is lower than that at which the drug will produce side effects. It is desirable whenever possible to use a bacteriocidal antibiotic rather than a bacteriostatic one. This is perhaps not so important in the treatment of cystitis as inhibition of growth may be sufficient to permit normal micturition to dispose of the infection. (4). This mechanism cannot be relied upon to eradicate infection in renal tissue, and therefore when treating pyelonephritis, a cidal antibiotic should always be used.

One possible method of controlling urinary tract infection is to change the pH and specific gravity of the urine so that the conditions become unsuitable for bacterial multiplication. Unfortunately, however, changes in the specific gravity within the physiological range have little effect on bacterial growth. Changes in pH do have some effect, but to be useful, the pH of the urine must be lowered below 5. This is very difficult to do.

Mandelic acid is an effective antibacterial agent when the pH of the urine is below 5.5 and the concentration of mandelic acid can be maintained between 0.5 and 1 %. Kass reports that this drug gives effective control of about 84 % of urinary tract infections. Most of the failures occurred in patients with some abnormality of the urinary tract. It is effective against a wide range of organisms but there is a tendency to
relapse in about 30%. If given in doses of over 12 G per day, it tends to produce gastro-intestinal symptoms and haematuria.

The suphonamides are widely used in general practice for the treatment of urinary tract infection. Murdoch (24) finds that 61% of Esch. coli are resistant to sulphonamides and hence condemns their use. Williams and his colleagues (34), however, find that 88% of the organisms which they isolate are sensitive to sulphadimidine in a concentration of less than 50 mcg. per ml. They therefore use sulphonamides regularly and achieve good results with this therapy. They quote cure rates, both clinical and bacteriological, of over 75% after 1 week's treatment. When sulphonamides fail, treatment with a second course of sulphonamides is unlikely to be effective, a cure rate of 20% being reported in this situation. They point out, however, that sulphonamides may be dangerous to use during pregnancy as they are bound to plasma proteins. This prevents the binding of unconjugated bilirubin. There is then a danger of the free bilirubin producing kernicterus in the foetus.

The penicillins are effective against enterococci, which are implicated only in a small number of urinary tract infections. Ampicillin is the drug of choice for infections caused by Proteus species, which are usually sensitive to it. Nitrofurantoin and cephaloridine are both valuable drugs when the organisms are sensitive to them. Like sulphonamides, tetracyclines are commonly used in general practice, with the result that there is a high incidence of resistance to these drugs. Murdoch considers colistin to be the drug of choice in the treatment of infections caused by Ps. pyocyanea. As Murdoch finds a high incidence of resistance to sulphonamides among the Esch. coli isolated from his patients, he prefers to use cycloserine for
the treatment of these infections. 4% of isolated organisms are resistant to this drug when the M.I.C. is 100 mcg. per ml. This concentration can be easily obtained in the tissues but even at this level, tends to produce side effects in 5% of patients to whom it is given. These side effects are, however, completely reversible when the drug is withdrawn.

Kanamycin is the drug of choice in the treatment of acute fulminating infections when the M.I.C. is less than 16 mcg. per ml. As this drug is oto- and nephro- toxic, the blood levels should be carefully monitored in all patients receiving it. These side effects are irreversible.

Kass (12) reports that during treatment with various antibiotics, 95% of the infections were eradicated. Other studies have shown similar figures. There is a high correlation between the sensitivity of organisms and their eradication during treatment, provided that the limits of sensitivity are set at suitable levels. A major problem in the treatment of pyelonephritis is the development during treatment of bacterial resistance to the drug employed. Some authorities think that this does not occur but Mond et al (23) report 4 cases being treated on a mixture of sulphonamides in whom the original organism was still present at the end of treatment, the M.I.C. having risen from less than 50 mcg. per ml. to over 200 mcg. per ml. In the series reported by Murdoch et al, it is noted that most of the patients had received sulphonamides, tetracycline or chloramphenicol before being referred to hospital. The efficacy of these drugs was not proven by the high incidence of persistent bacteriuria in these patients. It is noted that the lowering of bacterial counts, even when the urine is not sterilised, can often produce relief from dysuria and other lower tract
symptoms. It is therefore essential to perform follow-up quantitative counts after apparent clinical cure.

Urinary tract infections are notoriously liable to recur. In some cases, this reinfection is due to the same organism as the first infection, i.e. it is a relapse. In others, a different organism is implicated. In either case, there is likely to be some structural abnormality of the urinary tract. Repeated attacks of pyelonephritis leading to the chronic state are to be avoided if possible as it is with chronic pyelonephritis that hypertension and uraemia are associated. The relapse rate is reported to be 11% in uncomplicated acute infections, 36% in uncomplicated chronic infections and 74% in chronic infections complicated by an abnormality of the urinary tract. Because of this unfortunate tendency to relapse, Murdoch believes that long-term suppressive therapy is valuable in those patients who are at high risk. For this purpose, he uses cycloserine in 1/4 of the usual dosage and has reduced the relapse rate from 47% to 20%.

Braude, Siemienksi and Jacobs (3) suggest that, as relapses with the same strain of organism are common despite sterilisation of the urine having been achieved between infections, the organisms must persist in the urinary tract. They suggest that persistent forms must be some inactive form which is resistant to antibiotics and to the immune mechanisms of the body, is not detectable by normal culture methods and is capable of reconversion to the bacillary form under suitable conditions. They further suggest that this form may be the protoplast. Protoplast forms do not have a rigid cell wall and are not therefore protected from osmotic changes and undergo lysis in a hypotonic medium. Not having a cell wall, protoplasts are not affected by most antibiotics, by lysozyme
or by antibodies, all of which act on the cell wall. Protoplast formation can be induced in vitro in bacteria isolated from human urinary tract infections by exposure to penicillin in concentrations of $50 - 150$ mcg. per ml., concentrations which are easily obtainable in urine on standard dosages. Tetracycline will also cause protoplast formation in some strains of Esch. coli, P. mirabilis, K. pneumonia and A. aerogenes. None of the other antibiotics commonly used in the treatment of urinary tract infections produced protoplast formation in vitro. This conversion to protoplast forms can occur when hypertonic urine is used as the culture medium. Reconversion to bacillary forms can occur when the protoplasts are protected from osmotic lysis. This was shown by subculturing protoplasts obtained as described above on 2 media. One was the type of medium normally used in the laboratory; the second was a hypertonic modification of it. The number of colonies obtained from the protoplasts on the hypertonic medium was much greater than that obtained from the standard medium. Protoplast forms were shown to occur in the urine of a patient infected with P. mirabilis being treated with penicillin to which the organism had been reported sensitive. When therapy was stopped, gradual reconversion to the bacillary forms was demonstrated over the subsequent 31 hours. Protoplast formation was also induced in a patient, also with a P. mirabilis infection, by chloramphenicol, although this did not occur when the same strain was tested in vitro. It seems that protoplasts can survive phagocytosis and can grow and divide intracellularly. Alderman and Freedman (1) think that the production of protoplasts in vivo undoubtedly represents a means by which bacteria are destroyed in isotonic tissues, since lysis would occur in such regions. The renal medulla, being hypertonic, is ideally suited to the survival of protoplasts. Experimentally they have recovered more than $10^3$ Esch. coli from the test kidneys of 11
out of 17 animals which had been injected with protoplasts. In only 2 of these cases were organisms recovered from the non-injected kidney and in both of these cases large numbers of organisms were isolated from the test kidney. Guze and Kalmanson (7), studying experimentally induced pyelonephritis in rats, cultured a homogenate of renal tissue in normal and hypertonic media after the animals had been treated with penicillin. They found much higher isolation rates in the cultures grown in the hypertonic medium. They suggest that protoplast formation had occurred during treatment. They consider that lysozyme, antibodies and complement, all of which are known to induce protoplast formation, may be responsible for the maintenance of the protoplast state intrarenally. It is not known whether the protoplasts themselves are capable of producing renal damage or whether reconversion to bacillary form must occur first; certainly the lesions of pyelonephritis are commoner in the medulla.

It is suggested that reduction of the osmolarity of the renal medulla by a forced water diuresis may produce lysis in protoplasts lingering there. This remains to be studied, but if it is found to be true, then a forced water diuresis may become standard practice towards the end of a course of chemotherapy.

There may in fact be some evidence in favour of this hypothesis already: Smythe (32) has shown that a water diuresis reducing the osmolarity of the renal medulla in rats from 1200 milliOsmols per litre to 600 milliOsmols per litre reduces the susceptibility of these animals to experimental infection. Smythe suggests that this may be due to increasing the activity of the leucocytes in the medulla as their activity is reduced
in hypertonic surroundings. It has already been pointed out, however, that protoplasts seem to be able to survive phagocytosis. The reduced susceptibility to infection shown in this study may therefore be due more to providing an unsuitable medium for protoplasts than to increasing the leucocytic activity.
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